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Research report

Afferents to the central nucleus of the amygdala and functional subdivisions of the periaqueductal gray: neuroanatomical substrates for affective behavior

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Abstract

Evidence suggests the periaqueductal gray (PAG) is involved in the integration of behavioral and autonomic components of affective behavior. Our laboratory has shown that electrical stimulation of the ventrolateral periaqueductal gray (vl PAG) versus the dorsolateral periaqueductal gray (dl PAG), in the rabbit, elicits two distinct behavioral/cardiorespiratory response patterns. Furthermore, evidence suggests that the amygdaloid central nucleus (ACe) may influence cardiovascular activity during emotional states. The purpose of this study was to delineate the topography and determine the origin of forebrain projections to the PAG and the ACe, as well as commonalties and differences in the pattern of afferents. Examination of common afferents may lend insights into their function as components of a forebrain system regulating autonomic activity during emotional states. Separate retrograde tracers were injected into functional subdivisions of the PAG and the ACe in rabbits. PAG injections led to neuronal labeling in numerous cortical regions including the ipsilateral medial prefrontal and insular cortices. Additionally, bilateral labeling was observed in several hypothalamic nuclei including the paraventricular nucleus, the dorsomedial nucleus and the ventromedial nucleus as well as the region lateral to the descending column of the fornix. Sparse labeling was also seen in various basal forebrain regions, thalamic nuclei and amygdaloid nuclei. Many of these regions were also labeled following injections in the ACe. Although double-labeled cells were never observed, afferents to the ACe were often proximal to PAG afferents. Implications of these findings are discussed in terms of two functionally distinct behavioral/cardiovascular response patterns.

Theme: Neural basis of behavior

Topic: Stress

Keywords: Central nucleus of the amygdala; Periaqueductal gray; Hypothalamus; Medial prefrontal cortex; Neuroanatomical tracer; Emotional stress

Abbreviations: AC, anterior commissure; ACe, amygdaloid central nucleus; ad, anterior dorsal nucleus of thalamus; Aha, anterior hypothalamic area; Ahl, lateral hypothalamic area; Amg, amygdala; bic, brachium of the inferior colliculus; BL, basolateral nucleus of the amygdala; Bm, basomedial nucleus of the amygdala; BNST, bed nucleus of the stria terminalis; cd, caudate nucleus; cl, centralis lateralis nucleus of thalamus; cm, centre median nucleus of thalamus; Dhy, dorsal nucleus of hypothalamus; dl PAG, dorsal lateral periaqueductal gray; Dmh, dorsomedial nucleus of hypothalamus; gp, globus pallidus; IC, internal capsule; ip, interpeduncular nucleus; iam, inter-anteromedial nucleus of thalamus; La, lateral nucleus of the amygdala; la, lateral anterior nucleus of thalamus; Lg, lateral geniculate nucleus; Lh, lateral habenula nucleus; Ls, lateral septal nucleus; Ma, medial amygdaloid nucleus; md, medial dorsal nucleus of thalamus; Mg, medial geniculate nucleus; mrf, midbrain reticular formation; npc, nucleus of the posterior commisure; nr, nucleus raphis; NTM, nucleus of tubero-mamillaris; OT, optic tract; ox, optic chiasm; PAG, periaqueductal gray; Pb, parabrachial nucleus; pc, paracentral nucleus of thalamus; PED, cerebral peduncle; ph, posterior hypothalamus; pol, lateral preoptic area; pom, medial preoptic nucleus; prm, dorsal premamillary nucleus; Pvn, paraventricular nucleus of hypothalamus; Pvn(th), paraventricular nucleus of thalamus; pu, putamen; re, nucleus reunions; Ret, reticular nucleus of thalamus; SC, superior colliculus; si, substantia innominata; smm, supramamillary nucleus; sn, substantia nigra; so, supraoptic nucleus of hypothalamus; ST, stria terminalis; Sth, subthalamic nucleus; tbc, tuber cinereum; va, ventral anterior nucleus of thalamus; vb, ventrobasal nucleus of thalamus; vl, ventrolateral nucleus of thalamus; vlg, ventral lateral geniculate nucleus; vl PAG, ventrolateral periaqueductal gray; vm, ventromedial nucleus of thalamus; Vmh, ventromedial nucleus of hypothalamus; VTA, ventral tegmental area; zi, zona incerta

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1. Introduction

The midbrain PAG has long been implicated as a nodal region in the neural organization of an animal's response to stressful stimuli [1]. A number of studies have shown the importance of the PAG in the integration of behavioral and cardiovascular responses to aversive stimuli [6,26,59,65]. Behavioral responses, which tend to be species specific [20], include freezing, flight and aggression. Visceral responses include changes in cardiac output, heart rate, blood pressure, blood flow and respiration.

Our laboratory has focused on two integrated response patterns to stressful stimuli identified in New Zealand Albino rabbits [18,39]. One response pattern has been referred to as the defense reaction. Preferentially elicited from the dl PAG, this response pattern consists of a pressor response accompanied by tachycardia, gating of the baroreceptor reflex, and hind limb muscle vasodilatation serving to prepare the animal for 'fight or flight', or 'go' responses. Behavioral and cardiovascular response patterns similar to the defense reaction in the rabbit have also been elicited from the lateral and dorsolateral PAG in the rat [7,25,26] and the cat [14,59].

The other cardiovascular/behavioral response pattern exhibited by rabbits to threatening stimuli is a freezing, or 'no go' response referred to as the vigilance reaction. When responding in this manner, the rabbit becomes vigilant to environmental cues, and exhibits a pressor response, vasoconstriction, decreased blood flow in the hind limbs and viscera, augmentation of the baroreceptor reflex, bradycardia and inspiratory apnea or tachyponea [19,40]. Furthermore, neuroanatomical and electrical stimulation studies suggest that the vigilance reaction is preferentially elicited from a neuroanatomical region in the vl PAG distinct from the one responsible for mediating the defense reaction [17,39].

Neuroanatomical evidence suggests the PAG receive numerous afferent connections from cortical, limbic, diencephalic and midbrain structures [11,37,43]. Among these structures is the ACe [28,43,60]. The ACe is thought to be a key structure in the regulation of the autonomic nervous system [27,30,45]. The ACe also has been hypothesized to mediate learned cardiovascular responses to stressful stimuli [21,29] and has been suggested to be capable of modulating responses elicited from the PAG [60,61]. Furthermore, neuroanatomical evidence suggests forebrain afferents to the ACe originate from many of the same regions that project to the PAG.

Although there is a general consensus as to the afferent connections of the ACe and the PAG, a paucity of research has examined the extent of overlap in the afferent connections to these structures responsible for mediating cardiovascular responses to stressful stimuli. The present study, therefore, was designed to examine the afferent connections from the forebrain and midbrain to two distinct populations of neurons in the vl PAG and the dl PAG, shown to produce two different cardiovascular/behavioral response patterns to aversive stimuli. We also assessed the pattern of afferent connections of the ACe, observed to modulate these responses. Insights into the functions of these regions as components of a larger forebrain system responsible for attending and responding to stressful stimuli may be gained by examining similarities and differences in the topography and specific origin of forebrain projections to separate functional subdivisions of the PAG and the ACe.

2. Experimental design/method

2.1. Subjects

Thirteen male and female New Zealand Albino rabbits (*Oryctolagus cuniculus*) weighing 2–3 kg served as subjects for this experiment. Animals were housed individually in a room with artificially controlled temperature (22–24°C) on a 12 h light/dark cycle. All experiments and after-care handling was conducted during the light portion of this cycle. Water and food pellets were provided ad libitum. The rules and regulations for the care and use of experimental animals, as outlined by the National Institute of Health were strictly followed.

2.2. Retrograde neuroanatomical tracers

Since the introduction of horseradish peroxidase (HRP) retrograde axonal tract tracing technique by Kristensson and Olsson [34], many other neuroanatomists have attempted to develop alternative methodologies. Many of the new tracers gain access to the neuronal somata by retrograde transport of fluorescent organic dyes [56,64]. Several of these tracers have been found useful because of their sensitivity and the requisite tissue processing is minimal. Furthermore, the use of multiple fluorescent retrograde tracers with different emission characteristics allows the visualization and comparison of multiple subpopulations of neurons on a single histological section [3] to determine whether individual retrogradely labeled neurons send axon collaterals to the neural region.

Fluoro-Gold (FG) is a fluorescent organic dye capable of undergoing retrograde transport. FG was chosen for this experiment because it is flexible in terms of post-injection survival times, concentration range, tissue treatment and compatibility with other histochemical techniques [56]. Although fading poses a potential problem for many fluorescent markers, our use of FG clearly labeled cells with a distinct granular appearance. As such, we felt that appreciable fading of retrogradely labeled cells was not a serious problem. Furthermore, others have demonstrated that neither time nor prolonged exposure to UV light has been shown to cause significant fading of labeled tissue [56]. Similarly, Fluoro-Ruby (FR) has been found to be a

suitable partner in double-labeling protocols within the central nervous system [57].

