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Research report

The role of the habenular complex in the elevation of dorsal raphe nucleus serotonin and the changes in the behavioral responses produced by uncontrollable stress

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Abstract

Previous research indicates that the serotonergic neurons of the caudal dorsal raphe nucleus (DRN) are activated to a greater degree by inescapable shock (IS) as compared to escapable shock (ES), causing a greater release of serotonin (5-HT) in the DRN and in target regions. This differential activation is necessary for the behavioral changes that occur after exposure to IS, but not to ES (i.e. learned helplessness/behavioral depression). Although the critical role of the DRN in learned helplessness is clear, the neural inputs to the caudal DRN which result in this selective activation are unknown. One structure that may be involved in the activation of the DRN and the induction of learned helplessness/behavioral depression is the habenular complex. In experiment 1, habenula lesions eliminated the differential rise in DRN extracellular 5-HT levels in response to IS and ES exposure by severely attenuating the rise in 5-HT for both groups. In experiment 2, sham operated and habenula lesioned rats were exposed to either ES, IS or no stress (home cage control; HCC). Twenty-four hours later, sham rats previously exposed to IS exhibited longer escape latencies as compared to both ES and HCC rats (i.e. learned helplessness). The habenular lesion eliminated the differences in escape latency between groups, thus eliminating the induction of learned helplessness/behavioral depression. These results suggest that the habenula is necessary for the differential activation of the DRN and the escape deficits produced by IS.

Theme: Neural basis of behavior

Topic: Monoamines and behavior

Keywords: Habenula; Dorsal raphe nucleus; Serotonin; Stress; Learned helplessness; Escape learning

1. Introduction

The degree of behavioral control that an organism has over a stressor is an important factor in determining the nature of the physiological and behavioral reactions that follow [2]. Exposure to uncontrollable stressors produces a broad constellation of behavioral changes that do not occur in animals who have had control over the aversive event. For example, animals exposed to uncontrollable stressors subsequently exhibit an impaired ability to escape from an aversive event [29,14], increased fear conditioning [28], increased anxiety [34], a potentiated response to addictive drugs such as morphine [42], altered pain sensitivity [37,38], and decreased aggression [43]. The set of behavioral and physiological changes which occur in response to uncontrollable, but not controllable, stressors, has been referred to as learned helplessness [23] and behavioral depression [41].

Although a variety of neurotransmitter systems have been implicated in the mediation of uncontrollable stressor phenomena [19,41,4,31,32], recent work in our laboratory has focussed on serotonin (5-HT). A variety of evidence suggests that the occurrence of learned helplessness involves a selective activation of serotonergic neurons in the dorsal raphe nucleus (DRN) by uncontrollable stressors,

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with a consequent elevation of extracellular 5-HT within the DRN. This is indicated by the findings that inescapable shock (IS) induces greater cFos immunoreactivity in 5-HT neurons in the caudal region of the DRN than does equivalent escapable shock (ES; [11]), as well as greater 5-HT efflux as assessed by in vivo microdialysis [26]. Moreover, this activation of the serotonergic neurons in the caudal DRN appears to be necessary to produce the behavioral sequelae that follow IS. Both lesions of the DRN [22] and pharmacological inhibition of DRN serotonergic activity during IS blocks the occurrence of the behavioral changes that normally follow IS [21,24]. This intense activation appears to sensitize these caudal DRN neurons so that subsequent input produces an exaggerated release of 5-HT in projection regions [1]. This exaggerated release of 5-HT into the projection regions of the caudal DRN at the time of subsequent behavioral testing appears to be critical in producing the behavioral consequence of IS, as pharmacological blockage of this 5-HT release at the time of testing prevents the occurrence of helplessness behaviors [24].

Although the evidence suggests that selective DRN activation by uncontrollable stressors is critical, the neural inputs to the caudal DRN which result in this selective activation are unknown. Structures that supply excitatory amino acid input to the DRN are likely candidates since the intra-DRN microinjection of excitatory amino acid antagonists blocks the behavioral effects of IS [10]. The habenular complex is one such structure. The habenula has been shown to project to the DRN [17,3,30], and this projection appears to utilize the excitatory amino acid aspartate as a transmitter [17,16,15]. As would be expected, electrical stimulation of the lateral habenula excites DRN neurons [8], and has been shown to increase extracellular 5-HT levels in DRN projection areas [15]. This increase in 5-HT levels can be blocked by transecting the habenular–raphe pathway or by infusing the excitatory amino acid antagonist kynurenic acid into the DRN [15]. In addition, neurons in the habenula are responsive to peripheral nociceptive stimulation [9], and cFos (either protein or mRNA) is elevated in the habenula after exposure to a stressor [44,13,5]; although see Ref. [6]. The present study, therefore, examined the role of the habenular complex in the efflux of 5-HT in the DRN and the interference with escape behavior produced by IS.

2. Materials and methods

2.1. Experiment 1: The role of the habenula in the rise of 5-HT in the DRN during exposure to IS and ES

2.1.1. Subjects and surgery

The subjects were male Sprague–Dawley rats (225–250 g; Harlan Laboratories) and were maintained on an ad libitum food and water schedule. After 1 week of acclimatization, one half of the rats received electrolytic lesions of the habenular complex, while the other half served as sham operated controls. For the lesioned group, rats were anesthetized with halothane and a small hole was drilled through the skull directly dorsal to the site of lesion. An electrode was lowered to the level of the habenula bilaterally (AP: −3.7; LM: ±0.5; DV: −4.3 mm with respect to bregma), and current was passed (1 mA for 10 s). Sham operated rats were treated as above except no current was passed. In addition, all rats were implanted with guide cannula aimed just dorsal to the caudal aspect of the DRN (AP: −8.2; LM: 0.0; DV: 5.1 mm with respect to bregma at a 0° angle). Dummy probes were inserted into the cannula which then were anchored to the skull using skull screws (4) and dental acrylic. Screw caps of 15 ml Eppendorf tubes were also fastened with acrylic to allow protection of the microdialysis assemblies during treatment. Rats were placed in a recovery chamber and monitored until the anesthetic dissipated.

