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Review of the cytology and connections of the lateral habenula, an avatar of adaptive behaving

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Abstract

The cytology and connections of the lateral habenula (LHb) are reviewed. The habenula is first introduced, after which the cytology of the LHb is discussed mainly with reference to cell types, general topography and descriptions of subnuclei. An overview of LHb afferent connections is given followed by some details about the projections to LHb from a number of structures. An overview of lateral habenula efferent connections is given followed by some details about the projections from LHb to a number of structures. In considering the afferent and efferent connections of the LHb some attention is given to the relative validity of regarding it as a bi-partite structure featuring ‘limbic’ and ‘pallidal’ parts. The paper ends with some concluding remarks about the relative place of the LHb in adaptive behaving.

Keywords

ventral tegmental area; substantia nigra compacta; dopamine; median raphe; dorsal raphe; lateral preoptic area; lateral hypothalamus; ventral pallidum

Introduction

The habenula (Hb) is a phylogenetically ancient, paired structure that straddles the midline of the brain atop the thalamus just in front of the pineal body, together with which it comprises the ‘epithalamus’. In fish and amphibians, the Hb exhibits such marked asymmetry that in some species there are two Hb nuclei to the left of the midline and only one to the right (Braitenberg and Kemali, 1970; Kemali and Guglielmotti, 1977; Kemali et al., 1980; Aizawa et al., 2005; Amo et al., 2010). The asymmetry is buttressed in some species, but not others, by unilateral connections of the Hb to a photoreceptive parapineal organ (Ya ez and Anadón (1996; Concha and Wilson, 2001). The amniote (reptiles, birds

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and mammals) Hb displays apparent bilateral symmetry (Figs. 1 and 2), comprising, on each side of the midline, a paramedian medial habenula (MHb) of tightly packed small neurons abutted laterally by a larger lateral habenula (LHb) comprising more loosely packed, bigger ones (Nissl, 1889; Cajal, 1911; Iwahori, 1977; see also, e.g., Ahumada-Galleguillos et al., 2016, and Hetu et al., 2016, regarding anatomical and functional asymmetry, respectively, of the human habenula). The present review is concerned mainly with the cytology and connections of the mammalian LHb, the MHb being briefly touched upon only where the comparison highlights or clarifies aspects of LHb organization.

The mammalian Hb is the central relay in what Sutherland (1982) has called the dorsal diencephalic conduction system (DDC), a multi-synaptic pathway worked out by Nauta (1958; 1960), which he conceived as an adjunct to the medial forebrain bundle (mfb) by which limbic forebrain structures, including the hippocampus (Papez, 1937; Brodal, 1947; McLean, 1950; Nauta, 1956), might influence his paramedian 'limbic midbrain area' (Nauta, 1958; Nauta and Kuypers, 1958). The DDC originates mainly with forebrain neurons that project to or through (Morissette et al., 2008; Gifuni et al., 2012) the Hb via the stria medullaris (sm), a loosely organized bundle of fibers that sweeps upward from the rostral forebrain and traverses the rostradorsal convexity of the thalamus.¹ Just before it enters the Hb, the sm is joined by the inferior thalamic peduncle (itp), which transmits additional forebrain afferents to the Hb through the body of the thalamus. Axons traversing the sm and itp mostly make synaptic contact with Hb neurons, although some by-pass the Hb either entirely without functional contact or having given off collaterals that have synaptic contacts in the Hb (Nauta, 1958; Cragg, 1961; Herkenham and Nauta, 1977). Hb outputs mainly join the fasciculus retroflexus (fr), which comprises a compact central core of fibers projecting from MHb to the interpeduncular nucleus and a more loosely composed sheath containing fibers that descend to or ascend from other midbrain structures. A few Hb axons course downward and rostralward through the thalamic mediodorsal (MD) and central medial nuclei enroute to the mfb within which they ascend to hypothalamus and forebrain (Araki et al., 1988).

Broadest caudally, the fusiform Hb narrows to a small rostral tip. The epithelial roof of the third ventricle attaches along the dorsomedial convexity of the Hb such that the medial and dorsal surfaces of the Hb contribute, respectively, to the wall of the dorsal part of the third ventricle and epithalamic brain surface (Figs. 1 and 2). Hb is bounded rostrally by the parataenial nucleus. The thalamic paraventricular nucleus forms its ventromedial boundary and the MD bounds its ventrolateral-lateral convexity. The dorsal part of the Hb is traversed by the band-like lateral and medial fascicles of the sm, which loop across the midline in front of the pineal body as the habenular commissure. The fr descends through the parafascicular nucleus from the posterolateral aspect of the LHb (Figs. 1E and F and 2C and F), such that numerous MHb fibers pass through the LHb before descending.

¹As recounted recently by Geisler and Trimble (2008) and Viswanath (2013), the word habenula derives from the latin for 'little rein', which likely gained attribution not so much due to the physical elongation of the habenular nuclei themselves, but rather the gross appearance in human brain of the remnant attachments of the roof of the third ventricle and associated paired fasciculi of stria medullaris arching forward from the habenular commissure, strand-like, along the dorsal surface of the thalamus, indeed much resembling a horse's reins (see, e.g., Figs. 2–18 and 15–7 in Carpenter, 1976).

The mammalian MHb and LHb are starkly distinguished by connectivity. Nearly all MHb inputs come from one structure, the supracommissural septum, and almost all of its outputs go to another, the interpeduncular nucleus. In contrast, the larger LHb is richly interconnected with widespread parts of the brain, not least by massive outputs that strongly influence dopaminergic (DA), serotonergic, cholinergic, noradrenergic and GABAergic brainstem nuclei. For example, LHb stimulation produces mainly profound inhibition of midbrain DA neurons (Christoph et al., 1986; Ji and Shepard, 2007; Matsumoto and Hikosaka, 2007; 2008; 2009; but see Brown and Shepard, 2016), consistent with an LHb role as a final arbiter of negative modulation of affect in response to aversive stimuli and reward omission (Brady and Nauta, 1955; Caldecott-Hazard et al., 1988; Chastrette et al., 1991; Nagao et al., 1993; Wirtshafter et al., 1994; Vale-Martinez et al., 1997; Sica et al., 2000; Amat et al., 2001; Timofeeva and Richard, 2001; Villarreal et al., 2002; Huang and Cheng, 1998; Yakabi et al., 2002; Shumake et al., 2003; Ullsperger and Cramon, 2003; Kazi et al., 2004; Wang et al., 2004; Pobbe and Zangrossi, 2008; Smith and Lonstein, 2008; Yang et al., 2008; Shumake et al., 2010; Hong et al., 2011; Winter et al., 2011; Zhou et al., 2013; Hennigan et al., 2015; Li et al., 2016a). This major influence of LHb on the activity of DA neurons, in combination with its interactions with other brainstem ascending modulatory projection systems (e.g., Aghajanian and Wang, 1978; Pasquier et al., 1978; Reisine et al., 1982; Gottesfeld, 1983; Goto et al., 2001; Olucha-Bordinau et al., 2003; Sego et al., 2014; Ma and Gundlach, 2015; Zhou et al., 2017), fits with the kaleidoscopic breadth of reported LHb functional involvements in, e.g., olfactory-guided behavior, addiction, ingestion, endocrine control, brain stimulation reward, stress response, pain, maternal behavior, nociception, circadian rhythms, learning and memory, attention, sleep, motor activity, anxiety and depression (reviewed, together with then current information on LHb connections, in Sutherland, 1982; Risold and Swanson, 1997; Andres et al., 1999; Klemm, 2004; Lecourtier and Kelly, 2007; Bianco and Wilson, 2008; Geisler and Trimble, 2008; Hikosaka et al., 2008; Meye et al., 2013; Proulx et al., 2014). A reasonable expectation that the remarkable diversity of LHb function should be accompanied by a complex organization both in terms of its cytology and axonal connections (Andres et al., 1999) will be addressed in the following sections.

Cytology and subnuclei

The earliest cytological descriptions of the Hb, reviewed and embellished by Ramon y Cajal (1911), recognized LHb and MHb as distinct. Whereas Cajal (1911) maintained that the cells of the MHb are smaller and more tightly packed than those of the LHb, the question of which nucleus contains what size cells was much debated (Gurdjian, 1925; Tsai, 1925; Clark, 1930; Rioch, 1929; 1931; Ingram et al., 1932; Papez and Aronson, 1934; Rose, 1942; Marburg, 1944), possibly reflecting the uneven quality of brain preservation in those early days, but also perhaps a harbinger of actual cellular heterogeneity indicative of subnuclear organization of the Hb. Cajal's viewpoint has been borne out and was further elaborated by the Golgi study of Iwahori (1977), who identified large sparsely spiny neurons mainly in rostral and ventral parts of the cat LHb, small sparsely spiny neurons in caudomedial LHb, medium-sized densely spiny neurons throughout the LHb and small presumptive interneurons with a bushy dendritic arbor and short axon. Alternatively, Kim and Chang

(2005) recognized Type I and II LHb neurons with, respectively, larger and smaller varicose dendritic arbors, whereas Weiss and Veh (2011) identified four main categories of LHb projection neuron on the basis of somatodendritic characteristics, including spherical, fusiform, polymorphic and vertical, and, in addition, a neurogliaform cell type, which they regarded as interneuronal.

Modern studies have consistently demonstrated non-uniform LHb distributions of immunoreactivity against various neuropeptides, mainly due to peptidergic enrichments in a small-celled (Pert and Atweh, 1976; Iwahori, 1977) medial core (e.g., opiates – Pert and Atweh, 1976; Atweh and Kuhar, 1977; substance P – Cuello, 1978; vasopressin – Buijss, 1978; Sofroniew and Weindl, 1978; somatostatin - Vincent et al., 1985; neurotensin - Ray and Price, 1990). Aside from the peptides, LHb neurons are reported to be mainly glutamatergic, expressing either vesicular glutamate transporter 2 (Geisler et al., 2007; Brinschwitz et al., 2010) or vesicular glutamate transporter 3 (Herzog et al, 2004). A small subset of LHb neurons expresses GAD67 (Brinschwitz et al., 2010; Zhang et al., 2016), thereby synthesizing GABA, but there is as yet no confirmation that LHb GAD-expressing neurons also contain vesicular GABA transporter (VGAT), the vesicular machinery that packages GABA into synaptic vesicles for synaptic release.

The subnuclear organization of the Hb was analyzed in earnest by Andres et al. (1999) in a painstaking study that utilized high resolution light microscopy of plastic-embedded, toluidine blue-stained, 0.8 μ m serial sections spanning the rat Hb from its rostral to caudal pole, supplemented with transmission electron microscopy. Taking into consideration the size and morphology of habenular neurons, dendrites, glia, cell nuclei, axons, axon fascicles and synapses, they defined 10 distinct subnuclei in the LHb (Table 1, Fig. 1B–F), and 5 in the MHb, which prompted them to substitute the term ‘complex’ for ‘nucleus’ in reference to the LHb and MHb. They pointed out that certain Hb subnuclei are in register with the origins or terminations of known Hb connections or sites of stimulus-elicited immediate early gene expression (see Abbreviations and Table 1 for LHb subnucleus designations). Their MHbS and MHbI coincide with superior and inferior parts of the MHb that are replete with substance P-expressing and cholinergic neurons, respectively (Contestabile et al., 1987), and project relatively specifically to distinct parts of the interpeduncular nucleus (Contestabile and Flumerfelt, 1981; Kawaja et al., 1988). They also noted, based on a report by Behzadi et al. (1990), that LHb projections to the median raphe that take up radiolabeled D-aspartate arise in their LHbMS, which contains neurons that express Fos after persistent noxious stimulation (Nagao et al., 1993). They further cited work indicating that major DA innervation of the LHb is relatively limited to their LHbMPc (Skagerberg et al., 1984), the small-celled site of striking peptidergic enrichments mentioned above, where also neurons in which Fos-immunoreactivity is elicited by daytime stress are concentrated (Chastrette et al., 1991), and which was early reported to be the LHb origin of projections to the dorsal raphe nucleus (Aghajanian and Wang, 1977; Pasquier et al., 1977; Sego et al., 2014).

