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Projections from the Subfornical Region of the Lateral Hypothalamic Area

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Abstract

The L-shaped anterior zone of the lateral hypothalamic area's subfornical region (LHASfa) is delineated by a pontine nucleus incertus input. Function evidence suggests the subfornical region and nucleus incertus modulate foraging and defensive behaviors, although subfornical region connections are poorly understood. A high resolution *Phaseolus vulgaris*-leucoagglutinin (PHAL) structural analysis is presented here of the LHASfa neuron population's overall axonal projection pattern. The strongest LHASfa targets are in the interbrain and cerebral hemisphere. The former include inputs to anterior hypothalamic nucleus, dorsomedial part of the ventromedial nucleus, and ventral region of the dorsal premammillary nucleus (defensive behavior control system components), and to lateral habenula and dorsal region of the dorsal premammillary nucleus (foraging behavior control system components). The latter include massive inputs to lateral and medial septal nuclei (septo-hippocampal system components), and inputs to bed nuclei of the stria terminalis posterior division related to the defensive behavior system, intercalated amygdalar nucleus (projecting to central amygdalar nucleus), and posterior part of the basomedial amygdalar nucleus. LHASfa vertical and horizontal limb basic projection patterns are similar, although each preferentially innervates certain terminal fields. Lateral hypothalamic area regions immediately medial, lateral, and caudal to the LHASfa each generate quite distinct projection patterns. Combined with previous evidence that major sources LHASfa neural inputs include the parabrachial nucleus (nociceptive information), defensive and foraging behavior system components, and the septo-hippocampal system, the present results suggest that the LHASfa helps match adaptive behavioral responses (either defensive or foraging) to current internal motivational status and external environmental conditions.

Indexing terms

amygdala; behavioral activation; defensive behavior; hypothalamus; lateral habenula; motivation; nucleus incertus

INTRODUCTION

The lateral hypothalamic area's anterior zone of the subfornical region (LHASfa) lies ventral to the fornix at a transverse level that includes the caudal end of the anterior hypothalamic

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nucleus and the rostral end of the ventromedial hypothalamic nucleus. The LHAsfa was recognized and delineated in rats by a dense, restricted input from the nucleus incertus (NI) of the pons (Goto et al., 2001; Olucha-Bordonau et al., 2003). In those studies it was also shown that the NI forms a highly interconnected neural network with two other midline cell populations of the brainstem—the interpeduncular nucleus (IPN) and superior central nucleus (CS, also known as the median raphe nucleus)—that are thought to play a role in modulating behavioral activity levels. Functional evidence suggests that the IPN and CS are involved in controlling behavioral inhibition, whereas several analyses indicate that Fos expression in the NI is upregulated in certain challenging situations (see Goto et al., 2001).

Two experiments from our own group also suggest that Fos expression in the NI reflects at least in part the general level of an animal's exploratory or foraging behavior, although the relationship is not necessarily simple (Goto, 1998). First, naive rats placed in a water tank for 10 minutes initially display vigorous swimming, followed by a characteristic immobile posture, and they show much less Fos-immunoreactivity in the NI as compared to animals that simply swim for 10 minutes searching for a platform they have learned to expect. And second, rats trained on a restricted food access paradigm display impressive Fos increases in the NI immediately before food delivery, coinciding with a period of hyperactivity and increased aggressiveness.

One way the midline brainstem NI-CS-IPN network could influence goal-oriented or motivated responses during behavioral activation is through the LHAsfa. In addition to receiving direct axonal inputs from all three brainstem network components—NI, CS, and IPN (Groenewegen et al., 1986; Vertes et al., 1999; Goto et al., 2001)—the LHAsfa is also innervated by the parabrachial nucleus (Bester et al., 1997); the ventral region of the dorsal premammillary nucleus (M. Goto and N.S. Canteras, personal observations) and dorsal division of the periaqueductal gray (S.R. Mota-Ortiz and N.S. Canteras, personal observations), both components of the defensive behavior system mediating innate fear responses (Canteras, 2002); and the septohippocampal system via an input from the dorsolateral zone of the rostral part of the lateral septal nucleus (Risold and Swanson, 1977).

Here we analyze systematically with the anterograde tracer, PHAL, the overall pattern of LHAsfa axonal projections, which are essentially unknown, although a broader lateral hypothalamic region including the LHAsfa was examined previously (Roeling et al., 1994). The results demonstrate that the LHAsfa projection pattern to interbrain (diencephalon) and cerebral hemisphere is quite distinct from those generated by adjacent LHA regions, thus providing additional evidence that the LHAsfa is an anatomically distinct LHA cell group. Of particular interest, the results indicate that the LHAsfa neuron population generates topographically ordered inputs to the septal and amygdalar regions of the cerebral hemisphere, and to the hypothalamus and thalamus. The LHAsfa's ventrolateral or horizontal limb preferentially innervates the posterior basomedial and intercalated amygdalar nuclei, and the medial hypothalamic defensive behavior control network (anterior hypothalamic nucleus, dorsomedial part of the ventromedial nucleus, and ventral region of the dorsal premammillary nucleus), whereas the dorsomedial or vertical limb innervates preferentially the lateral habenula and dorsal region of the dorsal premammillary nucleus. Overall, the results suggest that the LHAsfa is positioned to influence neural activity in the septo-hippocampo-amygdalar system, and to modulate a variety of defensive (fight or flight) and exploratory or foraging behavioral responses.

MATERIALS AND METHODS

Adult male Wistar rats (n=25) weighing about 280–300g were used in this study. The animals were kept under controlled temperature (23°C) and illumination (12-hour cycle)

conditions in the animal quarters and had free access to water and standard laboratory diet (Purina). Conditions of animal housing and all experimental procedures were conducted under institutional guidelines, and in compliance with *NIH Guidelines for the Care and Use of Laboratory Animals*.

Each animal received a single injection of PHAL (Vector) into the LHAsfa or adjacent LHA regions. They were anesthetized with a mixture of ketamine and xylazine (v/v; 1 ml/kg body weight), and the iontophoretic injection of a 2.5% solution of PHAL in 0.1 M sodium phosphate-buffered saline, pH 7.4 (Gerfen and Sawchenko, 1984), was made over a 10 min period through a stereotactically positioned glass micropipette (tip diameter 10 μ m) by applying a +5 μ A current, pulsed at 7 second intervals, with a constant-current source (Midgard Electronics, model CS3). After a survival time of 14 to 16 days, the animals were perfused and the brains were processed according to procedures described in detail elsewhere (Canteras et al., 1992). Briefly, each animal was deeply anesthetized with sodium pentobarbital (40 mg/kg, intraperitoneal) and perfused transcardially with a solution of 4.0% paraformaldehyde in 0.1 M sodium borate buffer at pH 9.5%; the brains were removed immediately and postfixed for 3 hours in the same fixative containing 20% sucrose. The brains were then frozen, and 6 series of 25 μ m-thick sections were cut on a sliding microtome in the transverse (frontal) plane and collected from the caudal medulla through the rostral tip of the prefrontal cortex. One complete series was processed for immunohistochemistry with an antiserum directed against PHAL (Dako; Carpintera, CA) at a dilution of 1:5,000. The antigen-antibody complex was localized with a variation of the avidin-biotin complex system (ABC; Hsu and Raine, 1981), with a commercially available kit (ABC Elite kit, Vector). The sections were mounted on gelatin-coated slides and then treated with osmium tetroxide to enhance visibility of the reaction product. Slides were then dehydrated and coverslipped with DPX. An adjacent series was always stained with thionin to serve as a reference for cytoarchitecture.

Sections were examined under a microscope with bright- and darkfield illumination. PHAL-labeled cells in the injection sites, and the distribution of anterogradely labeled fibers, were plotted with the aid of a camera lucida onto maps prepared from adjacent thionin-stained sections. For illustration purposes the distribution of anterogradely labeled fibers was transferred onto a reference atlas of the rat brain (Swanson, 2004). The figures were prepared for publication using Adobe Photoshop (version 5.5; Adobe Systems, San Jose, CA) for photomicrographs, and Adobe Illustrator (version 10, Adobe Systems) for line drawings. Unless otherwise indicated, parcellation of the rat brain follows Swanson (2004). Terminology for describing morphological features of PHAL-labeled axons, and mapping strategies and procedures, are detailed by Swanson (2004).

RESULTS

Nomenclature

In transverse sections, the LHAsfa is an L-shaped differentiation of the LHA that descends just below the fornix and then extends laterally, just dorsal to the supraoptic commissure (Fig. 1). It is delimited precisely by a heavy input from the NI (Goto et al., 2001) (Fig. 1A2–C2), and it extends rostrocaudally for about 400–600 μ m, from the rostral pole of the ventromedial nucleus (Fig. 1A) to the anterior part of the dorsomedial hypothalamic nucleus (Fig. 1C). The LHAsfa reaches its maximal extent at the level of the lateral parvicellular part of the paraventricular nucleus and the tiny dorsal part of the anterior hypothalamic nucleus (Fig. 1B; Atlas Level 27 in Swanson, 2004). The LHAsfa is not homogeneous when examined in Nissl-stained preparations. Instead, there are clear dorsomedial (vertical) and ventrolateral (horizontal) limbs. The former is characterized by small to medium-sized fusiform neurons with a vertical orientation that are somewhat more darkly stained than

those in surrounding areas (Fig. 1A1–C1). The latter is characterized by larger multipolar neurons with a somewhat greater packing density than in its surroundings, although it is difficult to distinguish in Nissl preparations (Fig. 1A1–C1), darkfield illumination being more helpful than brightfield. The LHA's subfornical region also has separate posterior (LHAsfp) and then premammillary (LHAsfpm) zones (see Swanson, 2004).

Injection sites

In 15 experiments the PHAL deposit is confined almost entirely to the LHAsfa. In three experiments (LHA57, 88, and 94) the PHAL injection is relatively large and labels neurons throughout much of the LHAsfa. In nine other experiments the injection site is smaller and most PHAL-labeled neurons are centered either dorsomedially (LHA43, 89, and 95) or ventrolaterally (LHA53, 54, 56, 63, 65, and 93). Figures 2 and 3 illustrate a representative example of each group.

The projections labeled in Experiment LHA94 are described in detail because it labels neurons throughout most of the LHAsfa, and the projection pattern is similar to that labeled by other injections centered in the LHAsfa. The projection pattern seen in Experiment LHA94 is summarized in Table 1. Because a few labeled neurons spread outside the borders of the LHAsfa as defined here, major projection differences seen with injections centered medial (LHA52), lateral (LHA45) or caudal (LHA22) to the LHAsfa (Fig. 2) are also considered.

Projections from the LHAsfa

Axons labeled from PHAL injections centered in the LHAsfa (exemplified by experiment LHA94, Figs. 2 and 3) follow six main pathways to innervate cell groups in the cerebral hemispheres and brainstem (Fig. 4). Individual pathways are distinguished by the spatial distribution of labeled axons.

