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THE CENTRAL AMYGDALA MODULATES HYPOTHALAMIC–PITUITARY–ADRENAL AXIS RESPONSES TO SYSTEMIC INTERLEUKIN-1β ADMINISTRATION

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Abstract—In the present study we examined the role of the central nucleus of the amygdala in hypothalamic–pituitary–adrenal axis responses to an immune challenge in the form of systemic administration of the proinflammatory cytokine interleukin-1β (1 μg/kg). We found that bilateral ibotenic acid lesions of the central amygdala substantially reduced adrenocorticotropin hormone release and hypothalamic corticotropin-releasing factor and oxytocin cell c-*fos* expression responses to interleukin-1β suggesting a facilitatory role for this structure in the generation of hypothalamic–pituitary–adrenal axis responses to an immune challenge. Since only a small number of central amygdala cells project directly to the paraventricular nucleus, we then examined the effect of central amygdala lesions on the activity of other brain nuclei that might act as relay sites in the control of the hypothalamic–pituitary–adrenal axis function. We found that bilateral central amygdala lesions significantly reduced interleukin-1β-induced c-*fos* expression in cells of the ventromedial and ventrolateral subdivisions of the bed nucleus of the stria terminalis and brainstem catecholamine cell groups of the nucleus tractus solitarius (A2 noradrenergic cells) and ventrolateral medulla (A1 noradrenergic and C1 adrenergic cells).

These findings, in conjunction with previous evidence of bed nucleus of the stria terminalis and catecholamine cell group involvement in hypothalamic–pituitary–adrenal axis regulation, suggest that ventromedial and ventrolateral bed nucleus of the stria terminalis cells and medullary catecholamine cells might mediate the influence of the central amygdala on hypothalamic–pituitary–adrenal axis responses to an immune challenge. Thus these data establish that the central amygdala influences hypothalamic–pituitary–adrenal axis responses to a systemic immune challenge but indicate that it primarily acts by modulating the activity of other control mechanisms. © 1999 IBRO. Published by Elsevier Science Ltd.

Key words: bed nucleus of the stria terminalis, brainstem catecholamine cells, central amygdala, corticotropin-releasing factor, hypothalamic–pituitary–adrenal axis, interleukin-1β.

An immune challenge, in the form of peripheral infection, inflammation or tissue injury, initiates the release of proinflammatory cytokines into the circulation thereby setting in motion a series of reciprocal interactions between the immune and central nervous systems. Numerous studies have shown that proinflammatory cytokines such as interleukin-1β (IL-1β), tumour necrosis factor-α and interleukin-6 can act as signalling molecules capable of activating CNS mechanisms which contribute to the acute phase response of an immune challenge.[27,30,44,45]

An important and widely studied neuroendocrine component of the acute phase response is the activation of the hypothalamic–pituitary–adrenal (HPA) axis. The HPA axis response to an immune challenge consists of a cascade of events primarily dependent on the integrity of a population of tuberoinfundibular-projecting corticotropin-releasing factor (CRF) cells within the medial parvocellular division of the paraventricular nucleus (mPVN).[4,43,46,52] Systemic administration of the cytokine IL-1β produces robust mPVN CRF cell activation in rats[8,19] and this is followed by the release of adrenocorticotropin hormone (ACTH) from the anterior pituitary into the circulation.[8,28,55] In rodents CRF cell activation is often accompanied by release of oxytocin (OT) and there is evidence that OT release from axons of hypothalamic magnocellular cells passing through the median eminence can enhance ACTH release from corticotrophs.[22,37,38] It is thus notable that there is a concurrent increase in OT cell activation with ACTH release in response to IL-1β administration.[8]

Although the importance of the mPVN CRF cells in the HPA axis response to systemic IL-1β is well documented, the afferent pathways that converge on this cell group and trigger their response to an immune challenge are less well characterized. Brainstem catecholamine cell groups of the nucleus tractus solitarius (NTS) and ventrolateral medulla (VLM) are likely to constitute the primary afferent mPVN input responsible for the generation of HPA axis responses following an immune challenge since destruction of PVN catecholaminergic terminals[10,25,33,56] and interruption of catecholamine projections from the medulla oblongata to the hypothalamus[19] attenuate IL-1β-induced HPA axis responses. However, this pathway may not fully account for HPA axis responses observed following an immune challenge. In particular the amygdala, and more specifically the central amygdala (CeA), is robustly activated by systemic IL-1β[8,19] and has previously been shown to have a role in HPA axis function. Electrical stimulation of the amygdala has been reported to increase ACTH release while lesions of the amygdala, and particularly the CeA, have been shown to attenuate HPA axis responses to various stimuli.[1,2,20,39,53] Conversely the CeA does not appear to alter HPA axis regulation following restraint stress or ether exposure.[20,40] Furthermore we have recently proposed that different amygdala nuclei may mediate

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Abbreviations: ACTH, adrenocorticotropin hormone; BNST, bed nucleus of the stria terminalis; CeA, central amygdala; CRF, corticotropin-releasing factor; HPA axis, hypothalamic–pituitary–adrenal axis; IL-1β, interleukin-1β; MeA, medial amygdala; mPVN, medial parvocellular division of the paraventricular nucleus; NTS, nucleus tractus solitarius; OT, oxytocin; PBS, phosphate-buffered saline; PNMT, phenyl-N-methyl transferase; PVN, paraventricular nucleus; SON, supraoptic nucleus; TH, tyrosine hydroxylase; VLM, ventrolateral medulla.

HPA axis responses to different categories of stressor; the CeA being important for physical and MeA for psychological stressors.[15] Thus it seems possible that the amygdala could hold an important position in generating, or at least modulating, HPA axis activation during an immune challenge.

Even though there is a direct neural projection from the CeA to the mPVN it involves only a small number of cell bodies.[3,23,31,41,48] This, coupled with evidence that IL-1β-activated neurons in the CeA do not correspond to those that project directly to the PVN,[19] suggests that if the CeA does contribute to the HPA axis response to immune challenge it may do so via indirect pathways. Retrograde tracing and lesion studies implicate two regions as potential relay pathways for CeA regulation of HPA responses: brainstem catecholamine cell groups and the bed nucleus of the stria terminalis (BNST). As described above, brainstem noradrenergic and adrenergic cell groups of the NTS and VLM have already been demonstrated to play a pivotal role in generating HPA axis responses to systemic IL-1β administration.[19] Moreover the CeA projects to regions of the NTS and VLM that contain catecholamine nuclei,[35,36,54] although to date there has been no investigation, to our knowledge, of the contribution of descending amygdala pathways to HPA axis responses. The amygdala could also potentially influence HPA axis responses via the BNST. The amygdala projects to the BNST via the stria terminalis[51,57] and the BNST is believed to be involved in modulating HPA axis responses.[16,21,24] In addition, it has been demonstrated that BNST lesions block HPA axis responses elicited by amygdala stimulation.[21] The CeA projects specifically to lateral components of the BNST including the oval nucleus, ventrolateral and ventromedial subdivisions and the latter two subdivisions have been shown to project to the mPVN cell group.[12,32,48] Although there is evidence to suggest that the oval nucleus does not contribute to IL-1β-induced HPA axis activation[19] the involvement of the ventromedial and ventrolateral subdivisions of the BNST has not been studied.