However valuable was the laborious approach of Andres et al. (1999) in having permitted the discovery of Hb subnuclei, one would prefer that mapping the Hb subnuclear organization be accomplished more conveniently and directly, particularly if one is seeking to relate subnuclei to particular connections or functional correlates. Toward this end,

Geisler et al. (2003), used conventional Nissl staining and immunohistochemistry in free floating frozen sections to screen antisera against 42 markers of classical and peptidergic synaptic transmission, transmitter reuptake, ion channels, calcium buffering and cytoskeleton, of which all are either themselves functionally informative or compatible in double-labeling protocols with other approaches that reflect morphology or function. They observed that one or more LHb subnucleus was relatively well defined by immunoreactivities against one or more of 4 of these proteins, neurofilament H, Kir3.2 protein, GABA B receptor and tyrosine hydroxylase, whereas 22 other immunoreactivities also marked subnuclei, but with less precision. 'Patchy' distributions of none of the rest conformed particularly well to the subnuclear organization. Typically markers might exhibit tantalizing subnuclear correspondence at one rostrocaudal level, which would then be not demonstrable at other levels. Nonetheless, the cytological analysis (Andres et al., 1999) and the precise, albeit limited, correspondence of certain immunohistochemical markers (Geisler et al., 2003; Weiss and Veh, 2011; Aizawa et al., 2012; Schmidt et al., 2014; Wagner et al., 2016a; b) provided forceful evidence in support of LHb subnuclei. The existence of fairly numerous other markers that delineated LHb subnuclei less precisely or not at all, only suggests that the subnuclei possess additional inobvious levels of chemical neuroanatomical organization or are themselves compartmentalized.

Afferent Connections

Overview

Understanding of LHb inputs has progressed in part on the strength of at least ten separate retrograde labeling studies, of which five mapped labeled neurons throughout all or much of the CNS (Table 2). In the seminal study in the rat, Herkenham and Nauta (1977), upon injecting horseradish peroxidase (HRP) into the lateral part of the LHb, observed a very dense accumulation of retrogradely labeled neurons in the EPN (rodent homologue of the primate internal segment of the globus pallidus [GPi], a basal ganglia output structure) from which almost equivalently dense labeling extended into the adjacent lateral hypothalamus so far medialward as to envelope the fornix bundle. A rostralward extension of this labeling within what Herkenham and Nauta (1977) called the lateral preoptico-hypothalamic continuum invaded the lateral preoptic area (LPO) and continued into the ventral pallidum (VP), lateral septum (LS) and vertical and horizontal limbs of the diagonal band (Figs. 1 and 2). Modest labeling spread caudally into the lateral hypothalamus (LH), ventral tegmental area (VTA), median raphe, and dorsolateral tegmental region, all of these structures being aligned along the mfb. The injections made by Herkenham and Nauta (1977) into the medial part of the LHb labeled an equivalent or perhaps greater abundance of neurons, perhaps shifted a bit medially, within the aforementioned group of forebrain and brainstem structures, but fewer and more medially disposed neurons in the EPN. Thus, they viewed lateral preoptico-hypothalamic afferents as extending across the entire LHb (Figs. 1 and 2), but input from the EPN as occupying its lateral half nearly exclusively and, accordingly, consistent with the earlier designation of HJ Nauta (1974), they parcelled LHb into medial limbic and lateral 'pallidal' sectors, establishing what has become a persistent conceptualization of LHb organization as partitioned.

Subsequent retrograde labeling studies have confirmed that projections from the EPN and lateral preoptic-hypothalamic continuum constitute robust projections to the LHb, but not necessarily that those from EPN are dominant or confined within the lateral half of the LHb. Parent et al. (1981), also using HRP, but in the monkey and cat rather than rat, observed predominant retrograde labeling in neurons of the lateral preoptico-hypothalamic continuum, rather than the GPi/EPN, even after injections into the lateral part of the LHb, suggesting that limbic-extrapyramidal partitioning of the LHb is subject to significant species differences. A number of reports in rats followed on LHb injections of more efficacious retrograde tracers (than HRP), wherein the lateral preoptico-hypothalamic continuum also contained the predominant retrograde labeling and, in addition, labeled neurons were present in several previously unreported sites, especially, e.g., the cerebral cortex, medial preoptic area (MPO), dorsal raphe nucleus (DR) and pedunculopontine and laterodorsal tegmental nuclei (Li et al., 1993; Felton et al., 1999; Kowski et al., 2008). Consistent with these findings, a recent study of LHb afferents labeled with the very efficacious retrograde tracer cholera toxin β subunit revealed that by far the most abundant projections to the LHb arise within the lateral preoptico-hypothalamic continuum (Yetnikoff et al., 2015). Indeed, Tables 3 and 4 (modified from Yetnikoff et al., 2015) show that nearly 40% of all LHb-projecting neurons occupy a stretch of basal forebrain extending along the mfb from the ventral pallidum to the perifornical lateral hypothalamus. Actually, numerous CNS structures exhibited greater amounts of labeling (expressed as % total) than did the EPN following tracer injections that encompassed medial and lateral parts of the LHb (Table 4). Thus, the lateral preoptico-hypothalamic continuum dominates LHb input, at least in numbers of LHb-projecting neurons, even in LHbL, and in the rat as well as the monkey and cat. Ultimately, it seems that the species difference that most characterizes Hb connections concerns fewer outputs to the LHb from the monkey GPi and cat EPN, as compared to the rat EPN (see also below).

Consideration of retrograde tracing exclusively, however, provides a potentially misleading picture of inputs to a CNS structure. Certain LHb inputs might give rise to uncharacteristically exuberant, synapse-dense axonal arborations capable of exerting disproportionately robust functional impacts. Alternatively, axons passing through the LHb without functional contacts or projecting to adjacent structures impinged upon by the misplaced injections might pick up retrograde tracers, in both cases with the effect of producing artifactual labeling. Thus, LHb afferents identified by retrograde labeling require further study, especially with anterograde tracing and electrophysiology, and, where possible, in pathways characterized by chemogenetic or optogenetic technology. Details of this nature that exist for a number of presumptive LHb-projecting structures are provided in following sections.

Frontal cortex

A projection from the frontal cortex to the LHb was first documented by Greatrex and Phillipson (1982), who utilized transmission electron microscopy to show that lesions of the prefrontal cortex produce axonal degeneration, including within boutons possessing axodendritic synapses, predominantly in LHbM. Sesack et al. (1989) also illustrated and briefly noted a few axons in LHbM that were anterogradely labeled following injections of

the very efficacious anterograde tracer Phaseolus vulgaris-leucoagglutinin (PHA-L) into the PL, whereas Vertes (2002; 2004) charted light labeling in LHb following PHA-L injections into PL, infralimbic cortex (IL) and anterior cingulate cortex (ACg). Kim and Lee (2012) produced anterograde labeling in the LHb following large injections of biotinylated dextran amine (BDA) into agranular insular cortex (AI), ACg and PL and IL together. They showed a very dense aggregation of anterograde labeling in the MD beneath the LHb from which numbers of labeled axons that were estimable, but orders of magnitude fewer, penetrated the LHb. Interestingly, labeling produced by injections into the AI exhibited some subnuclear specificity, insofar as a focus of labeled terminations was concentrated in LHbMC and LHbMPc. Fibers labeled by combined PL-IL injections occupied mainly LHbM, consistent with earlier findings cited above, and likely reflected projections from the PL, insofar as no labeling was observed in the LHb in an earlier study of large PHA-L injections limited to the IL (Hurley et al., 1991; but see also Vertes, 2002; 2004). In contrast, labeled cingulohabenular fibers extended without obvious specificity across the width of LHbL. Interestingly, connections resembling those reported by Kim and Lee (2012) have been demonstrated in the human brain with diffusion tensor imaging (Vadovicova, 2014).

Lateral septum (LS)

Findings supporting a LS-LHb projection are intriguing. The early axonal degeneration studies were divided on such a projection, it being detected by Powell (1963, 1968) and Knook (1966), but not Nauta (1958), Ban and Zyo (1962), Powell (1966) or Raisman (1966). In early retrograde tracing studies in which the relatively insensitive tracer HRP was injected into the LHb, retrogradely labeled neurons were not reported in the rat septum (Herkenham and Nauta, 1977; Parent et al., 1981), although a few were found there in the monkey (Parent et al., 1981). However, subsequent investigators with access to more sensitive tracers, such as cholera toxin β subunit (Li et al., 1993) and Fluorogold (Felton et al., 1999) reported moderate to abundant retrograde labeling in the rat lateral septum after LHb injections. However, only slight anterograde labeling was detected in the LHb following injections of PHA-L into all but the more ventral parts of the lateral septum, which gave rise to but a few more labeled septo-LHb axonal terminations, specifically in LHbM (Jakab and Leranth, 1995; Risold and Swanson, 1997). Mok and Mogenson (1972a) recorded antidromically verified monosynaptic field potentials in LHb neurons after LS stimulations, indicating that the anatomical evidence for a septohabenular projection, although scant, nonetheless reflected a functionally potent connection. They reported in a companion paper (Mok and Mogenson, 1972b) that more than 90% of LHb neurons activated by LS stimulation also were fired monosynaptically by stimulations in the LPO, which is itself a recipient of major outputs from the LS (Jakab and Leranth, 1995; Zahm et al., 2013).

The LS-LHb projection story might end with a seemingly insignificant LS-LHbM projection had not Yetnikoff et al. (2015) recently completed yet another tracing study and calculated that LHb-projecting LS neurons represent more than 5% of all neurons in the hemisphere retrogradely labeled from the LHb. Interestingly, however, labeled neurons in that study were quite widely and relatively evenly spaced in the LS, such that their relatively large aggregate numbers reflect more the size of the LS as compared to other brain structures than

a remarkable density of LHb-projecting LS neurons. Furthermore, our own injections of BDA and PHA-L into LS, consistent with those of our predecessors, failed to label but a few LHb-projecting axons and these all had poorly arborized axonal terminations (Zahm, unpublished results). The diminutive axon terminal arbors of PHA-L-labeled axons in the LHb might be related to the reported incapacity of the LS-LHb projection to take up detectable amounts of the insensitive retrograde tracer HRP (Herkenham and Nauta, 1977; Parent et al., 1981). Considering all of it together, one may envision a massive, transforical, topographically organized projection from the subiculum that finds in the LS an evenly and widely spaced network of neurons serving to convey discrete bits of topographically organized information from the entire hippocampus to LHbM. Alternatively, the near negative PHA-L data may indicate simply that tracer was taken up in the retrograde labeling studies by fibers passing through the LHb (Powell, 1966, 1968). Additional work would be needed to adequately address these alternatives, but, at the least, the question of LS input to the LHb by now appears to be non-trivial.

Accumbens (Acb)

Although reported in various studies through the years (Conrad and Pfaff, 1976; Herkenham and Nauta, 1977; Troiano and Siegel, 1978a; Felton et al., 1999; Yetnikoff et al., 2015) any projection to LHb from the Acb seems to be small, such that only speculation regarding the function of such a pathway appears possible. Acb LHb-projecting neurons are restricted to the Acb shell (Yetnikoff et al., 2015), and they possibly could represent displaced LS Hb-projecting neurons (Zahm et al., 2013).

Ventral pallidum (VP)

A projection to LHb from the VP was reported early on and consistently thereafter (Herkenham and Nauta, 1977; Troiano and Siegel, 1978b; Groenewegen et al., 1993; Haber et al., 1993; Zahm et al., 1996; Tripathi et al., 2013), but the pathway has been little investigated otherwise. Root and colleagues (2015) reported projections from rostral and ventromedial subdivisions of the VP to medial portions of the LHb, noting that some of these are collaterals of ventral pallidal projections to the mediodorsal nucleus. Alternatively, at least a part of the VP-LHb projection terminates, possibly preferentially, in LHbLO (Zahm, unpublished observation). While overall the volume of the projection from VP to the LHb is considerably less than that of LPO (Tables 3 and 4), it nonetheless seems worth reiterating here that retrograde labeling in the VP following tracer injections into the LHb extends with no discernable interruption into the LPO and that in the LPO extends into the LH, consistent with the designation of a main body of LHb afferents being localized in a lateral preoptico-hypothalamic continuum (Herkenham and Nauta, 1977; Parent et al., 1981; Swanson, 1976; Saper et al., 1979), which Yetnikoff et al. (2015) suggested can be expanded to a VP-LPO-LH continuum.