2.1.2. Microdialysis and behavioral procedure

After 1 week of recovery, the dummy probes were replaced with CMA 12 microdialysis probes (0.5 mm in diameter; M_r cut-off, 20 000 Da; with membranous tip length of 1 mm; CMA/Micro dialysis, Acton, MA) such that the membranous tip of the probe extended beyond the end of the cannula and entered into the DRN. Twenty-four hours later, a portion of the 15-ml Eppendorf tube was screwed onto the mounted screw cap, through which the dialysis tubing entered and attached to the probe. This allows for protection of the probes and connectors during IS and ES treatment. Rats were then placed in separate infusion bowls (BAS, IND) and continuously infused with Ringer's irrigation fluid, with a flow rate of 0.8 μl/min using a Baby bee pump (BAS, IND).

After a minimum of 2 h to allow for stabilization, samples were collected every 20 min throughout the experimental day. At no point during any of the procedures were the infusions stopped or interrupted. After stabilization, four baseline samples were taken and then rats were moved to the shock room and given either IS or ES, as described below. The rats were then placed back into the infusion bowls in the original room and four samples were collected during the next 80 min. Each sample was immediately frozen at −80°C until analyzed. At the end of the day the dialysis tubing was unhooked, and the rats were returned to their home cages.

For both IS and ES exposure, each rat was placed into a Plexiglas wheel-turn box (14×11×17 cm). A wheel consisting of two 14-cm disks connected by 9-cm metal rods was mounted on the front wall. The rats tail protruded through the rear wall and was secured with tape to a rod extending from the box. Wheel turn boxes were placed into sound attenuating chambers, and shock electrodes were attached to the tail.

Rats received shocks in yoked ES–IS pairs such that the

shock began simultaneously for both rats in a pair and terminated for both rats when the escape requirement was met by the ES rat. In this manner both rats received the same pattern of shock in terms of duration, intensity, and intertrial interval, and differed only in terms of control (ability to escape shock) over the stressor. Initially, the shock could be terminated by a one-quarter turn of the wheel. When each of three consecutive trials were completed in less than 5 s, the response requirement was increased by a one-quarter turn. Subsequent latencies under 5 s increased the response requirement by 50% until a maximum of four full turns of the wheel were required to terminate the shock. If a trial was not completed in less than 5 s, the requirement was reduced. If the requirement was not reached in less than 30s, the shock was terminated and the response requirement was reduced to a single one-quarter turn of the wheel. The procedure was used to insure that the ES rat could not terminate the shock by a reflexive movement elicited by shock onset and instead had to learn an operant response. Sessions consisted of 100 trials (average ITI at 60 s), with a shock intensity of 1.0 mA.

2.1.3. 5-HT analysis

5-HT was measured by electrochemical detection using a BAS LC-4C Amperometric Controller in combination with a Unijet glassy carbon electrode (3 mm) directly connected to a Unijet stainless steel microbore column internally lined with fused silica (ODS, C_{18}, 3 μm, 100× 1.0 mm; BAS, MF-8949). The oxidation reduction potential was 0.65 V. The mobile phase (pH adjusted to 3.62) was 0.09 M citric acid, 0.07 M sodium phosphate, 0.10 mM ethylenediamine tetraacetic acid, 2.62 mM octane sulphonic acid, 10 mM sodium chloride and 13% methanol. The flow rate of the pump (BAS PM-80) was 70 μl/min. Quantitative determinations were made by comparisons with appropriate external standards.

2.1.4. Histological analysis

To verify cannulae placement and extent of habenula lesion, rats were anesthetized with 65 mg/kg sodium pentobarbital and transcardially perfused with heparinized saline. The skulls were then removed and partially dissected to expose the encased brain to 10% formalin–30% sucrose for at least 5 days. The brains were then removed from the skulls and cryostat sectioned (30 μm) at −20°C. Sections were mounted on gelatin-treated slides, stained with cresyl violet, and cover slipped to allow assessment of placement and lesion using light microscopy.

2.1.5. Data analysis and statistics

Rats with probe placement with less than 70% of the probe tip within the DRN were excluded. For the habenular lesion, rats with unilateral lesions, or lesions that extended substantially beyond the bounds of the habenular complex, were excluded. Extra care was taken to exclude rats whose lesion encroached into the dorsal medial nucleus of the thalamus (see Fig. 1). The final sample sizes for each group (ES–Sham; IS–Sham; ES–Lesion; IS–Lesion) were 11, 11, 10, and 10, respectively. Basal levels of 5-HT were calculated for each subject by averaging together the baseline samples collected before the introduction of the stressor, and expressed as pg per 5-μl sample. In order to detect differences in basal levels, a 2×2 ANOVA was performed with Lesion (sham vs. lesion) and Stressor type (ES vs. IS) as factors. Neurotransmitter levels before, during and after stressor exposure were expressed as percent of baseline for each subject. A 2×2×