2.3. Surgical procedures

Animals were initially anaesthetized through a mask placed over the face with 5% halothane in oxygen, and maintained throughout the procedure at 1–2% halothane in oxygen. Following induction of anesthesia the animal's head was secured firmly in a Kopf stereotaxic instrument. A local anesthetic (Lidocaine HCl 2%) and a topical antiseptic (betadine) were then administered to the scalp. A midline incision was made, extending 4 mm anterior to bregma to just beyond lambda. Bregma was positioned 2.5 mm dorsal to lambda. Subsequently, the skull was trephined above the ACe and the PAG.

A Hamilton syringe was advanced to either a dorsolateral (L 1.0, P 9.5–10.5, V 9.0–9.5) or a ventrolateral (L 1.0, P 9.5–10.5 V 9.5–0.5) site within the PAG. An additional Hamilton syringe was advanced to deposit a second retrograde tracer within the ACe (L 5.8–6.0, A 0.5–P 0.5, V 12). In 9 animals 50–100 nl of Fluoro-Gold (Fluorochrome) at a concentration of 8% was injected at sites targeting either the vl PAG or the dl PAG. Fifty–100 nl of a second retrograde tracer, Fluoro-Ruby (Molecular Probes), at a concentration of 10% was also deposited within the ACe. In 4 animals, the placement of the retrograde tracers was reversed such that the injection of FG targeted the ACe and the injection of FR targeted sites within the PAG. All deposits were delivered at an infusion rate of 10 nl every 5 min via a pressure injection. The Hamilton syringes were left in place for an additional 15 min to minimize the spread of tracer along the syringe tract following its withdrawal.

Following retraction of the Hamilton syringe, bone wax was placed in the skull opening and the wound sutured with wound clips. Additional doses of lidocaine and the antibiotic, Crystiben, were administered as needed. The animal was returned to its home cage following recovery from general anesthesia.

2.4. Histological procedures

Given the length of the pathways under examination, as well as suggestions by the manufacturer (Fluorochrome) and others [56,57], a 14-day survival period was chosen. Subsequently, the animals were sacrificed using an overdose of pentobarbital and perfused transcardially with a 0.9% saline solution, followed by a 4% formalin/saline solution. The brains were removed and stored overnight in a sucrose/phosphate buffered 4% formalin solution and later frozen in a cryostat. Forty μm sections were taken using a Jung Frigocut 2800 E. Every third or fourth section was mounted on a gelatin-coated slide and coverslipped in DPX (Fluka). Slides were saved for later observation. Additionally, adjacent sections were also collected and counter-stained with hematoxylin and eosin to aid in identification of labeled neurons.

2.5. Data analysis

Unstained sections were viewed under epifluorescence using a UV filter (excitation-323 nm, emission – 408 nm), when scanning for neurons retrogradely labeled with FG, or under a green filter (excitation – 555 nm, emission – 580 nm) when scanning for neurons retrogradely labeled with FR. All observations were made at 200× with a Nikon Optiphot microscope to determine the number and distribution of retrogradely labeled neurons rostral to PAG injection sites. Outlines of serial coronal sections were sketched with the aid of a microprojector. The positions of retrograde labeled neurons were plotted with reference to a variety of neural landmarks and with the aid of Nissl-stained sections as well as a rabbit atlas [62]. In each section, the number and location of all labeled cells were identified in order to calculate the total number of labeled neurons. In order to be counted as a retrograde labeled neuron, fluorescent lysosome-like granules of FG or FR must have been observed in the perikaya of the neuron (Fig. 1). Granules of FG or FR observed in the axon or proximal dendrites were considered insufficient evidence of retrograde labeling. Furthermore, the granules of FG or FR needed to be visible only under the appropriate light source to be considered a retrogradely labeled neuron. All labeled cells within the same nucleus on multiple adjacent sections were added to calculate the total number of projections to a targeted region.

3. Results

3.1. Overview

Deposits of fluorescent retrograde tracers into different functional areas of the PAG resulted in qualitatively and quantitatively distinct patterns of labeling. Additionally, an injection into the ACe resulted in a quantitatively distinct number of retrogradely labeled neurons observed in regions also sending projections to the vl PAG and/or the dl PAG. Although the origins of afferents targeting different regions often overlapped, it was still possible to distinguish the location of the injection site by comparing the density of retrogradely labeled neurons in multiple common sites.

3.2. Injection sites

The location and size of the FG and FR deposits within the vl PAG, the dl PAG and the ACe as well as control animals are depicted in Fig. 2 and listed in Table 1. Typically, the injections involved a small core region of dye adjacent to the syringe tract. Immediately adjacent is a highly compact area in which the tissue exhibits a brilliant

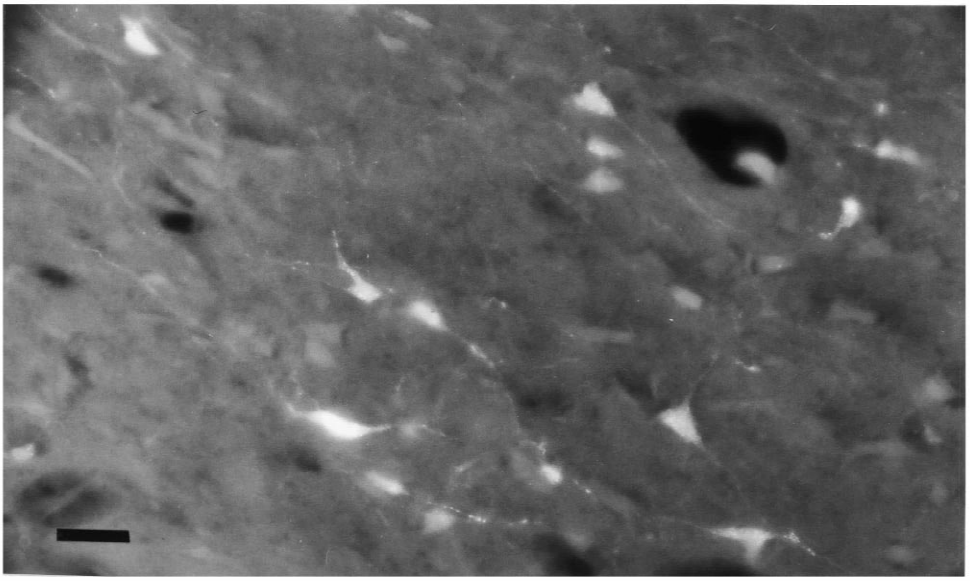


Fig. 1. A photomicrograph of a coronal section depicting neurons in the lateral hypothalamus labeled with Fluoro-Gold following a deposit in the PAG. 200×, U.V. excitation. Bar=50 μm.

fluorescence (Fig. 3). As seen in Fig. 2, two animals (nos. 41 and 47) received injections concurrently in the dl PAG and the ACe. Three additional animals (nos. 34, 36 and 48) had injection sites located in the dl PAG; injections aimed at the ACe in these animals were localized to the ventral amygdalofugal pathway (VAF, #34), the internal capsule (#36) and the basolateral amygdaloid complex (#48) instead. Injection sites were localized to the vl PAG and the ACe in four animals (nos. 38, 43, 44 and 45). Retrograde tracer deposits were observed in the lateral PAG, overlapping areas associated with both the defense reaction and the vigilance reaction, in two animals. This deposit was paired with a deposit in the ACe in one subject (#39), but was paired with an injection encompassing the medial nucleus of the amygdala (Ma), VAF, and the optic tract in the other subject. Two additional animals (nos. 40 and 42) had injections in neither the ACe nor the PAG, and served as controls.

3.3. Sources of afferents

Tables 2 and 3 list selected forebrain and midbrain regions containing retrogradely labeled neurons following injections in the ACe and the PAG together with their laterality (i.e. ipsi- versus contralateral) and the total number of labeled neurons in each region. Central nucleus of the amygdala afferents along with inputs to the vl PAG versus the dl PAG are depicted graphically in Fig. 4. Each structure shown projecting to the vl PAG, the dl PAG or the ACe was observed in at least 3 experimental animals. Although bilateral labeling was observed in many areas, ipsilateral labeling was predominant. The following commentary supplies detailed information on the distribution of the retrogradely labeled neurons in selected telencephalic, diencephalic and midbrain areas.

3.3.1. Telencephalon

Retrogradely labeled neurons were observed primarily in layer V of the cortex following injections in the vl PAG, the dl PAG, as well as the ACe. Although labeled cells were found widely dispersed throughout the cortex, the actual cortical area and the density of labeling within it were dependent upon the location of the injection.