Bed nucleus of the stria terminalis (BST)

Most retrograde tracing studies from LHb injection sites done with sensitive tracers show labeled neurons in the BST. However, in an extensive series of studies of the outputs from BST subnuclei done with PHA-L, Dong and Swanson identified only two BST subnuclei that produced labeled axons in the LHb. The dorsomedial (Dong and Swanson, 2006a) and

magnocellular (Dong and Swanson, 2006b) subnuclei provide moderate and slight inputs, respectively, to LHbM and LHbL. Interestingly, both of these subnuclei are nestled close to LPO, MPO or VP, all of which provide dense inputs to the LHb, suggesting that PHA-L injections in the dorsomedial and magnocellular BST subnuclei could involve displaced VP, LPO or MPO neurons or neurons occupying transition regions between them and the BST. Accordingly, Dong and colleagues reported no projections to LHb from BST subnuclei further removed from the VP, LPO and MPO, including the oval and fusiform (Dong et al., 2001), juxtacapsular (Dong et al., 2000), rhomboid (Dong and Swanson, 2003), anterolateral (Dong and Swanson, 2004a), posterior (Dong and Swanson, 2004b) or anteromedial (Dong and Swanson, 2006c).

Lateral (LPO) and medial (MPO) preoptic area, lateral (LH) and anterior hypothalamus

Projections to LHb from the preoptic region and rostral hypothalamus were suspected on the basis of early axon degeneration studies (e.g., Nauta, 1958; Cragg, 1961; Ku Sama and Hagino, 1961; Zyo et al., 1963; Knook, 1966), but were regarded skeptically due to lesion unspecificity and the inevitability of damage to fibers of passage. Confirmation of the pathway came by way of the Mok and Mogenson (1972a; b) electrophysiological demonstrations of monosynaptic, LPO-evoked field potentials (1972a) and single unit responses (1972b) in the LHb. Soon after, the projections were demonstrated by anterograde axonal tracing (Troiano and Siegel, 1975; 1978 [cat]; Swanson, 1976 [rat]; Saper et al., 1979 [rat]). Numerous studies followed in which retrogradely labeled neurons were mapped in the LPO-LH continuum after retrograde tracers were injected in the LHb (Herkenham and Nauta, 1977; Parent, 1981; Li et al., 1993; Felton et al., 1999; Kowski et al., 2008; Yetnikoff et al., 2015), all of which, together, lead to the present consensus that the LPO-LH continuum ranks among the strongest inputs to the LHb. The densest accumulation of neurons that project to the LHb in this part of forebrain occupies what Kowski et al. (2008) called the posterior or caudal LPO and others have referred to as the anterior or rostral lateral hypothalamus (Herkenham and Nauta, 1977; Saper et al., 1979; Li et al., 1993; Felton et al. 1999) or LPO-lateral hypothalamus transition (Yetnikoff et al., 2015). Irrespective of the nomenclature, neurons projecting to the LHb are densest in the caudal half of the LPO and rostral one-third of the LH, drop off decisively in the tuberal part of the LH and continue to decline in the posterior hypothalamus (Swanson, 1976; Saper et al., 1979). Swanson and colleagues have provided an abundance of superb PHA-L studies of the projections from various sites in the rostral lateral hypothalamus, indicating that robust projections to LHbM and LHbL arise in the juxtaparaventricular nucleus (Hahn and Swanson, 2010) and the near-adjacent juxtadorsomedial (Hahn and Swanson, 2012) and subfornical nuclei (Goto et al., 2005), whereas a relatively light projection mainly to LHbM arises in the juxtaventromedial nucleus (Hahn and Swanson, 2015). In this regard, Yetnikoff et al. (2015) had commented on the great density of retrogradely labeled neurons in the juxtaparaventricular lateral hypothalamic area following injections of retrograde tracer into the LHb.

Kowski et al. (2008) described a topography in which the rostral and caudal subfields of the LPO project laterally and medially in the LHb (Figs. 2A–C and 1), respectively. The former pathway, however, tends to avoid LHbMS and LHbLO, and the latter excludes LHbMPc. Pertinent to these findings, Mok and Mogenson (1972b) observed that single units in the

LHb exhibit monosynaptic discharges to stimulations of the LS and LPO, indicative of convergence of septal and LPO projections onto single LHb neurons, whereas Garland and Mogenson (1983) used similar methods to detect convergence of EPN and LPO projections onto LHb neurons. Alternatively, Yetnikoff et al. (2015) showed abundant divergent outputs from the LPO by co-localizing two retrograde tracers in single LPO neurons after the tracers had been injected into the LHb and rostromedial tegmental nucleus (RMTg) or RMTg and VTA.

Poller et al. (2013) combined tract tracing data with transmitter immunohistochemistry at the light and electron microscopic level to demonstrate that the main transmitter of the LH-LHb projection, surprisingly, is glutamate. Stamatakis et al. (2016) have confirmed those findings in transgenic mouse preparations in which LH neurons were transfected with photosensitive excitatory or inhibitory ion channels controlled by the VGluT2 promoter. Optogenetic stimulation of the VGluT2 controlled pathway produced mostly glutamate-mediated excitatory postsynaptic potentials. Optogenetic inhibition of the pathway increased feeding, particularly of palatable food, and produced real-time place preference, whereas excitation of the pathway produced opposite effects. In line with these findings, Barker et al. (2016a) utilized combined tract-tracing and *in situ* hybridization histochemistry to show that most LPO neurons projecting to the LHb have a glutamatergic phenotype, whereas a small subpopulation are GABAergic.

The anterior hypothalamic area is variously reported to project lightly (Saper et al. 1978; Saper et al., 1979; Risold et al., 1994) or robustly (Conrad and Pfaff, 1975; 1976b) to the LHb, but the large tracer injections illustrated in the Conrad and Pfaff studies suggest involvement of LPO-LH. Nor is there much evidence from some anterograde tracing studies for a projection from the MPO to the LHb (Swanson, 1976; Swanson et al., 1978; Simerly and Swanson, 1988), whereas others (Conrad and Pfaff, 1976a; Anderson and Shen, 1980) report an MPO-LHb projection, mainly to LHbM. Relatively abundant labeled neurons are observed in the MPO following injections of retrograde tracer into the LHb (Yetnikoff et al., 2015).

Other hypothalamic nuclei and the retina

Injections of sensitive retrograde tracers into the LHb have led to reports of retrograde labeling in widespread hypothalamic sites, including the periventricular, arcuate; ventromedial, dorsomedial, posterior, premammillary, suprachiasmatic, supraoptic and paraventricular hypothalamic nuclei (Li et al., 1993; Felton et al., 1999; Yetnikoff et al., 2015; Zhang et al., 2016). Some, but not all, of these have been confirmed with supplemental methods. For example, anterograde tracing verifies projections to LHb from the dorsomedial nucleus (Thompson et al., 1996) and from vasopressin-expressing neurons in the medial parvocellular part of the paraventricular nucleus (Sofroniew and Weindl, 1984). More recently, Hernandez et al. (2015) have provided additional compelling evidence in favor of a PVN-LHb pathway and have gone on to show that it exerts potent behavioral effects due presumably to actions of vasopressin in the LHb (Zhang et al., 2016, see also below). Another vasopressin-expressing projection to the LHb, from parvocellular neurons in the suprachiasmatic nucleus, has been reported in rats (Buijss et al., 1978; Sofroniew and

Weindl, 1978) and primates (Sofroniew et al., 1981) and was supported by one anterograde tracing study (Alonso et al., 1986). However, other anterograde tracing studies have not confirmed projections to the LHb from hypothalamic structures that contain vasopressin-expressing neurons (Saper et al., 1976; Mason et al., 1984; Luiten et al., 1985; Watts et al., 1987). Moreover, anterograde tracing from neither the ventromedial hypothalamic nucleus (Canteras et al., 1994) nor ventral premammillary nucleus (Canteras et al., 1992) produced labeling in LHb. Clearly, the situation remains fluid as regards presumptive hypothalamic projections to the LHb.

Several investigators have illustrated strong retinofugal projections to the suprachiasmatic nucleus (Cooper et al., 1993; Qu et al., 1996; Morin and Blanchard, 1997; Reuss and Decker, 1997; Matteau et al., 2003), which has a presumptive vasopressinergic projection to the LHb discussed in the preceding paragraph. In addition, each of these reports makes note of a direct projection to the LHb from the retina. Qu et al. (1996) saw labeled neurons in retinal ganglion cells in rat following injections of retrograde tracer into the LHb, but observed only sparse anterograde degeneration in the LHb following ocular enucleation. Others provided mainly diagrammatic illustrations of very slight labeling in the LHb following injections of various anterograde tracers into the eye (Cooper et al., 1993; Matteau et al., 2003), except for a micrograph of some anterogradely transported FG said to be in the LHb (Reuss and Decker, 1997). Alternatively, Morin and Blanchard (1997), using Ct β , and Hattar et al. (2006), who utilized promoter-driven expression of green fluorescent protein marking specifically the projections of melanopsin-expressing retinal ganglion cells, showed strong anterograde labeling in the posterior limitans nucleus, a pretectal structure (Morin and Blanchard, 1997) situated just outside the dorsolateral extremity of the LHb. Thus, connections of the retina with the LHb seem to be negligible to nil.

Nucleus basalis

An extensive formation of large hyperchromic corticopetal neurons extends between the septum and globus pallidus in rodents and primates (Mesulam et al., 1983; Mesulam, 1995). In primates, large cholinergic and GABAergic neurons belonging to this formation form a discrete structure, nucleus basalis, that occupies a substantial, central part of the subcommissural and sublenticular basal forebrain (Heimer et al., 1999; Sakamoto et al., 1999; Saper, 1990). Nauta and Herkenham (1977) attributed to nucleus basalis some of the labeling they observed in the rat basal forebrain after HRP injections into the LHb. While anterograde tracing of fibers from the cholinergic neuron-rich basal forebrain nuclei produced labeling mainly in MHb (Domesick, 1976), recent support has emerged for a potential cholinergic projection from nucleus basalis to LHb, insofar as muscarinic receptors in LHb together with GABA receptors, play an important role in impulsive responding for cocaine (Zapata et al., 2017). In this regard, it turns out that subsets of cholinergic neurons co-release GABA (Saunders et al., 2015), suggesting a possible interplay between transmitter systems in the diagonal band projection to LHb. Furthermore, the diagonal band nuclei have a major projection from GAD-expressing neurons to LHb that is involved in regulating theta rhythm across the median raphe-hippocampus axis (Aizawa et al., 2013). Another study done in mice showed that aggression reward is enhanced and mitigated, respectively, by optogenetic stimulations and inhibitions in the LHb of GABAergic axon

terminals after transfections in the basal forebrain of photosensitive channelrhodopsin 2 (excitatory) and archaeorhodopsin (inhibitory) ion channels (Golden et al., 2016). However, their large viral transfections in mouse involved neurons in so many basal forebrain structures as to render indeterminate which one(s) may have mediated the observed responses.

Epithalamus (Hb itself) and thalamus

Herkenham and Nauta (1979) provided evidence suggesting that LHb gets input from MHb. However, insofar as MHb fibers pass through LHb enroute to the fr and passing axons and terminal fields are poorly differentiated by anterograde tracing with audioradiography, the question had remained open. Recently, however, Lima et al. (2017) have provided tracing evidence for reciprocal connections between MHb and LHbM (but see Kim and Chang, 2005). Similarly, Kim (2009) has demonstrated convincingly with complementary retrograde and anterograde tract tracing that homologous parts of the LHb on the two sides of the midline are robustly related by reciprocal connections.