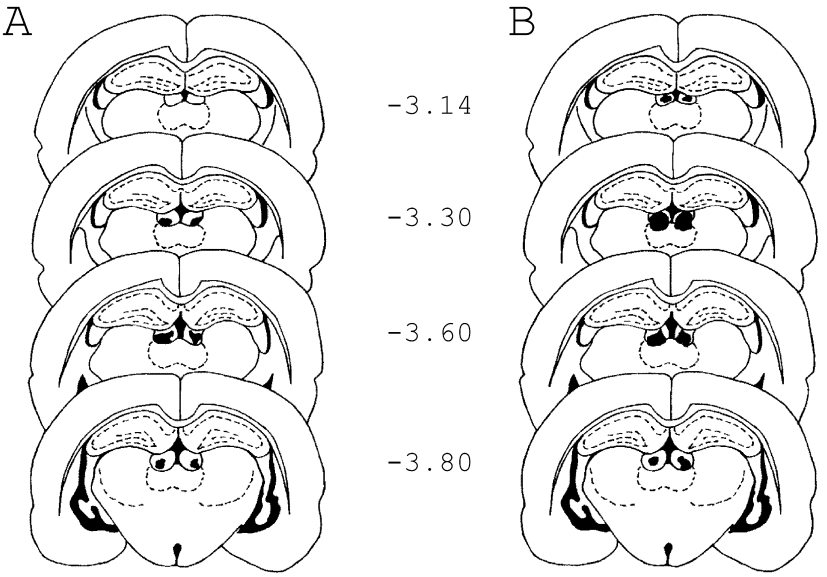

Fig. 1. Schematic drawing of the smallest (A) and largest (B) habenula lesion. Anterior–posterior coordinates are in millimeters with respect to bregma. See text for details.

12 ANOVA with Stressor (ES vs. IS), Lesion (Sham vs. Lesion) and the repeated measure Sample (BL1 through PS3) was performed, and was followed by secondary ANOVAs or the Fisher's PLSD post-hoc test, when appropriate.

2.2. Experiment 2: The role of the habenula on escape deficits produced by prior IS

2.2.1. Subjects, surgery, and paradigm

Male Sprague–Dawley rats were housed and habenula lesioned as described in experiment 1. One week after surgery, lesioned and sham operated animals were divided into three groups, two of which were exposed to either IS or ES as described in experiment 1. The third group was simply handled and placed back into their home cage (HCC).

2.2.2. Shuttlebox testing

Escape testing was conducted in a shuttlebox 24 h later. The floors were constructed of stainless steel rods, 0.3 cm in diameter and spaced 1.4 cm apart, while the end walls were aluminum and the side walls and tops were clear Plexiglas. An aluminum wall with a 5.5×7.5 cm archway cut out of it divided the shuttleboxes into two equal compartments. Scrambled shocks (ITI 60 s, 0.8 mA) were delivered to the grid floors by shockers modeled after the Grason–Stadler model 700. The first 5 trials were FR-1 escape trials, in which the rat was required to move from one side of the shuttlebox to the other in order to terminate the shock. The remaining 25 trials were FR-2 escape trials, in which the rat had to run from one side of the box to the other, and then back again in order to terminate the shock. Shocks were terminated automatically after 30s if escape had not occurred. IS induced escape deficits typically are revealed during the FR-2 trails, but not the FR-1 trials [25].

2.2.3. Histological analysis

After shuttlebox testing, lesioned rats were perfused with saline. The brains were fixed in 10% formalin, 30% sucrose solution for at least 3 days, and then were removed and cryostat sectioned (30 μm) at −20°C. Sections were mounted on slides, stained with cresyl violet, and cover-slipped. Lesion placement accuracy was verified using light microscopy. Data from rats not found to have bilateral lesions restricted to the habenular complex (both lateral and medial habenula) were excluded.

2.2.4. Data analysis and statistics

As in experiment 1, rats with unilateral lesions, or lesions that extended substantially beyond the bounds of the habenular complex, were excluded. The final sample sizes for each group (ES–Sham; IS–Sham; HCC–Sham; ES–Lesion; IS–Lesion; HCC–Lesion) were 17, 17, 15, 8, 8, and 10, respectively. For each subject, shuttlebox behavior was expressed as the average latency for every five escape trials. Thus, the first set reflects the latency for the FR-1 trials, and the remaining five sets reflect the behavior on FR-2 trials. The data for the FR-1 and FR-2 trials were analysed separately. For the FR-1 data, a 3×2 ANOVA with Stressor (ES, IS, and HCC), Lesion (Sham vs. Lesion) was performed. For the FR2 data, a 3×2×5 ANOVA was performed with the additional repeated factor Trial (2–6). The overall ANOVAs were followed by secondary ANOVAs or the post-hoc test Fisher's PLSD, when appropriate.

3. Results

3.1. Experiment 1

3.1.1. Histological analysis

The schematic drawing of the largest and smallest extent of habenular damage is presented in Fig. 1. The damage included both the lateral and medial nuclei, and in most cases, did not extend beyond the extent of the habenular complex. In some cases (<30%), damage extended into the dorsal medial nucleus of the thalamus. In these cases, damage only extended into the very dorsal aspect of this nucleus. In a very few cases ($n=2$), damage extended into the ventral medial aspect of the dorsal hippocampus, directly dorsal to the habenula. The results in animals whose lesion extended outside of the habenula did not differ from that of animals with lesions restricted to the habenula. For the placement of the dialysis probe into the DRN, animals for whom the 1-mm probe tip was not at least 70% within the confines of the DRN were excluded from analysis. Therefore, probe placement did not vary along the lateral–medial extent of the DRN. All probes were placed in the intermediate or caudal region of the DRN (approximately from −7.8 to −8.3 with reference to bregma).