In view of the lack of knowledge on the role of the amygdala during an immune challenge we sought to examine its involvement in IL-1β-induced HPA axis activation in the rat. We have used a bilateral CeA lesion approach, in combination with measuring plasma ACTH levels and c-*fos* expression (an indicator of neuronal activation) in mPVN CRF cells, to address the importance of the CeA in generating HPA axis responses. In addition, because of evidence that OT

(1:25,000; Peninsula Lab. Inc., CA), OT (1:250; OT-neurophysin directed monoclonal gift from D. Pow, University of Queensland), TH (1:15,000, monoclonal, Incstar, U.S.A.) or PNMT (1:1200, monoclonal, Incstar, U.S.A.). The sections were incubated for 2 h in biotinylated anti-mouse (for OT and TH; 1:400, Jackson Immuno-Research, West Grove, U.S.A.) or anti-rabbit (for PNMT and CRF; 1:400, Jackson ImmunoResearch) followed by an avidin–biotin–horseradish peroxidase complex solution (Vector Elite Kit, CA) for 2 h. Horseradish peroxidase activity was visualized with diaminobenzidine alone (nickel omitted) producing an amber cytoplasmic deposit. To minimize possible variations in immunocytochemistry, sections from each experimental group were processed simultaneously and therefore incubated for equal lengths of time in diaminobenzidine. Sections were mounted on chrome-alum slides, dehydrated in a series of alcohols, cleared in xylene and coverslipped.

Data analysis

All results are expressed as the mean ^ S.E.M. Fos-positive cells were identified by their black nuclear deposit whereas those immunolabelled for TH, PNMT, OT or CRF were distinctive by their amber cytoplasmic deposit. Counts were done blind to treatment. In the SON the total number of OT-positive cells co-localizing Fos was counted at 160 μm intervals at eight rostrocaudal levels. For the PVN, the numbers of OT- and CRF-positive cells co-localizing Fos were determined at two rostrocaudal levels corresponding to the posterior magnocellular and medial parvocellular subdivisions of the PVN.[14] These PVN divisions are where the majority of magnocellular OT and parvocellular, tuberoinfundibular-projecting CRF cells are located. The Fos-positive CRF cell count is a good estimate of cell activation in the mPVN as we have previously shown a high correlation of Fos-positive versus Fos-positive CRF mPVN cells for a variety of stimuli.[7,15] Furthermore this is consistent with others who, by combining *in situ* hybridization with Fos-immunolabelling, have shown that in animals subjected to an ACTH releasing stimulus, over 70% of Fos-positive mPVN cells expressed CRF.[19] The numbers of Fos-positive cells within the CeA and medial amygdala (MeA) were determined at 160 μm intervals at five rostrocaudal levels. In the BNST the number of Fos-positive cells were counted at 160 μm intervals at four, two and four rostrocaudal levels in the oval, ventrolateral and ventromedial subdivisions, respectively. The ventromedial and ventrolateral subdivisions correspond to regions located ventral to the anterior commissure while the oval nucleus is located ventral to the lateral ventricle.[19,41] In the VLM and NTS, TH- and PNMT-positive cells co-localizing Fos were counted at 250 μm intervals from 2.5 mm caudal to 2 mm rostral to obex. The VLM and NTS contain partially overlapped populations of noradrenergic cells, the A1 and A2 groups, and adrenergic cells, the C1 and C2 groups. As PNMT only occurs in adrenergic cells these were taken to be C1 or C2 cells. However, since TH is a marker for both noradrenergic and adrenergic cells the number of activated noradrenergic A1 or A2 cells was estimated by subtracting the number of double-labelled PNMT cells in one section from the number of double-labelled TH cells in the adjacent section. To determine ACTH plasma concentrations a radioimmunoassay was performed using a commercial radioimmunoassay kit was used (Diagnostic Product Corporation, CA). All plasma samples were analysed in the same assay and the intra-assay coefficients of variation were less than 10%. The extent of the amygdala lesions was assessed by determining the percentage reduction in Fos-positive cells in the CeA and MeA and also on the basis of gliosis in adjacent Nissl-stained amygdala sections. Only animals with a discrete CeA lesion were included in the present study. The effect of bilateral CeA lesions on IL-1β-induced c-*fos* expression in neuronal populations and plasma ACTH levels were analysed by comparing values from CeA-lesioned versus sham-lesioned groups using unpaired Student's *t*-tests (two-tailed).

RESULTS

Controls

In animals that received only an intra-arterial injection of saline 2 h prior to sacrifice very few Fos-positive cells were observed in any of the regions examined. For example, Fig. 1A illustrates the paucity of Fos-labelled cells in the CeA of a vehicle-injected animal. These findings indicate that variables such as surgery, housing and mode of drug delivery had no