A modest but consistent number of retrogradely labeled cells were found along the medial wall of the frontal lobe. Labeled neurons were observed in cortical area 24 in all but one instance (#38) following deposits within the vl PAG. In contrast, labeling was not observed following injections in the dl PAG. Injections ventral (#40) and lateral (#42) to the PAG also did not result in any retrograde labeling. Although afferents to the ACe were observed, there was no overlap or systematic organization to afferents targeting the ACe and the vl PAG from cortical area 24.

Retrogradely labeled cells were observed in cortical area 32 following all injections into the vl PAG and the dl PAG, but not from areas outside the PAG (nos. 40 and 42). Interestingly, labeled neurons were dispersed within cortical area 32 following deposits in the vl PAG, but were concentrated primarily in the ventral region following deposits in the dl PAG. Afferents to the ACe were also observed. Although afferents to the vl PAG were observed, there was no overlap or topographical organization between afferents to the ACe and the vl PAG originating in cortical area 32. In contrast, projections from cortical area 32 to the ACe tended to originate caudal to those targeting the dl PAG.

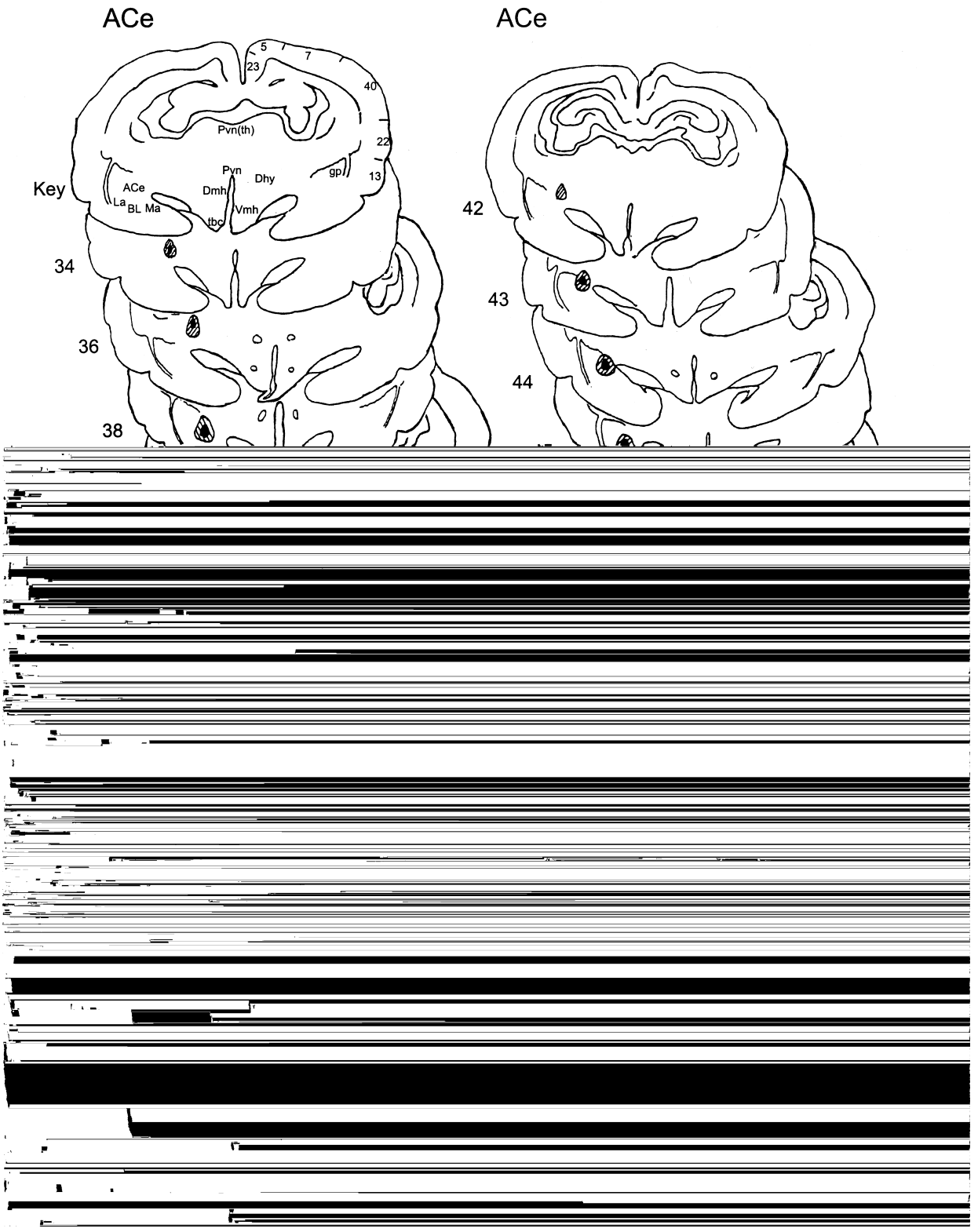


Fig. 2. Diagrammatic representations of coronal sections depicting the location of the deposit of retrograde tracer. Experimental numbers are given corresponding to Table 1.

Labeled neurons were consistently observed in cortical area 25 following deposits in the dl PAG, but not in the vl PAG. They were also observed in the dorsal region of cortical area 25 following deposits within the ACe. These projections tended to be located rostral to those directed towards the dl PAG.

Table 1
Table of retrograde tracer injections[a]

Experiment number	Location of the injection site	Retrograde tracer used	Concentration (%)	Volume (nl)
34	dl PAG	Fluoro-Gold	8	100
	IC/VAF	Fluoro-Ruby	10	100
36	dl PAG	Fluoro-Gold	8	100
	IC	Fluoro-Ruby	10	100
38	vl PAG	Fluoro-Gold	8	100
	ACe	Fluoro-Ruby	10	100
39	lateral PAG	Fluoro-Gold	8	100
	ACe	Fluoro-Ruby	10	100
40	ventral to vl PAG	Fluoro-Gold	8	100
	IC	Fluoro-Ruby	10	100
41	dl PAG	Fluoro-Gold	8	100
	ACe	Fluoro-Ruby	10	100
42	lateral to L PAG	Fluoro-Gold	8	50
	IC	Fluoro-Ruby	10	100
43	vl PAG	Fluoro-Gold	8	50
	ACe	Fluoro-Ruby	10	100
44	vl PAG	Fluoro-Gold	8	50
	ACe	Fluoro-Ruby	10	100
45	vl PAG	Fluoro-Ruby	10	50
	ACe	Fluoro-Gold	8	50
46	lateral PAG	Fluoro-Ruby	10	50
	Ma/ST	Fluoro-Gold	8	50
47	dl PAG	Fluoro-Ruby	10	50
	ACe	Fluoro-Gold	8	50
48	dl PAG	Fluoro-Ruby	10	50
	La/BL	Fluoro-Gold	8	50

[a] The location of the injection site within the periaqueductal gray or in the central nucleus of the amygdala and in control experiments with an indication of the concentration, volume injected and whether the retrograde tracer used was Fluoro-Gold or Fluoro-Ruby.

Sparse labeling was observed in cortical areas 13 and 14, corresponding to the insular cortex, following some deposits in the PAG. Retrograde labeling was observed in all animals except case 38 following deposits in the vl PAG, whereas lighter labeling was observed in less than half of the animals with a deposit localized within the dl PAG. In contrast, robust labeling was seen throughout the rostral–caudal extent of the insular cortex following injections in the ACe. However, it was somewhat sparser in the rostral and caudal poles. Although there was a large difference in the density of projections to the ACe versus the vl PAG, retrogradely labeled neurons were in close proximity at multiple rostral-caudal levels (Fig. 4).

Cortical areas 7, 21, 22, 36 and 41 along the lateral region of the cerebral hemisphere, as well as cortical areas 23 and 29 along the medial wall, provided a scattered but modest number of inputs to the ACe. Labeled cells were also identified in cortical areas 1–5, 17, 20, 23 and 36 following deposits in the PAG. However, much of this labeling was sporadic and inconsistent.

In the basal forebrain, the vertical nucleus and the horizontal nucleus of the diagonal band contained scattered retrogradely labeled neurons following deposits in either the PAG or the ACe. Caudally these nuclei merge with the substantia innominata. Within the substantia innominata a modest number of projections to the ACe were distributed among projections targeting the vl PAG and the dl PAG.

Labeling of neurons in the lateral septal nucleus indicated a differential pattern of afferents to distinct functional areas of the PAG. Labeling consistently followed vl PAG injections, but sparse labeling was observed in a single animal following deposits localized within the dl PAG (data not shown). Labeled neurons were uncommon in septal areas following ACe injections.

The bed nucleus of stria terminalis (BNST) appears to send differential projections to the vl PAG versus the dl PAG. Sparse to moderate labeling was observed in all subjects receiving a deposit into the vl PAG. In contrast, four retrogradely labeled neurons were observed in a single subject (≠36) following deposits in the dl PAG. Additionally, the BNST contained a small number of neurons projecting to the ACe. These neurons were often found in close proximity to neurons with fibers terminating in the vl PAG.