3.1.2. 5-HT analysis

Basal levels of 5-HT in the DRN were not affected by the damage to the habenular complex. The basal levels of 5-HT were 0.380±0.119 pg for the ES–sham group, 0.456±0.153 pg for the IS–sham group, 0.730±0.267 pg for the ES–lesion group and 0.342±0.095 pg for the IS–lesion group. A 2×2 ANOVA showed that neither the main effects of Lesion [$F(1,32)=0.421$, $P=0.5210$] or Stressor [$F(1,32)=0.744$, $P=0.3948$], nor the two-way interaction [$F(1,32)=1.825$, $P=0.1862$] were significant.

The effect of the stressor on the levels of 5-HT in the DRN for Lesioned and Sham operated rats is presented in Fig. 2. For Sham animals, ES and IS exposure produced a rapid and large increase in the level of extracellular 5-HT in the DRN (Fig. 2A). However, levels of 5-HT decreased to nearly basal levels as the shock continued in the ES condition. In contrast, extracellular levels of 5-HT were

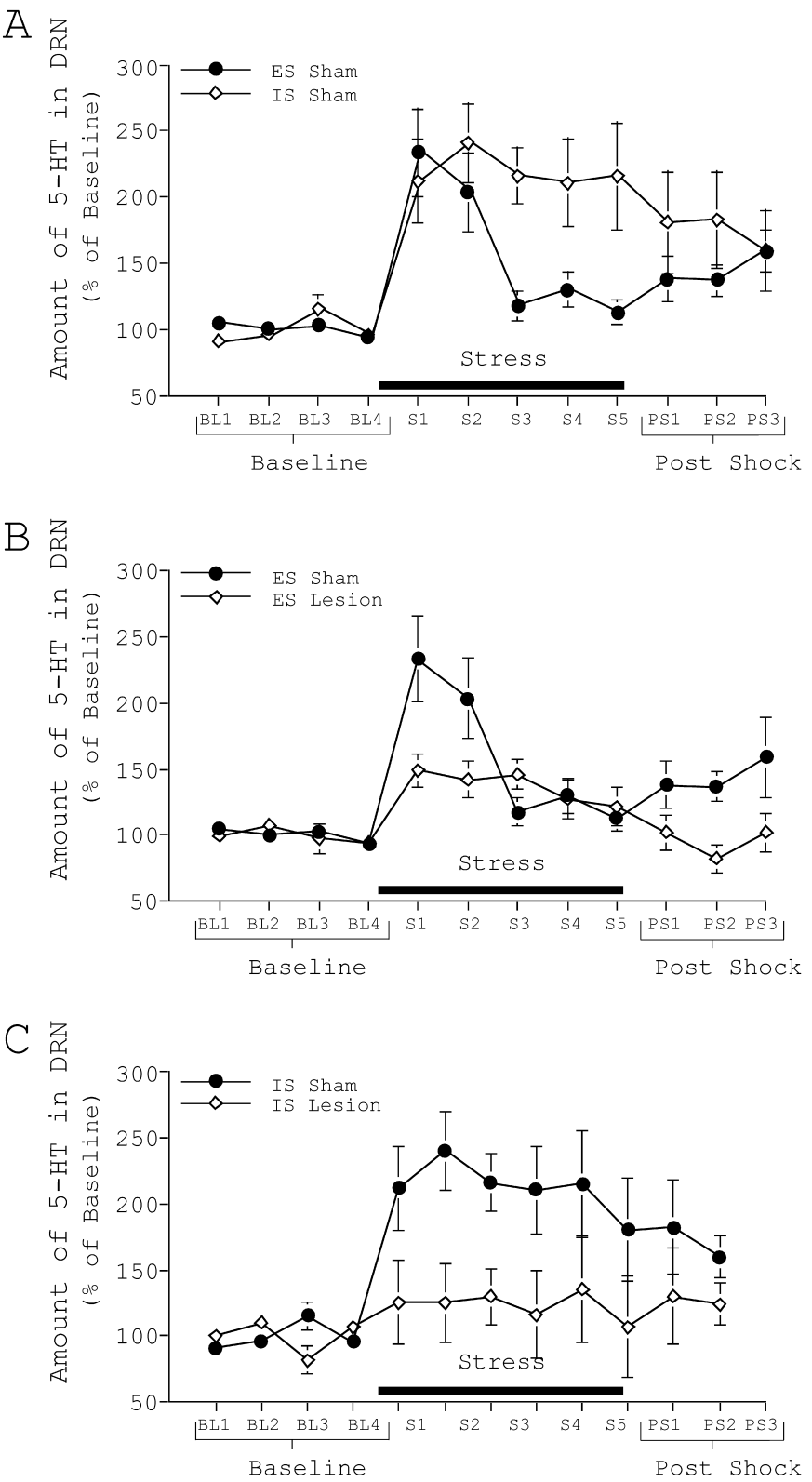

Fig. 2. 5-HT release within the DRN before, during and after exposure to ES or IS stress. In sham operated rats, both IS and ES caused an increase in DRN 5-HT during the initial stage of the shock session. The levels of 5-HT returned to near baseline in ES rats, while remaining elevated throughout the duration of the stressor for the IS rats (A). Habenula lesions significantly attenuated the increase in 5-HT in response to shock for both the ES (B) and the IS (C) groups.

maintained at elevated levels throughout the duration of shock presentation when the shock was inescapable. These observations replicate those reported by Maswood et al. [26]. Importantly, the habenula lesions almost completely blocked the increase in extracellular 5-HT produced by both ES (Fig. 2B) and IS (Fig. 2C), thereby eliminating the difference in the levels of 5-HT produced by IS and ES.