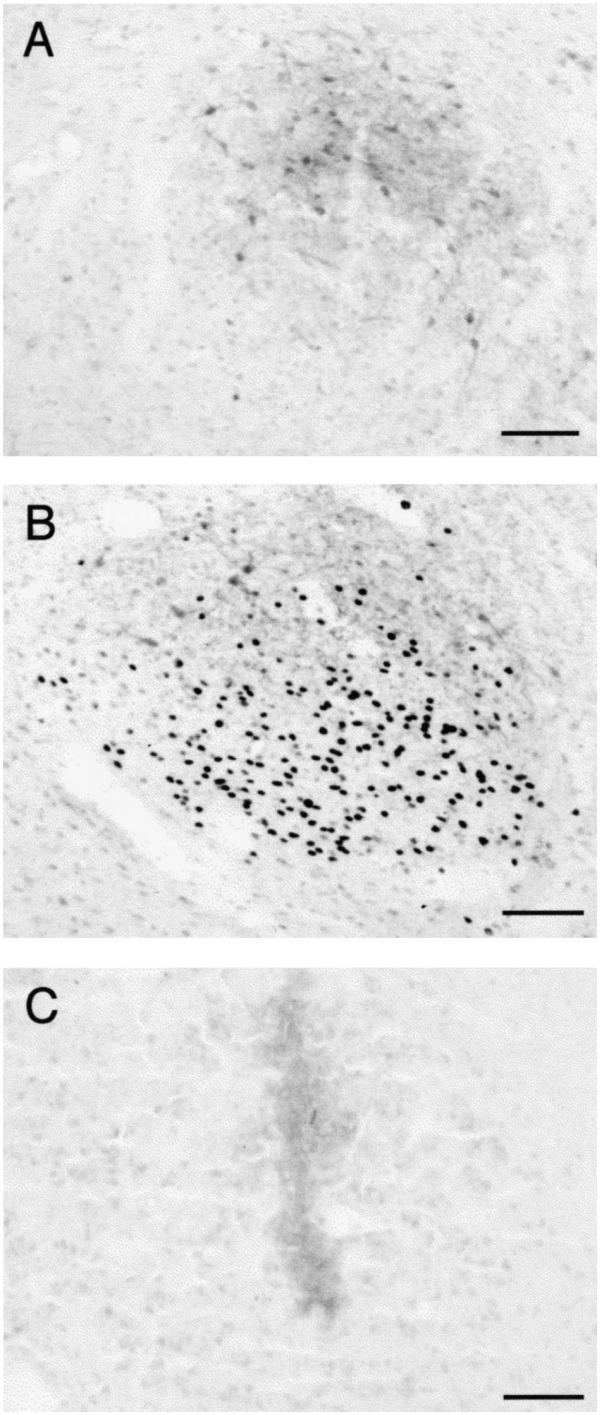

Fig. 1. Photomicrographs of coronal CeA sections (40 μm) taken from animals that had received systemic vehicle injection only (A), CeA sham lesion followed by systemic IL-1β injection (B) and CeA ibotenic acid lesion followed by systemic IL-1β (C). Sections have been immunolabelled for the detection of nuclear Fos and cytoplasmic CRF. Virtually no Fos-positive cells were apparent in vehicle-injected animals whereas there was a large recruitment of CeA cells in sham-lesioned animals following IL-1β administration. In sham-lesioned animals Fos-positive cells were concentrated within the lateral region of the CeA, a pattern analogous to that seen in intact animals. It is also notable that none of the CRF-positive cells were Fos-positive. Ibotenic acid injected into the CeA (Alcian Blue dye seen as grey smudge in the centre of the CeA) resulted in a significant loss of both Fos-positive and CRF-positive cells within the CeA. Scale bar = 100 μm.

Table 1. Effects of bilateral central amygdala lesions on the number of Fos-positive amygdala cells, hypothalamic oxytocin and corticotropin-releasing factor cells, bed nucleus of the stria terminalis and brainstem catecholamine cells following systemic interleukin-1β administration

	Sham lesion	CeA lesion
Amygdala		
Central amygdala	325.43 ^ 29.83	50.14 ^ 12.05***
Medial amygdala	61.29 ^ 8.37	57.57 ^ 8.16
Hypothalamus		
mPVN CRF cells	146.57 ^ 18.55	18.43 ^ 9.18***
SON OT cells	202.57 ^ 17.43	51.86 ^ 24.12***
PVN OT cells	43 ^ 6.41	4 ^ 2.67***
Bed nucleus of the stria terminalis		
Ventromedial nucleus	103 ^ 22.65	18.25 ^ 3.17**
Oval nucleus	96.75 ^ 27.63	100.2 ^ 12
Ventrolateral nucleus	67.6 ^ 18.29	21 ^ 7.58*
Brainstem		
VLM A1 cells	88.14 ^ 9.54	35 ^ 9.38**
VLM C1 cells	60.14 ^ 8.54	21.86 ^ 4.87*
NTS A2 cells	112.14 ^ 9.10	63.71 ^ 13.42*
NTS C2 cells	6.71 ^ 1.55	6.29 ^ 1.73

In the amygdala sham-lesioned animals showed typical patterns of c-*fos* expression after immune challenge with the majority of cells recruited within the CeA and relatively few in the MeA. Bilateral CeA lesions resulted in an 85% reduction in the number of CeA cells recruited by systemic IL-1β but had no effect on the number of cells recruited within the MeA. In the hypothalamus bilateral CeA lesions resulted in significant reductions in the number of Fos-positive mPVN CRF cells and magnocellular OT cells of the SON and PVN. In the BNST bilateral CeA lesions resulted in a significant reduction in the number of Fos-positive cells in the ventromedial and ventrolateral cell groups but had no effect on the number of cells recruited in the oval nucleus of the BNST. In the brainstem bilateral CeA lesions resulted in a significant reduction in IL-1β-induced Fos expression in the A1 and A2 noradrenergic and C1 adrenergic cell groups but had no effect on the small number of C2 adrenergic cells recruited.

Values are mean ^ S.E.M. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared with sham-lesioned values.

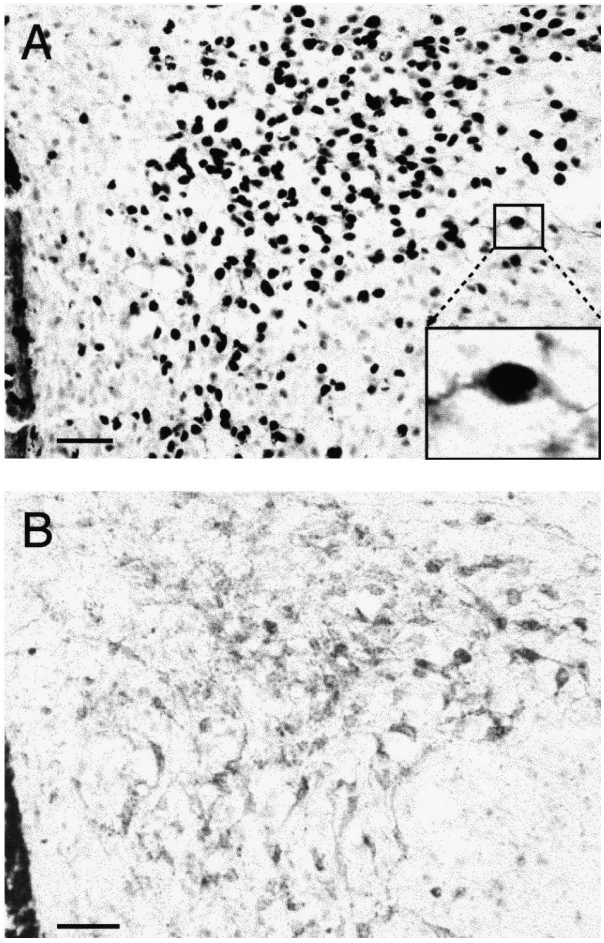

Fig. 2. Photomicrographs of coronal sections (40 μm) taken through the mPVN CRF cell division from sham- (A) and CeA-lesioned (B) animals administered systemic IL-1β. Sections have been immunolabelled for the detection of nuclear Fos and cytoplasmic CRF. In sham-lesioned animals, there was substantial recruitment of mPVN CRF cells following systemic IL-1β as shown by the dense population of Fos-positive CRF cells (A). The inset of A presents a Fos-positive CRF immunolabelled cell. In contrast, in bilateral CeA-lesioned animals there was a significant loss of Fos-positive CRF cells within the mPVN. Scale bars = 50 μm.

detectable effects on baseline Fos levels in the brain areas examined.

To determine whether the surgical procedures required to perform CeA lesions might influence IL-1β-induced activation of the nuclei of interest, IL-1β was administered to animals that had received a sham CeA lesion. In these animals the localization and number of Fos-positive cells for all brain regions examined were indistinguishable from those we have previously reported for intact animals which had not undergone neurosurgery.[8] Furthermore the baseline and IL-1β-induced plasma ACTH levels measured in sham-lesioned animals were comparable to those seen in intact animals.[8] Consequently we conclude that the neurosurgical procedures used in the present study had no effect on IL-1β-induced responses.