Another interesting presumptive epithalamic neuroendocrine action on the LHb has been considered by Pavel, Goldstein and colleagues (e.g., Goldstein, 1983; Pavel and Eisner, 1984). They observed that the bioactive peptide arginine vasotocin, which is synthesized by the pineal body, when microinjected into the pineal recess induces slow wave sleep, and this effect is transiently abolished (for about eight days) by destruction of the LHb. They showed further that stimulation of the LHb in and of itself induces slow wave sleep and that the paracrine and stimulation effects are both associated with decreased serotonin metabolites in the DR and blocked by intraventricular infusion of picrotoxin. They hypothesized that vasotocin released by the pineal body activates a GABAergic pathway that descends from the LHb to the DR nucleus and inhibits neurons there. But GABAergic LHb outputs have not been described, LHb efferents having been shown instead to be largely glutamatergic (Geisler et al., 2007; Brinschwitz et al., 2010), thus requiring that the hypothesized habenulo-raphé pathway should end in relation to a GABAergic interneuron. Another pineal influence on LHb may arise through melatonin receptor activation, which enhances the excitability neurons in the medial part of the LHb (Evely et al., 2016).

An anterograde tracing study using PHA-L revealed projections to the LHb from the thalamic parataenial nucleus, but not the paraventricular nucleus (Vertes and Hoover, 2008). In addition, moderate retrograde labeling was observed by Felton et al. (1999) in the thalamic reticular nucleus (Rt), which in retrospect appears to be where Yetnikoff et al. (2015) also saw retrograde labeling, although they called it the anteroventral thalamus. On the other hand, no study of which the author is aware appears to show labeling in the LHb following injections of anterograde tracer into the Rt (e.g., Jones, 1975). However, given the extent that particular parts of the Rt are connected selectively to particular nuclei in the dorsal thalamus (reviewed in Price, 1995), it seems possible that a part of Rt presumptively associated with LHb may as yet not have been the subject of a published injection of anterograde tracer.

Entopeduncular nucleus (EPN) and internal segment of the globus pallidus (GPi)

Tentative early evidence for a pallidohabenular projection emerged from evaluation of normal material and studies in which anterograde axonal degeneration was traced to the LHb following destruction of tissue at various forebrain sites (Wilson, 1914; Ranson et al., 1941; Nauta and Mehler, 1966). Such a pathway was regarded skeptically, however, because of the likelihood that such lesions might damage LHb-projecting fibers arising from neurons in other forebrain sites (e.g., Nauta, 1956; 1958; 1960; Cragg, 1961). Furthermore, such a pathway would have established the habenula as a point of convergence of limbic and basal ganglia outputs, which at that time was a counter-intuitive proposition. Doubt was laid to rest, however, by a bevy of subsequent studies revealing that the GPi and its homologue in non-primates (EPN) provide a robust projection to the LHb. There were autoradiographic demonstrations of anterogradely transported tritiated amino acids (HJ Nauta, 1974; 1979; Carter and Fibiger, 1978; McBride, 1981 [cat]; Kim et al., 1976 [monkey]), retrograde tracing with HRP and fluorescent tracers in rats (Herkenham and Nauta, 1977; van der Kooy and Carter, 1981; Araki et al., 1984; Felton et al., 1999) and monkeys (A Parent et al., 1981), anterograde transport of dextran biotin in rats (Kha et al., 2000) and monkeys (Parent et al., 2001) and electrophysiology in cats (Filion and Harnois, 1978; Larsen and Sutin, 1978) and rats (Garland and Mogenson, 1983).

Retrograde tracer injected into the LHb in rats and cats typically produces a large, dense aggregation of labeled neurons centered over the boundary between the EPN and LH and extending far into both (Herkenham and Nauta, 1977; McBride, 1981; Parent et al., 1981; Zahm, 2016). Anterograde tracers centered in the rat LH are reported to produce labeling in the medial half of the LHb (see also Saper et al., 1979), whereas injections into the lateral extremity of the LH label axons across the full mediolateral breadth of the LHb (personal observations, Fig. 2D–F). Alternatively, all injections into the EPN are reported to produce anterograde labeling relatively exclusively in LHbL, leading to the idea that the projection from EPN to the LHb maintains a mediolateral topography compressed relatively exclusively within LHbL (Nauta, 1974; 1979; Herkenham and Nauta, 1977; Carter and Fibiger, 1978; McBride, 1981). However, the available published EPN injections are almost invariably located near or lateral to the mediolateral midpoint of the EPN, presumably to avoid the confounding involvement of lateral hypothalamus, but such injections also exclude the medial half of the EPN *per se*. One available published injection into the medial EPN [in the cat] shows robust projections to LHbM (HJ Nauta, 1979), which renders as untenable the viewpoint that pallidohabenular projections avoid LHbM. Accordingly, Garland and Mogenson (1983), studying rats, recorded responses to EPN stimulation in 48% of units in LHbM as compared to 76% in LHbL and noted that the proportions of LHbM neurons influenced by stimulation of the medial and lateral halves of the EPN were not different.

The rodent pallidohabenular pathway has GABAergic, cholinergic and, according to more recent findings, glutamatergic components. EPN and GPi neurons contain glutamate decarboxylase (GAD), the synthetic enzyme of GABA (Oertel et al., 1984; Stephenson et al., 2005) and lesions of the EPN produced significant decreases in LHb glutamate decarboxylase (Gottesfeld et al., 1977; Nagy et al., 1978) and GABA-transaminase (Vincent et al., 1982) immunoreactivity, the latter of which was also identified in EPN neurons that

contained retrograde tracer following injections into the LHb (Araki et al., 1984). In a separate study, choline acetyltransferase immunoreactivity, a marker of cholinergic neurons, was detected in LHb-projecting EPN neurons (Moriizumi and Hattori, 1992). More recently, direct evidence for a glutamatergic component to the pallidohabenular pathway has come by way of optogenetic activation of EPN neurons in the rat, which generates not only GABAergic, inhibitory potentials in LHb neurons, but also depolarizing potentials that can be blocked with NBQX, an antagonist of glutamatergic AMPA receptors (Shabel et al., 2012). Further investigation has revealed that GABA and glutamate are co-released in mouse by unique somatostatin-expressing LHb-projecting EPN neurons (Wallace et al., 2017) that target LHb neurons that in turn project to midbrain GABA neurons (Meye et al., 2016). Furthermore, depression-like (Shabel et al., 2014) and psychostimulant-related behaviors (Meye et al., 2016) vary in relation to the proportion of co-released GABA and glutamate within the pallidohabenular pathway. Further deconstruction of the pallidohabenular pathway has revealed a parvalbumin-expressing EPN-LHb pathway that only releases glutamate (Wallace et al., 2017).

The cat and monkey pallidohabenular pathways have many fewer neurons than the same pathway in the rat (A Parent et al., 1981) and, consistent with the situation in the rat (van der Kooy and Carter, 1981) and cat (Filion and Harnois, 1978), the monkey pallidohabenular pathway arises at least partially from a subpopulation of pallidal output neurons distinct from those that project to the mesopontine tegmentum and thalamus (M Parent et al., 2001). For example, Filion and Harnois (1978) determined that, of EPN neurons activated by antidromic impulses resulting from electrode placements in the thalamus, brainstem or LHb, only about 25% responded to LHb stimulations and, of these, only about a third responded to LHb stimulations exclusively. Early on, van der Kooy and Carter (1981) had found that pallidohabenular and pallidothalamic neurons are sequestered rostrally and caudally, respectively, in the rat EPN and they designated rostral limbic and caudal basal ganglia districts in the rat EPN. Interestingly, Kim et al. (1976) had commented along similar lines that injections of anterograde tracer into the monkey GPi that give rise to labeled pallidohabenular projections appeared to be associated with the rostral pallidal medullary laminae and underlying substantia innominata. Accordingly, M Parent et al. (1981) went on to show that pallidohabenular neurons in monkey are concentrated in the rostral pole of the GPi and subjacent substantia innominata and, further caudally, in or near the medullary laminae. In the M Parent et al. (2001) study, 3-D reconstructions of individual labeled fibers revealed a subpopulation of GPi output fibers with abundantly arborizing axonal terminations exclusively in the LHb that is distinct from GPi outputs to the mesopontine tegmentum and thalamus. Partially consistent with this anatomy, Hong and Hikosaka (2013) electrophysiologically identified neurons projecting to the LHb only in a part of the rostral GP so ventral that they have referred to it as basal GPi (GPb) and in the substantia innominata beneath, which in monkey and human brain contains the sublenticular extended amygdala and nucleus basalis (Martin et al., 1991; Heimer et al., 1999; Sakamoto et al., 1999). Thus, the question arises as whether the primate projection from GPi to LHb constitutes, at least in part, displaced extended amygdala neurons.

Further as regards the phenotype of primate pallidohabenular neurons, Hong and Hikosaka (2008) showed that, whereas increased firing to reward omissions in the monkey GPi and

LHb is near coincident, firing typically occurs slightly earlier in the GPi, foreshadowing evidence from rats for abundant glutamatergic pallidohabenular projections (Shabel et al., 2012). Consequently, the notion has emerged that excitatory projections from the GPi to the LHb carry signals that excite the LHb in response to reward omission or the anticipation or actual experience of aversive stimuli (Matsumoto and Hikosaka, 2007; 2009; Hong and Hikosaka, 2008; 2013; Bromberg-Martin and Hikosaka, 2011; Shabel et al., 2012; 2014; Ishikawa and Kenny, 2016; Meye et al., 2016; Stephenson-Jones et al., 2016; Munte et al., 2017). It remains to be determined if co-localization and inversely co-regulated release of GABA and glutamate observed in mouse and rat LHb-projecting EPN neurons (Shabel et al., 2014; Meye et al., 2016) also obtains in primate LHb-projecting GPi neurons (see Hong and Hikosaka, 2013, for an alternative). However, neurons expressing vesicular markers of glutamate and GABA co-releasing neurons have been detected in human GPi (Wallace et al., 2017).

Ventral tegmental area (VTA)

The well documented projection from the VTA to the LHb has some surprising details. A monoaminergic innervation of the LHb was first described by Lindvall and Bjorklund (1974) and soon shown to originate in the VTA with the aid of tract-tracing and lesion-associated reductions of LHb DA and DA markers (Hökfelt et al., 1976; Kizer et al., 1976; Beckstead et al., 1979; Herkenham and Nauta, 1977; Simon et al., 1979; Phillipson and Pycock, 1982; Swanson, 1982; Skagerberg et al., 1984; Bjorklund and Lindvall, 1984). However, to what extent the projection is actually DA became a problem early on. Whereas Kizer et al. (1976) had reported that a 75% decrease in LHb DA is associated with electrolytic lesions in the rostral midline of the VTA, the histology indicated that the projection comprises relatively few DA neurons. For instance, Skagerberg et al. (1984) saw 20–30% of retrogradely labeled neurons with co-localized glyoxylic acid-induced catecholamine fluorescence, whereas Swanson (1982) saw less than 1% double-labeling using TH immunofluorescence as the second marker. The discrepancy between the two reports stems from the fact that Skagerberg et al. (1984) had taken into account that DA-fluorescing axons are concentrated in LHbM, particularly in what Andres et al. (1999) later identified as LHbMC and LHbMPc, and centered their injections there. Li et al. (1993) also deposited tracer in the medial division of the LHb and reported that TH immunoreactive neurons comprise about 10% of those projecting from the VTA to the LHb, but also observed retrogradely labeled neurons with co-localized TH immunoreactivity in the BST, MPO, periventricular, ventromedial and dorsomedial hypothalamic nuclei, interfascicular nucleus, substantia nigra compacta, ventrolateral periaqueductal gray and dorsal raphe nucleus. Li et al. (1993) reported further that injections of anterograde tracer into many of these sites resulted in labeled terminations mainly in LHbM. Gruber et al. (2007), utilizing sensitive reagents and also injecting in LHbM, produced retrograde labeling of neurons that were distributed mainly in the ventral paramedian field of the anterior half of the VTA and of which 45–50% were double labeled for TH immunoreactivity. Also, consistent with Li et al. (1993), Gruber et al. (2007) found double-labeled neurons in the incerto-hypothalamic and periventricular areas, but not other extra-VTA sites reported by Li et al. (1993).