Ascending projections—Rostrally directed axons leaving the LHAsfa may be divided conveniently into dorsomedial, dorsal, ventromedial, and ventrolateral pathways, as well as those coursing through the medial forebrain bundle.

Dorsomedial ascending pathway: Many PHAL-labeled axons extend dorsomedially from the injection site (Figs. 3-1 and 5G–H). Most of them end within the hypothalamus, although some continue into the thalamus, along with axons in the dorsal and ventromedial pathways (see below). The former generate a moderate terminal field in the anterior hypothalamic nucleus (Fig. 5F–H) and dorsomedial part of the ventromedial nucleus (Fig. 5H). Axons that extend slightly rostrally in the dorsomedial pathway richly innervate the LHA juxtapaaraventricular region along with axons in the dorsal pathway (Fig. 5F,G). Only a very light input to the paraventricular nucleus itself is observed.

Dorsal ascending pathway: Moderate numbers of PHAL-labeled axons course dorsally from the LHAsfa through and to the zona incerta (Figs. 4-2, 5G–I). Next, some of the axons continue dorsally into the dorsal thalamus, whereas others veer medially into the dorsal thalamic midline nuclei, along with axons in the dorsomedial and ventromedial pathways (Fig. 5F–I). A larger number of dorsal pathway axons enter rostral levels of the dorsal thalamus, along with axons in the dorsomedial and ventromedial ascending pathways. Together, they innervate moderately the paratenial and mediodorsal nuclei and ventral part of the nucleus reuniens (Fig. 5F–J), and lightly the paraventricular (Fig. 5E–M), central medial (Fig. 5G–M), and intermediodorsal (Fig. 5I–M) nuclei.

Other dorsal pathway axons enter the stria medullaris to innervate the lateral habenula (especially caudally), along with axons entering the thalamus caudally from the posterior hypothalamic nucleus. According to the parceling of Andres and colleagues (1999) moderate terminal fields are established in the caudal magnocellular, parvocellular, and marginal subnuclei of the lateral habenula lateral division, and in the parvocellular and central subnuclei of the medial division (Figs. 5H–M and 6).

Ventromedial ascending pathway: A small group of LHAsfa axons courses ventrally and medially to innervate the ventromedial hypothalamic nucleus (Figs. 4-3 and 5G,H). Some of them continue dorsally to enter the paraventricular hypothalamic nucleus, where they generate a few branches with terminal boutons, and then enter the thalamus, where they merge with the dorsomedial and dorsal ascending pathways. Another component of the ventromedial ascending pathway courses through the tuberal nucleus and retrochiasmatic area, where it crosses the midline to reach the contralateral LHAsfa (Figs. 4-3 and 5F,G).

Ventrolateral ascending pathway: These axons run through the caudal ansa peduncularis to the amygdalar region (Fig. 5G,H), where they are joined by axons coursing through more rostral levels of the ansa peduncularis and the stria terminalis (Fig. 4--4). These axons generate a moderately dense input to the posterior part of the basomedial nucleus (Figs. 5I–N and 6B). Many labeled axons with terminal boutons accumulate near the dorsal border of the main intercalated nucleus and the central nucleus (Fig. 5G,H), and in the paracapsular part of the intercalated nucleus (Millhouse, 1986), between the central nucleus and the basolateral and lateral nuclei (Figs. 5H,J and 6B). The lateral nucleus itself receives a light input (Figs. 5G–L and 6B), along with the posterior amygdalar nucleus (Fig. 5K–N). A few axons and terminal boutons are observed in caudoposterolateral (Fig. 5K–M) and posteromedial (Fig. 5L–N) regions of the anterior cortical nucleus and in rostral regions of the medial nucleus (Fig. 5F–H) of the amygdalar region.

Ascending medial forebrain bundle: A clear majority of LHAsfa ascending axons course through the medial forebrain bundle (Fig. 4–5). They heavily innervate more anterior regions of the LHA (Fig. 5E–F) and moderately innervate the lateral preoptic area (Fig. 5D).

At the anterior hypothalamic nucleus level, ascending LHAsfa axons in the medial forebrain bundle diverge to follow different routes. One fiber group enters the bed nuclei of the stria terminalis (Fig. 5E), where they split into three smaller bundles: a) moderately innervating the interfascicular and medial transverse nuclei of the bed (Fig. 5E), b) entering the stria terminalis (Fig. 5E), and c) entering the fornix (Fig. 5D,E) to innervate caudal regions of the lateral septal nucleus (Fig. 5D) along with axons coursing through rostral levels of the medial forebrain bundle. A second fiber group courses through and to the substantia innominata, in the ansa peduncularis, to reach the amygdalar region (Fig. 5E–I), along with axons in the ventrolateral ascending pathway that reach the amygdalar region through the caudal ansa peduncularis and stria terminalis (see above). A third ascending medial forebrain bundle group of axons enters the lateral preoptic area, where it generates a moderately dense terminal field (Fig. 5D). This bundle then splits, with one component coursing dorsomedially to enter and innervate densely caudal regions of the medial septal nucleus and the ventral zone of the caudal part of the lateral septal nucleus (Fig. 5D). From there a few axons course through the septofimbrial nucleus and enter the fimbria to generate light inputs to ventral regions of hippocampal field CA1 (Fig. 5M,N). The other component enters the substantia innominata and diagonal band nucleus before extending rostrally (Fig. 5D) and richly innervating the medial septal nucleus (Figs. 5B,C and 6C). A group of axons in the third ascending component of the medial forebrain bundle also extends dorsally to generate very dense branching and terminal boutons in the dorsolateral zone of the rostral

part of the lateral septal nucleus (LSr.dl), where many pericellular baskets of terminal boutons are observed (Fig. 5B,C).

A few labeled axons in the medial forebrain bundle proceed more rostrally, passing through the anterior olfactory nucleus and ventral regions of the nucleus accumbens to enter the tenia tecta and eventually reach the infralimbic area of medial prefrontal cortex, where some branching and terminal boutons are observed (Figs. 5A and 6A).

Descending projections—Most descending PHAL-labeled axons from the LHAsfa course initially through the medial forebrain bundle (Fig. 4–6), before diverging to specific terminal fields or to branches of the bundle. One group of axons extends dorsomedially and many of them enter the dorsomedial hypothalamic nucleus (Fig. 4–6a), where they generate a moderate terminal field in the rostral tip of its anterior part (Fig. 5I). Another group of medial forebrain bundle fibers enters the posterior hypothalamic nucleus (Fig. 4–6b), where there is also a relatively moderate terminal field (Fig. 5J–M) and some labeled axons cross the midline to enter the contralateral LHA. Other labeled fibers in the posterior hypothalamic nucleus extend dorsally into the thalamus (Fig. 4–6c). They branch abundantly and generate many boutons in the lateral habenula, especially caudally, along with axons from the dorsomedial ascending pathway described above (Figs. 5K,L and 6C).

Many LHAsfa axons extend farther caudally. At mammillary levels they generate a dense terminal field in the dorsal premammillary nucleus (Figs. 4–6d, 5L,M, and 6D). Some axons then extend laterally from the dorsal premammillary nucleus region to innervate moderately the LHAsfpm, and even farther laterally the LHA lateral zone's ventral region (LHA_{vl}; Fig. 5M). Slightly more caudally, axons in the medial forebrain bundle enter the supramammillary nucleus, where some cross the midline to enter the contralateral medial forebrain bundle (Fig. 5N). Some axons in the supramammillary nucleus turn dorsally and join axons in the posterior hypothalamic nucleus to enter the periaqueductal gray (Figs. 4–6e and 5N). This dorsal branch of the medial forebrain bundle lightly innervates the precommissural nucleus (Fig. 5N)—and the medial, dorsolateral, and lateral periaqueductal gray divisions—and relatively moderately innervates the ventrolateral division (Fig. 5O–R), along with the dorsal raphe nucleus (Fig. 5Q,R). Terminal fields in the latter two regions seem to be reinforced by axons ascending from the ventral continuation of the medial forebrain bundle (see below), and together these axons generate a moderate input to the cuneiform nucleus (Fig. 5Q,R).

Caudal to the supramammillary nucleus, LHAsfa axons in the medial forebrain bundle proper enter the ventral tegmental area (Figs. 4–6f and 5O,P). Some of them course laterally through and near the compact substantia nigra to then arch dorsomedially through the midbrain reticular nucleus and its retrorubral area differentiation to enter the periaqueductal gray, as mentioned above (Fig. 5O,P). Other axons in the ventral tegmental area extend dorsally through the rostral raphe region to enter the periaqueductal gray (Fig. 5O,P). However, most labeled axons in the ventral tegmental area continue caudally through the raphe region, where they provide moderately dense inputs to the superior central nucleus (Fig. 5Q,R), and then the dorsal raphe nucleus and adjacent periaqueductal gray ventrolateral region, together with axons described above that course through the dorsal branch of the medial forebrain bundle (Fig. 5Q,R). A few labeled axons in the midbrain ventral extension of the medial forebrain bundle take a dorsolateral course to enter the retrorubral area and cuneiform nucleus (Fig. 5Q,R), and it should also be mentioned that a few labeled axons cross the midline to innervate the contralateral periaqueductal gray (Fig. 5Q,R).

Finally, some LHAsfa axons reach the caudal brainstem central gray where they generate relatively light inputs to the region of pontine central gray bordering the dorsal tegmental nucleus and the nucleus incertus (Fig. 5S).

Projections from LHAsfa vertical and horizontal limbs

Analysis of experiments with PHAL injections centered in either the dorsomedial or ventrolateral limbs of the LHAsfa indicates that whereas the overall projection pattern from each is similar, relative terminal field strengths reveal a certain topographic organization to projections innervating septal, amygdalar, hypothalamic, and thalamic cell groups.

The LHAsfa horizontal limb—As mentioned above, six PHAL injections are centered in ventrolateral regions of the LHAsfa, and Experiment 93 (Figs. 2, 3, and 7F) was chosen to illustrate projection patterns typical of all six.

The lateral septal nucleus receives a massive input to the ventrolateral two-thirds of the LSr.dl (Fig. 7A) and an only slightly less dense input to ventral regions of the LSc.v (Fig. 7B). Ventral regions of the septofimbrial nucleus also receive a moderately dense terminal field, and as in experiment LHA94 there is a dense input to the medial septal nucleus (Fig. 7A).

In the amygdalar region, the paracapsular part of the intercalated nucleus, and the posterior basomedial nucleus, are densely innervated (Fig. 7C), whereas the lateral nucleus has a sparser terminal field (Fig. 7C). The hypothalamus displays a rich terminal field in the LHA's juxtaparaventricular region (Fig. 7E), and substantial terminal fields are also observed in the anterior hypothalamic nucleus (Fig. 7E,F), dorsomedial part of the ventromedial nucleus (Fig. 7F,G), and dorsomedial nucleus (Fig. 7G). These amygdalar and hypothalamic neuron populations are only lightly labeled by PHAL injections centered in the LHAsfa dorsal limb.