Effects of central amygdala lesions on the amygdala

In sham-lesioned animals systemic administration of IL-1β resulted in a substantial recruitment of cells within the CeA (Fig. 1B; Table 1). Fos-positive cells were concentrated primarily within the lateral portion of the CeA and negligible Fos-positive or Fos-positive CRF cells were apparent in the medial CeA. Besides the CeA, the amygdala of sham-lesioned animals was largely devoid of Fos-positive cells except for a very small number of cells in the MeA (Table 1). Bilateral injections of ibotenic acid into the CeA resulted in a very localized pattern of neuronal loss and glial infiltration between the internal limb of the external capsule and the optic tract. Nuclei bordering the CeA such as the lateral amygdala were not affected by ibotenic acid. Bilateral CeA lesions significantly reduced the number of Fos-positive cells by 85% compared to sham-lesioned animals (Fig. 1C; Table 1). The number of Fos-positive cells seen in the MeA was not altered in CeA-lesioned animals (Table 1) thus further indicating that the lesions were confined to the CeA.

Effects of central lesions on medial parvocellular division of the paraventricular nucleus corticotropin-releasing factor cells and adrenocorticotropin release

In sham-lesioned animals, systemic IL-1β produced a substantial number of Fos-positive mPVN CRF cells (Fig. 2A; Table 1). Compared to sham-lesioned animals, bilateral CeA lesions significantly reduced the number of mPVN Fos-positive CRF cells recruited in response to systemic IL-1β (Fig. 2B; Table 1). Systemic administration of IL-1β also produced a significant release of ACTH into the plasma 30 min after IL-1β delivery in sham-lesioned animals

Table 2. Plasma adrenocorticotropin values (pg/ml) in sham- and central amygdala-lesioned animals measured 30 min before and after systemic administration of interleukin-1β

	Sham lesion	CeA lesion
30 min before IL-1β	18.46 ^ 2.44	17.02 ^ 2.95
30 min after IL-1β	143.91 ^ 23.44	74.21 ^ 12.07*

In sham-lesioned animals IL-1β administration resulted in a pronounced release of ACTH into the plasma. Compared to sham-lesioned values, bilateral CeA lesions resulted in significant reductions in the plasma ACTH level measured 30 min after IL-1β administration. Note that compared to sham-lesioned animals, bilateral CeA lesions had no effect on baseline plasma ACTH levels measured 30 min before IL-1β was administered.

Values are mean ^ S.E.M. *$P < 0.05$ compared with sham-lesioned values.

(Table 2). Bilateral CeA lesions significantly attenuated the rise in plasma ACTH normally observed 30 min after systemic injection of IL-1β (Table 2). There was no difference between sham- and CeA-lesioned animals in the baseline plasma ACTH values obtained 30 min before IL-1β administration (Table 2).

Effects of central amygdala lesions on hypothalamic neurosecretory oxytocin cells

In sham-lesioned animals, systemic administration of IL-1β resulted in a large number of Fos-positive OT cells in the magnocellular divisions of both the SON and PVN (Table 1). Bilateral CeA lesions significantly reduced the numbers of SON and PVN Fos-positive OT cells recruited by IL-1β compared to sham-lesioned animals (Table 1).

Effects of central amygdala lesions on bed nucleus of the stria terminalis cells

In sham-lesioned animals, systemic IL-1β administration resulted in Fos-positive cells in three distinct bilateral regions of the BNST; the ventromedial (Fig. 3A), ventrolateral and oval nuclei (Fig. 3C). Bilateral CeA lesions significantly reduced the number of Fos-positive cells within both the ventromedial and ventrolateral subdivisions but there was no significant difference between the sham- and CeA-lesioned animals for the oval nucleus (Fig. 3B, C; Table 1).

Effect of central amygdala lesions on ventrolateral medulla catecholamine cells

In sham-lesioned animals IL-1β administration resulted in the recruitment of Fos-positive A1 noradrenergic and C1 adrenergic cell groups of the VLM cell column (Fig. 4; Table 1). Rostrocaudal plots of the VLM A1 and C1 cell recruitment patterns indicate that the majority of cells were located just caudal and rostral to obex, respectively (Fig. 5A, B). Bilateral CeA lesions resulted in significant reductions in the recruitment of both A1 noradrenergic and C1 adrenergic cells (Table 1, Fig. 5A, B).

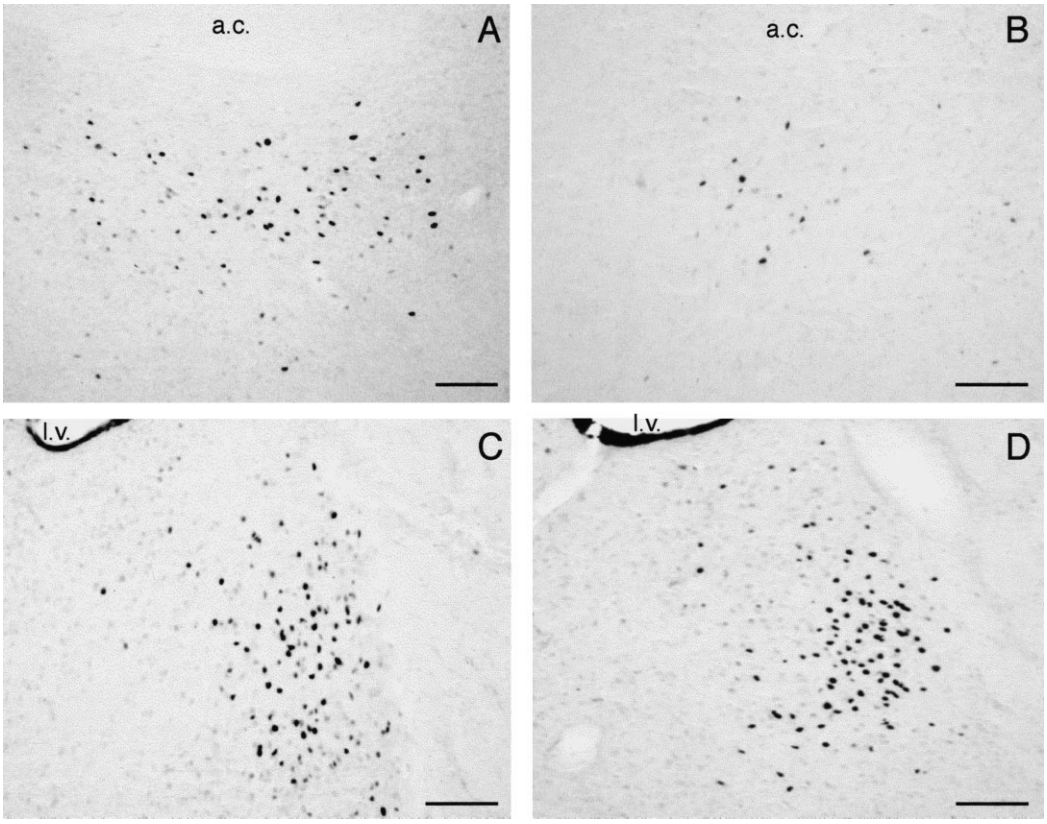

Fig. 3. Photomicrographs illustrating the effects of CeA lesions on BNST cells recruited by systemic IL-1β administration. Sections (40 μm) have been immunolabelled for nuclear Fos and taken from the ventromedial (A, B) and oval nucleus subdivisions (C, D) of the BNST. Systemic IL-1β administration resulted in recruitment of cells within both the ventromedial and oval nucleus in sham-lesioned animals (A, C). Bilateral CeA lesions significantly reduced the number of Fos-positive cells within the ventromedial BNST (B) but not the oval nucleus (D). Scale bars = 100 μm. a.c., anterior commissure; l.v., lateral ventricle.