Swanson (1982), who had reported very few (<1%) TH-expressing mesohabenular neurons, had injected mainly in LHbL, where DA-fluorescing and TH immunoreactive (H kfelt et al., 1976) axons are fewer, and, in contrast to both Skagerberg et al. (1984) and Gruber et al. (2007), observed dense retrograde labeling in the *dorsal* (rather than ventral) paramedian field of the VTA mainly adjacent to the rostral linear nucleus. Subsequently, Del-Fava et al. (2007) injected the rostral linear nucleus with the anterograde tracer PHA-L and found that the densest terminations of this mainly non-DA component of the mesohabenular pathway occupy specifically the oval nucleus of Andres et al. (1999).

It then turned out that what had appeared, at least in part, to be a mesohabenular DA projection to LHbM actually exhibits incompetent vesicular release of DA (Root et al., 2015; reviewed in Barker et al., 2016b). Stamatakis et al. (2013) had determined that optogenetic stimulation of the mesohabenular pathway under the control of the TH promoter results in release in the LHb of GABA and glutamate, not DA, and, by inhibiting LHb output neurons, elicits a reward-like behavioral phenotype. The lack of DA release by TH-expressing VTA neurons that innervate the LHb is reported to be due to an absence of the vesicular monoamine transporter (VMAT) in these axons in the mouse and rat (Stamatakis et al., 2013; Li et al., 2013; Root et al., 2015) of which most presumably project to LHbMC/LHbMPc, where the TH-expressing innervation is most concentrated in the rat LHb. Notably, this proposed mechanism is distinct from that in the mesostriatal projection, wherein VMAT2 co-packages DA with GABA (Tritsch, et al., 2012).

In any event, the ‘feigned’ DA (but actually GABAergic and glutamatergic) mesohabenular pathway thus may disproportionately affect LHb outputs to the DR, which mainly arise from LHbMC and LHbMPc (Aghajanian and Wang, 1977; Pasquier et al., 1977; Sego et al., 2014). In line with this connectivity, Luo et al. (2015) hypothesized that loss of DA neurons in Parkinson’s disease (PD) may produce a disinhibition of LHb neurons (due to loss of GABA transmission), which itself is dysphoric and may, in turn, act to decrease serotonin levels – another deficit likely to be associated with behavioral depression in PD patients. Accordingly, Luo et al. (2015) went on to demonstrate that the changes in behavioral and neurochemical markers that they predicted occur in PD actually do occur in a rodent model of PD. Of further interest, Schmidt et al. (2014) have provided evidence that the targeting of LHbMPc by developing TH-expressing mesohabenular axons is guided such that axons growing out of LHbMC/LHbMPc interact with axons growing in from the VTA. That such a mechanism could specify targeting of the DA mesohabenular phenotype suggests an explanation as to why TH expression is retained in an otherwise non-DA projection. Nevertheless, DA receptors are expressed in LHb and appear to play important roles in aversive memory retrieval (Chan et al., 2017), the aversive effects of ethanol (Zuo et al., 2017) or cocaine (Shelton et al., 2016) and cue-induced reinstatement of nicotine seeking (Kaled et al., 2014).

The LHb neurotransmission plot thickens further upon consideration of the larger, non-TH-expressing mesohabenular projection (Swanson, 1982; Del-Fava et al., 2007). Root et al. (2014b) report that about 80% of neurons that give rise to this pathway express both the glutamatergic marker vesicular glutamate transporter 2 (VGluT2) and the GABAergic markers glutamate decarboxylase (GAD) and vesicular GABA transporter (VGAT). In

addition, single mesohabenular axon terminals release glutamate at asymmetric synapses and GABA at symmetric synapses, each with post-synaptic specializations bearing transmitter-appropriate glutamate and GABA receptors, resulting, respectively, in excitatory and inhibitory postsynaptic potentials. Whether the relative outputs of glutamate vs. GABA may be regulated in mesohabenular fibers by experience as reported for the pallidohabenular pathway (Shabel et al., 2014; Meye et al., 2015) remains to be determined. Interestingly, Root et al. (2014a) also show that optogenetic stimulation of mesohabenular axon terminals under the control of the VGluT2 promoter results in aversive conditioning that is blocked by AMPA and NMDA receptor antagonism. Alternatively, Yoo et al. (2016) have reported that optogenetic stimulation of mesohabenular axon terminals under control of the VGluT2 promoter supports mild self-stimulation, although these same mice avoid chambers that are paired with optogenetic stimulation of VGluT2 mesohabenular axon terminals. Furthermore, Shen et al. (2012) had reported that single pulse stimulation of the VTA-substantia nigra compacta (SNc) inhibits 90% of pain-elicited LHb responses, whereas tetonic stimulations tended to increase neuronal population activity in the LHb (a physiological substrate of aversion). These results suggest that multiple motivational states may be reflected by differential recruitment of GABA and glutamate components of the mesohabenular pathway (see also Li et al., 2013). Further research into the mechanisms underlying co-released glutamate and GABA in LHb may provide novel insights into such motivational states as well as disorders involving the LHb. Some recent observations include that excitatory and inhibitory LHb synapses exhibit differential plasticity modulated by distinct mechanisms (Valentinova and Mameli, 2016), that glutamate and GABA release are differentially regulated by *mu* opioid receptor activation (Margolis and Fields, 2016), that GABA receptors are specifically internalized in response to stress (Lecca et al., 2016), that AMPA receptors are enhanced at excitatory synapses following cocaine exposure (Marteau and Mamelli, 2012; Meye et al., 2015), and that LHb neurotransmission undergoes circadian modulations (Park et al., 2017).

Raphe nuclei

Retrograde labeling is detected in the raphe nuclei following injections of retrograde tracers into the LHb (Herkenham and Nauta, 1977; Li et al., 1993; Felton et al., 1999; Yetnikoff et al., 2015; Muzerelle et al., 2016) and strong bilateral projections from the median raphe to the LHb have been mapped by autoradiographic demonstration of anterogradely transported tritiated amino acids (Conrad and Pfaff, 1976a; Vertes and Martin, 1988) and PHA-L (Vertes et al., 1999). In contrast, Vertes (1991) was able to detect with PHA-L only a light ipsilateral projection to the LHb from the DR. Morin and Meyer-Bernstein (1999), studying the hamster, have made the interesting point that the MHb has a moderately dense serotonin terminal plexus, but does not receive significant input from the median or dorsal raphe, whereas LHb, which exhibits little serotonergic immunoreactivity, receives substantial input from the raphe nuclei (mainly median raphe) presumably of a non-serotonergic phenotype. The raphe nuclei have many vesicular glutamate transporter 3 (VGluT3) - expressing neurons (Geisler et al., 2007) that co-express serotonergic markers, but also an abundance of VGluT3-expressing neurons that lack such markers (reviewed in Sego et al., 2014) and thus project to a variety of brain sites, including the LHb, strictly as glutamatergic neurons (Hioki et al., 2010). This being said, serotonin is not without effect in the LHb,

insofar as bath application of serotonin in brain slice preparations suppresses EPN stimulation-induced LHb depolarizations, which *in vivo* are thought to be aversive (Shabel et al., 2012). The effects of 5-HT on LHb neurons is complex and may have roles in addiction and depression (Meye et al., 2013). 5-HT activation of 5-HT_{2/3} receptors results in excitation of LHb neurons (Xie et al., 2016) and 5-HT₇ as well as 5-HT_{2C} receptors are involved in regulating depression-like behaviors in PD models (Han et al., 2015; 2016).

Laterodorsal tegmental nucleus and nucleus incertus

A few retrogradely labeled neurons are present in the pedunculo pontine tegmental nucleus (PPTg) and midbrain extrapyramidal area (MEA, Rye et al., 1983) and more occupy the laterodorsal tegmental nucleus (LDTg) following injections of tracer into the LHb (Li et al., 1993; Yetnikoff et al., 2015). However, PHA-L injections into neither the compact part of the PPTg nor the MEA produced significant anterograde labeling in the LHb (Halanger and Wainer, 1988). In contrast, anterograde tracing confirms that the LHb is related to the LDTg by relatively robust, reciprocal connections (Cornwall et al., 1990).

Similarly, robust reciprocal connections link the LHb with the nucleus incertus (Goto et al., 2001; Olucha-Bordonau et al., 2003; Ma and Gundlach, 2015), a strongly GABAergic structure (Ford et al., 1995) with widespread projections that occupies the medial part of dorsal tegmental region just behind the dorsal raphe (see below under Efferent connections, Nucleus incertus).

Locus coeruleus

The potential importance of a norepinephrine (NE) innervation of the LHb is emphasized by a recent study showing that D₄ DA receptors, which excite LHb neurons giving rise to habenular outputs (Good et al., 2013; see also Kowski et al., 2011), are stimulated by NE and not VTA DA (Root et al., 2015). Injections of retrogradely transported tracers into the LHb label a few neurons in the locus coeruleus (Phillipson and Pycock, 1982; Gruber et al., 2007; Yetnikoff et al., 2015), of which some were reported to exhibit TH immunoreactivity, a marker of monoaminergic phenotype (Gruber et al., 2007). Earlier histofluorescence and lesion studies (e.g., Kobayashi et al., 1974; 1975), particularly that of Gottesfeld (1983), had detected reductions in norepinephrine (NE) levels in the LHb after transections of the dorsal noradrenergic bundle and dorsal longitudinal fasciculus, both of which carry NE projections ascending from the locus coeruleus, but not after ablation of the superior cervical ganglion, from which postganglionic sympathetic fibers ascend to the pineal body and MHb. The functional significance of NE neurotransmission in the LHb in the absence of psychostimulant drug on board remains obscure, however, insofar as NE release in the LHb is impulse independent and detected electrophysiologically only in conditions of blocked DA transporter and impaired monoamine oxidase function (Root et al., 2015).

Efferent connections

Overview

The seminal description of LHb efferent connections was provided by Herkenham and Nauta (1979). They described three main efferent pathways – caudal, lateral and rostral – of

which all descend in the loosely organized sheath of the fr. Fibers of the caudal pathway, by far the more robust of the three, leave the fr in the VTA and turn caudalward toward the serotonergic median and dorsal raphe, which, respectively, are richly and more lightly innervated. Also targeted are the cholinergic LDTg, ventral and dorsal tegmental nuclei of Gudden (subsequently shown by Kim [2009] to be avoided by dense LHb inputs to the surrounding medial pontine periventricular central gray) and nucleus reticularis tegmenti pontis. There is an additional, very dense accumulation of terminations in the tegmentum of the caudal midbrain and rostral pons just lateral to the raphe zone from which less labeling extends into the medial half of the parabrachial nucleus. The modest lateral pathway reaches the VTA in the fr and turns to pass through and above the SNc and overlying midbrain tegmentum and, ultimately, upward through the retrorubral area and into the ventrolateral quadrant of the periaqueductal gray substance, following a pathway identical to that described for efferents of the Acb (Nauta et al., 1978), LH (Nauta and Domesck, 1978) and, actually, several other forebrain structures, including the BST, VP, LPO and PVN (Geerling et al., 2010; Zahm et al. 2011). Thus, the caudal and lateral pathways convey LHb influence to brainstem regions containing the DA, cholinergic and serotonergic ascending modulatory projections. The rostral pathway also is modest. Upon having joined the mfb, it ascends to lightly innervate posterior and dorsomedial hypothalamic nuclei, ventromedial thalamic nucleus, LH, LPO, substantia innominata and what they referred to as a basolateral septal zone lying between the Acb and nuclei of the diagonal band.

LHb output is bilateral. Herkenham and Nauta (1979) estimated that the density of labeled fibers and terminals in the forebrain and medial brainstem observed a 3:2 ipsilateral to contralateral ratio, with decussations occurring in the VTA, supramammillary region, linear raphe nuclei and median raphe. In contrast, they pointed out that dense labeling in the reticular region abutting the raphe zone is less strongly bilateral at about a 10:1 ipsilateral to contralateral ratio. Literature cited in this review supports contralateral projections that vary between these extremes depending upon the particular structures.