More caudally, abundant PHAL-labeled branches and boutons are observed in and around the ventral region of the dorsal premammillary nucleus, whereas the dorsal region is more lightly innervated (Fig. 7H).

In the thalamus, a sparse terminal field is observed in lateral regions of the medial habenula, and in the parvocellular and superior parts of the lateral habenula's medial division (Fig. 7D).

The LHAsfa vertical limb—Experiment LHA89 (Figs. 2, 3, and 8F) was chosen to illustrate the three PHAL injections mentioned above that are centered in dorsomedial regions of the LHAsfa.

In the lateral septal nucleus a dense input is observed in the dorsal third of the LSr.dl (Fig. 8A). More ventrally, anterograde labeling intensity decreases and is concentrated in more medial regions of the LSr.dl—avoiding more lateral regions that are massively labeled in experiments with injections centered in the LHAsfa horizontal limb (Fig. 8A). In the LSc, anterograde labeling is concentrated in the intermediate third of the ventral zone (Fig. 8B), like that described above for experiment LHA94—and just dorsal to the region labeled in experiments with injections centered in the LHAsfa horizontal limb. PHAL labeling in the medial and septofimbrial nuclei is lighter than that observed in horizontal limb injections (Fig. 8A,B).

In the amygdalar region, anterograde labeling in the posterior basomedial nucleus and paracapsular part of the intercalated nucleus is much lighter than that observed in experiments with injections centered in the horizontal limb (Fig. 8C).

In the hypothalamus only very light inputs are seen in the anterior, ventromedial, and dorsomedial nuclei, and LHA juxtaparaventricular region, all of which are densely labeled by horizontal limb injections (Fig. 8E–G). In the dorsal premammillary nucleus, the dorsal region and areas surrounding the ventral region are richly innervated—very few boutons are observed within the latter (Fig. 8H). In contrast, the ventral region of the dorsal premammillary nucleus is densely innervated by the LHAsfa horizontal limb.

In the thalamus, the marginal and parvicellular parts of the lateral habenular medial division are densely innervated, along with the magnocellular and parvicellular parts of the lateral division (Fig. 8D).

Control injections

PHAL injections centered in LHA regions outside the border of the LHAsfa were obtained and the main differences with LHAsfa experiments are described briefly because they control for scattered labeled neurons in some experiments with injections centered in the LHAsfa, and because they help clarify the organization of projections from LHA regions surrounding the LHAsfa. In these experiments, very distinct projection pattern are observed to the septal region, amygdalar region, hypothalamus, thalamus, and lower brainstem, as described below and summarized in Table 1.

The LHA juxtaventricular region (LHAjv)—In Experiment LHA52 the PHAL injection is centered in the rostral end of the LHAjv (just medial to the LHAsfa), although some PHAL-labeled neurons also lie within the LHAsfa (Fig. 2).

At intermediate levels of the lateral septal nucleus a dense terminal field is labeled in the ventral third of the medial region of the LSr.dl (Fig. 9A), with lighter labeling in lateral regions of the LSr.dl that are massively labeled by PHAL injections centered in the LHAsfa horizontal limb. Labeled axons with boutons are also observed in the LSr.m (Fig. 9A), which is only lightly labeled in experiments with injections centered in the LHAsfa. A substantial terminal field is also observed in the interfascicular nucleus of the bed nuclei of the stria terminalis's posterior division, as well as in the anteromedial area of the anterior division (Fig. 9B). The latter is not labeled in experiments with injections centered in the LHAsfa.

In the amygdalar region, a dense terminal field is labeled in the posterior nucleus (Fig. 9F), which is only lightly labeled in LHAsfa experiments, and in caudal regions of the posterior basomedial nucleus (Fig. 9F). The intercalated nucleus is free of labeling, starkly contrasting with the dense terminal fields labeled after LHAsfa injections. Furthermore, a moderate input is observed in temporal regions of hippocampal field CA1 (Fig. 9E,F) and the medial entorhinal area.

In the hypothalamic preoptic region, moderately dense anterograde labeling is seen in the medial preoptic area (Fig. 9B), near the border with the lateral preoptic area (which only contains what appear to be fibers of passage). In LHAsfa experiments only a few axons and boutons are observed in the medial preoptic area, and a moderate to dense terminal field is found in the lateral preoptic area. More caudally, the LHA juxtaparaventricular region is heavily labeled (Fig. 9C), and substantial labeling is also observed in the dorsal zone of the anterior region of the LHA. Moderate labeling is seen in the anterior parvicellular part of the paraventricular nucleus. In the ventromedial nucleus, the rostral pole and the dorsomedial,

central, and ventrolateral parts are all heavily labeled (Fig. 9D), whereas in LHAsfa experiments the anterograde labeling is restricted to the rostral pole and dorsomedial part. A moderately dense terminal field occupies the ventral region of the dorsal premammillary nucleus.

In the thalamus, conspicuous anterograde labeling is observed in far medial regions of the mediodorsal nucleus's medial part (bordering the paraventricular nucleus), and moderate labeling is observed in the paraventricular nucleus and rostral regions of the paratenial nucleus.

Finally, inputs to the periaqueductal gray are denser than after LHAsfa injections. Moderately dense terminal fields occupy the lateral and ventrolateral divisions and moderately dense labeling is also found in the midbrain reticular, cuneiform, and superior central raphe nuclei.

The LHA anterior and dorsal regions (LHAa/d)—In experiment LHA45 the PHAL deposit is centered just lateral to the LHAsfa, in a region that appears to include the caudomedial tip of the LHA anterior region and rostromedial tip of the LHA dorsal region (Fig. 2; Atlas Levels 26 and 27 of Swanson, 2004).

In the lateral septal nucleus, anterograde labeling is densest in the caudal part (Fig. 10A,B), and moderate labeling is seen in the ventrolateral zone of the rostral part. The LSr.dl, which is heavily labeled in LHAsfa experiments, is only lightly innervated. A moderately dense terminal field is also observed in the medial septal nucleus (Fig. 10A).

In the amygdalar region, a moderately dense input is observed in the central nucleus's capsular part, whereas no labeling is seen in the posterior part of the basomedial nucleus, and only light labeling is found in the intercalated nucleus (both heavily labeled by LHAsfa injections).

Moving to the hypothalamus, there is a moderately dense terminal field in the lateral preoptic area, which is most obvious rostrally (Fig. 10A). A moderately dense input occupies the supraforncal region of the LHA, and moderate labeling is seen in the dorsal part of the anterior region of the LHA. Caudally, there is a massive input to the dorsal region of the dorsal premammillary nucleus (Fig. 10D). The posterior hypothalamic nucleus is moderately densely innervated, whereas the ventral and posterior regions of the LHA, and the supramammillary nucleus, receive moderate inputs.

In the thalamus, a massive input is observed in the lateral habenula's medial and lateral divisions. In the former, the marginal and anterior parts are especially involved, whereas labeling is much lighter in the parvicellular and central parts (Fig. 10E–G). In the lateral division massive terminal fields are labeled in the magnocellular, parvicellular, marginal, and basal parts, with the oval part being avoided (Fig. 10F,G). Moderate inputs are seen in the nucleus reuniens, lateral part of the mediodorsal nucleus, central medial and paraventricular nuclei, and caudal regions of the intermediodorsal nucleus.

Finally, the precommissural nucleus, and the rostromedial, medial, lateral, and ventrolateral divisions, of the periaqueductal gray receive moderately dense inputs.

The LHAsfp—In Experiment LHA22 the PHAL injection is centered just caudal to the LHAsfa, in the LHAsfp, although some labeled neurons extend beyond the limits of the LHAsfp.

The dorsolateral zone of the LSr (heavily labeled in the LHAsfa experiments) contains very few terminals in this experiment, whereas moderate labeling is observed in the medial zone, which is not labeled in LHAsfa experiments. In addition, a moderate terminal field is labeled in caudomedial regions of the nucleus accumbens, commencing a short distance but clear distance below the dorsomedial pole of the nucleus at this level; it is not labeled in LHAsfa experiments. In the bed nuclei of the stria terminalis, the posterior division's interfascicular and transverse nuclei are clearly labeled (Fig. 11C), and the anterior division's anteromedial and anterolateral areas, along with the rhomboid, dorsomedial, magnocellular, and ventral nuclei, are densely innervated (Fig. 11B). The anterior division is not labeled in our LHAsfa experiments. In the amygdala, the medial part of the central nucleus is densely innervated (Fig. 11F), and a substantial terminal field is observed in the posterior nucleus. Neither amygdalar terminal field is labeled in the LHAsfa experiments. The intercalated nucleus is only lightly labeled, also in contrast to the LHAsfa experiments.

In the hypothalamic preoptic region, heavy terminal fields are labeled in the medial preoptic area and in medial and rostral regions of the lateral preoptic area (Fig. 11A), and substantial inputs are also seen in the anteroventral preoptic, anterodorsal preoptic, and parastrial nuclei (Fig. 11A). Only the lateral preoptic area appears significantly innervated by the LHAsfa. Farther caudally, the paraventricular hypothalamic nucleus's anterior, medial, and lateral parvicellular parts, and the fornical part, are densely labeled (Fig. 11C,D), in clear distinction to injections centered in the LHAsfa. The dorsomedial nucleus's anterior (Fig. 11E) and ventral parts contain rich terminal plexuses. A moderate terminal field is seen in the juxtaventromedial, anterior, supraformical, and ventral regions of the LHA, as well as in the posterior hypothalamic nucleus. Little or no anterograde labeling is observed in the anterior (Fig. 11D), ventromedial (Fig. 11D), and dorsal premammillary nuclei.

Moderately dense terminal fields are observed in the paraventricular thalamic nucleus and in the lateral habenula medial division's parvicellular and central parts. More caudally, the periaqueductal gray's medial and ventrolateral divisions are innervated considerably more substantially than after LHAsfa PHAL injections, and moderately dense inputs are seen in the pontine central gray and in Barrington's nucleus. In addition, moderate anterograde labeling is observed in the retrorubral area and dorsal raphé nucleus.

Experiments involving the dorsal premammillary nucleus

The results described above indicate that the LHAsfa horizontal and vertical limbs project differentially to the dorsal premammillary nucleus, with the former innervating preferentially the ventral region of the nucleus, and, in contrast, the latter preferentially generating terminals in the dorsal region and around the ventral region. Because we previously analyzed projections from the dorsal premammillary nucleus as a whole (Canteras and Swanson, 1992b), and demonstrated an important role for it in the expression of defensive behavior (Canteras et al., 1997), we next made tiny PHAL injections centered in either dorsal or ventral regions of the nucleus to determine whether they generate distinguishable projection patterns that are subsets of the overall projection pattern.

Figure 12 illustrates two PHAL injection sites, one centered in the dorsal region (Fig. 12A), and the other in the ventral region (Fig. 12B), of the dorsal premammillary nucleus.