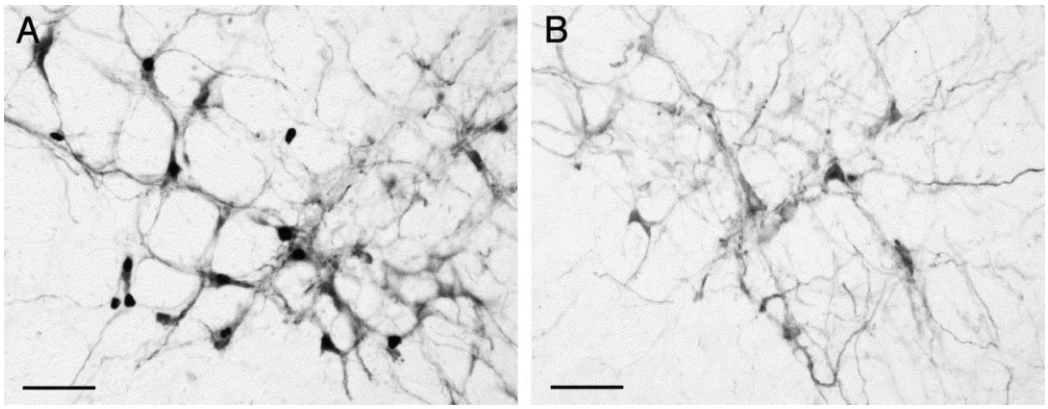

Fig. 4. Photomicrographs illustrating the effects of CeA lesions on VLM catecholamine cells following systemic IL-1β administration. Sections (50 μm) have been immunolabelled for nuclear Fos and cytoplasmic TH and have been taken from approximately 750 μm caudal to obex corresponding to a rostrocaudal level containing A1 noradrenergic cells. In sham-lesioned animals systemic IL-1β typically recruited large numbers of VLM A1 cells (A) while in bilateral CeA-lesioned animals there was a significant reduction in the number of Fos-positive TH cells in the VLM (B). Scale bars = 50 μm.

Effects of central amygdala lesions on nucleus tractus solitarius catecholamine cells

In sham-lesioned animals, systemic IL-1β administration also resulted in the recruitment of both A2 noradrenergic and C2 adrenergic cells however the majority of NTS cells recruited were A2 noradrenergic cells (Table 1; Fig. 6A, B). Rostrocaudal plots of Fos-positive NTS A2 noradrenergic cells showed that most recruited A2 cells were concentrated at the level of obex. Bilateral CeA lesions resulted in significant reductions in the recruitment of A2 cells while the very small number of C2 cells activated by IL-1β remained unchanged compared to sham-lesioned animals (Table 1; Fig. 6).

DISCUSSION

The present study provides the first evidence for the involvement of the CeA in the generation of HPA axis responses to immune challenge. As well as suppressing HPA axis responses, lesions of the CeA greatly reduced the activation of BNST and brainstem catecholamine cells. Since these populations have been implicated in the control of the HPA axis, and because direct connections between the CeA and the hypothalamic apex of the HPA axis are scarce, it appears likely that the CeA can modulate the activity of other central pathways that normally provide the primary drive to the HPA axis after detection of an increase in circulating levels of proinflammatory mediators such as IL-1β.

Role of the central amygdala in the hypothalamic–pituitary–adrenal axis responses to interleukin-1β

In agreement with previous reports we found that in sham-lesioned animals the majority of Fos-positive cells within the amygdala were located in the lateral CeA.[8,19] The failure to observe more than minor levels of c-*fos* expression in other regions of the amygdala cannot be construed as evidence that they were not also affected, although it is noteworthy that other studies have established that almost all divisions of the amygdala do contain substantial numbers of cells capable of expressing c-*fos*.[5]

Many previous studies have implicated the CeA in the control of HPA axis responses to stressful stimuli[1,2,20,39,53] although none have investigated its involvement in the response to immune challenge. The present data show that CeA lesions reduced IL-1β-induced c-*fos* expression in both mPVN CRF cells and neurosecretory OT cells, the latter

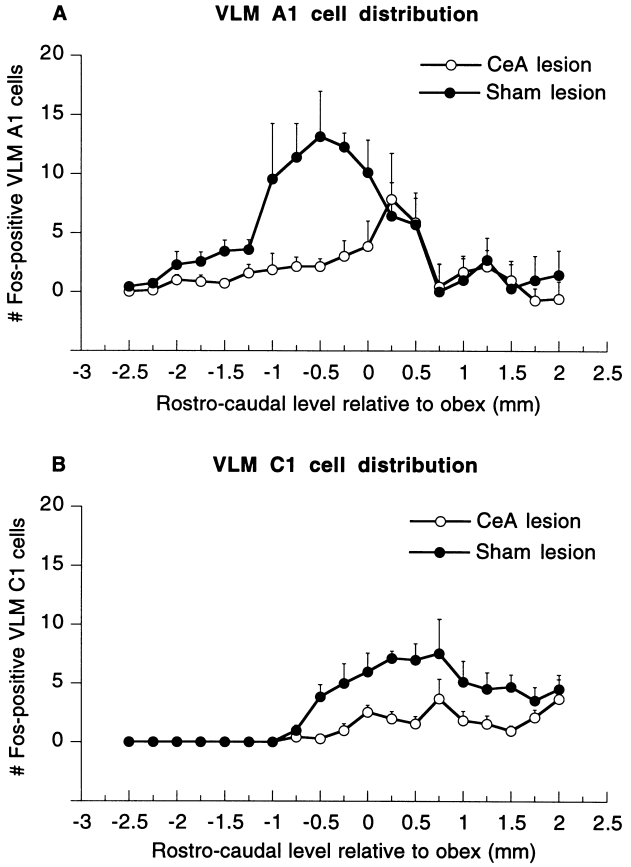

Fig. 5. Effects of bilateral CeA lesions on the rostrocaudal distribution of IL-1β-induced Fos-positive A1 noradrenergic cells (A) and C1 adrenergic cells (B) in the VLM. Note that because TH is a marker for both noradrenergic and adrenergic cells the number of activated noradrenergic A1 cells was estimated by subtracting the number of double-labelled PNMT cells in one section from the number of double-labelled TH cells in the adjacent section. Systemic IL-1β administration resulted in the recruitment of both A1 noradrenergic and C1 adrenergic cells along the VLM cell column. The majority of A1 cells recruited were found below obex whereas C1 cells were concentrated from 0–2 mm relative to obex. Bilateral CeA lesions resulted in a significant reduction in IL-1β-induced Fos expression in both the A1 noradrenergic and C1 adrenergic cell groups.