The contribution of afferents to the PAG from the amygdala appears limited. Furthermore, this source of afferents appears to project differentially to the vl PAG versus the dl PAG. Amygdaloid labeling was not observed following deposits of tracer in the dl PAG. In contrast, sparse but consistent labeling was observed in the amygdala following retrograde tracer deposits in the vl PAG. The ACe contained the largest number of retrogradely labeled amygdaloid cells following vl PAG injections. The medial, basolateral, basomedial and anterior

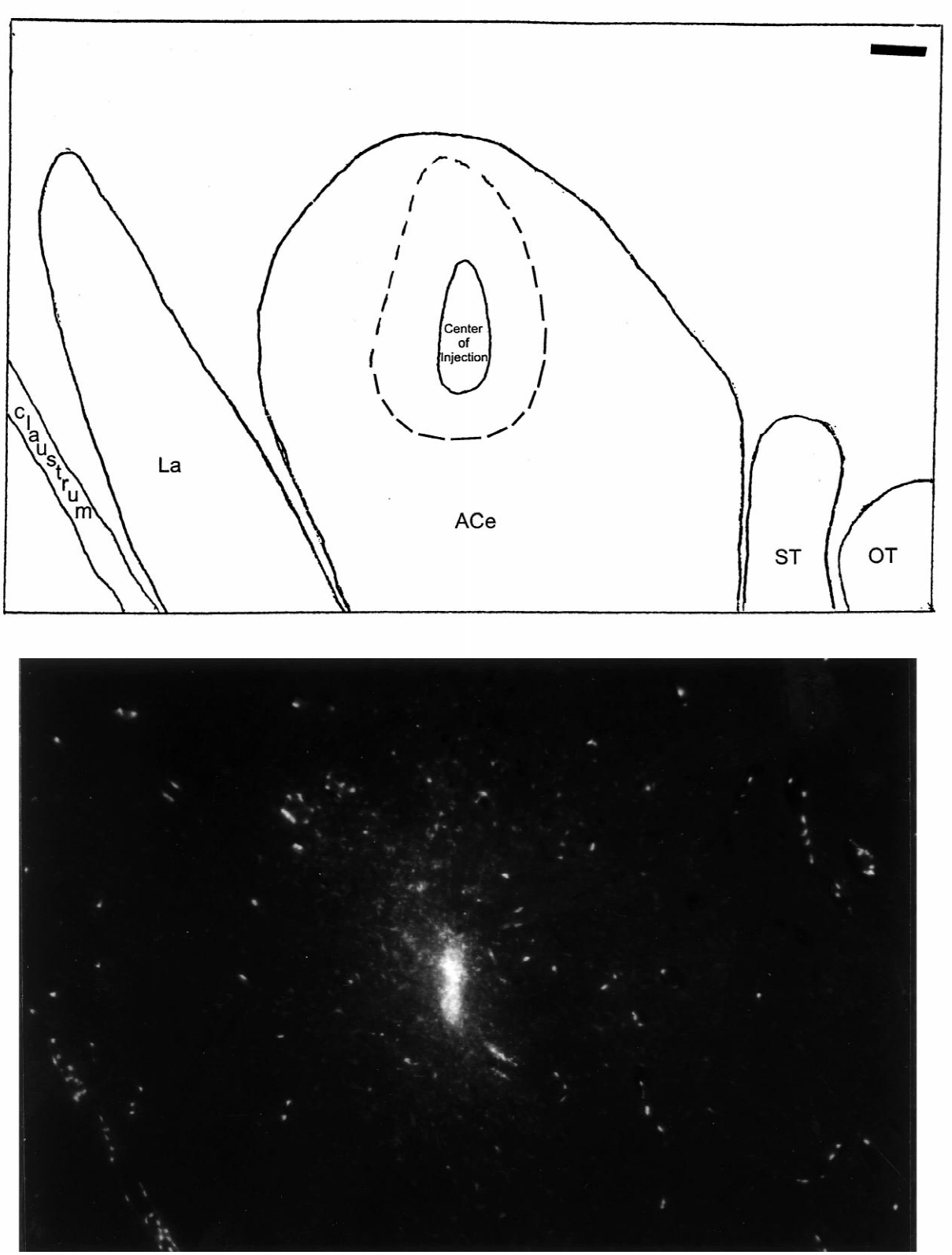


Fig. 3. (A) A schematic drawing of a coronal section of the rabbit brain and the location of a Fluoro-Ruby injection in the ACe. Borders of amygdaloid subnuclei are approximations. (B) Representative photomicrograph of the same field in the amygdala following a deposit of Fluoro-Ruby. The claustrum and optic tract are evident in the lower left and right corners, respectively. The photomicrograph was taken using a green filter (excitation – 555 nm, emission – 580 nm) at 40×. Bar=250 μm.

Table 2
Table depicting the distribution of retrogradely labeled neurons following injections targeting the periaqueductal gray

Injection site	dl PAG		dl PAG		vl PAG		L PAG		V to vl PAG		dl PAG		L to L PAG		vl PAG		vl PAG		vl PAG		L PAG		dl PAG		dl PAG	
Case #	#34		#36		#38		#39		#40		#41		#42		#43		#44		#45		#46		#47		#48	
Laterality	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C
Cortex																										
Area 25	**39**	**0**	**73**	**1**	–	–	**2**	**0**	–	–	**16**	**0**	–	–	–	–	–	–	–	–	**6**	**1**	**2**	**0**	**2**	**1**
Area 24	–	–	–	–	–	–	**4**	**0**	–	–	–	–	–	–	**6**	**0**	**1**	**0**	**14**	**1**	**49**	**3**	–	–	–	–
Area 32	**4**	**0**	**29**	**4**	**1**	**0**	**6**	**0**	–	–	**4**	**0**	–	–	**7**	**0**	**2**	**1**	**12**	**2**	**46**	**2**	**1**	**0**	**2**	**0**
Area 13	–	–	**1**	**1**	–	–	**1**	**0**	–	–	–	–	–	–	**3**	**0**	**3**	**0**	**5**	**0**	**9**	**0**	–	–	**2**	**0**
Hypothalamus																										
Pom	–	–	30	6	180	68	13	7	1	0	3	0	–	–	25	3	2	0	4	1	5	0	2	0	–	–
Aha	19	9	47	6	56	36	31	6	1	0	2	1	1	0	24	3	3	0	2	1	3	0	3	0	1	0
So	1	0	11	4	42	7	10	3	6	1	3	0	2	0	8	2	4	0	4	0	7	2	–	–	2	1
ph	50	1	23	7	158	81	71	6	5	0	15	0	7	0	68	5	42	12	15	0	36	2	3	0	2	0
Ahl	**68**	**14**	**38**	**4**	**306**	**159**	**149**	**20**	**6**	**0**	**26**	**5**	**25**	**1**	**188**	**35**	**32**	**5**	**27**	**0**	**38**	**6**	**5**	**2**	**6**	**1**
Vmh	**101**	**31**	**149**	**55**	**312**	**162**	**192**	**49**	**3**	**0**	**53**	**19**	**21**	**4**	**147**	**22**	**38**	**4**	**21**	**4**	**35**	**4**	**6**	**1**	**6**	**2**
Dmh	**116**	**23**	**194**	**35**	**223**	**144**	**105**	**11**	**1**	**0**	**98**	**15**	**19**	**1**	**95**	**15**	**20**	**3**	**10**	**0**	**17**	**1**	**15**	**1**	**3**	**1**
Pvn	**23**	**1**	**37**	**4**	**83**	**36**	**20**	**1**	**4**	**0**	**38**	**5**	**13**	**0**	**41**	**10**	**10**	**3**	**8**	**1**	**12**	**0**	**3**	**0**	**6**	**1**
Amygdala																										
BNST	–	–	4	0	32	12	2	1	–	–	–	–	–	–	7	0	2	0	2	0	–	–	–	–	–	–
ACe	–	–	–	–	**6**	**0**	**13**	**0**	–	–	–	–	–	–	**21**	**0**	**5**	**0**	**2**	**0**	**5**	**0**	–	–	–	–
Amg	–	–	–	–	**4**	**1**	**2**	**0**	**1**	**0**	–	–	–	–	**14**	**0**	**4**	**0**	**2**	**0**	**1**	**0**	–	–	–	–
Midbrain																										
sn	12	2	57	0	59	28	129	2	5	0	17	2	6	0	49	1	32	3	24	3	46	4	5	0	4	0
PAG	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*

amygdaloid nuclei also were labeled occasionally. In contrast, the ACe received many afferents from within the amygdala. These local intrinsic connections were often observed adjacent to vl PAG afferents (Fig. 4).

3.3.2. Hypothalamus

The hypothalamus provided the largest source of afferents to both subdivisions of the PAG as well as a significant source of afferents to the ACe. Retrograde labeling from the PAG suggests some topographical organization and overlap with hypothalamic areas that have been defined functionally using electrical and chemical stimulation techniques. Afferents to the ACe were organized in a way such that there was abundant overlap with hypothalamic projections to the vl PAG and the dl PAG. Interestingly, hypothalamic regions that tended to differentially provide more input to the vl PAG versus the dl PAG also provided more abundant input to the ACe.