The clearest difference between these two experiments is in the periaqueductal gray. Injections centered dorsally label a very dense terminal field in the lateral division, especially in far lateral regions, and in the dorsal division (Fig. 12B). Inputs to the dorsolateral division are lighter and restricted to medial regions (Fig. 12B). In contrast, injections centered ventrally in the dorsal premammillary nucleus label a massive terminal

field in the periaqueductal gray's dorsolateral division, a dense terminal field in the dorsal division, and a lighter terminal field in the lateral division (Fig. 12D).

Finally, ventral regions of the dorsal premammillary project very heavily back to the LHAsfa.

DISCUSSION

Overall, the structural results presented here indicate that the LHAsfa, which was initially distinguished by a circumscribed input from the pontine NI, generates an axonal projection pattern that is distinct from LHA regions immediately lateral, medial, and caudal to it—and that the LHAsfa vertical and horizontal limbs generate topographically organized projections to the septal and amygdalar regions of the cerebral hemisphere on one hand, and to the hypothalamus and thalamus on the other.

An earlier PHAL study (Roeling et al., 1994) analyzed the projections of a related LHA region, Nieuwenhuys and colleagues's (1982) intermediate hypothalamic area, or hypothalamic "aggression area," as defined by electrical stimulation. However, the latter is more extensive and only its rostradorsal region overlaps with the LHAsfa as defined here. Two experiments (207 and 334) mentioned by Roeling et al. (1994) may have been centered in the LHAsfa's vertical limb, but comparisons with our data are difficult because their results were not illustrated. Nevertheless, comparisons with their results will be made where feasible.

Projections from LHAsfa and adjacent regions

As summarized in Figure 4, a majority of LHAsfa axons innervate forebrain structures, whereas a minority innervate cell groups in the midbrain and pons.

Projections to cerebral hemisphere—Our results indicate that by a wide margin the septal region is the most heavily innervated target of the LHAsfa. Furthermore, the LHAsfa, along with LHA regions immediately medial and lateral to it (the LHAjv and LHAa/d), generate a topographically organized projection to the lateral septal nucleus. The LHAsfa itself massively innervates the LSr.dl, and less densely the LSc—with the horizontal and vertical limbs targeting ventral and dorsal regions of the LSr.dl, respectively. The LHAa/d regions (in our experiments including the caudomedial anterior region and the adjacent rostromedial dorsal region) innervate densely the LSc and less densely the LSr. The LHAjv (in our experiment the rostromedial LHAjv) innervates more medial regions of the LSr.dl and the LSr.m. The latter is also innervated by the LHAsfp (just caudal to the LHAsfa), which also innervates the ventral zone of the LSc. Roeling and colleagues (1994) described a dense projection from their intermediate LHA to dorsolateral regions of the intermediate part of the lateral septal nucleus. Our results extend their observation by showing that the projections from the LHA to the lateral septal nucleus are topographically organized.

It is clear that the region of the LHAsfa and the LSr.dl share strong bidirectional connections (Luiten et al., 1982; Veening et al., 1987; Staiger and Nürnberger, 1989; Risold et al., 1994; Roeling et al., 1994; Risold and Swanson, 1997b; present results), and it is interesting to note that the LHAsfa projection forms pericellular baskets around LSr.dl neurons (Fig. 5A), which are presumably GABAergic medium spiny stellate neurons (Risold and Swanson, 1997a). A projection from the region of the LHAsfa to the LSc was demonstrated with retrograde labeling (Risold and Swanson, 1997b).

The LHAsfa also projects densely to the medial septal nucleus, which may also project "back" to the LHAsfa (Swanson and Cowan, 1979). A moderately dense projection to the

nucleus was observed in PHAL experiments with injections centered in the LHAA/d and LHAjv. The LHAsfp does not seem to innervate the medial septal nucleus. Roeling and colleagues (1994) also reported a dense projection to the medial septal nucleus after injections centered in dorsal regions of their intermediate hypothalamic area.

In the bed nuclei of the terminalis's posterior division, the LHAsfa innervates moderately the interfascicular and transverse nuclei. The latter are heavily innervated by the LHAjv, which also innervates the bed nuclei anterior division's anteromedial area. The LHAsfp also heavily innervates the bed nuclei's anterior division, which is not innervated by the LHAsfa. It should also be mentioned that the LHAsfa generates a moderately dense terminal field in the subjacent substantia innominata, like the LHAA/d and LHAsfp. Roeling and colleagues (1994) reported projections to the BST from their intermediate hypothalamic area that were denser after PHAL injections centered medially.

In the amygdalar region, the LHAsfa clearly innervates the posterior basomedial nucleus and paracapsular region of the intercalated nucleus, with less dense terminal fields in the lateral nucleus, and a few terminal boutons in the medial and central nuclei. The LHAsfa horizontal limb innervates the posterior basomedial and paracapsular intercalated nuclei more heavily than the vertical limb. Ottersen (1980) demonstrated retrograde labeling in the vicinity of the LHAsfa after retrograde tracer injections in an area that included ventral regions of the central and intercalated nuclei. In contrast, the rostromedial LHAjv does not innervate the paracapsular intercalated nucleus. Instead, dense anterograde labeling is observed in the posterior amygdalar nucleus and caudal regions of the posterior basomedial nucleus. In striking contrast, PHAL injections in the LHAsfp richly innervate the medial part of the central amygdalar nucleus. Similarly, Roeling and colleagues (1994) described projections from medial regions of their intermediate hypothalamic area to the posterior amygdalar nucleus. However, they did not report projections from dorsal regions to the intercalated and basomedial nuclei.

Projections to hypothalamus—In the preoptic region, the LHAsfa innervates moderately the lateral preoptic area. PHAL injections in the LHAA/d label a dense input to the lateral preoptic area, although, in contrast, it is most dense rostrally. Importantly from a topographic point of view, the LHAjv innervates the medial rather than lateral preoptic area—as does the LHAsfp, which also innervates the anteroventral preoptic, anterodorsal preoptic, and parastrial nuclei. In agreement, Roeling and colleagues (1994) described a projection to the medial preoptic area from medial regions of their intermediate LHA.

The major intrahypothalamic target of the LHAsfa is the dorsal premammillary nucleus, bilaterally. Moderately dense terminal fields are also established in the LHA juxtapaaraventricular region, anterior hypothalamic nucleus, dorsomedial part of the ventromedial nucleus, and anterior part of the dorsomedial nucleus—and they arise preferentially in the LHAsfa horizontal limb. The LHAsfa-dorsal premammillary nucleus projection was described with anterograde and retrograde methods (Roeling et al., 1994; Comoli et al., 2000). The observations are extended here by showing a topographic organization in the projection. The LHAsfa vertical limb innervates preferentially the dorsal region, and an area surrounding the ventral region, of the dorsal premammillary nucleus, whereas the LHAsfa horizontal wing innervates preferentially the ventral region of the dorsal premammillary nucleus. This is interesting because, as shown here with PHAL, the dorsal and ventral regions of the dorsal premammillary nucleus display a complementary projection pattern to the periaqueductal gray, with the former preferentially innervating the lateral division and the latter preferentially innervating the dorsolateral division (see below). Curiously, the LHAA/d projects densely to the dorsal region of the dorsal premammillary

nucleus, whereas the LHA_{jv} projects densely to areas surrounding the ventral region of the dorsal premammillary nucleus.

The LHA_{sf} projects selectively to the dorsomedial part of the ventromedial nucleus, whereas the LHA_{jv} innervates the entire nucleus. In contrast, the LHA_{sf} avoids the ventromedial and anterior hypothalamic nuclei and instead richly innervates the paraventricular nucleus, including its anterior, medial, and lateral parvicellular parts—which are not labeled by LHA_{sf} injections. The LHA_{a/d} only very sparsely innervates the ventromedial and anterior hypothalamic nuclei, instead projecting substantially to the LHA supraforical region. The latter also receives substantial inputs from the LHA_{sf}, which also innervates the juxtaventricular, and the dorsal and ventral zones of the anterior, regions of the LHA. The dorsal zone of the LHA's anterior region is also an important target of the LHA_{jv}. Roeling and colleagues (1994) reported only sparse to moderate inputs to the LHA from their intermediate hypothalamic area.

A projection from LHA_{sf} to dorsomedial hypothalamic nucleus was observed with retrograde labeling (Thompson and Swanson, 1998). The LHA_{sf} projects substantially to the anterior and ventral parts of the dorsomedial hypothalamic nucleus, whereas the LHA_{jv} sends moderate inputs to the anterior part, both of which are only lightly labeled by the LHA_{a/d}. Essentially the same findings were reported by Roeling and colleagues (1994).

Finally, the LHA_{sf} and LHA_{jv} provide moderate terminal fields to the posterior hypothalamic nucleus, as do the LHA_{a/d} and LHA_{sf}.

Projections to thalamus—The LHA_{sf} sends moderately dense inputs to caudal levels of the lateral habenula, somewhat lighter inputs to the paratenial and mediodorsal nuclei, and ventral regions of the nucleus reuniens; and even lighter inputs to the paraventricular, intermediodorsal, and central medial nuclei. The heaviest projection to the lateral habenula arises in the LHA_{sf} vertical limb. Projections from LHA_{sf} to the paratenial, paraventricular, mediodorsal, and reuniens nuclei were identified with retrograde tracer methods (Groenewegen, 1988; Chen and Su, 1990; McKenna and Vertes, 2004). Roeling and colleagues (1994) reported dense projections from dorsal regions of their intermediate hypothalamic area to the mediodorsal and paratenial nuclei.

Heavier, topographically organized projections to the lateral habenula's lateral division arise from the LHA_{a/d}, where they innervate massively more lateral regions of the magnocellular and marginal parts.

Curiously, the region of the LHA_{jv} injected here innervates a conspicuous region lying between the paratenial and mediodorsal nuclei.

Projections to midbrain and pons—The LHA_{sf} generates light projections to the precommissural nucleus, and to the dorsal, medial, dorsolateral, and lateral divisions of the periaqueductal gray; and moderate projections to caudal regions of the periaqueductal gray's ventrolateral division, the dorsal and superior central nuclei of the raphe, and the cuneiform nucleus. The periaqueductal gray is innervated more densely by LHA regions immediately surrounding the LHA_{sf}. Thus, the LHA_{jv} generates moderately dense terminal fields in the lateral and ventrolateral periaqueductal gray divisions; the LHA_{a/d} innervates substantially the precommissural nucleus, and the PAG medial and rostromedial divisions; and the LHA_{sf} projects heavily to the PAG ventrolateral division and pontine central gray, as well as to Barrington's nucleus. Moderate projections to the retrorubral area arise in the LHA_{jv}, LHA_{a/d}, and LHA_{sf}. The latter two also provide moderate innervation to the dorsal raphe nucleus.

Evidence for some of the projections reported here has already been adduced. Following noxious visceral stimulation and retrograde tracer injections in the periaqueductal gray's ventrolateral division, labeled neurons also exhibiting Fos immunoreactivity were identified in the LHAsfa region (Snowball et al., 2000). Projections from the LHAsfa to the dorsal and superior central nuclei of the raphé were reported with retrograde tracer methods (Behzadi et al., 1990; Peyron et al., 1998), and electrical stimulation of the cuneiform nucleus increases c-fos mRNA levels in the region of the LHAsfa (Lam et al., 1997).