Importantly, Herkenham and Nauta (1979) further emphasized that the patterns of anterograde labeling produced by LHbM and LHbL are distinct. LHbM gives rise to the descending projections to the raphe nuclei and central gray substance and to the ascending projections. LHbL projections produced the very dense anterograde labeling in the midbrain reticular formation abutting the raphe zone and its extension into dorsal parts of the pontomesencephalic tegmentum. Insofar as LHbM was distinguished in their earlier retrograde labeling study (Herkenham and Nauta, 1977) by its principally 'limbic' inputs from, e.g., the raphe nuclei, VTA, LH and LPO, whereas LHbL received basal ganglia inputs via the EPN, they regarded the different patterns of outputs from medial and lateral LHb as fully consistent with their earlier conceptualization of an LHb partitioned into medial 'limbic' and lateral 'pallidal' divisions. In contrast, they found that the main lateralward output pathway of the LHb through the SNc and overlying tegmentum comprised fiber contributions from LHbM and LHbL.

A subsequent PHA-L study of LHb efferent projections (Araki et al., 1988) confirmed much of what Herkenham and Nauta (1979) had reported and provided additional details. Similarly bilaterally disposed axon terminations were observed in the dorsal, parafascicular

and ventromedial thalamus, LH, ventral pallidum, substantia innominata and nucleus of the horizontal limb of the diagonal band. Particularly dense, more ipsilaterally disposed anterograde labeling (the densest they observed in their study) was seen in the oral pontine reticular formation abutting the raphe zone and in the median raphe itself, as was modest labeling in the central gray substance, dorsal raphe, deep mesencephalic nucleus, LDTg and nucleus raphe pontis. Araki et al. (1988) also reported modest labeling, not noted by Herkenham and Nauta (1979), in the deep layers of the superior colliculus and they found the PHA-L labeling in the thalamic mediodorsal and central medial nuclei to be more impressive than they had been led to anticipate by the earlier study. Furthermore, in contrast to Herkenham and Nauta (1979), they identified the dorsal thalamus as a site through which efferents not bound in the fr descend before joining the mfb enroute to rostral forebrain. More importantly, Araki et al. (1988) were concerned that, despite having injected only the lateral two thirds of the LHb, they observed projections covering “*almost all areas previously reported for neurons of the whole LHb.*” They concluded that neurons outside the medial zone project to limbic sites, e.g., the raphe nuclei, and argued that “*the LHb of the rat should be considered as an intermixed structure rather than one with distinct compartmentation.*”

Kim (2009), tracing anterogradely from the LHb with biotinylated dextran amine (BDA), also opted for a topographic description of LHb outputs. He designated medial, central and lateral parts of the LHb. The three parts project, respectively, to the median raphe, paramedian raphe and adjacent oral pontine tegmentum. Emphasizing the tremendous overlap of projections from adjacent parts of the LHb, Kim (2009) questioned if a two-fold medio-lateral partitioning, i.e., as ‘limbic’ and ‘pallidal’, best describes LHb organization. To be sure, Herkenham and Nauta (1979) themselves had commented that bi-partite mediolateral organization “*...offers little more than a caricature of the afferent relationships of the LHb. ...limbic afferents, although terminating principally in the M-LHb (LHbM here), are distributed throughout the width of the LHb. Conversely afferents of pallidal origin terminate in great density in the L-LHb but in smaller numbers also in more medial parts of the LHb.*” Thus, inasmuch as virtually any point in the LHb blends some combination of limbic and pallidal synaptic interactions, it seems worth reconsidering whether the termination patterns of LHb inputs and the origins of LHb outputs are described better in terms of gradients than compartments. As our knowledge of LHb cell types increases (Proulx et al., 2014; Lecca et al., 2014) we may find that specific LHb circuits are formed by molecularly-defined phenotypes in addition to specific subregional localizations.

Quina et al. (2015) used serial two-photon tomography combined with adeno-associated virus-mediated expression of green fluorescent protein to map LHb efferent connections in the mouse and produced data consistent with the classical tracing studies described above.

A note on the bilaterality of LHb connections

Afferent and efferent connections of the LHb have bilateral origins and terminations, respectively. The abundance of contralateral projections varies greatly from structure to structure, but is always subordinate relative to the ipsilateral side, usually substantially so. As regards the numbers of neurons giving rise to LHb inputs, Figure 2 in Yetnikoff et al.

(2015) provides a useful summary. Original literature should be consulted for details regarding specific structures.

Rostromedial tegmental nucleus (RMTg) and VTA

Investigators studying LHb outputs with anterograde tract-tracing were unanimous in their appreciation of the great density of axon terminal labeling in the mesopontine tegmentum abutting the raphe zone (Herkenham and Nauta, 1979; Araki et al., 1988; Kim, 2009), which, viewed in retrospect, foreshadowed additional interesting developments to come. Accordingly, the site of this labeling was soon found to harbor an aggregation of neurons that robustly expresses the immediate-early gene product, Fos (Scammell et al., 2000; Jhou, 2005; Zahm et al., 2010), and its long-lived splice variant, FosB (Perrotti et al., 2005), following administration of psychostimulant drugs. It was shown soon after that said Fos-expressing neurons are GABAergic, contacted by the dense innervation in question and the neurons of origin of a very strong projection to the VTA and SNc (Chou et al, 2004 [Chou is the same person as Jhou, cited elsewhere in this paper]; Geisler et al., 2008; Jhou et al., 2009a; b; Kaufling et al., 2010). Called the 'tail of the VTA' by one group (Perrotti et al., 2005, Kaufling et al., 2009), these neurons are now better known as the rostromedial tegmental nucleus (RMTg; Jhou et al., 2009a; b). The timely discovery of the RMTg provides an explanation as to how activation of LHb neurons by aversive stimuli (Christoph et al, 1986; Ullsperger and von Cramon, 2003; Brown et al., 2017) elicits profound inhibition of VTA DA neurons (Ji and Shephard, 2007; Shepard et al., 2006; Matsumoto and Hikosaka, 2007). The opposing valences of LHb and VTA/SNc responses to reward omission and aversive stimuli previously had been difficult to reconcile because the axonal projection from the LHb to the VTA is almost exclusively excitatory (Geisler et al., 2007; Brinschwitz et al., 2010), which dictated a need for an inhibitory intermediary – the RMTg, it turned out - to account for the functional findings (see the discussion in Ji and Shephard, 2007). RMTg has since been reviewed on numerous recent occasions (Barrot and Thome, 2011; Lavezzi and Zahm, 2011; Bourdy and Barrot, 2012; Sanchez-Catalan et al., 2014; Yetnikoff et al., 2014; Barrot et al., 2016; Zahm, 2016). Interestingly, while LHb lesions indisputably impair specific aspects of reward prediction error signaling in midbrain DA neurons, LHb may not be the exclusive source of such error signals (Tian and Uchida, 2015).

So, the GABAergic RMTg gets strong excitatory inputs from the LHb (Balcita-Pedicino et al., 2011; Stamatakis and Stuber, 2012) and projects strongly to the VTA and SNc (Jhou et al., 2009a; b; Kaufling et al., 2010), preferentially contacting DA neurons (Balcita-Pedicino et al., 2011), which accordingly are (mainly) strongly inhibited by LHb activation (Hong et al., 2011; Jalabert et al., 2011; Lecca et al., 2011; 2012; Stamatakis and Stuber, 2012; Brown et al., 2016). Notably, LHb projections to the VTA also elicit inhibition of DA neurons by contacting intermediary GABAergic interneurons in the VTA (Stamatakis and Stuber, 2012), but, most likely, as argued by Petzel et al. (2016), toward a functional end point different from that mediated by RMTg-elicited inhibition. Interestingly, however, evidence has emerged recently that this now well-known, potent, indirect inhibitory effect of LHb activation on mesotelencephalic DA neurons is complemented by a significant, direct excitatory effect, i.e., a direct excitatory projection from LHb neurons to DA neurons (Brown and Shepherd, 2016), suggestive of perhaps somewhat more complex processes by

which signaling of aversive stimuli and salience is conveyed from LHb to midbrain DA neurons. Activation of this pathway is aversive and regulated by mesocortical dopamine receptors (Lammel et al., 2012). Petzel et al. (2017) have made the related observation that more LHb neurons project to the VTA than RMTg, consistent with abundant direct LHb effects on VTA DA and local GABAergic neurons. However, the size of the terminal arbors (Omelchenko et al., 2009) and the physiological potency (Ji and Shepard, 2007) of direct LHb-VTA/SNc inhibitory projections are reported to be subordinate to those of the RMTg-VTA/SNc connection (Ji and Shepard, 2007; Jhou et al., 2009a;b; Balcita-Pedicino et al., 2011; Hong et al., 2011). The exuberant axonal arbors of RMTg projections to the VTA/SNc, in contrast, may amplify LHb signal strength, which, in turn, may be important for the aversive effects of cocaine (Jhou et al., 2013).

The notion that LHb outputs to the RMTg are topographically organized has been challenged by the argument that LHbM and LHbL project, respectively, to the VTA and RMTg relatively exclusively (Gonçalves et al., 2012; Petzel et al., 2017). While there is clearly a tendency along these lines, were the connectivity to be as selective as advocated by these investigators, injection of retrograde tracer into the VTA should produce robust labeling in LHbM and none in LHbL, which is contrary not only to our results (Jhou et al., 2009b), but also theirs (see Fig. 8 in Gonçalves et al., 2012 and Figs. 4–7 and 9 in Petzel et al., 2017). Indeed, there is a moderately strong projection from the medial LHb to the ipsilateral and contralateral RMTg (Jhou et al., 2009b). Thus, this (and any) model of LHb connections that emphasizes specificity of projections weakens when considering the 60–80% of the LHb between its most medial and lateral extremities.

To the extent that the RMTg is thought of simply as a relay of LHb signals, two additional facts should be recognized. [1] the RMTg also has numerous non-habenular afferents and, while these much resemble those of the LHb itself (Yetnikoff et al., 2015), modulation of the activity of RMTg and thus its output cannot be attributed exclusively to the LHb (Zahm, 2016). [2] RMTg outputs are not limited only to midbrain DA-expressing cell groups, but rather comprise, in addition, dense descending projections to the serotonergic raphe nuclei and other parts of the mesopontine reticular formation, such as the cholinergic MEA and laterodorsal tegmental nuclei (Jhou et al., 2009b; Gonçalves et al., 2012; Lavezzi et al., 2012; Zahm, 2016), as well as modest ascending projections to the parafascicular thalamic nucleus, entopeduncular nucleus, LH, thalamic midline and intralaminar cell groups, LPO, diagonal band complex, VP, dorsal hippocampus and frontal cortex. Among these structures is a significant proportion to which the LHb also projects, and which, like the VTA/SNc (Petzel et al., 2017), thus get major excitatory input directly from the LHb in combination with an LHb inhibitory influence relayed indirectly via the RMTg. Some of these structures include the raphe nuclei, mesopontine tegmentum, parafascicular nucleus, LH, LPO, VP and diagonal band nuclei.

Raphe nuclei

In an excellent series of papers, Metzger and colleagues have refined and expanded our knowledge of connections linking the LHb and DR (see also Zhou et al., 2017). Accordingly, and consistent with earlier studies (Aghajanian and Wang, 1977; Pasquier et

al., 1977), Sego et al. (2012) have shown that a direct projection from the LHb to DR arises mainly from LHbM, specifically in the vicinity of LHbMPc and LHbMC, but they went further to specify that it terminates in the caudalmost, midline part of the dorsal raphe nucleus. They had previously argued that LHbL projects relatively specifically to the RMTg (Gonçalves et al., 2012), which, in turn, projects strongly and specifically to the core of the dorsal part of the rostral DR complex, a structure enriched in VGluT-3-expressing glutamatergic neurons. These they have shown provide a reciprocal input to the RMTg (Sego et al., 2014). Thus, in this model, LHb 'limbic' and 'pallidal' sectors of Herkenham and Nauta (1979) give rise to projections to distinct parts of the dorsal raphe nucleus, the former via direct, presumably glutamatergic, projections from LHbM and the latter utilizing the RMTg as an inhibitory relay that is in turn modulated by a reciprocal glutamatergic projection from the rostradorsal core of the dorsal raphe to the RMTg (see Fig. 13 in Sego et al., 2014). Enthusiasm for this seminal result is tempered only to the extent that these pathways likely reflect enrichments in a broader connectional topography than the focal connectional specificity advocated by Metzger and colleagues; i.e., the pathways may be not quite so specific as promoted. Monosynaptic viral vector tracing and electrophysiological studies have shown a variety of pathways from LHb to DR and median raphe, including glutamatergic projections to 5-HT neurons and GABAergic neurons, and that the LHb innervation of GABAergic DR neurons provides feedforward inhibition to 5-HT neurons (Pollack-Dorocic et al., 2014; Ogawa et al., 2014; Zhou et al., 2017). These mechanisms by which the LHb regulates the serotonergic DR and median raphe have been proposed to be involved in comorbidity of a variety of disorders with depression (Li et al., 2017; Wang et al., 2017; Zhang et al., 2016) and defensive behavior (Pobbe and Zangrossi, 2010).

