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Molecular mapping of mouse brain regions innervated by leptin receptor-expressing cells

Christa M. Patterson^a, Rebecca L. Leshan^{a,1}, Justin C. Jones^a, and Martin G. Myers Jr.^a

^a Division of Metabolism, Endocrinology and Diabetes, Department of Internal Medicine and Department of Molecular and Integrative Physiology, University of Michigan, 1000 Wall St., Ann Arbor MI, 48105

Abstract

Leptin acts via the long form of the leptin receptor (LepRb) on specialized sets of neurons in the brain to modulate diverse functions in concert with energy stores. Previous studies have revealed the distribution of LepRb-expressing neurons in the brain but not the regions to which LepRb neurons project to mediate downstream leptin actions. We utilized LepRb-cre in combination with cre-inducible enhanced green fluorescent protein (EGFP) and farnesylated EGFP (EGFPf) mouse reporter strains to visualize LepRb neurons and their projections, respectively, throughout the brain. The areas containing LepRb soma and projections were relatively circumscribed, as many brain regions contained no detectable EGFP or EGFPf. The highest concentrations of LepRb neurons and LepRb-projections were found in the hypothalamus, where the ventral premammillary (PMv), dorsomedial (DMH) and arcuate (ARC) nuclei contained the greatest number of cell bodies, in addition to substantial EGFPf-reactivity. Furthermore, both LepRb soma and projections were present in a few midbrain and brainstem nuclei. Several brain regions including the hypothalamic paraventricular nucleus (PVH), the anteroventral periventricular nucleus (AVPe), and the central nucleus of the amygdala (CeA) contained few LepRb neurons but substantial EGFPf, suggesting that these regions represent targets of LepRb neurons that lie elsewhere in the brain. In some nuclei that contained both soma and projections, the distribution of soma and projections differed, suggesting that these areas transmit leptin-encoded information in a neuroanatomically directional manner.

Keywords

leptin; cre recombinase; enhanced green fluorescent protein; axon

Correspondence: Martin G. Myers, Jr., M.D., Ph.D., Division of Metabolism, Endocrinology and Diabetes, Department of Medicine, University of Michigan Medical School, 1000 Wall Street, Brehm Tower, Ann Arbor, MI 48105, PH: 734-647-9515, Fax: 734-232-8175, mgmyers@umich.edu.

¹Present Address: Rebecca L. Leshan, PhD, Neurobiology and Behavior, The Rockefeller University, Box 275, 1230 York Avenue, New York, NY 10021

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1. Introduction

Leptin is a primarily adipose-derived hormone that modulates diverse behaviors and physiologic functions in line with energy balance (Ahima et al., 1999; Bouret and Simerly, 2007; Friedman and Halaas, 1998; Myers et al., 2009; Williams et al., 2009). Mice null for leptin (*Lep^{ob/ob}*) display severe hyperphagia and obesity, along with diabetes, decreased sympathetic tone, impaired thyroid function, infertility, and cold intolerance (Chua et al., 1996; Elmquist et al., 1999; Friedman and Halaas, 1998; Vaisse et al., 1996). Exogenous leptin reverses these defects and restores energy balance in *Lep^{ob/ob}* animals, as well as diminishing appetite and supporting energy expenditure in food restricted animals and humans (Ahima et al., 1996; Rosenbaum et al., 2002). Additionally, leptin promotes antidepressant and anxiolytic effects and modulates the mesolimbic dopamine system in rodents (Fulton et al., 2006; Hommel et al., 2006; Leininger et al., 2009; Lu, 2007; Lu et al., 2006). Thus, leptin signals the repletion (or, by its absence, the depletion) of adipose energy stores to modulate diverse behaviors and energy expenditure in concert with energy status. Since both intracerebroventricular (icv) and intraperitoneal (ip) administration of leptin decrease appetite and increase energy expenditure in rodents (Campfield et al., 1995), the major effects of leptin appear to be mediated via the CNS. Indeed, disruption of the leptin receptor (LepR) in the CNS promotes hyperphagia and decreased energy expenditure (Balthasar et al., 2004; Bates et al., 2004; Bingham et al., 2008; Cohen et al., 2001; Dhillon et al., 2006).