Roeling and colleagues (1994) observed dense projections to the precommissural nucleus and other regions of the periaqueductal gray after PHAL injections in medial regions of their intermediate hypothalamic area. Our experiments confirm that these midbrain cell groups are substantially labeled after PHAL injections centered in LHA regions immediately medial to the LHAsfa.

Functional implications of LHAsfa circuitry

It is instructive to start a brief consideration of the most obvious possible LHAsfa functional significance with an overview of what is known about sources of axonal inputs, which fall into three broad categories (see Fig. 13). First, the LHAsfa was defined originally by a dense, circumscribed input from the NI (Goto et al., 2001; Swanson, 2004), and in this context it is significant that two other brainstem midline nuclei that share bidirectional connections with the NI, and with each other—the superior central (median raphé) and interpeduncular nuclei—also provide clear inputs to the LHAsfa (Groenewegen et al., 1986; Vertes et al., 1999). These three midline nuclei form a highly interconnected network (they are all bidirectionally connected) involved in modulating levels of behavioral activity, arousal, or foraging behavior (for references see Goto et al., 2001).

The midline network integrates neural inputs from prefrontal cortex (directly) and hippocampal formation (via a septo-habenular pathway), and is strategically placed to control behavior-gating mechanisms through its axonal projections to mediodorsal thalamic nucleus and prefrontal cortex, hippocampal formation, and nucleus accumbens. In addition, midline network activity may be influenced by corticotropin-releasing hormone (CRH) in the cerebrospinal fluid, through abundant type 1 CRH receptors expressed on NI neurons. It is therefore likely that under certain challenging conditions, the LHAsfa receives information from a midline brainstem neural network involved in modulating behavioral arousal. The LHAsfa may also receive important affective information via direct axonal projections from the ventral region of the dorsal premammillary nucleus (here) and the periaqueductal gray's dorsolateral division (S.R. Mota-Ortiz and N.S. Canteras, personal observations), which are integral parts of the neural network that mediates innate fear responses (see Canteras, 2002).

To summarize, the strongest “descending” projections of the LHAsfa are to neural networks that control the expression of defensive (also see below) and foraging behaviors, and key parts of this circuitry (the nucleus incertus, dorsolateral periaqueductal gray, and dorsal premammillary nucleus) provide major “feedback” inputs to the LHAsfa.

The only evidence to date for a relatively direct source of sensory information to the LHAsfa involves a direct projection from the parabrachial nucleus's superior lateral part. It probably relays noxious information, at least in part (Bester et al., 1997).

A third—and strongest—source of input to the LHAsfa is the septal region (Fig. 13). The LSr.dl is the single greatest source of this projection to the LHAsfa (Risold and Swanson, 1997b), and it is important to note that the LSr.dl in turn is the single densest target of LHAsfa projections (Swanson and Cowan, 1979; Roeling et al., 1994; Risold and Swanson,

1997b; present results). The LSr.dl “relays” neural activity from specific regions of hippocampal field CA1, and to a somewhat lesser extent field CA3, that may be related to environmental, spatial, and navigational cues (see Risold and Swanson, 1997b). Thus, specific regions of Ammon’s horn project to the LSr.dl, which then establishes massive bidirectional connections with the LHAsfa. The LHAsfa in turn is positioned to influence septo-hippocampal system activity (see Swanson et al., 1987) by way of a dense projection to the medial septal nucleus, and a less dense projection to the diagonal band nucleus.

LHAsfa interactions with the septo-hippocampal system are complemented by LHAsfa projections to the amygdalar region, in particular the posterior basomedial and intercalated nuclei (Fig. 13). On one hand, the posterior basomedial nucleus shares bidirectional connections with hippocampal field CA1 and the subiculum (Canteras and Swanson, 1992a; Petrovich et al., 1996, 2001; Cenquizca, 2004), and on the other it shares bidirectional connections with the lateral amygdalar nucleus (Krettek and Price, 1978; Pitkanen et al., 1995; Petrovich et al., 1996), thus integrating auditory, visual, and polymodal sensory information from various sources (Canteras and Swanson, 1992a; Romanski and LeDoux, 1993; McDonald and Jackson, 1987; McDonald, 1998). Finally, the posterior basomedial nucleus projects massively to virtually the entire ventromedial hypothalamic nucleus and the medial and capsular parts of the central amygdalar nucleus (Petrovich et al., 1996), positioning it to influence the expression of various reproductive and defensive behaviors (see Swanson, 2000) and autonomic responses (see Loewy, 1991), respectively.

The intercalated nucleus (Millhouse, 1986), another major target of LHAsfa projections, is formed by tiny GABAergic neurons (Nitecka and Ben-Ari, 1987; McDonald and Augustine, 1993; Pare and Smith, 1993; Royer et al., 1999) that receive presumed sensory-related inputs from the basolateral amygdalar complex (Royer et al., 1999), and orbitofrontal, visual, and auditory regions of cerebral cortex (Ghashghaei and Barbas, 2002)—and project to the central amygdalar nucleus (Royer et al., 1999).

The final set of connections with possible functional relevance, based on data currently available, involves intrahypothalamic LHAsfa projections, mentioned above in relation to defensive and foraging behaviors. Interestingly, the experimental PHAL analysis suggests that the LHAsfa horizontal (ventrolateral) and vertical (dorsomedial) limbs preferentially innervate functionally different neural networks.

The LHAsfa horizontal limb preferentially innervates the anterior hypothalamic nucleus, dorsomedial ventromedial nucleus, and ventral dorsal premammillary nucleus—integral components of the medial hypothalamic circuit controlling defensive (fight or flight) behavior (Canteras, 2002), which in turn is part of the rostral behavior control column (Swanson, 2000). As reviewed by Canteras (2002), the LHAsfa is a primary intrahypothalamic source of neural inputs to the behavior control column defensive behavior component, which also receives major inputs from the bed nuclei of the stria terminalis posterior division’s interfascicular and transverse nuclei (Dong and Swanson, 2004) and posterior basomedial amygdalar nucleus (Petrovich et al., 1996)—both of which receive inputs from the LHAsfa. Thus, the LHAsfa is positioned to influence the medial hypothalamic defensive behavior circuit, which appears to mediate behavioral responses to innate threats like exposure to natural predators (Canteras, 2002). Correspondingly, selective lesions including the LSr.dl (which shares strong bidirectional connections with the LHAsfa) are followed by hyperdefensiveness characterized by agonistic responses to the experimenter and hyperreactivity to a variety of sensory stimuli (Albert and Walsh, 1984).

In contrast, the LHAsfa vertical limb projects preferentially to medial regions of the lateral habenula and the dorsal part of the dorsal premammillary nucleus. Lateral habenular lesions

disrupt integration of contextual cues and selection of adaptive behavioral responses under stress (Thornton and Evans, 1982, 1984; Thornton et al., 1990), perhaps through projections to the ventral tegmental area, the NI-CS-IPN midline network (see Goto et al, 2001), and other areas. As shown here, dorsal regions of the dorsal premammillary nucleus project strongly to the periaqueductal gray's lateral division, which has been implicated in the modulation of foraging or exploratory responses (Comoli et al., 2005). In line with this possibility, NMDA lesions of the periaqueductal gray's lateral division appear to disrupt foraging activity during predatory hunting (S.R. Mota-Ortiz and N.S. Canteras, personal observations).

Overview

Clearly, the LHAsfa is embedded within exceptionally complex circuitry, both structurally and functionally, and only highlights of the apparently relevant literature available today can be reviewed here. As summarized in Figure 13, the LHAsfa is positioned to integrate a complex set of input information from the nociception system, brainstem networks involved in coordinating behavioral responses (particularly foraging and defensive behaviors), and the septo-hippocampal system. On the output side, LHAsfa projections to cerebral hemisphere target especially the hippocampal formation and amygdalar region, and its projections to interbrain target neural networks controlling a variety of behavioral responses related most directly to defense (fight or flight) and foraging (exploration).

Supporting this view, a number of studies implicate LHA regions ventral to the fornix, including the LHAsfa, in controlling defensive behavior because either attack or escape responses can be evoked there by electrical stimulation (Roberts, 1969; Kruk et al., 1983; Lammers et al., 1988a; Kruk, 1991; Roberts and Nagel, 1996). Furthermore, the same general LHA region also has been associated with nondefensive behaviors like upward flight and exploratory behavior that includes sniffing, nosing, looking around, and locomotion (Lammers et al., 1988b; Roberts and Nagel, 1996).

Overall, the present analysis supports the hypothesis that the LHAsfa helps match adaptive autonomic and behavioral responses (either defensive or foraging) to current internal motivational status and external environmental conditions.

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ABBREVIATIONS

AAA	anterior amygdalar area
ab	angular bundle
ACAd	anterior cingulate area, dorsal part
ACB	nucleus accumbens
aco	anterior commissure, olfactory limb
AD	anterodorsal nucleus
ADP	anterodorsal preoptic nucleus
AHA	anterior hypothalamic area
AHN	anterior hypothalamic nucleus

AHNa	anterior hypothalamic nucleus, anterior part
AHNc	anterior hypothalamic nucleus, central part
AHNd	anterior hypothalamic nucleus, dorsal part
AHNp	anterior hypothalamic nucleus, posterior part
alv	alveus
AM	anteromedial nucleus thalamus
amc	amygdalar capsule
AMd	anteromedial nucleus thalamus, dorsal part
AMv	anteromedial nucleus thalamus, ventral part
AONpv	anterior olfactory nucleus, posteroventral part
APN	anterior pretectal nucleus
AQ	cerebral aqueduct
ARH	arcuate nucleus hypothalamus
AV	anteroventral nucleus thalamus
AVP	anteroventral preoptic nucleus
AVPV	anteroventral periventricular nucleus
BA	bed nucleus accessory olfactory tract
BLA	basolateral nucleus amygdala
BLAa	basolateral nucleus amygdala, anterior part
BLAp	basolateral nucleus amygdala, posterior part
BMAa	basomedial nucleus amygdala, anterior part
BMAp	basomedial nucleus amygdala, posterior part
bsc	brachium of the superior colliculus
BST	bed nuclei of the stria terminalis
BSTal	bed nuclei of the stria terminalis, anterior division, anterolateral area
BSTam	bed nuclei stria terminalis, anterior division, anteromedial area
BSTdm	bed nuclei of the stria terminalis, anterior division, dorsomedial nucleus
BSTfu	bed nuclei of the stria terminalis, anterior division, fusiform nucleus
BSTif	bed nuclei of the stria terminalis, posterior division, interfascicular nucleus
BSTju	bed nuclei of the stria terminalis, anterior division, juxtacapsular nucleus
BSTmg	bed nuclei of the stria terminalis, anterior division, magnocellular nucleus
BSTov	bed nuclei of the stria terminalis, anterior division, oval nucleus
BSTpr	bed nuclei of the stria terminalis, posterior division, principal nucleus
BSTrh	bed nuclei of the stria terminalis, anterior division, rhomboid nucleus
BSTtr	bed nuclei of the stria terminalis, posterior division, transverse nucleus
BSTv	bed nuclei of the stria terminalis, anterior division, ventral nucleus