Nucleus incertus (NI)

NI is a paired, mainly GABAergic (Ford et al., 1995) structure wedged between the ventromedian boundary of the pontine periventricular gray substance and the dorsal tegmental nucleus just behind the dorsal raphe. NI has widespread, robust ascending projections to many structures (Goto et al., 2001; Olucha-Bordonau, 2003; Ma and Gundlach, 2015), including median and dorsal raphe nuclei, LDTg, periaqueductal gray substance, superior colliculus, interpeduncular nucleus, VTA/medial SNc, mammillary and supramammillary nuclei, medial and lateral zones of the hypothalamus, dorsomedial hypothalamus, zona incerta, LPO, dorsal and ventromedial thalamic nuclei, the diagonal band nuclei, medial, lateral and supracommissural septal nuclei, hippocampus, entorhinal area, endopiriform nucleus, claustrum and prefrontal cortex.

The LHb is reciprocally interconnected with NI. LHb gets moderate direct input from the NI as well as robust inputs relayed via, e.g., median and dorsal raphe nuclei, LDTg, LH and LPO. The subfornical part of LH, in particular, receives a very dense input from NI and provides the strongest LH projection to LHb seen by Goto et al. (2001). LHbM, in turn, has robust direct projections to NI, which also gets major inputs from the LPO, LH, RMTg (see Fig. 10 in Zhou et al., 2009b), periaqueductal and central gray substance and median and dorsal raphe, to all of which LHb projects at least moderately. Thus, LHb is securely embedded in a network of connections that controls the activity of NI, which, in turn, is hypothesized to modulate locomotor activity by its control over prefrontocortical and

hippocampal activity (Goto et al., 2001), particularly as regards arousal and synchronization-desynchronization of the theta rhythm (Olucha-Bordonau et al., 2003). NI thus is the origin of yet another state-setting system modulated by the LHb.

Concluding comments

The anatomy of the LHb submits to superficial conceptualization in two ways. On one hand, it may be regarded as partitioned, comprising medial 'limbic' and lateral 'pallidal' parts (Fig. 3, right side), each with different patterns of afferent and efferent connectivity (HJ Nauta, 1974; Herkenham and Nauta, 1977; 1979). On the other hand, LHb comprises a complex of cytologically distinct subnuclei (Fig. 3, left side), each, perhaps, with different connections, functions (Andres et al. 1999) and cell types (Proulx et al., 2014). There certainly is no mutual exclusivity between these alternatives, inasmuch as five of ten described LHb subnuclei are mainly medial and the other five are mainly lateral (Table 1). However, as it turns out, neither alternative, nor both together, is able to serve as more than a skeletal framework beneath what abundant studies reviewed here have shown to be a much less physically constrained cytological and connectional interface. Studies with sensitive axonal tracers have shown that extrapyramidal afferents of the LHb are not limited to its lateral half, nor are most limbic inputs constrained to the medial half of the LHb, which, notwithstanding, is not to say that the medial part of the LHb is not different than the lateral. Indeed some limbic afferents, such as, e.g., from the PL, lateral septum and VTA, are segregated medially, but others, e.g., from the rostral linear nucleus, and certainly the LH, terminate laterally. As regards the subnuclei, the early hope that they would generally possess afferent and efferent connectional specificity has gone largely unrealized, although hints of specificity abound. Enrichments of or lacunae in afferent terminations that coincide tantalizingly with a particular subnucleus are common, but they are typically limited to a level or two, only to degrade to at best an approximate correspondence or be lost entirely at a distance. As regards outputs, there is a consensus that a projection to the raphe nuclei arises in the medial, small-celled district of LHb, but not much evidence for strict specificity of other LHb outputs. It is difficult to escape the idea that there exist in the LHb fundamentals of cytological and connectional organization still to be discovered.

Having acknowledged our rather blurred understanding of principles underlying LHb connectivity, i.e., the integration of its inputs and organization of its outputs, one may also take note of relatively new ground being broken in the LHb with regard to the neuromessenger milieu. Abundant peptide-classical transmitter co-transmission aside, recent discoveries in the LHb include quite novel modes of neurocommunication – a projection from VTA DA neurons that lacks the capability to package DA in vesicles (Root et al., 2015); VTA and EPN axons exhibiting both structural and neurochemical differentiations consistent with both glutamatergic and GABAergic transmission at different synapses (Root et al., 2014; Shabel et al., 2015) – the regulation and physiological and behavioral impacts of which are still to be fully determined. Indeed, there are modes of neurotransmission extant in the LHb, e.g., glycinergic (Li et al., 2016b), the connectional infrastructure and implications of which have barely been touched.

Level of neuronal activity in the LHb is extraordinarily consequential. It is a snapshot of what is going on at any given moment in the entire cerebral cortex, conveyed by the several basal forebrain macrosystems (Alheid et al., 1988), including the dorsal and ventral striatum, extended amygdala and lateral septal-preoptic system, via the lateral preoptico-hypothalamic-ventral pallidal continuum (Yelnikoff et al., 2015; Zahm, 2016). It is, in turn, efficiently transmitted by abundant, powerful LHb outputs mainly to a number of diverse brainstem nuclei of which each gives rise to a unique system of ascending modulatory projections engaged in pervasive behavioral 'state-setting'. Subtle changes in LHb firing rates thus are likely reflected in subtle responses of the organism to subtle changes in internal and external circumstances, rendering the LHb as a sort of embodiment, an avatar if you will, of adaptive behavior. LHb may be just one of several, or perhaps one of many, brain structures so entrained to the behavioral imperatives of the environment, but it is currently seeming to be among the more important of these.

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Abbreviations

III	third ventricle
III_d	dorsal part of third ventricle
arIII	attachment of the roof of the third ventricle
Acb	nucleus accumbens
ACg	anterior cingulate cortex
AI	agranular insular cortex
AMPA	α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor, an ionotropic glutamate receptor
BDA	biotinylated dextran amine, an anterogradely transported axonal tracer
BST	bed nucleus of the stria terminalis
CNS	central nervous system
Ctβ	cholera toxin β subunit, a retrogradely transported axonal tracer
DA	dopamine, dopaminergic
DDC	dorsal diencephalic conduction system
DR	dorsal raphe
EPN	entopeduncular nucleus

FG	FluoroGold, a retrogradely transported axonal tracer
Fos	protein product of <i>c-fos</i> , an immediate early gene
FosB	long lasting splice variant of Fos
fr	fasciculus retroflexus
GABA	gamma-aminobutyric acid
GAD67	glutamic acid decarboxylase 67kD
Glu	glutamate
GPI	globus pallidus, internal segment
Hb	habenula
HRP	horseradish peroxidase, a retrogradely transported axonal tracer
ic	internal capsule
IL	infralimbic cortex
itp	inferior thalamic peduncle
LH	lateral hypothalamus
LHb	lateral habenula
LHbL	lateral division of LHb
LHbLB	basal subnucleus of LHbL
LHbLMc	magnocellular subnucleus of LHbL
LHbLMg	marginal subnucleus of LHbL
LHbLO	oval subnucleus of LHbL
LHbLPc	parvocellular subnucleus of LHbL
LHbM	medial division of LHb
LHbMA	anterior subnucleus of LHbM
LHbMC	central subnucleus of LHbM
LHbMMg	marginal subnucleus of LHbM
LHbMPc	parvocellular subnucleus of LHbM
LHbMS	superior subnucleus of LHbM
LPO	lateral preoptic area
LS	lateral septum

LDTg	laterodorsal tegmental nucleus
MD	mediodorsal nucleus of the thalamus
MEA	midbrain extrapyramidal area
mfb	medial forebrain bundle
MHb	medial habenula
MHbI	medial habenula, inferior subnucleus
MHbS	medial habenula, superior subnucleus
MPO	medial preoptic area
NBQX	2, 3-dihydroxy-6-nitro-7-sulfamoyl-endo[f]quinoxaline-2, 3-dione, an AMPA receptor antagonist
NI	nucleus incertus
ot	optic tract
PPTg	pedunculopontine tegmental nucleus
PHA-L	<u>Phaseolus vulgaris-leucoagglutinin</u> , an anterogradely transported axonal tracer
PL	prelimbic cortex
PVN	hypothalamic paraventricular nucleus
RMTg	rostromedial tegmental nucleus
Rt	reticular nucleus of the thalamus
sm	stria medullaris
SNe	substantia nigra compacta
VGaT	vesicular GABA transporter
VGluT	vesicular glutamate transporter
VP	ventral pallidum
VTa	ventral tegmental area

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Highlights

1. Description, cytology and subnuclear organization
2. Afferent and efferent connections
3. Functional correlates
4. Perspective

Figure 1.

Micrographs illustrating a PHA-L injection into the caudal part of the lateral preoptic area (A, inset in A shows additional landmarks) and several frontal sections cut through the habenula (Hb) in rostrocaudal sequence from B to E. Outlines of lateral habenula subnuclei have been approximated on the contralateral side relative to the injection according to the original drawings of Andres et al. (1999). Note also the locations of the attachments of the roof of the dorsal part of the third ventricle (arIII). Panels B–E show anterograde labeling (brown reaction product) from the injection site shown in A. Sparse anterograde labeling is observed in the LHb contralateral to the injection. Note on the side ipsilateral to the injection

that dense labeling is present in LHb, but not in MHb, and spreads across its entire mediolateral extent, although the labeling is 'patchy' with some areas being relatively devoid of reaction product (* and **). Areas exhibiting reaction product lacunae do not correspond precisely or consistently to subnuclei, however. For example, central area with lesser density of reaction product in B and C (*) has no obvious subnuclear correspondence. That in D –F may imprecisely approximate LHbMC and LHbMPc. A ventrolateral area lacking dense labeling (**) would seem to include LHbLO, consistent with the findings of Kowski et al. (2008), but is invariably much larger than that subnucleus. Alternatively, dense labeling appears to fill LHbLPc and LHbLMg. Additional abbreviations: arIII – attachment of the roof of the third ventricle; f – fornix; fr – fasciculus retroflexus; LPO – lateral preoptic area; LHbLB – basal subnucleus of LHbL; LHbLMc – magnocellular subnucleus of LHbL; LHbLMg – marginal subnucleus of LHbL; LHbLO – oval subnucleus of LHbL; LHbLPc – parvocellular nucleus of LHbL; LHbMA – anterior nucleus of LHbM; LHbMC – central subnucleus of LHbM; LHbMMg – marginal subnucleus of LHbM; LHbMPc – parvocellular subnucleus of LHbM; LHbMS – superior subnucleus of LHbM; MHb – medial habenula; ot – optic tract; PV – paraventricular nucleus of the thalamus; sm – stria medullaris; v – ventricle; IIIId = dorsal part of the third ventricle. Scale bar: 1 mm

Figure 2.