The vl PAG receives a dense projection from the lateral hypothalamic area (Ahl) and the ventromedial nucleus of the hypothalamus (Vmh) along with a somewhat weaker projection from the dorsomedial nucleus of the hypothalamus (Dmh). In contrast (Fig. 4 and Table 2), the dl PAG receives a dense projection from the Dmh whereas the Ahl and the Vmh provide a smaller source of afferents. Both the vl PAG and the dl PAG received similar but a more modest number of projections from the paraventricular nucleus of the hypothalamus (Pvn).

Two prominent afferent projections to the ACe originated in the Vmh and the Ahl. These neurons were found intermingled and often adjacent to neurons projecting to the vl PAG and the dl PAG (Fig. 4). Although no individual neuron was observed to be double-labeled, overlap between hypothalamic projections to the PAG and the ACe was greatest in these two nuclei. However, slightly more overlap occurred between afferents to the vl PAG and the ACe versus the dl PAG and the ACe. This may have been related to the differentially denser projection from these two nuclei to the vl PAG versus the dl PAG.

Slightly fewer projections to the ACe originated in the Dmh and the Pvn. Following injections in the ACe, retrogradely labeled neurons were observed scattered through the Dmh, but tended to cluster in the medial region of the Pvn. Deposits localized in the dl PAG labeled proportionately more neurons in the Dmh than deposits localized in the vl PAG. However, this did not translate into a greater degree of overlap between afferents to the ACe and afferents to the vl PAG versus the dl PAG from the Dmh (Fig. 4). Additionally, there were no differences in the amount of overlapping afferents between the ACe and the vl PAG and the overlap of afferents between the ACe and the dl PAG from the Pvn. Interestingly, most afferents to the ACe originating in the Pvn were juxtaposed to neurons targeting the PAG. Double-labeled neurons were never observed in the Dmh or the Pvn.

Table 3
Table depicting the distribution of retrogradely labeled neurons following injections targeting the central nucleus of the amygdala[a]

Injection site	IC/VAF		IC		ACe		ACe		IC		ACe		IC		ACe		ACe		BL/ACe		Ma/ST		ACe/Ma		La/BL	
Case #	#34		#36		#38		#39		#40		#41		#42		#43		#44		#45		#46		#47		#48	
Laterality	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C
Cortex																										
Area 25	**9**	**0**	**2**	**0**	**2**	**0**	**3**	**1**	**2**	**0**	**5**	**0**	**2**	**1**	**2**	**0**	**5**	**0**	**2**	**0**	**4**	**0**	**2**	**0**	–	–
Area 24	–	–	**1**	**1**	**1**	**1**	**2**	**0**	**1**	**0**	**3**	**0**	**1**	**0**	**2**	**0**	**2**	**0**	**4**	**0**	**2**	**0**	**1**	**0**	–	–
Area 32	**4**	**0**	**3**	**0**	**2**	**0**	**3**	**2**	**2**	**0**	**2**	**0**	**2**	**0**	**3**	**0**	**6**	**1**	**7**	**0**	**3**	**0**	**3**	**0**	–	–
Area 13	**146**	**12**	**109**	**0**	**45**	**2**	**128**	**1**	**38**	**0**	**112**	**2**	**227**	**3**	**104**	**0**	**25**	**0**	**25**	**7**	**24**	**2**	**3**	**2**	**4**	**0**
Hypothalamus																										
Pom	11	1	2	0	4	0	2	0	4	0	11	1	9	0	5	0	3	0	8	0	24	1	2	0	2	0
Aha	4	0	1	0	1	0	7	0	7	1	7	1	9	0	1	0	2	0	4	0	39	4	–	–	1	0
So	9	1	8	2	11	2	7	0	27	0	21	0	19	2	15	0	7	0	17	1	18	2	1	0	6	0
ph	–	–	1	0	10	0	8	2	12	0	18	1	16	0	6	0	3	0	15	3	32	6	–	–	3	0
Ahl	**48**	**10**	**4**	**1**	**10**	**3**	**15**	**0**	**44**	**0**	**23**	**2**	**32**	**1**	**21**	**5**	**3**	**0**	**14**	**1**	**99**	**4**	**1**	**0**	**12**	**1**
Vmh	**226**	**41**	**6**	**0**	**7**	**0**	**51**	**5**	**38**	**4**	**22**	**1**	**38**	**12**	**20**	**5**	**10**	**1**	**12**	**0**	**60**	**7**	**2**	**0**	**3**	**0**
Dmh	**79**	**8**	**3**	**1**	**4**	**0**	**21**	**0**	**9**	**2**	**12**	**1**	**16**	**2**	**6**	**2**	**2**	**0**	**4**	**2**	**48**	**4**	–	–	–	–
Pvn	**51**	**9**	**1**	**0**	**1**	**1**	**10**	**0**	**12**	**0**	**4**	**0**	**17**	**5**	**14**	**3**	**1**	**0**	**6**	**2**	**51**	**18**	–	–	**4**	**1**
Amygdala																										
BNST	18	1	1	0	1	0	14	2	28	0	5	0	28	1	7	0	2	0	3	0	169	14	–	–	3	0
ACe	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Amg	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*
Midbrain																										
sn	37	1	12	1	65	10	18	0	43	0	71	14	26	2	12	0	20	0	84	15	10	0	–	–	30	5
PAG	37	0	4	0	2	0	27	4	15	0	9	0	14	0	6	0	4	0	29	6	69	39	–	–	10	2

[a] A table summarizing the distribution of retrogradely labeled neurons in the cortex, hypothalamus, amygdala, thalamus and midbrain following a pressure injection of either Fluoro-Gold or Fluoro-Ruby targeting the central nucleus of the amygdala. The case number and the injection site are listed at the top of the table and selected nuclear groups within the forebrain and midbrain which contained retrogradely labeled neurons are indicated on the left. The laterality (I=ipsilateral, C=contralateral) of labeling for each area is also indicated.

Retrogradely labeled neurons were consistently observed in the medial preoptic nucleus (pom) and to a lesser extent the lateral preoptic nucleus, following injections localized within the vl PAG. Although a similar pattern of retrogradely labeled cells were observed following dl PAG injections; consistently fewer cells were found (Fig. 4). The preoptic nuclei also contained a few retrogradely labeled neurons following injections in the ACe. Afferents to the ACe showed a similar topographical pattern that was similar to the one observed for the vl PAG and the dl PAG. Although afferents to the ACe from the pom were often proximal to afferents to the vl PAG and the dl PAG, no adjacent or double-labeled cells were seen.