Although alternative splicing leads to the production of multiple LepR variants, only the so-called “long” LepR form (LepRb) contains a fully signaling-competent intracellular domain; *Lepr^{db/db}* mice specifically null for the LepRb isoform largely recapitulate the phenotype of *Lep^{ob/ob}* animals, suggesting that LepRb mediates the majority of physiologic leptin action (Chua et al., 1996; de Luca et al., 2005; Robertson et al., 2008). Furthermore, transgenic expression of LepRb in the brains of mice null for all LepR isoforms restores most leptin action (Chua et al., 2004). Thus, LepRb-expressing neurons in the brain represent the major cellular mediators of leptin action.

The expression of LepRb within the brain has been mapped by a variety of means, including functional and genetic assays and *in situ* hybridization, and these studies have revealed large numbers of LepRb neurons within discrete hypothalamic nuclei, as well as in circumscribed regions of the midbrain, brainstem, and a few other brain regions (Elmquist et al., 1998; Leshan et al., 2006; Scott et al., 2009). While the functional analysis of the many populations of LepRb neurons remains incomplete, a variety of data suggest important roles for several sets of hypothalamic LepRb neurons in the response to leptin. Deletion of LepRb specifically in subsets of LepRb-expressing ARC neurons promotes weight gain and hyperleptinemia (Balthasar et al., 2004; van de et al., 2008). Similarly, mice lacking LepRb specifically in SF1-expressing neurons in the VMH display increased susceptibility to obesity (Dhillon et al., 2006). Overall, the effects of these ARC/VMH-specific manipulations are modest compared to the phenotype of *Lepr^{db/db}* animals, however, suggesting that leptin action is more widely distributed. Indeed, important functional roles have been described for hypothalamic LepRb neurons, including elsewhere in the hypothalamus, including the lateral hypothalamic area (LHA), as well as in the midbrain and brainstem (Grill et al., 2002; Hayes et al., 2010; Hommel et al., 2006; Leininger et al., 2009; Leininger and Myers, 2008; Leshan et al., 2009; Leshan et al., 2010; Ring and Zeltser, 2010).

While the identification of LepRb neurons reveals the direct targets of leptin action in the brain, it is also important to identify the brain regions that are innervated by LepRb neurons, as these sites likely represent important output nuclei for leptin action. In this study, we have

analyzed the mouse brain for LepRb-containing neural soma and their projections, in order to identify such regions.

2. Results

2.1 LepRb^{EGFP} and LepRb^{EGFPf} mice

To effectively study the sites and mechanisms of leptin action in the brain, it is necessary to determine the brain regions where leptin acts- including not only those that contain LepRb-expressing soma, but also those regions to which LepRb neurons project to effect trans-synaptic regulation. Due to the lack of sensitive LepRb detection in the brain by immunocytochemistry, we and others have employed genetic models to detect LepRb soma (Leshan et al., 2006; Scott et al., 2009). Use of a mouse model (*Lep^r^{cre}*) that expresses cre recombinase from the LepRb-specific mRNA in combination with a cre-inducible enhanced green fluorescent protein (EGFP; LepRb^{EGFP} mice) or similar reporter alleles promotes reporter gene expression in LepRb-expressing cells to facilitate their detection (Figure 1) (Leshan et al., 2006). While EGFP accumulates in the cytoplasm and serves well to identify soma, cytoplasm-poor neural processes (such as axons) are poorly labeled by standard EGFP in many cases. Since it is also important to identify which brain regions are indirectly influenced by leptin, we also generated a reporter allele (*Gt(ROSA)26Sor^{EGFPf}*) that drives the expression of farnesylated EGFP (EGFPf; which is localized to the membrane and allows for the visualization of axonal projections) in cre-expressing cells (Figure 1)(Leshan et al., 2009).