Micrographs illustrating additional injections of PHA-L into the lateral preoptic area-lateral hypothalamus continuum, one more rostral (A–C) and one more caudal (D–F) than the PHA-L injection shown in Figure 1. Injection sites are shown in the insets in A and D and frontal sections through the habenula are shown at rostral (A and D), middle (B and E) and caudal (C and F) levels. The medial habenula (MHb), lateral habenula (LHb) and stria medullaris (sm) are outlined for reference on one side of the midline. Subnuclei have been left out. Note also the locations of the attachments of the roof of the dorsal part of the third ventricle (arIII). Panels A–C and D–F show anterograde labeling resulting from injections of

PHA-L into the rostralmost part of LPO where it transitions to ventral pallidum (A–C) and the rostralmost dorsolateral part of the lateral hypothalamus (D–F). Both injections produced sparse anterograde labeling in the LHb contralateral to the injection. Note in panels A–C, however, that dense labeling (black reaction product) on the side ipsilateral to the injection is present in LHb, but not in MHb, and spreads into the lateral half of the LHb as previously reported by Kowski et al. (2008) as marked by ** in B. Lacunae in the immunolabeling in B and C, the latter possibly corresponding to the position of LHbLO. Alternatively, in panels D–F ipsilateral labeling spreads densely, but in a ‘patchy’ disposition, across the entire LHb. In D, a central area in LHbL relatively devoid of labeling can be seen (*) as can a spread of dense labeling across dorsal parts of LHb curving around to its lateral parts (**). A small lacuna in the labeling of unclear correspondence, possibly part of the LHbMC/LHbMPc complex is also evident (* in E). Additional abbreviations: ac – anterior commissure; arIII – attachment of the roof of the third ventricle; EPN – entopeduncular nucleus; f – fornix; fr – fasciculus retroflexus; LH – lateral hypothalamus; LHb – lateral habenula; MHb – medial habenula; ot – optic tract; PV – paraventricular nucleus of the thalamus; sm – stria medullaris; v – ventricle; VP – ventral pallidum; IIIId = dorsal part of the third ventricle. Scale bar 1 mm.

Figure 3.

Diagram of a cross-section through about the middle of the lateral habenula (LHb) illustrating the two ways that it has been conceptualized. On the right, LHb is shown comprising two divisions, a medial 'limbic' part (LHbM) and a lateral 'pallidal' part (LHbL). Various axonal projections are described as preferentially arising or terminating in LHbM or LHbL, although, as developed in the text, this is strictly true of but few of them. Subnuclei are depicted on the left side of the diagram. Upon discovery of the subnuclei, it was anticipated that they would reflect functional and connectional compartmentation of the LHb, but this has been realized only in small part, mainly as pertains to LHbMC and LHbMPC (green), which are small-celled, project preferentially to the raphe nuclei and receive input enriched in tyrosine hydroxylase immunoreactivity. Functional and connectional correlates are much more difficult to relate to other LHb subnuclei. Note on the left that subnuclei representing LHbL and LHbM are separated by a heavier, finely stippled line and, at this rostrocaudal level, only eight of ten LHb subnuclei are represented. Additional color codes: dark gray – stria medullaris (sm) ; light gray – medial habenula (MHb). Other abbreviations: LHbLMc – magnocellular subnucleus of the lateral division of the LHb; LHbLMg – marginal subnucleus of the lateral division of the LHb; LHbLO – oval subnucleus of the lateral division of the LHb; LHbLPC – parvocellular subnucleus of the lateral division of the LHb; LHbMC – central subnucleus of the medial division of the LHb; LHbMMg – marginal subnucleus of the medial division of the LHb; LHbMPC – parvocellular subnucleus of the medial division of the LHb; LHbMS – superior subnucleus of the medial division of the LHb.

Table 1Lateral Habenula Subnuclei[/]

LHb division			
medial	lateral	descriptor (abbrev.)	abbreviated name
x		anterior (A)	LHbMA
x		superior (S)	LHbMS
x		central (C)	LHbMC
x	x	parvocellular (Pc)	LHbMPc, LHbLPc
x	x	marginal (Mg)	LHbMMg, LHbLMg
	x	magnocellular (Mc)	LHbLMc
	x	oval (O)	LHbLO
	x	basal (B)	LHbLB

Full names: **LHbMA** – anterior subnucleus of the medial division of the LHb; **LHbMS** – superior subnucleus of the medial division of the LHb; **LHbMC** – central subnucleus of the medial division of the LHb; **LHbMPc** – parvocellular subnucleus of the medial division of the LHb; **LHbMMg** – marginal subnucleus of the medial division of the LHb; **LHbLPc** – parvocellular subnucleus of the lateral division of the LHb; **LHbLMg** – marginal subnucleus of the lateral division of the LHb; **LHbLMc** – magnocellular subnucleus of the lateral division of the LHb; **LHbLO** – oval subnucleus of the lateral division of the LHb; **LHbLB** – basal subnucleus of the lateral division of the LHb.

Shading: Distinct parvocellular and marginal subnuclei occupy medial and lateral LHb divisions.

[/] According to Andres et al. (1999) and Geisler et al. (2003).

Table 2

Investigations of lateral habenula afferent connections done by retrograde tracing showing the tracer(s) used and region(s) studied

Citation	Tracer(s)	Region(s) studied
Herkenham and Nauta, 1977	HRP	CNS
Parent et al., 1981	HRP	CNS
Swanson, 1982	true blue	VTA
Skagerberg et al., 1984	true blue	VTA
Li et al., 1993	Ct β	CNS
Felton et al., 1999	FG, pseudorabies virus	CNS
Gruber et al., 2007	au WGA	VTA
Kowski et al., 2008	FG, Ct β	LPO
Kim and Lee, 2012	Ct β	PfC
Yetnikoff et al., 2015	Ct β	CNS

Abbreviations: au WGA - colloidal gold-coupled wheat germ agglutinin; Ct β - cholera toxin β subunit; CNS – central nervous system; Fluorogold - FG; HRP – horseradish peroxidase; LPO – lateral preoptic area; PfC – prefrontal cortex; VTA – ventral tegmental area

Table 3

Numbers of labeled neurons produced by injections of tracer into the LHb expressed as % total and listed by brain region

A. Cortex		D. Thalamus, epithalamus, and hypothalamus	
medial orbital	1.04±0.18	anteroventral thalamic n	<i>1.69±0.94</i>
ventral orbital	0.91±0.53	paraventricular thalamic n	0.29±0.21
lateral orbital	0.18±0.12	laterodorsal thalamic n	0.065±0.02
tenia tecta	0.42±0.14	ventrolateral geniculate n	0.39±0.17
navicular n	0.12±0.03	parafascicular thalamic n	0.24±0.11
cingulate	<i>3.17±1.3</i>	paraventricular hypothalamic n	0.28±0.13
prelimbic	<i>4.37±1.73</i>	lateral hypothalamus	<i>9.78±1.3</i>
infralimbic	<i>1.27±0.22</i>	juxtapaaventricular lat. hypothalamus	<i>4.8±2.05</i>
insular	0.55±0.37	ventromedial hypothalamus	<i>1.4±0.44</i>
dorsal peduncular	0.48±0.15	dorsal hypothalamus	<i>1.32±0.54</i>
claustrum/endopiriform	0.8±0.21	posterior hypothalamus	<i>2.55±0.44</i>
retrosplenial	0.8±0.84	premamillary region	0.34±0.22
B. Deep telencephalic nuclei (not amygdala)		E. Mesopontine region and cerebellum	
accumbens shell	0.25±0.24	dorsal periaqueductal gray	<i>3.22±1.42</i>
lateral septum	<i>5.85±2.13</i>	ventrolateral periaqueductal gray	<i>2.38±0.16</i>
medial septum	0.87±0.37	superior colliculus, ipsilateral	<i>3.66±1.65</i>
ventral pallidum	<i>4.23±1.76</i>	superior colliculus, contralateral	0.99±0.5
lateral preoptic area	<i>13.84±4.68</i>	substantia nigra	<i>1.22±0.68</i>
LPO-LH transition	<i>6.87±2.76</i>	ventral tegmental area	<i>1.25±0.4</i>
medial preoptic area	<i>2.61±0.15</i>	retrotrubral field	0.1±0.03
diagonal band, horizontal limb	<i>2.37±1.37</i>	deep mesencephalic n	0.6±0.39
magnocellular preoptic area	0.19±0.05	dorsal raphe	0.2±0.03
entopeduncular n	<i>1.65±1.2</i>	median raphe	0.89±0.30
C. Amygdala and extended amygdala		cuneiform n	0.83±0.54
bed nucleus of stria terminalis	<i>1.69±0.16</i>	pedunclopontine tegmental n	0.31±0.07
sublenticular extended amygdala	<i>2.38±0.35</i>	laterodorsal tegmental n	0.43±0.23
anterior amygdaloid area	0.18±0.05	pontine reticular formation	0.4±0.16
medial amygdaloid n	1.01±0.18	pontine central gray	0.14±0.06
		locus ceruleus	0.15±0.10

Values are means±SEM reflecting % of total labeling from 3 cases in which retrograde tracer, cholera toxin β subunit, was injected into the LHb (Yetnikoff et al., 2015). Values from 1.125 to 2.249 are *italicized*, values from 2.5 to 4.999 are ***italicized and bolded***, values at 5.0 and above are ***italicized, bolded and underlined***.

Table 4

Numbers of labeled neurons produced by injections of tracer into the LHb expressed as % total and listed in descending order of magnitude

lateral preoptic area	<u>13.84±4.68</u>	median raphe	0.89±0.30
lateral hypothalamus	<u>9.78±1.3</u>	medial septum	0.87±0.37
LPO-LH transition	<u>6.87±2.76</u>	cuneiform n	0.83±0.54
lateral septum	<u>5.85±2.13</u>	claustrum/endopiriform	0.8±0.21
juxtaparaventricular lat. hypothalamus	4.8±2.05	retrosplenial cx	0.8±0.84
prelimbic cx	4.37±1.73	deep mesencephalic n	0.6±0.39
ventral pallidum	4.23±1.76	insular cx	0.55±0.37
superior colliculus, ipsilateral	3.66±1.65	dorsal peduncular cx	0.48±0.15
dorsal periaqueductal gray	3.22±1.42	laterodorsal tegmental n	0.43±0.23
cingulate cx	3.17±1.3	tenia tecta	0.42±0.14
medial preoptic area	2.61±0.15	pontine reticular formation	0.4±0.16
posterior hypothalamus	2.55±0.44	ventrolateral geniculate n	0.39±0.17
sublenticular extended amygdala	<i>2.38±0.35</i>	premammillary region	0.34±0.22
ventrolateral periaqueductal gray	<i>2.38±0.16</i>	pedunculopontine tegmental n	0.31±0.07
diagonal band, horizontal limb	<i>2.37±1.37</i>	paraventricular thalamic n	0.29±0.21
bed nucleus of stria terminalis	<i>1.69±0.16</i>	paraventricular hypothalamic n	0.28±0.13
anteroventral thalamic n	<i>1.69±0.94</i>	accumbens shell	0.25±0.24
entopeduncular n	<i>1.65±1.2</i>	parafascicular thalamic n	0.24±0.11
ventromedial hypothalamus	<i>1.4±0.44</i>	dorsal raphe	0.2±0.03
dorsal hypothalamus	<i>1.32±0.54</i>	lateral orbital cx	0.18±0.12
infralimbic cx	<i>1.27±0.22</i>	magnocellular preoptic area	0.19±0.05
ventral tegmental area	<i>1.25±0.4</i>	anterior amygdaloid area	0.18±0.05
substantia nigra	<i>1.22±0.68</i>	locus ceruleus	0.15±0.10
medial orbital cx	1.04±0.18	pontine central gray	0.14±0.06
medial amygdaloid n	1.01±0.18	navicular n	0.12±0.03
superior colliculus, contralateral	0.99±0.5	retrotrubral field	0.1±0.03
ventral orbital cx	0.91±0.53	laterodorsal thalamic n	0.065±0.02

Values are means±SEM reflecting % of total labeling from 3 cases in which retrograde tracer, cholera toxin β subunit, was injected into the LHb (Yetnikoff et al., 2015). Values from 1.125 to 2.249 are *italicized*, values from 2.5 to 4.999 are ***italicized and bolded***, values at 5.0 and above are ***italicized, bolded and underlined***.