A 2×2×12 repeated measures ANOVA revealed a significant three-way interaction between Sample, Stressor, and Lesion [$F(11,418)=2.331$, $P<0.0087$]. In order to further examine the effect of type of stressor on the amount of 5-HT, the data were analyzed separately for Sham and Lesioned rats. For the Sham group, a 2×12 repeated measures ANOVA yielded a significant main effect of Sample [$F(11,220)=12.613$, $P<0.001$] and interaction between Sample and Stressor [$F(11,220)=2.983$, $P<0.001$]. The main effect of Stressor was marginally reliable [$F(1,20)=2.945$, $P=0.1011$]. A post hoc analysis revealed significant differences between IS and ES groups for samples S3, S4 and S5 ($P<0.02$), indicating that while both groups exhibited similarly elevated levels of 5-HT during the initial response to the stressor, levels in the ES group significantly differed from that of IS rats as extracellular 5-HT levels returned to near basal levels during the later portion of the stress period (Fig. 2A). For the lesion groups, a 2×12 repeated measures ANOVA yielded a significant main effect of Sample [$F(11,198)=3.691$, $P<0.0001$], but the main effect of Stressor and the interaction were not significant ($P>0.1482$ and $P>0.8364$, respectively). Thus, the differential exposure to ES or IS did not cause a difference in extracellular 5-HT levels in rats with habenular damage.

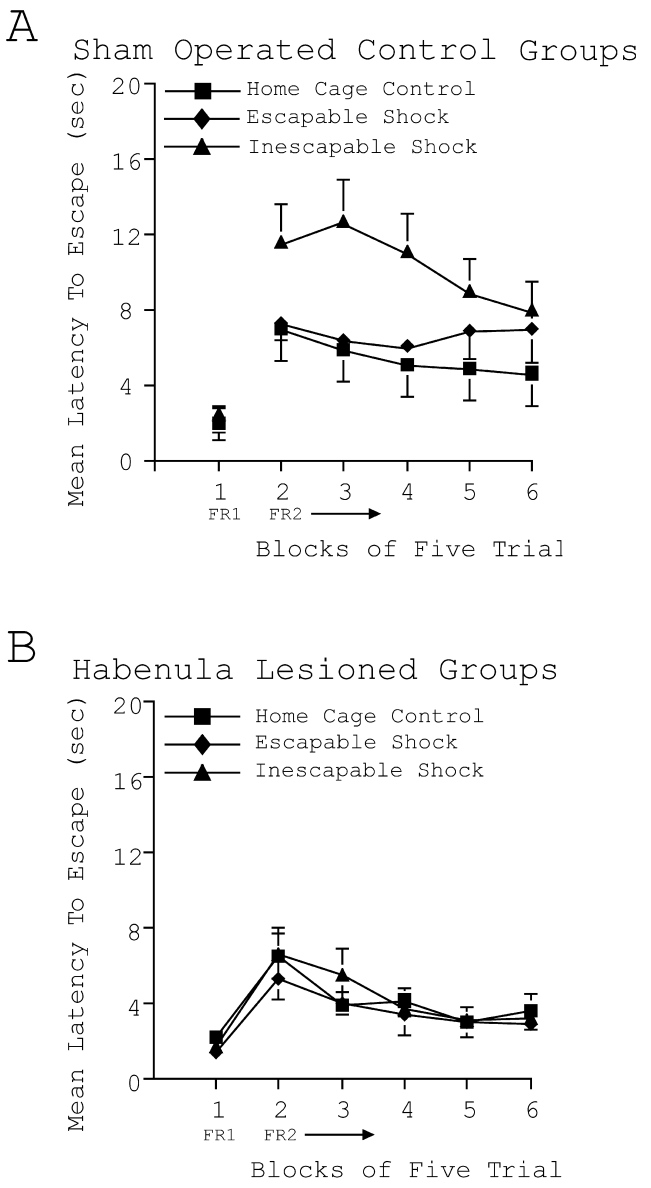

Fig. 3. Shuttle box escape latencies for sham operated (A) and habenula lesioned (B) rats. Exposure to IS caused a increase in the latency to escape in sham operated animals as compared to rats exposed to ES or HCC (A). The habenula lesion reduced escape latencies of IS rats to levels comparable to ES rats and home cage controls (B).

In order to further evaluate the effect of habenular damage, the data were analyzed separately for ES and IS groups. For both the IS and the ES groups, a 2×12 ANOVA yielded a significant main effect of Lesion ([$F(1,19)=7.432$, $P<0.0134$] and [$F(1,19)=5.213$, $P<0.0341$], respectively), and Sample ([$F(11,209)=7.765$, $P<0.0001$] and [$F(11,209)9.405$, $P<0.0001$], respectively), and significant interactions ([$F(11,209)=3.496$, $P<0.0002$] and [$F(11,209)=3.311$, $P<0.0003$), respectively). Thus, for both the ES and IS groups, damage of the habenula attenuated the rise of 5-HT in response to the stressor (Fig. 2B and C). Lastly, while there no significant differences were found in basal extracellular levels between groups, there were slight differences. In order to see if these small differences contributed to differences between groups during shock, a 2×2×5 ANOVA was performed (lesion, stress and the five shock samples), with basal 5-HT level as a covariate. This analysis indicates that the small but insignificant differences in basal 5-HT level did not contribute or impact the effect of the lesion and/stress on the level of extracellular 5-HT during shock.

3.2. Experiment 2

3.2.1. Histological analysis

As in experiment 1, the damage to the habenular complex involved both the medial and lateral habenula nucleus, and for most cases did not extend beyond the bounds of the habenula. Habenular damage in experiment 2 did not differ qualitatively from that of experiment 1 (see histological analysis for experiment 1 for a more detailed description of the extent of damage).