We generated *Lep^r^{cre};ROSA^{EGFPf}* (LepRb^{EGFPf}) mice for comparison to LepRb^{EGFP} mice to systematically define the brain regions that can be modulated by leptin via innervation from LepRb neurons relative to areas in which leptin acts directly on LepRb neurons, respectively. Fixed brains from LepRb^{EGFP} and LepRb^{EGFPf} mice were immunostained for EGFP-immunoreactivity (–IR) and imaged to determine the brain regions containing LepRb neural soma (soma with EGFP-IR in LepRb^{EGFP} mice) and those containing neural projections from LepRb neurons (diffuse EGFP-IR in LepRb^{EGFPf} mice) (Tables 1–2; Supplemental Material).

2.2 Hypothalamic regions containing both LepRb neurons and projections

Overall, the hypothalamus contained both the greatest number of LepRb/EGFP-IR neurons and the greatest density of LepRb (EGFP-IR) projections (Figure 2; Tables 1–2; Supplemental Material). The largest numbers of EGFP-IR neurons in LepRb^{EGFP} mice were found in the ventral premamillary (PMv), arcuate (ARC), and dorsomedial (DMH) nuclei of the hypothalamus (Figure 2; Table 1). While EGFP-containing LepRb soma were distributed throughout the ARC, most neurons in the PMv lay in the middle portion of the nucleus (relative to the dorsoventral axis). In the DMH, EGFP expression was markedly lower in the compact portion. Substantial numbers of LepRb neurons were also present in the posterior hypothalamus (PH), VMH (specifically the dorsomedial portion), the LHA, and the preoptic area (POA) (Table 1; Supplemental Material).

Most hypothalamic regions containing EGFP-IR soma in LepRb^{EGFP} mice also contained substantial EGFP-IR projections in LepRb^{EGFPf} mice (Table 2; Figure 2; Supplemental Material). While the distribution of somatic EGFP in LepRb^{EGFP} mice roughly approximated that of EGFPf in LepRb^{EGFPf} mice in most hypothalamic regions (Figure 2; see, e.g., DMH), this was not universally true: In the ARC of LepRb^{EGFPf} mice, copious EGFP-IR projections were observed streaming ventral and lateral to the nucleus in addition to within the main cluster of ARC LepRb soma, and in the PMv little EGFPf was detected except in the area ventral and medial to the main cluster of LepRb neurons (in the region of

the ARC). These findings not only reveal the ability of EGFPf to identify projections relative to soma, but also suggest that the area containing PMv LepRb neurons may be sparsely innervated by other LepRb neurons and that PMv LepRb neurons project outside of the PMv, while the opposite may be true for DMH LepRb neurons.

2.3 Midbrain regions containing both LepRb neurons and projections

Within the midbrain, the largest concentration of LepRb soma and LepRb projections were found in the dorsal raphe (DR) and periaqueductal gray (PAG) with lower amounts in the ventral tegmental area (VTA), the Edinger-Westphal nucleus (EW), and linear raphe (LR) (Figure 3; Tables 1–2; Supplemental Material). LepRb and LepRb projections were also present in the substantia nigra (SN) primarily in the pars compacta, with many fewer cells and projections in the pars reticulata (Tables 1–2; Supplemental Material). A small number of LepRb neurons and projections were also localized in the intermediate geniculate nucleus, the cuneiform nucleus, and in the A8 dopamine cells (Tables 1–2). Overall, the distribution of LepRb soma and projections were similar in these regions, suggesting the presence of projections from local LepRb neurons and/or the innervation of these areas from distant populations of LepRb neurons.

2.4 Hindbrain regions containing both LepRb neurons and projections

In the hindbrain, substantial numbers of LepRb neurons and projections were located in the lateral parabrachial nucleus (LPBN) and within and surrounding the nucleus of the solitary tract (NTS) (Figure 4; Tables 1–2). While LepRb soma and projections were similarly distributed in the LPBN, the density of EGFPf-IR fibers was lower than in other regions with a similar density of EGFP-IR LepRb soma (compare, e.g., to the DMH, Figure 2A), suggesting that, like the PMv, the LPBN receives relatively few projections from local or distant LepRb neurons, and that LPBN LepRb neurons predominantly project outside the LPBN.