CA1	field CA1, Ammon's horn
CA1slm	field CA1, stratum lacunosum-moleculare
CA1so	field CA1, stratum oriens [Sala]
CA1sp	field CA1, pyramidal layer
CA1spd	field CA1, pyramidal layer, deep
CA1sps	field CA1, pyramidal layer, superficial
CA1sr	field CA1, stratum radiatum
CA2	field CA2, Ammon's horn
CA3	field CA3, Ammon's horn
CA3slu	field CA3, stratum lucidum
CA3so	field CA3, stratum oriens
CA3sp	field CA3, pyramidal layer
CA3sr	field CA3, stratum radiatum
cc	corpus callosum, genu
CEA	central nucleus amygdala
CEAc	central nucleus amygdala, capsular part
CEAl	central nucleus amygdala, lateral part
CEAm	central nucleus amygdala, medial part
cic	inferior colliculus commissure
CL	central lateral nucleus thalamus
CLA	claustrum
CLI	central linear nucleus raphé
CM	central medial nucleus thalamus
COA	cortical amygdalar nucleus
COAa	cortical amygdalar nucleus, anterior part
COApl	cortical nucleus amygdala, posterior part, lateral zone
COApm	cortical nucleus amygdala, posterior part, medial zone
COM	commissural nucleus, periaqueductal gray
CP	caudoputamen
cpd	cerebral peduncle
csc	commissure superior colliculus
CS	superior central nucleus raphé
CSl	superior central nucleus raphé, lateral part
CSm	superior central nucleus raphé, medial part
CUN	cuneiform nucleus
DG	dentate gyrus

DGIb	dentate gyrus, lateral blade
DGIb-mo	dentate gyrus, lateral blade-molecular layer
DGIb-po	dentate gyrus, lateral blade-polymorph layer
DGIb-sg	dentate gyrus, lateral blade-granule cell layer
DGmb	dentate gyrus, medial blade
DMH	dorsomedial hypothalamic nucleus
DMHa	dorsomedial hypothalamic nucleus, anterior part
DMHp	dorsomedial nucleus hypothalamus, posterior part
DMHv	dorsomedial hypothalamic nucleus, ventral part
DR	dorsal nucleus raphé
dtd	dorsal tegmental decussation
DTN	dorsal tegmental nucleus
ec	external capsule
em	external medullary lamina thalamus
ENTm1-6	entorhinal area, medial part, layers 1–6
ENTmv	entorhinal area, medial part, ventral zone
EPd	endopiriform nucleus, dorsal part
EPv	endopiriform nucleus, ventral part
EW	Edinger-Westphal nucleus
fa	corpus callosum, anterior forceps
FF	fields of Forel
fi	fimbria
fr	fasciculus retroflexus
FS	fundus of the striatum
fx	columns of the fornix
fxpr	precommissural fornix
GPe	globus pallidus, external segment
GPI	globus pallidus, internal segment
hf	hippocampal fissure
IA	intercalated nuclei amygdala
IAD	interanterodorsal nucleus thalamus
IAM	interanteromedial nucleus thalamus
IC	inferior colliculus
IF	interfascicular nucleus raphé
IGL	lateral geniculate complex, intergeniculate leaflet
III	oculomotor nucleus

ILA	infralimbic cortical area
IMD	intermediodorsal nucleus thalamus
INC	interstitial nucleus of Cajal
int	internal capsule
IPN	interpeduncular nucleus
IPNc	interpeduncular nucleus, central subnucleus
IPNlr	interpeduncular nucleus, lateral subnucleus, rostral part
IPNr	interpeduncular nucleus, rostral subnucleus
IV	trochlear nucleus
IVn	trochlear nerve
LA	lateral amygdalar nucleus
LC	locus ceruleus
LD	lateral dorsal nucleus thalamus
LDT	laterodorsal tegmental nucleus
LGd	lateral geniculate complex, dorsal part
LGvl	lateral geniculate complex, ventral part, lateral zone
LGvm	lateral geniculate complex, ventral part, medial zone
LH	lateral habenula
LHA	lateral hypothalamic area
LHAad	lateral hypothalamic area, anterior region, dorsal zone
LHAai	lateral hypothalamic area, anterior region, intermediate zone
LHAav	lateral hypothalamic area, anterior region, ventral zone
LHAd	lateral hypothalamic area, dorsal region
LHAjd	lateral hypothalamic area, juxtadorsomedial region
LHAjp	lateral hypothalamic area, juxtaparaventricular region
LHAjv	lateral hypothalamic area, juxtaventromedial region
LHAjvd	lateral hypothalamic area, juxtaventromedial region, dorsal zone
LHAjvv	lateral hypothalamic area, juxtaventromedial region, ventral zone
LHAm	lateral hypothalamic area, magnocellular nucleus
LHAp	lateral hypothalamic area, posterior region
LHApc	lateral hypothalamic area, parvicellular region
LHAs	lateral hypothalamic area, supraforinal region
LHAsf	lateral hypothalamic area, subforinal region
LHAvl	lateral hypothalamic area, ventral region, lateral zone
LHAvm	lateral hypothalamic area, ventral region, medial zone
LM	lateral mammillary nucleus

lot	lateral olfactory tract
LP	lateral posterior nucleus thalamus
LPO	lateral preoptic area
LS	lateral septal nucleus
LSc	lateral septal nucleus, caudal part
LSc.d	lateral septal nucleus, caudal part, dorsal zone
LSc.v	lateral septal nucleus, caudal part, ventral zone
LSc.v.l	lateral septal nucleus, caudal part, ventral zone, lateral region
LSc.v.l.d	lateral septal nucleus, caudal part, ventral zone, lateral region, dorsal domain
LSc.v.l.v	lateral septal nucleus, caudal part, ventral zone, lateral region, ventral domain
LSc.v.m.d	lateral septal nucleus, caudal part, ventral zone, medial region, dorsal domain
LSc.v.m.v	lateral septal nucleus, caudal part, ventral zone, medial region, ventral domain
LSr	lateral septal nucleus, rostral part
LSr.dl	lateral septal nucleus, rostral part, dorsolateral zone
LSr.dl.l	lateral septal nucleus, rostral part, dorsolateral zone, lateral region
LSr.dl.l.d	lateral septal nucleus, rostral part, dorsolateral zone, lateral region, dorsal domain
LSr.dl.l.v	lateral septal nucleus, rostral part, dorsolateral zone, lateral region, ventral domain
LSr.dl.m	lateral septal nucleus, rostral part, dorsolateral zone, medial region
LSr.dl.m.d	lateral septal nucleus, rostral part, dorsolateral zone, medial region, dorsal domain
LSr.dl.m.v	lateral septal nucleus, rostral part, dorsolateral zone, medial region, ventral domain
LSr.m	lateral septal nucleus, rostral part, medial zone
LSr.m.d	lateral septal nucleus, rostral part, medial zone, dorsal region
LSr.m.v.c	lateral septal nucleus, rostral part, medial zone, ventral region, caudal domain
LSr.m.v.r	lateral septal nucleus, rostral part, medial zone, ventral region, rostral domain
LSr.vl.d.l	lateral septal nucleus, rostral part, ventrolateral zone, dorsal region, lateral domain
LSr.vl.d.m	lateral septal nucleus, rostral part, ventrolateral zone, dorsal region, medial domain
LSr.vl.v	lateral septal nucleus, rostral part, ventrolateral zone, ventral region

LSv	lateral septal nucleus, ventral part
MA	magnocellular preoptic nucleus
MD	mediodorsal nucleus thalamus
MDc	mediodorsal nucleus thalamus, central part
MDl	mediodorsal nucleus thalamus, lateral part
MDm	mediodorsal nucleus thalamus, medial part
MEA	medial nucleus amygdala
MEAad	medial amygdalar nucleus, anterodorsal part
MEAav	medial amygdalar nucleus, anteroventral part
MEPO	median preoptic nucleus
MEV	midbrain nucleus of the trigeminal
MGm	medial geniculate complex, medial part
MGv	medial geniculate complex, ventral part
MH	medial habenula
ml	medial lemniscus
MM	medial mammillary nucleus, body
MMme	medial mammillary nucleus, median part
MOp	primary motor area
moV	motor root of the trigeminal nerve
mp	mammillary peduncle
MPN	medial preoptic nucleus
MPO	medial preoptic area
MPT	medial pretectal area
MRN	midbrain reticular nucleus
MRNp	midbrain reticular nucleus, parvicellular part
MS	medial septal nucleus
mtt	mammillothalamic tract
NB	nucleus brachium inferior colliculus
ND	nucleus of Darkschewitsch
NDB	diagonal band nucleus
NI	nucleus incertus
NIc	nucleus incertus, compact part
NIId	nucleus incertus, diffuse part
NLL	nucleus of the lateral lemniscus
NPC	nucleus of the posterior commissure
och	optic chiasm

OP	olivary pretectal nucleus
opt	optic tract
OT	olfactory tubercle
PA	posterior nucleus amygdala
PAA	piriform-amygdalar area
PAG	periaqueductal gray
PAGd	periaqueductal gray, dorsal division
PAGdl	periaqueductal gray, dorsolateral division
PAGl	periaqueductal gray, lateral division
PAGm	periaqueductal gray, medial division
PAGrl	periaqueductal gray, rostromedial division
PAGrm	periaqueductal gray, rostromedial division
PAGvl	periaqueductal gray, ventrolateral division
PAR1-6	parasubiculum, layers 1–6
PARN	parvicellular reticular nucleus
PBl	parabrachial nucleus, lateral part
PBlc	parabrachial nucleus, central lateral part
PBlv	parabrachial nucleus, ventral lateral part
PBmm	parabrachial nucleus, medial medial part
pc	posterior commissure
PCG	pontine central gray
PCN	paracentral nucleus thalamus
PF	parafascicular nucleus
PH	posterior hypothalamic nucleus
PIR	piriform area
PL	prelimbic cortical area
pm	principal mammillary tract
PMD	dorsal premammillary nucleus
PMDd	dorsal premammillary nucleus, dorsal region
PMDv	dorsal premammillary nucleus, ventral region
PMv	ventral premammillary nucleus
PO	posterior complex thalamus
PP	peripeduncular nucleus
PPN	pedunculopontine nucleus
PR	perireuniens nucleus
PRC	precommissural nucleus, periaqueductal gray