3.2.2. Behavioral analysis

The results from experiment 2 are presented in Fig. 3. As can be observed in Fig. 3A and B, there does not

appear to be differences between groups for the FR-1 trial. A 3×2 ANOVA revealed no significant differences for Lesion [$F(1,69)=0.039$, $P<0.8435$], Stressor [$F(2,69)=1.643$, $P<0.2010$] or the two-way interaction [$F(2,69)=1.646$, $P<0.2004$]. For the FR-2 trials, Sham rats exposed to IS had longer latencies to escape the shock, as compared to rats exposed to either ES or those in the HCC group (Fig. 3A). Lesions of the habenula completely eliminated the escape deficit produced by exposure to IS (Fig. 3B). A 3×2×5 repeated measures ANOVA revealed a main effect of Lesion [$F(1,69)=10.864$, $P<0.0016$], Stressor [$F(2,69)=4.998$, $P<0.0094$], and Trials [$F(4,276)=8.511$, $P<0.0001$], as well as, a significant interaction between Lesion and Stressor [$F(2,69)=3.980$, $P<0.0231$]. No other interaction proved significant ($P>0.15$). For Sham groups, a Fisher's PLSD revealed that animals exposed to IS exhibited greater escape latencies (collapsed across trials) than did ES animals ($P<0.0185$) and HCC animals ($P<0.001$). The difference between ES and HCC groups approached significance ($P=0.0726$; see Fig. 3A). For rats with habenula damage, there was no difference in latency between stressor groups (all $P>0.05$; see Fig. 3B). In addition, a Fisher's PLSD yielded significant differences in latency between Sham and Lesioned rats for both the ES ($P<0.0362$) and IS ($P<0.0145$) conditions, but not for the HCC condition ($P>0.8094$).

4. Discussion

These experiments demonstrate a role for the habenular complex in mediating the neurochemical and behavioral response to stress. The level of 5-HT in the caudal region of the DRN was elevated dramatically during the initiation of both IS and ES. The responsiveness of 5-HT to stress, though, was sensitive to the degree of behavioral control that the rat had over the stressor. Levels of 5-HT returned to near basal levels when the stressor was controllable, but remained elevated when the stressor was uncontrollable. Damage to the habenula severely attenuated the elevation of 5-HT, thereby eliminating the differential 5-HT response to stress between ES and IS groups. This suggests that the habenula is involved in the activation of the 5-HT neurons in the caudal DRN in response to stress. In addition, exposure to IS stress dramatically increased the latency to escape a stressor 24 h later, a behavioral change not observed after exposure to ES. In this case as well, habenular damage eliminated the difference between stressor groups.

These data support and extend the conclusions of previous studies which suggest a functional relationship between the habenula and the DRN during stress. The habenula is activated by peripheral nociceptive stimulation [9] and stress [44,13,5]; although see Ref. [6], and projects to the DRN [12,17,3,30]. Furthermore, this projection appears to be excitatory [15–17,8]. The present study extends these findings by showing that the habenula is necessary for stress induced increases in 5-HT in the caudal DRN. In addition, since habenular damage did not alter the basal level of 5-HT (see also Ref. [35]), the attenuation of the rise of 5-HT is not likely to be due to a dramatic loss of basal tone. Rather, the habenula likely provides a phasic excitatory input to the DRN in response to environmental stress.

In addition, habenula lesions eliminated the difference in escape behavior produced by prior exposure to IS. Thus, the habenula appears to be necessary for the induction of learned helplessness/behavioral depression. Since the habenula lesion also attenuated the increase in DRN 5-HT normally observed during the exposure to IS, the present data further strengthen the tie between induction of 5-HT efflux within the DRN and the behavioral effects of IS. Prior experiments have indicated that manipulations which act directly on the DRN to inhibit increased levels of intra-DRN 5-HT during IS block the usual behavioral consequences of IS [24]. Here, elimination of a normal excitatory input to the DRN that prevented the typical elevation of intra-DRN 5-HT during IS also prevented the interference with escape behavior that normally follows IS.

It should be noted, of course, that the habenula was damaged during the escape learning task as well as during IS exposure. Therefore, it is possible that the elimination of the escape deficit was due to the lack of habenular function during escape testing. It has been proposed that the intense activation of DRN 5-HT neurons by IS sensitizes these cells so that they respond in an exaggerated fashion during behavioral testing [19]. The habenula could play a role in the activation of the DRN during escape learning, and habenula lesions could thus have eliminated an input to the DRN in response to the escape testing procedure. It also should be mentioned that the habenula regulates the activity of many other systems [see Refs. 7,33,36] and so these other systems may also play a role in the elimination of the escape deficit.

Interestingly, the habenula lesion reduced the escape latency of rats in the ES group. This could be taken to suggest that the lesion simply reduced escape latencies in general, rather than specifically eliminating the effect of IS on escape latencies. There is reason to doubt this interpretation. First, habenula lesions did not affect escape latencies in the HCC group. Second, ES produced a slight increase in escape latency, as compared to the HCC group. Thus, it would appear that ES exposure itself had a slight effect on escape latency, and it is this small increase that was eliminated by the habenula lesions, rather than the lesion reducing escape latencies per se.

Prior research also has suggested a role for the habenula in stress-related phenomena, although the exact nature of this role is not clear. Lesion of the habenula or its efferent pathway has been shown to increase exploration in an open field paradigm [27,18,35], with the increase in exploration being associated with an increase in grooming and a slight,

but not significant, reduction in freezing behavior [27]. These findings suggest that habenula lesions decrease anxiety. Similarly, lesions of the efferent path of the habenula attenuate the impact of prior stressors such as isolation and food deprivation on open arm exploration in the elevated plus maze [27]. However, basal open arm exploration in the elevated plus maze was not affected, and was associated with an increase in freezing behavior [27]. In addition, basal levels of plasma corticosterone were increased by lesions of the efferent path [27]. Lesions of the habenula also impair one-way avoidance learning, but only when physical effort or stress level is increased [39], and do not affect two-way avoidance [40]. The interference with escape behavior produced by IS has been argued to reflect anxiety induced by IS [20], and so the present data are consistent with a role for the habenula in anxiety. This general pattern, though, suggests that involvement of the habenula in anxiety-related processes is complex. However, the habenula clearly does play a key role in the neurochemical and behavioral response to uncontrollable stress.