In addition, LepRb projections into the area surrounding the LepRb soma in the NTS were substantially stronger than into the NTS region containing the LepRb soma, suggesting that the NTS LepRb neurons may project outside of the confined area in which they lie, and that, in contrast, the regions surrounding the circumscribed area containing NTS LepRb neurons receive copious innervation from LepRb neurons. Interestingly, the area postrema (AP) was devoid of LepRb soma and projections (Figure 4), suggesting that the integration of leptin action with signals that act via the AP must occur at other sites. Smaller numbers of neurons and projections were identified in the dorsal motor nucleus of the vagus (DMV), the parvocellular reticular nucleus, and the prepositus hypoglossal nucleus (Tables 1–2).

2.5 Additional regions containing LepRb neurons and projections

Substantial numbers of LepRb soma and projections were found in the bed nucleus of the stria terminalis (BNST). Comparatively few cortical regions contained LepRb soma and projections, and most that did were relatively circumscribed. Both LepRb neurons and projections were observed throughout the prefrontal cortex (PFC). The insular cortex, temporal cortex, auditory cortex, and somatosensory cortex all contained discrete pockets of LepRb soma and projections (Tables 1–2; Supplemental Material). Fewer LepRb neurons and sparse LepRb projections were found in the visual cortex. A few LepRb neurons were also detected in the hippocampal formation, along with projections from LepRb neurons, which showed the highest density in CA1–3 and lower concentrations in the dentate gyrus. Scattered LepRb neurons and projections were observed within the thalamus.

2.6 Brain regions predominantly containing projections from LepRb neurons

Our analysis of LepRb^{EGFP} compared to LepRb^{EGFPf} mice also revealed brain regions that contained few or no LepRb soma, but that received copious projections from LepRb neurons, suggesting that these regions represent important targets of LepRb neurons that lie elsewhere in the brain (Figure 5; Tables 1–2). These regions included the anteroventral periventricular nucleus (AVPe), the nucleus accumbens (NAc) subregion that borders the BNST, the paraventricular nucleus of the hypothalamus (PVH), and the central nucleus of the amygdala (CeA). Interestingly, the innervation of these regions by distant LepRb neurons was also detectable as diffuse EGFP-IR in LepRb^{EGFP} mice (although the axonal morphology was less well-defined with standard EGFP than with EGFPf), suggesting the dense innervation of these regions by LepRb neurons.

3. Discussion

Leptin regulates appetite, energy homeostasis, neuroendocrine function, and a variety of behaviors via LepRb in the brain (Ahima et al., 1999; Bouret and Simerly, 2007; Friedman and Halaas, 1998; Myers et al., 2009; Williams et al., 2009). Understanding the neural mechanisms of leptin action necessarily entails defining the brain sites on which leptin acts directly and indirectly. Here, we have utilized two molecular tools, the LepRb^{EGFP} and LepRb^{EGFPf} mouse models, to elucidate the distribution of LepRb neurons and their projections, respectively, throughout the brain.

3.1 LepRb soma

The distribution of LepRb neurons in the brain revealed by LepRb^{EGFP} mice is similar to that determined by examination of LepRb mRNA expression via *in situ* hybridization (Elmqvist et al., 1998) and a LepRb-EYFP reporter model (Scott et al., 2009) similar to our LepRb^{EGFP} mice. Large populations of LepRb neurons lie in the hypothalamus, especially in the ARC, DMH and PMv; other substantial populations reside in the LHA, VMH, and preoptic areas. The midbrain populations are generally smaller than those of the hypothalamus, with the exception of the PAG; the VTA and Raphe nuclei also contain substantial concentrations of LepRb neurons. Within the brainstem, the LPBN contains a large number of LepRb neurons; the NTS represents the only other hindbrain site with significant numbers of LepRb soma. Additionally, while most cortical sites contain no LepRb neurons, a few regions, including the PFC and other circumscribed areas, contain pockets of LepRb neurons. The thalamus contains relatively few, sparsely-distributed LepRb soma, while the BNST contains a substantial number of LepRb soma throughout the region.