PRE1-6	presubiculum, layers 1–6
PRNc	pontine reticular nucleus, caudal part
PRNr	pontine reticular nucleus, rostral part
PS	parastrial nucleus
PST	preparasubthalamic nucleus
PSTN	parasubthalamic nucleus of the lateral hypothalamic area
PSV	principal sensory nucleus of the trigeminal
PT	paratenial nucleus
PVa	periventricular hypothalamic nucleus, anterior part
PVH	paraventricular hypothalamic nucleus
PVHap	paraventricular hypothalamic nucleus, anterior parvicellular part
PVHf	paraventricular nucleus hypothalamus, forniceal part
PVHlp	paraventricular hypothalamic nucleus, lateral parvicellular part
PVHmpd	paraventricular hypothalamic nucleus, medial parvicellular part, dorsal zone
PVHpm	paraventricular hypothalamic nucleus, posterior magnocellular part, lateral zone
PVHpv	paraventricular hypothalamic nucleus, periventricular part
PVi	periventricular hypothalamic nucleus, intermediate part
PVp	periventricular hypothalamic nucleus, posterior part
PVT	paraventricular thalamic nucleus
py	pyramid
RCH	retrochiasmatic area, lateral hypothalamic area
RE	nucleus reuniens
REd	nucleus reuniens, rostral division, dorsal part
REm	nucleus reuniens, rostral division, median part
REv	nucleus reuniens, rostral division, ventral part
RH	rhomboid nucleus
RL	rostral linear nucleus raphé
RM	nucleus raphé magnus
RN	red nucleus
RPO	nucleus raphé pontis
RR	midbrain reticular nucleus, retrorubral area
RT	reticular nucleus thalamus
rust	rubrospinal tract
SC	superior colliculus

SCdg	superior colliculus, deep gray layer
SCdw	superior colliculus, deep white layer
SCH	suprachiasmatic nucleus
SCig	superior colliculus, intermediate gray layer
SCiw	superior colliculus, intermediate white layer
SCop	superior colliculus, optic layer
scp	superior cerebellar peduncle
SCsg	superior colliculus, superficial gray layer
sectv	ventral spinocerebellar tract
SCzo	superior colliculus, zonal layer
SF	septo-fimbrial nucleus
SGN	supragenulate nucleus
SH	septohippocampal nucleus
SI	substantia innominata
SLC	subceruleus nucleus
sm	stria medullaris
smd	supramammillary decussation
SMT	submedial nucleus thalamus
SNc	substantia nigra, compact part
SNr	substantia nigra, reticular part
SO	supraoptic nucleus
SPFm	subparafascicular nucleus thalamus, magnocellular part
SPFpl	subparafascicular nucleus thalamus, parvocellular part, lateral division
SPFpm	subparafascicular nucleus thalamus, parvocellular part, medial division
st	stria terminalis
STN	subthalamic nucleus
SUBv	subiculum, ventral part
SUBv-sp	subiculum, ventral part, pyramidal layer
SUMl	supramammillary nucleus, lateral part
SUMm	supramammillary nucleus, medial part
sup	supraoptic commissures
SUT	supratrigeminal nucleus
SUV	superior vestibular nucleus
TMv	tuberomammillary nucleus, ventral part
TR	postpiriform transition area
tsp	tectospinal pathway

TTd	tenia tecta, dorsal part
TTv	tenia tecta, ventral part
TU	tuberal nucleus
TUi	tuberal nucleus, intermediate part
TUI	tuberal nucleus, lateral part
TUsv	tuberal nucleus, subventricular part
TUte	tuberal nucleus, terete subnucleus
V3	third ventricle
V4	fourth ventricle
VAL	ventral anterior-lateral complex thalamus
vhc	ventral hippocampal commissure
VL	lateral ventricle
vlt	ventrolateral hypothalamic tract
VM	ventral medial nucleus thalamus
Vma	motor nucleus of the trigeminal, magnocellular part
VMH	ventromedial nucleus hypothalamus
VMHa	ventromedial nucleus hypothalamus, anterior part
VMHc	ventromedial nucleus hypothalamus, central part
VMHdm	ventromedial nucleus hypothalamus, dorsomedial part
VMHvl	ventromedial nucleus hypothalamus, ventrolateral part
VPL	ventral posterolateral nucleus thalamus
VPLpc	ventral posterolateral nucleus thalamus, parvicellular part
VPM	ventral posteromedial nucleus thalamus
VPMpc	ventral posteromedial nucleus thalamus, parvicellular part
VTa	ventral tegmental area
vtd	ventral tegmental decussation
VTN	ventral tegmental nucleus
ZI	zona incerta
ZIda	zona incerta, dopaminergic group

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Fig. 1.

Low (A–C) and high (A1–C1) power brightfield photomicrographs of transverse Nissl-stained sections showing cytoarchitectonic features of the left LHAsfa and surrounding parts of the hypothalamus. The boxed areas in A, B, and C are shown at higher magnification in A1, B1, and C1, respectively. (A2–C2) Darkfield photomicrographs of adjacent sections to show the LHAsfa terminal field after a PHAL injection in the NI. Scale bars = 100 μ m.

Fig. 2.

Camera lucida plots of PHAL-labeled neurons following injections centered in the LHASfa (Experiments LHASfa94, LHASfa89, LHASfa93), LHA region medial to the LHASfa (the LHAjv; Experiment LHA52), LHA region lateral to the LHASfa (LHAa/d; Experiment LHA45), and LHA region caudal to the LHASfa (the LHASfp; Experiment LHA22). In Experiments LHASfa89 and LHASfa93 the PHAL injection is centered in the vertical (dorsomedial) and horizontal (ventrolateral) limbs of the LHASfa, respectively. Transverse sections are arranged from rostral (left) to caudal (right).

Fig. 3. Brightfield photomicrographs showing PHAL deposits in the LHAsfa. (A) A large PHAL injection in the LHAsfa, Experiment LHA94; (B) a PHAL injection centered in the horizontal (ventrolateral) limb of the LHAsfa, Experiment LHA93; (C) a PHAL injection centered in the vertical (dorsomedial) limb of the LHAsfa, Experiment LHA 89. Scale bars = 100 μ m.

Fig. 4. General organization of projections from the LHAsfa. The magnitude of each pathway is roughly proportional to the thickness of the line representing it. The numbers 1–6 refer to main axonal pathways described in the text. The flatmap is adapted from Swanson (2004).

Fig. 5.

Projections from the LHAsfa. The distribution of PHAL-labeled axons (thin black lines) in experiment LHAsfa94 was plotted onto a series of rat brain reference templates derived from Swanson (2004), arranged from rostral to caudal (A-S). The black dots in G and H indicate the injection site.

Fig. 6. Darkfield photomicrographs showing the distribution of PHAL-labeled axons in experiment LHAsfa94, with a large injection centered in the LHAsfa. (A) Dorsolateral zone of the lateral septal nucleus's rostral part, with an abundance of pericellular baskets; (B) intercalated and posterior basomedial nuclei of the temporal lobe's amygdalar region; (C) lateral habenula; and (D) dorsal premammillary nucleus. Scale bars = 100 μ m.

Fig. 7.

Darkfield photomicrographs showing the distribution of PHAL-labeled axons in experiment LHAsfa93, with a PHAL injection centered in the LHAsfa's horizontal limb. (A) Dorsolateral zone of the lateral septal nucleus's rostral part, with abundant pericellular baskets, and medial septal nucleus; (B) caudal part of the lateral septal nucleus and septofimbrial nucleus; (C) intercalated and posterior basomedial nuclei of the amygdalar region; (D) lateral habenula; (E) LHA's juxtaparaventricular region and anterior hypothalamic nucleus; (F) anterior and ventromedial hypothalamic nuclei and the PHAL deposit centered in the LHAsfa's ventrolateral or horizontal limb; (G) dorsomedial and ventromedial hypothalamic nuclei; (H) ventral region of the dorsal premammillary nucleus. Scale bars = 100 μ m.

Fig. 8.

Darkfield photomicrographs showing the distribution of PHAL-labeled axons in experiment LHAsfa89, with a PHAL injection centered in the LHAsfa's vertical limb. (A) Dorsolateral zone of the lateral septal nucleus's rostral part, with abundant pericellular baskets, and medial septal nucleus; (B) caudal part of the lateral septal nucleus; (C) intercalated and posterior basomedial amygdalar nuclei; (D) lateral habenula; (E) juxtavaraventricular region of the LHA and anterior hypothalamic nucleus; (F) anterior and ventromedial hypothalamic nuclei, and the PHAL deposit in the dorsomedial or vertical limb of the LHAsfa; (G) dorsomedial and ventromedial hypothalamic nuclei; (H) dorsal region of the dorsal premammillary nucleus. Note dense terminal field in the lateral habenula and in dorsal regions of the dorsal premammillary nucleus, in contrast to very little labeling in the intercalated and posterior basomedial nuclei, LHA's juxtavaraventricular region, and anterior, ventromedial, and dorsomedial hypothalamic nuclei. Ventral regions of the dorsal premammillary nucleus are almost free of labeling as well. Scale bars = 100 μ m.

Fig. 9. Darkfield photomicrographs showing the distribution of PHAL-labeled axons in experiment LHA52, with a PHAL injection centered just medial to the LHAsfa, rostrolaterally in the LHA's juxtaventromedial region. (A) Lsr.dl.l and Lsr.dl.m, and the medial septal nucleus; (B) bed nuclei of the stria terminalis's anterior division and the medial preoptic area; (C) LHA juxtaparaventricular region; (D) ventromedial hypothalamic nucleus; (E) caudal region of the posterior basomedial nucleus, posterior amygdalar nucleus, and field CA1 of Ammon's horn; (F) caudal regions of the posterior amygdalar nucleus and field CA1. Note labeling in the bed nuclei's anterior division, and in the ventromedial nucleus's central and ventrolateral parts, which are not labeled in LHAsfa experiments—and a dense terminal field in the posterior amygdalar nucleus, which is only lightly labeled in LHAsfa experiments. Scale bars = 100 μ m.

Fig. 10.

Darkfield photomicrographs showing the distribution of PHAL-labeled axons in experiment LHA45, with a PHAL injection centered just lateral to the LHAsfa, in the LHA. (A) Caudal part of the lateral septal nucleus and medial septal nucleus, (B) caudal part of the lateral septal nucleus and septofimbrial nucleus, (C) lateral preoptic area, (D) dorsal region of the dorsal premammillary nucleus, and (E–G) lateral habenula, arranged from rostral to caudal. Note dense labeling in dorsal regions of the dorsal premammillary nucleus, contrasting with very sparse labeling in ventral regions of the nucleus, and a massive projection to the lateral habenula. Scale bars = 100 μ m.

Fig. 11.

Darkfield photomicrographs showing the distribution of PHAL-labeled axons in experiment LHA22 with a PHAL injection centered caudal to the LHAsfa, in the LHAsfp. (A) Medial preoptic area, and the parastrial, anterodorsal preoptic, and anteroventral preoptic nuclei; (B) anteromedial and anterolateral areas and the rhomboid, dorsomedial, and ventral nuclei of bed nucleus of the stria terminalis's anterior division; (C) interfascicular and transverse nuclei of bed nuclei's the posterior division, and the paraventricular hypothalamic nucleus's anterior parvocellular part; (D) paraventricular hypothalamic nucleus and lateral hypothalamic area; (E) dorsomedial hypothalamic nucleus and PHAL deposit centered in, but not restricted to, the LHAsfp; (F) medial part of the central amygdalar nucleus. Scale bars = 100 μ m.