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References

[1] J. Amat, P. Matus-Amat, L.R. Watkins, S.F. Maier, Escapable and inescapable stress differentially alter extracellular levels of 5-HT in the basolateral amygdala of the rat, Brain Res. 812 (1-2) (1998) 113–120.

[2] H. Anisman, S. Zalcman, N. Shanks, R.M. Zacharo, Multisystem Regulation of Performance Deficits Induced By Stressors: An Animal Model of Depression, Humana Press, Clifton, NJ, 1991.

[3] M. Araki, P.L. McGeer, H. Kimura, The efferent projections of the rat lateral habenular nucleus revealed by the PHA-L anterograde tracing method, Brain Res. 441 (1988) 319–330.

[4] S. Cabib, S. Puglisi-Allegra, Opposite responses of mesolimbic dopamine system to controllable and uncontrollable aversive experiences, J. Neurosci. 14 (5) (1994) 3333–3340.

[5] N. Chastrette, D.W. Pfaff, R.B. Gibbs, Effects of daytime and nighttime stress on Fos-like immunoreactivity in the paraventricular nucleus of the hypothalamus, the habenula, and the posterior paraventricular nucleus of the thalamus, Brain Res. 563 (1-2) (1991) 339–344.

[6] E.A. Del Bel, M.C. Silveira, F.G. Graeff, N. Garcia-Cairasco, F.S. Guimaraes, Differential expression of c-fos mRNA and Fos protein in the rat brain after restraint stress or pentylenetetrazol-induced seizures, Cell Mol. Neurobiol. 18 (3) (1998) 339–346.

[7] G. Ellison, Stimulant-induced psychosis, the dopamine theory of schizophrenia, and the habenula, Brain Res. Rev. 19 (1994) 223–239.

[8] G. Ferraro, M.E. Montalbano, P. Sardo, V. La Grutta, Lateral habenula and hippocampus: a complex interaction raphe cells-mediated, J. Neural Transm. 104 (1997) 615–631.

[9] D.M. Gao, D. Hoffman, A.L. Benabid, Simultaneous recording of spontaneous activities and nociceptive responses from neurons in the pars compacta of substantia nigra and in the lateral habenula, Eur. J. Neurosci. 8 (7) (1996) 1474–1478.

[10] R.E. Grahn, L.R. Watkins, S.F. Maier, Impaired escape performance and enhanced conditioned fear in rats following exposure to an uncontrollable stressor are mediated by glutamate and nitric oxide in the dorsal raphe nucleus, Behav. Brain Res. 112 (2000) 33–41.

[11] R.E. Grahn, M.J. Will, S.E. Hammack, S. Maswood, M.B. McQueen, L.R. Watkins, S.F. Maier, Activation of serotonin-immunoreactive cells in the dorsal raphe nucleus in rats exposed to an uncontrollable stressor, Brain Res. 826 (1) (1999) 35–43.

[12] M. Herkenham, W.J.H. Nauta, Efferent connections of the habenular nuclei in the rat, J. Comp. Neurol. 187 (1979) 19–47.

[13] T. Imaki, T. Shibasaki, M. Hotta, H. Demura, Intracerebroventricular administration of corticotropin-releasing factor induces c-fos mRNA expression in brain regions related to stress responses: comparison with pattern of c-fos mRNA induction after stress, Brain Res. 616 (1993) 114–125.

[14] J. Irwin, A. Suissa, H. Anisman, Differential effects of inescapable shock on escape performance and discrimination learning in a water maze escape task, J. Exp. Psychol. Animal Behav. Proc. 6 (1980) 21–40.

[15] P. Kalen, O. Lindvall, A. Bjorklund, Electrical stimulation of the lateral habenula increases hippocampal noradrenaline release as monitored by in vivo microdialysis, Exp. Brain Res. 76 (1) (1989) 239–245.

[16] P. Kalen, M. Pritzel, A. Nieoullon, L. Wiklund, Further evidence for excitatory amino acid transmission in the lateral habenular projection to the rostral raphe nuclei: lesion-induced decrease of high affinity glutamate uptake, Neurosci Lett. 68 (1) (1986) 35–40.

[17] P. Kalen, M. Karlson, L. Wiklund, Possible excitatory amino acid afferents to nucleus raphe dorsalis of the rat investigated with retrograde wheat germ agglutinin and D-[^{3}H]aspartate tracing, Brain Res. 360 (1-2) (1985) 285–297.

[18] E.H. Lee, S.L. Huang, Role of lateral habenula in the regulation of exploratory behavior and its relationship to stress in rats, Behav. Brain Res. 30 (3) (1988) 265–271.

[19] S.F. Maier, Learned helplessness, fear and anxiety, in: P. Stanford, K. Solomon (Eds.), Stress: From Synapse to Syndrome, Academic Press, London, 1993, pp. 207–248.

[20] S.F. Maier, Role of fear in mediating shuttle escape learning deficit produced by inescapable shock, J. Exp. Psychol. Animal Behav. Proc. 16 (1990) 137–149.