3.2 LepRb projections

In addition to confirming the presence of LepRb soma in these regions, our use of LepRb^{EGFPf} mice enabled us to examine the innervation of brain regions by LepRb neurons. Along with identifying brain areas that receive no “direct” leptin action either from LepRb soma or projections, this analysis revealed differences between the distribution of soma and projections in sites that contain both, or a relative abundance of one over the other.

3.3 Distinct patterns of soma relative to projections in different brain regions

Regions that contain both LepRb soma and projections fall into several distinct classes. Some of these areas, including the hypothalamic PMv and the hindbrain LPBN and NTS, reveal few EGFPf-containing LepRb projections in the vicinity of the LepRb soma relative to other areas with similar numbers of LepRb soma. This pattern reveals few projections (from local or distant sites) into the LepRb soma-containing area, suggesting that while leptin acts directly upon these LepRb neurons, these cells transmit leptin-encoded (and

other) information to other areas. This also implies that the soma in these LepRb projection-poor areas do not serve to integrate direct leptin action with information from other LepRb neurons.

3.4 Similarly distributed soma and projections

Other regions (including the hypothalamic ARC, DMH, and LHA, and most midbrain and cortical areas with LepRb soma) contain similarly distributed soma and projections, suggesting that these areas not only contain LepRb neurons, but also receive substantial input from local and/or distant LepRb neurons. These presumably represent areas that process/integrate information from multiple LepRb neurons, in addition to receiving direct leptin stimulation. For the ARC, from which axons can be observed streaming ventrally and laterally, an output function is also likely; indeed, such a function is consistent with the known projection patterns and roles of LepRb-containing POMC and AgRP/NPY neurons in this area (Elias et al., 1999; Elias et al., 1998; Fekete et al., 2000; King and Williams, 1998).

3.5 Projection targets with few or no soma

Areas that receive substantial projections from LepRb neurons, but which contain few LepRb soma, include the PVH, CeA, the subregion of the NAc bordering the BNST, and the region surrounding the LepRb soma-containing area of the NTS. These areas represent output targets of distant LepRb neurons. In the case of the PVH, this finding is consistent with data identifying this region as a target of distant (e.g., ARC and DMH) LepRb neurons, including the well-characterized ARC POMC and AgRP/NPY neurons (Bouret et al., 2004; Elmquist, 2001). Similarly, the LepRb projections to the CeA and NAc are consistent with our previous data demonstrating that the dense innervation of the CeA stems from LepRb neurons, including those in the VTA, while the relatively fewer projections into a small region of the NAc arise from midline midbrain LepRb neurons, such as those in the RLi (Leshan et al., 2010).

A number of conclusions follow from our findings in the hindbrain region containing the NTS and surrounding areas. First, the lack of LepRb soma or projections in the AP reveal that leptin itself does not act directly upon this area, suggesting that integration of leptin signals with those from peptides (e.g., amylin) that act on the AP occurs at other sites. LepRb neurons or projection targets in the NTS represent likely sites for the convergence of signals from leptin with those from the AP, since AP neurons project onto neurons in the NTS (Shapiro and Miselis, 1985). Indeed, while the innervation of the region containing the soma of NTS LepRb neurons contains relatively little EGFPf (few projections), the NTS regions adjacent to the AP and the DMV receive dense projections from LepRb neurons, suggesting the innervation of these areas by NTS LepRb neurons and/or distant (including ARC) LepRb neurons. These observations are consistent with a model in which the hindbrain areas surrounding the NTS LepRb soma integrate signals from substantial numbers of LepRb neurons.

In summary, we have utilized the LepRb^{EGFP} mouse model to verify and characterize the anatomical localization of brain-wide LepRb expression. Furthermore, using the LepRb^{EGFPf} mouse model we have systematically analyzed the regions of the brain receiving innervation directly from LepRb neurons. This analysis has also identified regions of the brain containing substantially greater number of LepRb soma relative to projections and vice-versa, suggesting that some areas that contain soma with few projections serve primarily as sensing sites for leptin, while other regions containing dense soma and projections integrate information from LepRb neurons.