Fig. 12.

(A,C) Brightfield photomicrographs showing the PHAL deposit centered in dorsal (A) and ventral (B) regions of the dorsal premammillary nucleus. (B,D) Darkfield photomicrographs showing the periaqueductal gray terminal fields labeled from the dorsally and ventrally centered injections, respectively. Note heavy labeling in the lateral division, and light labeling in the dorsolateral division, in the experiment with an injection centered dorsally in the nucleus (B), as compared to massive terminal labeling in the dorsolateral division, and lighter labeling in the lateral division, in the experiment with a PHAL deposit centered in ventral regions of the nucleus (D). Scale bars = 100 μ m.

Fig. 13.

Summary diagram illustrating the major axonal inputs and outputs of the LHAsfa, arranged to emphasize their possible functional significance, as discussed in the text. The overall projection pattern arising from the LHAsfa's horizontal limb (hl) and vertical limb (vl) is similar, although one or the other obviously projects more densely to certain terminal fields in a topographically ordered way, as indicated. For clarity, all of the connections of cell groups receiving inputs from, or projecting to, the LHAsfa are not shown.

TABLE 1

Semiquantitative analysis of PHAL labeling in the cerebrum and cerebrospinal trunk after a PHAL injection in the LHAsfa and adjacent LHA. The cell group hierarchy is from Swanson (2004).

Cell group	LHAsfa (LHA94)	LHAjv LHA52)	LHAa/d (LHA45)	LHAsfp (LHA22)
1 CEREBRUM				
1.1 CEREBRAL CORTEX				
1.1.1 Cortical Plate				
Visceral sensory-motor areas				
infralimbic área	+	-/+	-/+	-/+
agranular insular	-	+	-	-
Olfactory áreas				
cortical amygdalar area	+	-/+	-	-
Postpiriform amygdalar area	-	+	-	-
Polymodal association cortex				
prelimbic	-/+	-/+		-/+
anterior Cingulate	-	-	-	-
Hippocampal formation				
entorhinal area (medial)	+	++	-	-
subiculum (ventral part)	-	+/++	-	-
field CA1	+	++	-	-
1.1.2 Cortical subplate				
Lateral amygdalar nucleus	+	+	-	-
Basolateral amygdalar nucleus	-	-	-	-
Basomedial amygdalar nucleus				
anterior part	-	-	-	-
posterior part	++/+++	++/+++	-	-
Posterior amygdalar nucleus	-/+	+++	-	++/+++
1.2 CEREBRAL NUCLEI				
1.2.1 Striatum				
Nucleus accumbens	-	+	+	++
Lateral septal complex				
Lateral septal nucleus				
caudal part				
dorsal zone (LSc.d)	-/+	-	+++	-
ventral zone (LSc.v)	++/+++	++	++/+++	++/+++
rostral part				
medial zone (LSr.m)	-	++	-/+	++
ventrolateral zone (LSr.vl)	-	++	++	+
dorsolateral zone				
medial region (LSr.dl.m)	++	+++	+	-/+
lateral region (LSr.dl.l)	+++	+	-/+	-/+
ventral part (LSv)	-	-	-	+
Septofimbrial nucleus	-	+	+	-

Cell group	LHAsfa (LHA94)	LHAjv LHA52)	LHAa/d (LHA45)	LHAsfp (LHA22)
Anterior amygdaloid area	—	—/+	+	—
Central amygdalar nucleus				
medial part	—	—	—	+++
lateral part	—	—	—	—
capsular part	—/+	+	++	—
Medial amygdalar nucleus	—	+	—	—
Intercalated amygdalar nuclei	+++	—	+	+
1.2.2 Pallidum				
Substantia innominata	+/++	+	++	++
Medial septal complex				
Medial septal nucleus	+++	++	++/+++	—
Diagonal band nucleus	++	—	—/+	—
Bed nuclei of the stria terminalis				
Anterior division				
anterolateral area (BSTal)	—	—/+	—	++/+++
anteromedial area (BSTam)	—	++/+++	+	+++
oval nucleus (BSTov)	—	—	—	—
juxta-capsular nucleus (BSTju)	—	—	—	—
rhomboid nucleus (BSTrh)	—	+	—	+++
dorsomedial nucleus (BSTdm)	—	+	—	+++
fusiform nucleus (BSTfu)	—	—	—	—
ventral nucleus (BSTv)	—	+	—	++/+++
magnocellular nucleus (BSTmg)	—	+	—	++/+++
Posterior division				
principal nucleus (BSTpr)	—	—	—	—/+
interfascicular nucleus (BSTif)	++	++/+++	++	++
transverse nucleus (BSTtr)	++	++	+	++
2. CEREBROSPINAL TRUNK				
2.1. SENSORY SYSTEM				
2.1.1 Thalamus				
Sensory-motor cortex related				
Polymodal association cortex related				
Medial group of the thalamus				
Mediodorsal nucleus of the thalamus				
medial part	++	++/+++	+	+
central part	—	—	—	—
lateral part	—	—	++	—
Midline group of the dorsal thalamus				
paraventricular thalamic nucleus	+	++	++	++
parataenial nucleus	++	++	—	—
nucleus reuniens	++	+—/++	++	—
Intermediodorsal nucleus (?)	—/+	—	—	+

Cell group	LHAsfa (LHA94)	LHAjv LHA52)	LHAa/d (LHA45)	LHAsfp (LHA22)
Intralaminar group of the dorsal thalamus				
rhomboid nucleus	-	-	-/+	-
central medial nucleus of the thalamus	+	+	++	-
paracentral nucleus of the thalamus	-	-	-	-
central lateral nucleus of the thalamus	-	-	-	-
parafascicular nucleus	-	-	-	-
2.2 BEHAVIORAL STATE				
Subparaventricular zone	-	+ -/+	-	-
Hypothalamic lateral zone				
lateral hypothalamic area, dorsal region	++	-/+	++	++
Supramammillary nucleus	-	+	++	+
Raphé nuclei				
interfascicular nucleus raphé	-	-	-	+
interpeduncular nucleus	-/+	-	-	-
dorsal nucleus raphé	++	-/+	++	++
superior central nucleus raphé	++	++	++	+
nucleus incertus	+	-	-/+	-
2.3 MOTOR SYSTEM				
2.3.1 Behavioral Control Column				
Medial preoptic nucleus				
lateral part	-	+	-	+
medial part	-	-	-	-
central part	-	-	-	-
Anterior hypothalamic nucleus				
anterior part	++	+/++	+	++
Central	++	++	+	-
posterior part	++	+	-	-
Paraventricular hypothalamic nucleus, descending division				
medial parvicellular part	-	-	-	+++
dorsal parvicellular part	-	-	-	+
lateral parvicellular part	-	-	-	+++
forniceal part	-	-	-	++/+++
Ventromedial nucleus				
anterior	+	+++	-	-
dorsomedial part	++	+++	-	-
central part	-	++/+++	-	-
ventrolateral part	-	+/+++	-	-
Ventral premammillary nucleus	-	+/++	-	-
Dorsal premammillary nucleus				
dorsal part	+++	++	+++	-
ventral part	+++	++/+++	++	-
Ventral tegmental area	-	-	++	++

Cell group	LHAsfa (LHA94)	LHAjv LHA52)	LHAa/d (LHA45)	LHAsfp (LHA22)
Midbrain reticular nucleus, retrorubral area	—	++	++	++
Midbrain reticular nucleus	—	++	—/+	—
2.3.2 Central Gray				
Epithalamus				
Medial habenula	—	—	+	—
Lateral habenula				
medial part	++	++	+++	++
lateral part	+	+	++++	—
Posterior hypothalamic nucleus	++	++	++/+++	++/+++
Periaqueductal gray				
precommissural nucleus	+	+	++/+++	—
rostromedial division	—	+	++/+++	—/+
medial division	—	+	++/+++	—/+
dorsal division	—/+	++	+ /++	—/+
dorsolateral division	+	—/+	—	—/+
lateral division	—/+	++/+++	++	+
ventrolateral division	++	++/+++	++	++/+++
pontine central gray	—/+	+	+	++/+++
Barrington's nucleus	—	—/+	—	++/+++
2.3.3 Hypothalamic Periventricular Region				
Median preoptic nucleus	—	—	—	+
Anteroventral periventricular nucleus	—	—	—	—/+
Preoptic periventricular	—	—	—	+
Anterodorsal preoptic nucleus	—	—	—	+++
Anteroventral preoptic nucleus	—	++	+	+++
Parastrial nucleus	—	—	—	+++
Medial preoptic area	—/+	++/+++	—/+	+++
Anterior hypothalamic area	+	++	—/+	++
Dorsomedial hypothalamic nucleus				
anterior part	++	++	+	++/+++
posterior part	—	—/+	—	—
ventral part	—	—/+	—	++/+++
Periventricular hypothalamic nucleus, posterior part	—	—/+	—	++
2.3.4 Reticular Formation				
Hypothalamic lateral zone, motor related				
Lateral preoptic area	++	—/+	+++	+++
Lateral hypothalamic area, motor related				
juxtaparaventricular region	++/+++	+++	++	+ /++
juxtadorsomedial region	+	+ /++	—	+
juxtaventromedial	+		—	++/+++
anterior region				
dorsal zone	++	++/+++	++	++/+++

Cell group	LHAsfa (LHA94)	LHAjv LHA52)	LHAa/d (LHA45)	LHAsfp (LHA22)
intermediate zone	++/+++			++
ventral zone	++	+	+	++/+++
retrochiasmatic area	-/+	++	-/+	+/++
tuberal nucleus	-	-/+	-	++
supraforfical region	-	-	++/+++	++/+++
subforfical				
anterior zone		-	-	-
posterior zone	+	+	+	
premamillary zone	++	++	-/+	++
magnocellular nucleus	-	-	-	-
parvicellular region	-	-	+	+
ventral region	++	-	++	+/++
posterior region	-/+	-	++	++/+++
Preparasubthalamic nucleus	-	-	-	-
Parasubthalamic nucleus	-	-	-	+
Zona incerta	-/+	+	-/+	-/+
Cuneiform nucleus	++	++	+	-
2.3.5 Motoneuron Groups				
Neuroendocrine motor zone				
Parvicellular				
paraventricular hypothalamic nucleus				
anterior parvicellular part	-/+	++	+	+++
medial parvicellular part, dorsal zone	-	+	-	+++
periventricular part	-	+	-	++
periventricular hypothalamic nucleus, anterior part	-	+	-	+
periventricular hypothalamic nucleus, posterior part	-	-/+	-	+
arcuate nucleus	-	-	-	-

(-) absence of labeling; (-/+) very light labeling; (+) light labeling; (++) moderate labeling; (++) moderately dense labeling; (+++) dense labeling.