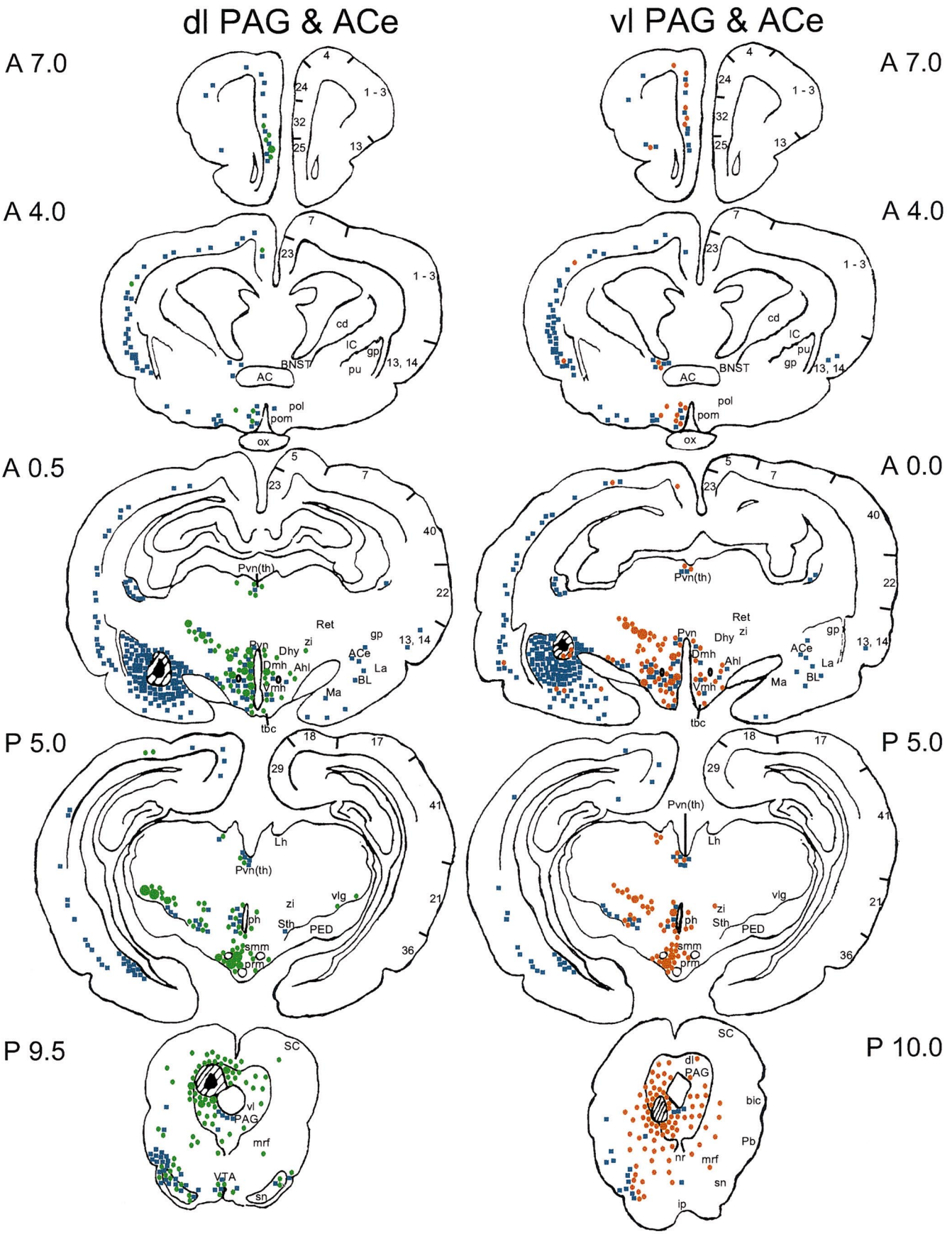
The supraoptic nucleus and the posterior nucleus of the hypothalamus also revealed evidence of a differential contribution to vl PAG afferents versus dl PAG afferents. Consistently more retrogradely labeled neurons were observed in these locations following vl PAG injections. The supraoptic nucleus provided one of the stronger sources of hypothalamic input to the ACe. Interestingly, these retrogradely labeled neurons showed evidence of topographical organization with respect to PAG afferents. Frequently, these neurons were found lateral to neurons labeled following injections to either the vl PAG or the dl PAG. Additionally, the posterior hypothalamus contained a moderate number of retrogradely labeled neurons following injections localized in the ACe. They were often observed interspersed among neurons targeting the vl PAG and the dl PAG (Fig. 4).

Most other hypothalamic nuclei contained a similar number and pattern of retrogradely labeled neurons following injections localized to either functional area of the PAG and/or the ACe. Following injections in either the vl PAG or the dl PAG similar displays of retrogradely labeled cells were observed in the anterior hypothalamic area (Aha), the suprachiasmatic nucleus, the tuber cinereum and the dorsal hypothalamic area (Dhy). Both the Aha and the Dhy provided a consistent source of afferents to the ACe. Often these labeled neurons were adjacent to neurons labeled following PAG injections.

The dorsal premamillary nucleus contained the greatest number of retrogradely labeled cells of any neural region examined following PAG injections. This projection's density was such that over 50 neurons could be observed on each 40 μm section through this nucleus following an injection in the dl PAG. The vl PAG received a similar but slightly smaller afferent contribution from this region.

3.3.3. Thalamus

Compared to the hypothalamus, thalamic input to the


dl PAG & ACe
vl PAG & ACe
A 7.0
A 4.0
A 0.5
A 0.0
P 5.0
P 9.5
P 10.0
4
24
1 - 3
32
25
13
7
23
cd
IC
gp
pu
13, 14
BNST
AC
pol
pom
ox
5
40
22
Pvn(th)
Ret
zi
Dhy
Dmh
Ahl
ACe
La
BL
Vmh
Ma
tbc
18
17
29
41
Lh
vlg
21
ph
Sth
PED
smm
prm
36
SC
vl
PAG
mrf
VTA
sn
dl
bic
Pb
nr
ip

PAG is less pronounced. Most thalamic afferents to the PAG originated in the reticular nucleus of the thalamus (Ret). However, slightly more retrogradely labeled neurons were observed following injections localized to the vl PAG. In contrast, more afferents to the dl PAG than afferents to the vl PAG originated in the medial and lateral geniculate nuclei. The lateral habenula and the paraventricular nucleus of the thalamus contained modest amounts of retrogradely labeled cells following any PAG injection, whereas the superior colliculus contained labeled neurons only when the injection site was in the dl PAG. Other thalamic nuclei contained sparse and inconsistent numbers of retrogradely labeled neurons.

In contrast to the general pattern of afferents to the vl PAG and the dl PAG, more ACe afferents originate in the thalamus than in the hypothalamus. Scattered labeling was observed in many midline and intralaminar thalamic nuclei, including the nucleus centralis lateralis, the centre median nucleus, the paracentral nucleus, nucleus reuniens and the nucleus subfasicularis.

Small, variable amounts of retrogradely labeled neurons were observed in the Ret, the lateral habenula and the lateral geniculate nucleus. However, only neurons in the Ret showed any signs of topographical organization following PAG injections. They were often observed immediately dorsal to neurons projecting to the PAG. A slightly greater number of afferents to the ACe were observed to originate from the paraventricular nucleus along the midline. These retrogradely labeled neurons were consistently found to be dispersed among neurons projecting to the vl PAG and the dl PAG (Fig. 4). The medial geniculate provided the ACe, with its strongest source of thalamic afferents. Although double-labeled cells were not observed, these afferents were often observed proximal to and interspersed with afferents to the vl PAG and the dl PAG.

The subthalamic nucleus (Sth) and the zona incerta (zi) were also labeled following injections the PAG and/or the ACe. Evidence of a differential projection from the zi but not from the Sth to the PAG was observed. Slightly more cells were observed in the zi following injections localized to the vl PAG versus the dl PAG. The ACe received a dense projection from the Sth, of which many neurons were observed intermingled amongst neurons projecting to the PAG. Although the zi contained a more modest number of retrogradely labeled neurons, these neurons were also found around and near neurons with projections to the PAG.

3.3.4. Midbrain

The PAG, itself, was a large contributor of afferents to vl PAG and dl PAG injection sites. Retrogradely labeled cells were observed bilaterally throughout the PAG rostral and caudal to injection sites in the vl PAG and the dl PAG. In addition, the substantia nigra (sn) and the peripeduncular nucleus (pp) were labeled thoroughly following deposits in either PAG subdivision. Retrogradely labeled neurons also were often observed in the ventral tegmental area, the sn and the pp following injections localized to the ACe. These labeled neurons often overlapped with other neurons labeled following injections in the PAG, with the pp containing the most numerous overlapping neurons.

Following injections localized in the ACe, retrogradely labeled cells in the PAG showed evidence of a topographical organization. Labeled neurons were observed primarily in the caudal two thirds of the PAG. These neurons were observed primarily in a row just ventral to the cerebral aqueduct (Fig. 4). A few scattered cells were also observed in the vl PAG and the dl PAG. Furthermore, the density of retrogradely labeled cells increased in a caudal direction. Although a few double-labeled neurons were observed in the ventral PAG, the majority of afferents to the ACe were adjacent to afferents to the vl PAG and the dl PAG.

3.4. Control experiments

Spread of tracer from the injection site to adjacent tissue is a common confound of retrograde neuroanatomical studies. Thus, it is important to determine if retrograde tracer spread from either the ACe or the PAG to adjacent tissue was a significant source of uptake and retrograde transport. As such, control injections were made adjacent to the ACe and the PAG in two animals (nos. 40 and 42).

As seen in Table 2, the control animals had a markedly different number and pattern of retrogradely labeled neurons following control injections. Following injections in the PAG, labeling was consistently observed in the cortex, particularly in cortical areas 13, 24, 25 and 32. In contrast, retrogradely labeled neurons were never observed in these regions following control injections. Although retrogradely labeled neurons were found in many of the same hypothalamic nuclei following either experimental or control

Fig. 4. A diagram of selected coronal sections through the forebrain, diencephalon and midbrain depicting the distribution of retrogradely labeled neurons following a deposit of Fluoro-Gold in the PAG and a deposit of Fluoro-Ruby in the central nucleus of the amygdala. Coronal sections from animal #41, which contained injections in the dl PAG and the ACe, are depicted on the left. Coronal sections from animal #43, which contained injections in the vl PAG and the ACe, are depicted on the right. The red circles represent the distribution of ventrolateral periaqueductal gray afferents highlighted with Fluoro-Gold, where • (brown)=1 neuron, ● (brown)=5 neurons and ⬤ (brown)=15 neurons projecting to the vl PAG. The green circles represent the distribution of dorsolateral periaqueductal gray afferents highlighted with Fluoro-Gold, where • (green)=1 neuron, ● (green)=5 neurons and ⬤ (green)=15 neurons projecting to the dl PAG. Finally, the blue rectangles represent the distribution of afferents to the ACe highlighted with Fluoro-Ruby, where ▪ (blue)=1 neuron, ■ (blue)=5 neurons and ■ (blue)=15 neurons projecting to the ACe.

injections, the extent and proportion of labeled cells differed. An injection ventral to the vl PAG (≠40) resulted in very little retrogradely labeled neurons in any hypothalamic nuclei. An injection lateral to the L PAG (≠42) also resulted in retrogradely labeled neurons. Despite some similarity to injections in the L PAG, this injection is clearly different from injections in the vl PAG (compare ≠42 to ≠43).