4. Methods

4.1 Animals

LepRb^{EGFP} (*LepR^{cre}; Gt(ROSA)26Sor^{tm2Sho}*) and LepRb^{EGFPf} (*LepR^{cre}; Gt(ROSA)26Sor^{EGFPf}*) mice (Figure 1) were bred in-house in the Unit for Laboratory Animal Medicine (ULAM) at the University of Michigan, as previously described (Leshan et al., 2009). All mice were housed in a 12h light/12h dark cycle, had *ad libitum* access to food and water. All care and procedures were in accordance with the guidelines and with the approval of the University of Michigan Committee on the Use and Care of Animals.

4.2 Perfusion and Immunolabeling

Forty week-old, female, LepRb^{EGFP} and LepRb^{EGFPf} mice were anesthetized with a lethal dose of intraperitoneal pentobarbital and immediately perfused with 0.1M PBS followed by 10% formalin. Brains were removed, postfixed, and cryoprotected as previously described (Munzberg et al., 2003). Coronal 30µm sections were collected from a sliding microtome in four representative series and stored in cryoprotectant at -20°C until use. For immunofluorescent (IF) labeling, free-floating sections were blocked in normal donkey serum and then incubated with chicken anti-EGFP (Abcam, 1:1000). Sections were then washed and incubated with donkey anti-chicken Alexa-488 (Cell Signaling, 1:200). Following washing, sections were mounted and coverslipped with ProLong Antifade mounting medium (Invitrogen).

4.3 Microscopy and Analysis

Multiple 4x images of each brain section were obtained using an Olympus BX-51 brightfield microscope with attached camera and merged using Adobe Photoshop software (Adobe Systems). For quantification of LepRb expressing neurons in LepRb^{EGFP} mice, the number of EGFP expressing neurons were counted and recorded for each brain region as defined by the Paxinos and Franklin mouse brain atlas (Paxinos and Franklin, 2001). Cell counts were performed on merged images and brain regions were categorized based on their number of EGFP expressing neurons. Similarly, LepRb projections were quantified using merged 4x images obtained from LepRb^{EGFPf} mice. Specific brain regions were defined as above and assessed for presence of EGFPf and were quantified using ImageJ software. Briefly, a 0.1 × 0.1 mm ROI was placed on the brightest region of each defined brain area and image density was measured by the image analysis software. Brain regions appearing in several sections were measured multiple times and the highest measurement was used for relative comparison between brain regions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

AVPe	anteroventral periventricular nucleus
Ac	anterior commissure
Aq	aqueduct
AP	area postrema
ARC	arcuate nucleus
BNST	bed nucleus of the stria terminalis

CeA	central nucleus of the amygdala
DMH	doromedial hypothalamic nucleus
DMV	dorsal motor nucleus of the vagus
DR	dorsal raphe
EW	Edinger-Westphal nucleus
EGFP	enhanced green fluorescent protein
EGFPf	farnesylated enhanced green fluorescent protein
icv	intracerebroventricular
ip	intraperitoneal
IR	immunoreactivity
LHA	lateral hypothalamic area
LPBN	lateral parabrachial nucleus
LepR	leptin receptor
LiR	linear raphe
LepRb	long form of the leptin receptor
NAc	nucleus accumbens
NTS	nucleus of the solitary tract
PVH	paraventricular nucleus
PAG	periaqueductal gray
PH	posterior hypothalamus
PFC	prefrontal cortex
POA	preoptic area
SN	substantia nigra
3v	third ventricle
PMv	ventral premamillary nucleus
VTA	ventral tegmental area

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Figure 1. *LepRb^{EGFP}* and *LepRb^{EGFPf}* reporter mice

a) Schematic diagram demonstrating cre-mediated EGFP and EGFPf expression in *LepRb* expressing cells of *LepRb^{EGFP}* and *LepRb^{EGFPf}* mice. In each line, cre-dependent excision of a transcription-blocking cassette induces the expression of EGFP or EGFPf in *LepRb* neurons of mice expressing cre recombinase from the *LepRb*-specific mRNA.