[21] S.F. Maier, B.A. Kalman, R.E. Grahn, Chlordiazepoxide microinjected into the region of the dorsal raphe nucleus eliminates the interference with escape responding produced by inescapable shock whether administered before inescapable shock or escape testing, Behav. Neurosci. 108 (1) (1994) 121–130.

[22] S.F. Maier, R.E. Grahn, B.A. Kalman, L.C. Sutton, E.P. Wiertelak, L.R. Watkins, The role of the amygdala and dorsal raphe nucleus in mediating the behavioral consequences of inescapable shock, Behav. Neurosci. 107 (2) (1993) 377–388.

[23] S.F. Maier, M.E.P. Seligman, Learned helplessness: Theory and evidence, J. Exp. Psychol. 105 (1976) 33–46.

[24] S.F. Maier, R.E. Grahn, L.R. Watkins, 8-OH-DPAT microinjected in the region of the dorsal raphe nucleus blocks and reverses the enhancement of fear conditioning and interference with escape produced by exposure to inescapable shock, Behav. Neurosci. 109 (3) (1995) 404–412.

[25] S.F. Maier, R. Albin, T. Testa, Failure to learn to escape in rats previously exposed to inescapable shock depends on the nature of the escape response, J. Comp. Physiol. Psychol. 85 (1973) 581–592.

[26] S. Maswood, J.E. Barter, L.R. Watkins, S.F. Maier, Exposure to inescapable but not escapable shock increases extracellular levels of 5-HT in the dorsal raphe nucleus of the rat, Brain Res. 783 (1) (1998) 115–120.

[27] C.A. Murphy, A.M. DiCamillo, F. Haun, M. Murray, Lesion of the habenular efferent pathway produces anxiety and locomotor hyperactivity in rats: a comparison of the effects of neonatal and adult lesions, Behav. Brain Res. 81 (1996) 43–52.
[28] F. Osborne, B. Mattingly, W. Redmon, J. Osborne, Factors affecting the measurement of classically conditioned fear in rats following exposure to escapable vs. inescapable signaled shock, J. Exp. Psychol. Animal Behav. Proc. 1 (1975) 364–373.
[29] J.B. Overmier, M.E.P. Seligman, Effects of inescapable shock on subsequent escape and avoidance behavior, J. Comp. Physiol. Psychol. 63 (1967) 28–33.
[30] C. Peyron, J.M. Petit, M. Rampon, M. Jouvet, P.H. Luppi, Forebrain afferents to the rat dorsal raphe nucleus demonstrated by retrograde and anterograde tracing methods, Neuroscience 82 (1998) 443–468.
[31] F. Petty, G.L. Kramer, J. Wu, Serotonergic modulation of learned helplessness, Ann. NY Acad. Sci. 821 (1997) 538–541.
[32] F. Petty, G. Kramer, L. Wilson, Y.L. Chae, Learned helplessness and in vivo hippocampal norepinephrine release, Pharmacol. Biochem. Behav. 46 (1) (1993) 231–235.
[33] R. Sandyk, Relevance of the habenular complex to neuropsychiatry: A review and hypothesis, Int. J. Neurosci. 61 (1991) 189–219.
[34] K.R. Short, S.F. Maier, Stressor controllability, social interaction, and benzodiazapine, Pharmacol. Biochem. Behav. 45 (4) (1993) 827–835.
[35] S.G. Speciale, L.M. Neckers, R.J. Wyatt, Habenular modulation of raphe indolamine metabolism, Life Sci. 27 (1988) 2367–2372.
[36] R.J. Sutherland, The dorsal diencephalic conduction system: a review of the anatomy and functions of the habenular complex, Neurosci. Biobehav. Rev. 6 (1982) 1–13.
[37] L.C. Sutton, S.E. Lea, M.J. Will, B.A. Schwartz, C.E. Hartley, J.C. Poole, L.R. Watkins, S.F. Maier, Inescapable shock-induced potentiation of morphine analgesia, Behav. Neurosci. 111 (5) (1997) 1105–1113.
[38] L.C. Sutton, R.E. Grahn, E.P. Wiertelak, L.R. Watkins, S.F. Maier, Inescapable shock-induced potentiation of morphine analgesia in rats: involvement of opioid, GABAergic, and serotonergic mechanisms in the dorsal raphe nucleus, Behav. Neurosci. 111 (4) (1997) 816–824.
[39] E.W. Thornton, G.E. Bradbury, Effort and stress influence the effect of lesion of the habenula complex in one-way active avoidance learning, Physiol. Behav. 45 (1989) 929–935.
[40] A. Vale-Martinez, M. Marti-Nicolovius, G. Guillazo-Blanch, M. Coll-Andreu, I. Morgado-Bernal, Effects of habenular lesions upon two-way active avoidance conditioning in rats, Neurobiol. Learn. Mem. 68 (1997) 68–74.
[41] J.M. Weiss, P.A. Goodman, B.G. Losito, S. Corrigan, J.M. Charry, W.H. Bailey, Behavioral depression produced by an uncontrollable stressor: Relationship to norepinephrine, dopamine, and serotonin levels in various regions of rat brain, Brain Res. Revs. 3 (1981) 167–205.
[42] M.J. Will, L.R. Watkins, S.F. Maier, Uncontrollable stress potentiates morphine's rewarding properties, Pharmacol. Biochem. Behav. 60 (3) (1998) 655–664.
[43] J.L. Williams, Influence of shock controllability by dominant rats on subsequent attack and defensive behaviors toward colony intruders, Anim. Learn. Behav. 10 (1982) 305–313.
[44] D. Wirtshafter, K.E. Asin, M.R. Pitzer, Dopamine agonists and stress produce different patterns of Fos-like immunoreactivity in the lateral habenula, Brain Res. 633 (1-2) (1994) 21–26.