Table 3 shows an abundance of overlapping projections to the ACe between control and experimental animals. The fact that there is common labeling is not surprising, since the internal capsule contains projections to and from many of the cortical and hypothalamic regions we examined. However, in one animal (≠48), the injection clearly fell outside the ACe (Fig. 2). Following an injection in the La/BL region of the amygdala, labeling was never observed in the medial prefrontal cortex (mPFC) with only minimal labeling of cortical area 13 (insular cortex). In contrast, the experimental injections always resulted in retrogradely labeled neurons observed in the mPFC as well as numerous retrogradely labeled neurons in cortical area 13. It is possible that the degree of common labeling might indicate spread of retrograde tracer from the injection site. A second alternative is that the different proportions of labeled neurons reflect the differential extent of the projections to each area. Because the similarity of our results with previous retrograde neuroanatomical tracing studies, the second possibility seems more plausible.

4. Discussion

The main findings of this study indicate that separate functional areas of the PAG, the vl PAG and the dl PAG, receive different patterns of afferent input. Furthermore, the ACe receives a pattern of afferents distinct from either region of the PAG. However, there are multiple neural regions with efferents to the ACe that sometimes overlap with projections destined for the PAG. Double-labeled neurons were never observed in neural regions associated with the control of autonomic function.

The vl PAG receives afferent projections from the amygdala and regions of the medial prefrontal cortex (cortical areas 24 and 32) as well as the lateral orbitofrontal cortex (cortical area 13); these areas are associated with the control of the autonomic nervous system. Afferents from the hypothalamus to the vl PAG originate primarily from a shell of neurons in the ventral and lateral regions of the hypothalamus, areas from which electrical stimulation has been observed to elicit the vigilance reaction [19].

In contrast to vl PAG injections, dl PAG injections resulted in retrograde labeling of cells in cortical area 25 extending into the ventral region of cortical area 32 along the medial wall of the prefrontal cortex, but not in the amygdala. Retrograde labeling was also more prominent in the dorsal and medial portion of the hypothalamus. This area of the hypothalamus has been referred to as the Hypothalamic Defense Area based on electrical and chemical stimulation studies [39,63,65].

Similarly, retrogradely labeled neurons were observed in cortical regions associated with autonomic control (cortical areas 13, 24, 25 and 32) following retrograde tracer deposits in the ACe. Additional retrograde labeling was found throughout the hypothalamus, often overlapping regions sending efferents to the vl PAG and/or the dl PAG. Reciprocal connections between the ventral PAG and the ACe were also found. These important findings provide evidence of different neuroanatomical substrates for the elicitation and control of two distinct response patterns to stressful stimuli.

4.1. Comparison with previous neuroanatomical reports

Historically, the PAG has been viewed as an integrative nodal output structure of many reflexive behaviors, whereas the ACe has been hypothesized to play a modulatory role in a forebrain system concerned with cardiovascular/autonomic regulation. Neuroanatomical research has identified widespread afferent connections between cortical, limbic, and diencephalic structures and the PAG [8,9,11,12,22–24,37,38,43,46]. Furthermore, ACe afferents, many of which originate in areas also projecting to the PAG, have been examined thoroughly in the rat [47–50], the cat [54,55] and the monkey [2]. Select afferents of the ACe have also been studied in rabbits [31,32,51], as well as other mammals [4,5,15,35,41,42].

An advantage of the present study is that afferents to multiple regions of a hypothesized system responsible for attending and responding to aversive environmental stimuli were examined within the same subject. Another particular advantage of the present study is that our PAG injection sites were associated with sites from which the defense and vigilance reactions are reliably elicited using electrical stimulation. The general pattern of forebrain and midbrain projections to the PAG and the ACe reported here is consistent with the results of other studies. However, some discrepancies exist between the results of the present study and those of some of the previous studies.

Although afferents to the ACe and the PAG observed in the present study were similar to the observations of others, small differences in the density of projections and the ratio of these projections to different PAG areas sometimes occurred [43]. Disparity between the present results demonstrating differential projections to functional subdivisions of the PAG and the lack of such a finding by Powell, Watson and Maxwell [52] can be reconciled. Following their anterograde experiments, they deposited HRP in the lateral PAG and observed its retrograde transport to the mPFC confirming their earlier results. Similarly, the present experiment observed retrograde labeled cells in all three subdivisions of the mPFC following deposits in the lateral PAG (Table 2; nos. 39 and 46).

Observations from the present study confirm suggestions that the ACe projects primarily to the vl PAG. Although reports of a smaller projection from the ACe to the dorsal PAG have been made [28,43,53], retrogradely labeled cells in the ACe were not observed following deposits in the dl PAG in the present study. This projection to the dorsal PAG has been reported to be much smaller than the projection to the ventral PAG in rabbits [43] and other mammals [28,53], as such, differences in the sensitivity of the neuroanatomical tracers may account for the present study's lack of finding this small projection.

4.2. Hypothalamic afferents to the ACe

The ACe has been shown to influence the elicitation of behavioral, neuroendocrine and autonomic responses from the hypothalamus [44]. Accordingly, most neuroanatomical studies concerned with connections between the ACe and the hypothalamus have examined ACe efferents to various hypothalamic nuclei. As such, afferents to the ACe from the hypothalamus have not been thoroughly examined, particularly in the rabbit. Both the hypothalamus and the ACe are believed to be involved in the regulation of emotion and motivation. Thus, the hypothalamic afferents to the ACe may provide an important substrate for hypothalamic modulation of activity in the ACe.

Hypothalamic afferents to the ACe of various densities were observed to originate from multiple hypothalamic nuclei following injections in the ACe. Sparse projections to the ACe originated in the medial preoptic nucleus, the anterior hypothalamic area and the paraventricular nucleus. A slightly greater number of projections originated in the posterior hypothalamus and the dorsomedial hypothalamic nucleus. Additional afferents originated in the supraoptic nucleus and the lateral hypothalamic area; the largest afferent projection originated in the Vmh. These results are in general agreement with results from similar studies done in other animals [2,4,5,16,48,55]. However, a minor disparity exists concerning which hypothalamic subdivision sends the largest projection to the ACe. The present study is in agreement with Cechetto and colleagues [16] conclusion that the largest set of afferents to the ACe from the hypothalamus originates in the Vmh. In contrast, findings from other laboratories [4,48] have suggested that afferents to the ACe originating in the lateral hypothalamic area are denser than afferents originating in the Vmh. Methodological and species differences most likely account for this discrepancy. Interestingly, similar to the vl PAG, the ACe receives most of its hypothalamic afferents from the lateral hypothalamic area and the ventromedial nucleus of the hypothalamus (Table 3).

4.3. Functional implications

The PAG has been implicated in a variety of functions, including lordosis, fear and anxiety, pain and analgesia, vocalization and cardiovascular control (see [10] for review). The common element among these functions seems to be a reflexive, unlearned response to stimuli. Our laboratory has focused on its role in the mediation of the behavioral and cardiovascular components of two specific responses patterns referred to as the defense reaction and the vigilance reaction. Fig. 5 depicts a heuristic model for the neural mediation of two separate, parallel forebrain systems, hypothesized to influence the activity and elicitation of these two response patterns to aversive stimuli. Superimposed on these two systems in Fig. 5 are select forebrain and midbrain afferents to the ACe. The ACe is hypothesized to be an important modulator of these systems, particularly with respect to learned responses to aversive stimuli.

Depicted in green is the forebrain system associated with the defense reaction, which prepares the animal for 'flight or fight' or 'go' responses. The cardiovascular component of this response pattern consists of a pressor response accompanied by tachycardia, gating of the baroreceptor reflex, and hind limb muscle vasodilatation. Concomitant behavioral manifestations include aggression and flight. It would appear that such a reflexive response would best serve an organism to escape from immediate danger in a natural context. This response pattern seems similar to the circa-strike defensive mode elicited from the dl PAG of rats that occurs when contact with a predator is imminent [20].

Many of the forebrain projections to the dl PAG depicted in Fig. 5 have been shown to have a similar purpose. Within the hypothalamus, most of these projections originate in the dorsomedial nucleus. This area seems to overlap with a region in the hypothalamus located dorsal and medial to the fornix (the Hypothalamic Defense Area) from which similar behavioral and cardiovascular responses have been elicited using electrical stimulation.