Figure 2. $\text{LepRb}^{\text{EGFP}}$ and $\text{LepRb}^{\text{EGFPf}}$ reporter mice reveal LepRb neurons and projections in the hypothalamus and surrounding regions

Representative fluorescent images (4x) of EGFP-IR of brain sections from $\text{LepRb}^{\text{EGFP}}$ (left panels) and $\text{LepRb}^{\text{EGFPf}}$ (right panels (')) mice in the mid-hypothalamus (A, A') at the level of the ARC and DMH and at the level of the PMv (B, B'). Higher magnification (10x) images of the indicated regions in A, A' show the DMH (Aa, Aa') and ARC (Ab, Ab'). Higher magnification (10X) images of the indicated regions in B, B' show the PMv (Ba, Ba'). 3v, third-ventricle.

Figure 3. LepRb^{EGFP} and LepRb^{EGFPf} reporter mice reveal LepRb neurons and projections in the midbrain

Representative fluorescent images (4x) of EGFP-IR of brain sections from LepRb^{EGFP} (first and third columns) and LepRb^{EGFPf} (second and fourth columns (')) mice in the midbrain (A, A') at the level of the VTA and at the level of the DR (B, B'). Higher magnification (10x) images of the indicated regions in A, A' show the LiR (Aa, Aa'), EW (Ab, Ab'), and VTA (Ac, Ac'). Higher magnification (10X) images of the indicated regions in B, B' show the DR (Ba, Ba') and PAG (Bb, Bb'). Aq, aqueduct.

Figure 4. LepRb^{EGFP} and LepRb^{EGFPf} reporter mice reveal LepRb neurons and projections in the hindbrain

Representative fluorescent images (4x) of EGFPf-IR of brain sections from LepRb^{EGFP} (left panels) and LepRb^{EGFPf} (right panels (')) mice in the hindbrain at the level of the LPBN (A, A') and at the level of the AP and NTS (B, B'). Higher magnification (10x) images of the indicated regions in A, A' show the LPBN (Aa, Aa'). Higher magnification (10X) images of the indicated regions in B, B' show the NTS (Ba, Ba'). Aq, aqueduct.

Figure 5. $\text{LepRb}^{\text{EGFP}}$ and $\text{LepRb}^{\text{EGFPf}}$ reporter mice reveal anatomical regions innervated by LepRb neurons and largely lacking LepRb soma

Representative fluorescent images (4x) of EGFP-IR of brain sections from $\text{LepRb}^{\text{EGFP}}$ (first and third columns) and $\text{LepRb}^{\text{EGFPf}}$ (second and fourth columns (')) mice in the rostral hypothalamus at the level of the POA and NAc (A, A'), at the level of the PVH (B, B') and the mid-hypothalamus/amygdala at the level of the CeA (C, C'). Higher magnification (10x) images of the indicated regions in A, A' show the POA (Aa, Aa'), and NAc (at the border with the BNST) (Ab, Ab'). Higher magnification (10X) images of the indicated regions in B, B' show the PVH (Ba, Ba'). Higher magnification (10X) images of the indicated regions in C, C' show the CeA (Ca, Ca'). 3v, third ventricle; Ac, anterior commissure.

Table 1

Localization of EGFP-IR soma in LepRb^{EGFP} reporter mice. Relative amounts of EGFP-IR LepRb soma in each brain area.