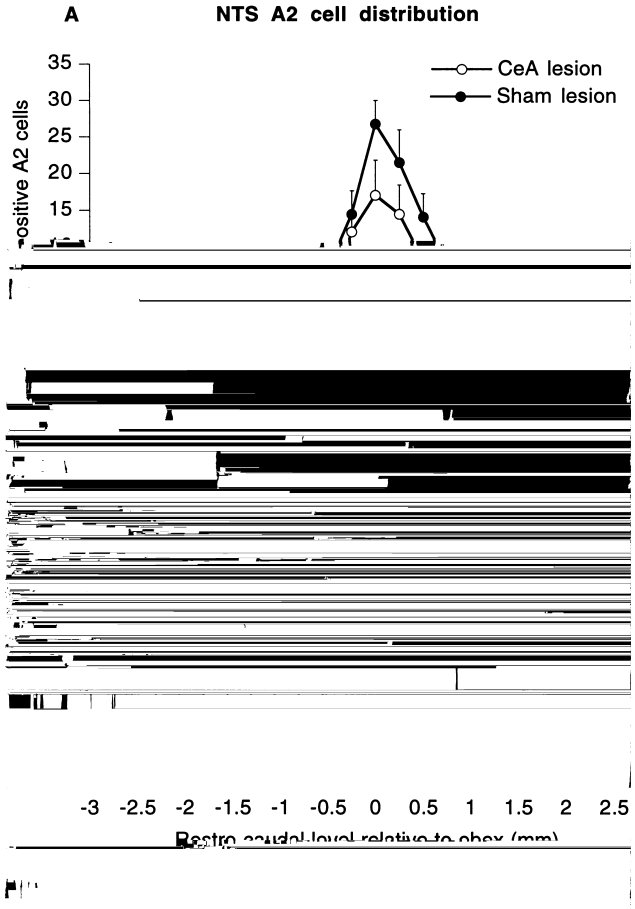

Fig. 6. Effects of bilateral CeA lesions on the rostrocaudal distribution of IL-1β-induced Fos-positive A2 noradrenergic cells (A) and C2 adrenergic cells (B) in the NTS. Note that because TH is a marker for both noradrenergic and adrenergic cells the number of activated noradrenergic A2 cells was estimated by subtracting the number of double-labelled PNMT cells in one section from the number of double-labelled TH cells in the adjacent section. Within the NTS the vast majority of catecholamine cells recruited following systemic IL-1β administration in sham-lesioned animals were A2 noradrenergic cells. Bilateral CeA lesions resulted in a significant reduction in IL-1β-induced Fos expression in A2 noradrenergic cells but had no effect on the small number of C2 adrenergic cells recruited.

being of interest due to evidence that OT can contribute to ACTH release through a direct action at the corticotroph.[22,37,38] Importantly, we were able to demonstrate that the effects of CeA lesions on CRF and OT cell activity were matched by a reduction in plasma ACTH responses to systemic IL-1β thus supporting the view that neuroendocrine cell c-*fos* expression can provide a useful index of HPA axis endocrine output during short-term activation. Furthermore we have recently shown an excellent correlation between the number of Fos-positive mPVN CRF cells and level of ACTH in response to systemic administration of IL-1β.[8] The CeA lesions produced in this study were quite discrete, as indicated both by the restricted extent of gliosis observed in the amygdala of the lesioned animals and the fact that these lesions had no effect on the small but consistent level of c-*fos* expression observed in the MeA after IL-1β administration. Interestingly we also found that in two animals where ibotenic acid lesions had been misplaced, and the MeA rather than the CeA had been destroyed, the HPA axis response to IL-1β was indistinguishable from that observed in sham-lesioned animals (data not shown).

Several lines of evidence suggest that direct projections between the CeA and the mPVN are not likely to be responsible for the altered HPA axis responses observed in the present study. Even though the CeA does have a small number of cells that project to the mPVN[3,23,31,41,48] a recent study shows that systemic IL-1β does not activate neurons in the CeA that project directly to the PVN.[19] Moreover some reports have specified that this small PVN-projecting CeA population originates primarily in the medial division of the CeA[23,31] whereas we found that neurons recruited by IL-1β administration were mostly concentrated in the lateral CeA. Consequently the significant attenuation of IL-1β-induced HPA axis responses in CeA-lesioned animals is very unlikely to be dependent on a direct pathway from the CeA. Similarly, although it is thought the amygdala can influence OT cell activity in the SON and PVN magnocellular regions,[11,34] it is unlikely that monosynaptic pathways mediate this influence.

Role of the bed nucleus of the stria terminalis in central amygdala-dependent hypothalamic–pituitary–adrenal axis responses

CeA lesions significantly reduced IL-1β-induced c-*fos* expression within the ventromedial and ventrolateral subdivisions of the BNST showing for the first time that the CeA can influence BNST cell responses to an immune challenge. Previous reports indicate that these BNST subdivisions project to the PVN[12,32,48] and selective anterior BNST lesions, that encompass the ventrolateral and ventromedial subdivisions, reportedly reduce the expression of ACTH secretagogues.[26] Although we and others also found Fos labelling in the oval nucleus of the BNST following systemic IL-1β,[8,19] CeA lesions did not alter this response and it has also been shown that IL-1β-activated neurons in the oval nucleus do not project to the PVN.[19] Based on the present data it seems plausible that the ventromedial and ventrolateral subdivisions of the BNST could mediate CeA influences on IL-1β-induced HPA axis activation although it remains to be determined whether it is the IL-1β-responsive neurons in these BNST subdivisions that project to the mPVN.

Role of brainstem catecholamine cells in central amygdala-dependent hypothalamic–pituitary–adrenal axis responses

It is plausible that brainstem catecholamine cells might be involved in mediating CeA effects on HPA axis responses to systemic IL-1β administration. In the present study we found that CeA lesions resulted in significant reductions in the recruitment of VLM A1 and C1 cells and NTS A2 cells. Whilst the CeA densely innervates the NTS A2 and C2 regions within the VLM it is thought to provide only a sparse innervation of C1 cell bodies.[35,36,54] Therefore, evidence that the CeA can influence VLM A1 and C1 cell activity is somewhat surprising. It may be that the CeA alters VLM catecholamine responses through NTS–VLM connections[29] or some other indirect pathway. PVN catecholamine inputs arising primarily from the VLM and NTS noradrenergic and adrenergic cell groups constitute major afferent pathways involved in generating HPA axis responses following systemic administration of IL-1β. Previous studies have shown that destruction of PVN catecholaminergic terminals or inputs to the PVN inhibit HPA axis responses to systemic IL-1[10,19,25,33,56]

and IL-1β-activated catecholamine cells project to the PVN.[19] Also with regard to magnocellular OT cell responses, there are substantial catecholaminergic projections to the SON and PVN[13,14,47] and knife cuts that interrupt the ascending catecholamine bundle have been shown to block PVN neurosecretory cell responses to IL-1β[19] suggesting that brainstem catecholamine cells play a major role in generating at least the PVN OT response to IL-1β. However, the role of brainstem catecholamine cells in IL-1β-induced SON OT cell recruitment remains to be elucidated.