Afferents from the mPFC, a region of the brain associated with response selection, are also depicted in Fig. 5. Specifically, dl PAG afferents originate in cortical areas 25 and 32. Interestingly, the two specific mPFC areas shown to project to the dl PAG have been shown to participate in sympathetic activities and learned cardiovascular responses to aversive stimuli, respectively [52]. Absent is a projection to the dl PAG from the ACe. Although the ACe has been shown to influence/modulate emotional reactivity to aversive stimuli, its efferents project primarily to parasympathetic autonomic regulatory nuclei in the brainstem [58].

The other behavioral pattern exhibited by rabbits in response to threatening stimuli is a freezing, or 'no go' response. When responding in this manner, the rabbit becomes vigilant to environmental cues, and exhibits a pressor response, vasoconstriction, decreased blood flow in the hind limbs and viscera, augmentation of the baroreceptor reflex, bradycardia and inspiratory apnea or tachyponea (vigilance reaction) [19,39]. This response pattern appears similar to the post-encounter defensive mode in rats described by Fanselow [20]. Characterized by

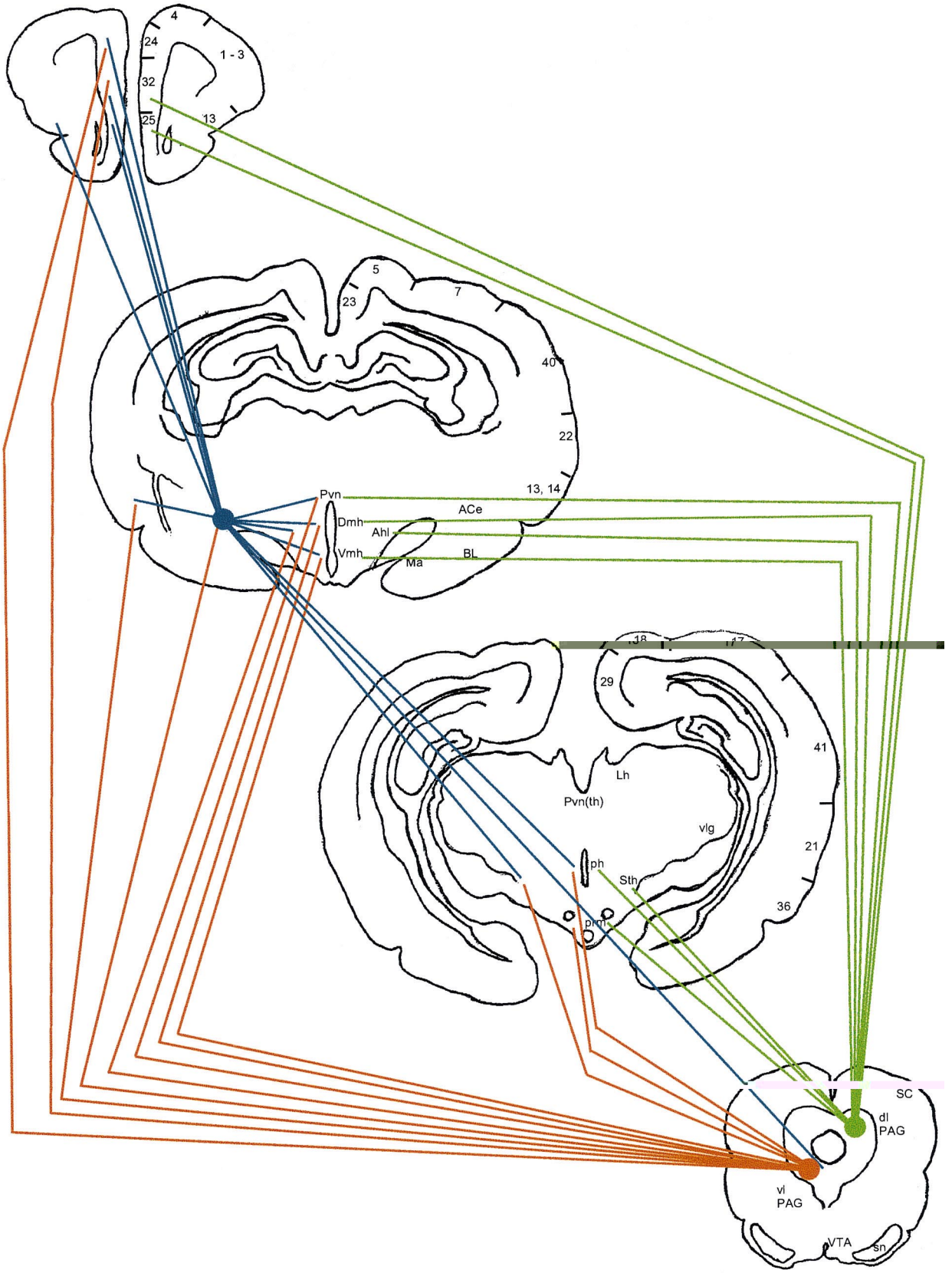


Fig. 5. Model depicting the origin of afferents to distinct periaqueductal gray regions and the central nucleus of the amygdala. The vl PAG and the dl PAG are capable of distinct responses to stressful stimuli. Many of the regions projecting to the PAG are hypothesized to modulate the defense reaction and/or the vigilance reaction.

behavioral freezing in response to the detection of a predator in the environment, this system includes the amygdala and ventral PAG. Although pressor responses have been elicited from the vl PAG of rabbits [19,39] and cats [13], not all laboratories have observed this effect on blood pressure. For example, stimulation of the vl PAG resulted in a depressor response in the rat [36] and the cat [13]. In fact, an inhibitory interaction between the vl PAG and the dl PAG has been proposed [36]. It is likely that species differences may account for the different blood pressure responses elicited from the vl PAG. However, as seen in the cat [13], it may also be possible that both types of blood pressure response can be elicited from the same region.

The Vmh and the Ahl send two of the densest hypothalamic projections to the vl PAG (Table 2). Interestingly, these hypothalamic projections seem to overlap with a region in the mediolateral hypothalamus located slightly ventral and lateral to the fornix, from which electrical stimulation yields a behavioral/cardiovascular response pattern similar to the one elicited from the vl PAG [18,40].

In contrast to the dl PAG, the present study demonstrated that the vl PAG receives a projection from the ACe. The ACe seems to mediate learned responses to aversive stimuli as well as a general enhancement of arousal and attention, which may facilitate information processing [33]. Vigilance serves to direct the organism's attention to the most important environmental stimuli. Upon the integration of this information, the ACe may act as a switch. It can either maintain the vigilance reaction or if the stimulus has been determined to be adverse, posing an imminent threat, switch to a more appropriate response such as the defense reaction.

Similar to the dl PAG, afferents to the vl PAG originate in the mPFC. In contrast, to the dl PAG, these afferents originate in different subdivisions of the mPFC. Depicted in Fig. 5 are afferents to the vl PAG from cortical areas 24 and 32, but not cortical area 25. As mentioned previously, damage to area 32 eliminates learned cardiovascular responses. Damage to cortical area 24 interferes with an animal's ability to discriminate between a learned aversive stimulus and a neutral stimulus [52]. This difference in mPFC projections provides an interesting contrast between these two systems. The vigilance reaction would appear to require more decision making and information processing as to which stimuli to respond. In contrast, the defense reaction may be a last line of defense. It is more of a sudden reaction to potentially pernicious contact, thus connections with neural regions involved in complex forebrain processing may not be as important to this response system.

Select afferents of the ACe have been superimposed on vl PAG and dl PAG afferents depicted in Fig. 5. As can be seen, ACe afferents originate in many of the same regions as afferents to the vl PAG and/or the dl PAG. Retrogradely labeled neurons in the mPFC were observed following injections in the ACe. The lack of double-labeled neurons or neurons juxtaposed to cells retrogradely labeled following vl PAG and dl PAG injections would suggest that the ACe and different functional subdivisions of the PAG receive different information about the stimuli that initially elicited a response.

Afferents to the ACe also originated in most hypothalamic nuclei. They were often found commingled with neurons projecting to either the vl PAG or the dl PAG in the Vmh, Dmh, Ahl and the Pvn. Due to the proximity of these neurons, it is plausible that the ACe and the vl PAG or the dl PAG are receiving similar information concerning hypothalamic autonomic and neuroendocrine output. Interestingly, the distribution of ACe afferents paralleled the distribution of vl PAG afferents more closely than the distribution of dl PAG afferents. The specific role these overlapping projections may play is unclear at the present time.

This study also supports earlier reports of reciprocal connections between the ACe and the PAG [53]. This projection gives the PAG access to a forebrain system that has been implicated in the expression of emotional behavior to learned and unlearned stimuli. This projection provides a pathway by which the PAG might gain the ability to influence its own modulation to some extent. Further studies that combine retrograde tracing methods and immunocytochemistry to peptides and neurotransmitters are required to further clarify the relationships between these functional neuroanatomical connections.

Acknowledgements

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