Despite there being no direct evidence of specific CeA connectivity with brainstem catecholamine cells which project to the hypothalamus, the present data suggest that the activity of PVN-projecting brainstem catecholamine cells that respond to immune challenge could be under the influence of the CeA. This proposal is consistent with a report that CeA ablation can result in a decrease in stress-induced noradrenergic activity in the PVN[2] and also with findings that stimulation of the amygdala results in concomitant increases in brain catecholamine levels.[42]

Modulatory control of hypothalamic–pituitary–adrenal axis function by the central amygdala

Recent findings indicate that in the case of systemic immune challenge the triggering of the HPA axis activation by the occurrence of a rise in circulating levels of proinflammatory mediators is transduced by prostaglandin-mediated mechanisms at the level of the area postrema and the vasculature of the NTS and VLM.[6,8,9,17,18] This is followed by an activation of medullary catecholamine cells which provide the primary excitatory input to tuberoinfundibular CRF and perhaps neurosecretory OT cells, thus resulting in HPA axis activation. However, the catecholaminergic response to an immune challenge, based on the present results, appears to be gated by a descending input from the CeA. Given the acknowledged role of the amygdala in emotions, this could conceivably constitute a mechanism by which the emotional and physical responses of an animal to an immune challenge are integrated. With regard to the current findings concerning the BNST there is no evidence that BNST activation is integral to the activation of the HPA axis by an immune challenge as has been established for medullary catecholamine cells.

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REFERENCES

1. Beaulieu S., Di Paolo T. and Barden N. (1986) Control of ACTH secretion by the central nucleus of the amygdala: implication of the serotoninergic system and its relevance to the glucocorticoid delayed negative feedback mechanism. *Neuroendocrinology* **44,** 247–254.
2. Beaulieu S., Di Paolo T., Cote J. and Barden N. (1987) Participation of the central amygdaloid nucleus in the response of adrenocorticotropin secretion to immobilization stress: opposing roles of the noradrenergic and dopaminergic systems. *Neuroendocrinology* **45,** 37–46.
3. Berk M. L. and Finkelstein J. A. (1981) Afferent projections to the preoptic area and hypothalamic regions in the rat brain. *Neuroscience* **6,** 1601–1624.
4. Berkenbosch F., Oers J. V., Rey A. D., Tilders F. and Besedovsky H. (1987) Corticotropin-releasing factor producing neurons in the rat activated by interleukin-1. *Science* **238,** 524–526.
5. Bhatnagar S. and Dallman M. (1998) Neuroanatomical basis for facilitation of hypothalamic–pituitary–adrenal responses to a novel stressor after chronic stress. *Neuroscience* **84,** 1025–1039.
6. Breder C. D. and Saper C. B. (1996) Expression of inducible cyclooxygenase mRNA in the mouse brain after systemic administration of bacterial lipopolysaccharide. *Brain Res.* **713,** 64–69.
7. Buller K. M., Smith D. W. and Day T. A. (1999) Differential recruitment of hypothalamic neuroendocrine and ventrolateral medulla catecholamine cells by non-hypotensive and hypotensive hemorrhages. *Brain Res*. (in press).
8. Buller K. M., Xu Y. and Day T. A. (1998) Indomethacin attenuates oxytocin and hypothalamic–pituitary–adrenal axis responses to systemic interleukin-1β. *J. Neuroendocr.* **10,** 519–528.
9. Cao C., Matsumura K., Yamagata K. and Watanabe Y. (1996) Endothelial cells of the rat brain vasculature express cyclooxygenase-2 mRNA in response to systemic interleukin-1 beta: a possible site of prostaglandin synthesis responsible for fever. *Brain Res.* **733,** 263–272.
10. Chuluyan H. E., Saphier D., Rohn W. M. and Dunn A. J. (1992) Noradrenergic innervation of the hypothalamus participates in adrenocortical responses to interleukin-1. *Neuroendocrinology* **56,** 106–111.
11. Cirino M. and Renaud L. P. (1985) Influence of lateral septum and amygdala stimulation on the excitability of hypothalamic supraoptic neurons. An electrophysiological study in the rat. *Brain Res.* **326,** 357–361.
12. Cullinan W. E., Herman J. P. and Watson S. J. (1993) Ventral subicular interactions with the hypothalamic paraventricular nucleus: evidence for a relay in the bed nucleus of the stria terminalis. *J. comp. Neurol.* **332,** 1–20.
13. Cunningham E. T. Jr., Bohn M. C. and Sawchenko P. E. (1990) Organization of adrenergic inputs to the paraventricular and supraoptic nuclei of the hypothalamus in the rat. *J. comp. Neurol.* **292,** 651–667.
14. Cunningham E. T. Jr. and Sawchenko P. E. (1988) Anatomical specificity of noradrenergic inputs to the paraventricular and supraoptic nuclei of the rat hypothalamus. *J. comp. Neurol.* **274,** 60–76.
15. Dayas C. V., Buller K. M. and Day T. A. (1999) Neuroendocrine responses to an emotional stressor: evidence for involvement of the medial but not the central amygdala. *Eur. J. Neurosci.* (in press).
16. Dunn J. D. (1987) Plasma corticosterone responses to electrical stimulation of the bed nucleus of the stria terminalis. *Brain Res.* **407,** 327–331.
17. Elmquist J. K., Breder C. D., Sherin J. E., Scammell T. E., Hickey W. F., Dewitt D. and Saper C. B. (1997) Intravenous lipopolysaccharide induces cyclooxygenase 2-like immunoreactivity in rat brain perivascular micoplia and meningeal macrophages. *J. comp. Neurol.* **381,** 119–129.
18. Ericsson A., Arias C. and Sawchenko P. E. (1997) Evidence for an intramedullary prostaglandin-dependent mechanism in the activation of stress-related neuroendocrine circuitry by intravenous interleukin-1. *J. Neurosci.* **17,** 7166–7179.
19. Ericsson A., Kovacs K. J. and Sawchenko P. E. (1994) A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. *J. Neurosci.* **14,** 897–913.
20. Feldman S., Conforti N., Itzik A. and Weidenfeld J. (1994) Differential effect of amygdaloid lesions on CRF-41, ACTH and corticosterone responses following neural stimuli. *Brain Res.* **658,** 21–26.
21. Feldman S., Conforti N. and Saphier D. (1990) The preoptic area and bed nucleus of the stria terminalis are involved in the effects of the amygdala on adrenocortical secretion. *Neuroscience* **37,** 775–779.
22. Gibbs D. M. (1984) Dissociation of oxytocin, vasopressin and corticotropin secretion during different types of stress. *Life Sci.* **35,** 487–491.

23. Gray T. S., Carney M. E. and Magnuson D. J. (1989) Direct projections from the central amygdaloid nucleus to the hypothalamic paraventricular nucleus: possible role in stress-induced adrenocorticotropin release. *Neuroendocrinology* **50,** 433–446.
24. Gray T. S., Piechowski R. A., Yracheta J. M., Rittenhouse P. A., Bethea C. L. and Van de Kar L. D. (1993) Ibotenic acid lesions in the bed nucleus of the stria terminalis attenuate conditioned stress-induced increases in prolactin, ACTH and corticosterone. *Neuroendocrinology* **57,** 517–524.
25. Harbuz M. S., Chowdrey H. S., Jessop D. S., Biswas S. and Lughtman S. L. (1991) Role of catecholamines in mediating messenger RNA and hormonal responses to stress. *Brain Res.* **551,** 52–57.
26. Herman J. P., Cullinan W. E. and Watson S. J. (1994) Involvement of the bed nucleus of the stria terminalis in tonic regulation of paraventricular hypothalamic CRH and AVP mRNA expression. *J. Neuroendocr.* **6,** 433–442.
27. Hopkins S. J. and Rothwell N. J. (1995) Cytokines and the nervous system. I: Expression and recognition. *Trends Neurosci.* **18,** 83–88.
28. Katsuura G., Gottschall P. E., Dahl R. R. and Arimura A. (1988) Adrenocorticotropin release induced by intracerebroventricular injection of recombinant human interleukin-1 in rats: possible involvement of prostaglandin. *Endocrinology* **122,** 1773–1779.
29. Kawano H. and Masuko S. (1996) Caudal ventrolateral medullary neurons projecting to the paraventricular nucleus receive synaptic inputs from the nucleus tractus solitarius: an anterograde and retrograde double-labeling study in the rat. *Neurosci. Lett.* **218,** 33–36.
30. Kluger M. J. (1991) Fever: role of pyrogens and cryogens. *Physiol. Rev.* **71,** 93–127.
31. Marcilhac A. and Siaud P. (1997) Identification of projections from the central nucleus of the amygdala to the paraventricular nucleus of the hypothalamus which are immunoreactive for corticotrophin-releasing hormone in the rat. *Expl Physiol.* **82,** 273–281.
32. Mulders W. H. A. M., Meek J., Hafmans T. G. M. and Cools A. R. (1997) Plasticity in the stress-regulating circuit: decreased input from the bed nucleus of the stria terminalis to the hypothalamic paraventricular nucleus in Wistar rats following adrenalectomy. *Eur. J. Neurosci.* **9,** 2462–2471.
33. Parsadaniantz S. M., Gaillet S., Lenoir V., Batsche E. and Barbanel G.E.A. (1995) Lesions of the afferent catecholaminergic pathways inhibit the temporal activation of the CRH and POMC gene expression and ACTH release induced by human interleukin-1β in the male rat. *Neuroendocrinology* **62,** 586–595.
34. Petrov T., Jhamandas J. H. and Krukoff T. L. (1994) Electrical stimulation of the central nucleus of the amygdala induces fos-like immunoreactivity in the hypothalamus of the rat: a quantitative study. *Molec. Brain Res.* **22,** 333–340.
35. Petrov T., Krukoff T. L. and Jhamandas J. H. (1995) Convergent influence of the central nucleus of the amygdala and the paraventricular hypothalamic nucleus upon brainstem autonomic neurons as revealed by c-fos expression and anatomical tracing. *J. Neurosci. Res.* **42,** 835–845.
36. Pickel V. M., Van Bockstaele E. J., Chan J. and Cestari D. M. (1995) Amygdala efferents form inhibitory-type synapses with a subpopulation of catecholaminergic neurons in the rat nucleus tractus solitarius. *J. comp. Neurol.* **362,** 510–523.
37. Plotsky P. M. (1991) Pathways to the secretion of adrenocorticotropin: a view from the portal. *J. Neuroendocr.* **3,** 1–9.
38. Plotsky P. M. and Sawchenko P. E. (1987) Hypophysial-portal plasma levels, median eminence content and immunohistochemical staining of corticotropin-releasing factor, arginine vasopressin and oxytocin following pharmacological adrenalectomy. *Endocrinology* **120,** 1361–1369.
39. Prewitt C. M. and Herman J. P. (1994) Lesion of the central nucleus of the amygdala decreases basal CRH mRNA expression and stress-induced ACTH release. *Ann. N.Y. Acad. Sci.* **746,** 438–440.
40. Prewitt C. M. and Herman J. P. (1997) Hypothalamo–pituitary–adrenocortical regulation following lesions of the central nucleus of the amygdala. *Stress* **1,** 263–279.
41. Prewitt C. M. F. and Herman J. P. (1998) Anatomical interactions between the central amygdaloid nucleus and the hypothalamic paraventricular nucleus of the rat: a dual tract-tracing analysis. *J. chem. Neuroanat.* **15,** 173–185.
42. Reis D. J. and Gunne L.-M. (1965) Brain catecholamines: relation to the defence reaction evoked by brain stimulation in the cat. *Science* **15,** 1768–1770.
43. Rivest S. and Rivier C. (1991) Influence of the paraventricular nucleus of the hypothalamus in the alteration of neuroendocrine functions induced by physical stress or interleukin. *Endocrinology* **129,** 2049–2057.
44. Rothwell N. J. and Hopkins S. J. (1995) Cytokines and the nervous system. II: Actions and mechanisms of action. *Trends Neurosci.* **18,** 130–136.
45. Saper C. B. and Breder C. D. (1992) Endogenous pyrogens in the CNS: role in the febrile response. *Prog. Brain Res.* **93,** 419–428.
46. Sapolsky R., Rivier C., Yamamoto G., Plotsky P. and Vale W. W. (1987) Interleukin-1 stimulates the secretion of hypothalamic corticotropin-releasing factor. *Science* **238,** 522–524.
47. Sawchenko P. E. (1988) The effects of catecholamine-depleting medullary knife cuts on CRF- and vasopressin-immunoreactivity in the hypothalamus of normal and steroid-manipulated rats. *Neuroendocrinology* **48,** 459–470.
48. Sawchenko P. E. and Swanson L. W. (1983) The organization of forebrain afferents to the paraventricular and supraoptic nuclei of the rat. *J. comp. Neurol.* **218,** 121–144.
49. Smith D. W., Buller K. M. and Day T. A. (1995) Role of ventrolateral medulla catecholamine cells in hypothalamic neuroendocrine cell responses to systemic hypoxia. *J. Neurosci.* **15,** 7979–7988.
50. Smith D. W. and Day T. A. (1993) Neurochemical identification of Fos-positive neurons using two-colour immunoperoxidase staining. *J. Neurosci. Meth.* **47,** 73–83.
51. Sun N., Roberts L. and Cassell M. D. (1991) Rat central amygdaloid nucleus projections to the bed nucleus of the stria terminalis. *Brain Res. Bull.* **27,** 651–662.
52. Uehara A., Gottschall P. E., Dahl R. R. and Arimura A. (1987) Interleukin-1 stimulates ACTH release by an indirect mechanism which requires endogenous corticotropin releasing factor. *Endocrinology* **121,** 1580–1582.
53. Van de Kar L. D., Piechowski R. A., Rittenhouse P. A. and Gray T. S. (1991) Amygdaloid lesions: differential effect on conditioned stress and immobilization-induced increases in corticosterone and renin secretion. *Neuroendocrinology* **54,** 89–95.
54. Wallace D. M., Magnuson D. J. and Gray T. S. (1992) Organization of amygdaloid projections to brainstem dopaminergic, noradrenergic, and adrenergic cell groups in the rat. *Brain Res. Bull.* **28,** 447–454.
55. Watanobe H., Nasushita R. and Takebe K. (1995) A study on the role of circulating prostaglandin E2 in the adrenocorticotropin response to intravenous administration of interleukin-1β in the rat. *Neuroendocrinology* **62,** 596–600.
56. Weidenfeld J., Abramsky O. and Ovadia H. (1989) Evidence for the involvement of the central adrenergic system in interleukin 1-induced adrenocortical response. *Neuropharmacology* **28,** 1411–1414.
57. Weller K. L. and Smith D. A. (1982) Afferent connections to the bed nucleus of the stria terminalis. *Brain Res.* **232,** 255–270.

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