FOREBRAIN	EGFP
Clastrum	++
Insular Cortex	++
Temporal Cortex, Association Area	++
Somatosensory Cortex	+++
Auditory Cortex	+++
Visual Cortex	+
Perirhinal Cortex	+
Ectorhinal Cortex	+++
Dorsal Endopiriform Nucleus	+++
Ventral Endopiriform Nucleus	+++
Dentate Gyrus	+
CA1, CA2, CA3	+/-
Lateral Septal Nucleus	++
Bed Nucleus of the Stria Terminalis	++
Nucleus Accumbens	+/-
Central Amygdala	+
Substantia Innominata	+/-
Paraventricular Thalamic Nucleus	++
Parafascicular Thalamic Nucleus	+
Ventromedial Thalamic Nucleus	+
Subparafascicular Thalamic Nucleus	+
Ventral Anterior Thalamic Nucleus	+
Paraventricular Thalamic Nucleus	+
Submedial Thalamic Nucleus	+/-
Reuniens Thalamic Nucleus	++
Reticular Thalamic Nucleus	+/-
Anteromedial Thalamic Nucleus	+
Central Medial Thalamic Nucleus	+/-
Anterior Hypothalamus	+
Preoptic Nuclei	+++
Parastrial Nucleus	+
Anteroventral Periventricular Nuc	+/-
Arcuate Nucleus	++++
Dorsomedial Hypothalamus	++++
Lateral Hypothalamus	+++
Paraventricular Nucleus	+

FOREBRAIN	EGFP
Premammillary nucleus, ventral	++++
Ventromedial Hypothalamus	+++
median eminence	+/-
Posterior hypothalamus	+++
Supramammillary Nucleus	++
Perifornical area	++
Suprachiasmatic nucleus	+
Medial Tuberal Nucleus	+
Lateral Habenula	+/-
Zona Incerta	+
Medial Mammillary	+
ventral tuberomammillary nucleus	+/-
MIDBRAIN	EGFP
nucleus of brachium of inferior colliculus	+/-
commissure of inferior colliculus	+++
intermed geniculate nuc/superior colliculus	+
Ventral Tegmental Area	+++
Substantia Nigra	+++
Edinger-Westphal Nucleus	+++
Linear Raphe	++
Dorsal Raphe	+++
A8 Dopamine Cells	+
Median Raphe	+/-
Periaqueductal Gray	++++
Lateral Dorsal Tegmental Nucleus	+/-
red nucleus	+
raphe cap	+
Precommissural Nucleus	++
Cuneiform Nucleus	+
medial terminal nuc of acc optic tract	+/-
interfascicular nucleus	+
interpeduncular nucleus	+/-
Deep Mesencephalic Nucleus	+++
Supraoculomotor Cap	+
Supraoculomotor Central Gray	+
nucleus posterior commissure	+
parvocellular oculomotor nucleus	+/-
HINDBRAIN	EGFP
Pontine Reticular Formation, Oral	++

FOREBRAIN	EGFP
Medullary Reticular Nucleus, dorsal	+
Nucleus of the Solitary Tract	++
area postrema	+/-
Dorsal Motor Nucleus of Vagus Nerve	+
Hypoglossal Nucleus	+/-
Parabrachial Nucleus	+++
Parvicellular Reticular Nucleus	+
Spinal Trigeminal Nucleus	+/-
nucleus ambiguus	+/-
cuneate nucleus	+/-
Intermediate Reticular Nucleus	+
Subcoeruleus Nucleus, Dorsal Part	+/-
paralemniscal nucleus	+/-
nucleus intercalatus	+/-
rostromedial reticular nucleus	+/-
prepositus hypoglossal nucleus	+

++++, highest; +++, high; ++, medium; +, low; +/-, sparse/absent.

Table 2

Intensity of EGFPf-IR in LepRb^{EGFPf} reporter mice. Relative maximum density of EGFP-IR in each region by ImageJ quantification.

FOREBRAIN	EGFP
Clastrum	+++
Insular Cortex	++
Temporal Cortex, Association Area	++
Somatosensory Cortex	+
Auditory Cortex	++
Visual Cortex	+/-
Perirhinal Cortex	+
Ectorhinal Cortex	+
Dorsal Endopiriform Nucleus	+++
Ventral Endopiriform Nucleus	+++
Dentate Gyrus	+
CA1, CA2, CA3	++
Lateral Septal Nucleus	+
Bed Nucleus of the Stria Terminalis	++
Nucleus Accumbens	+++
Central Amygdala	++
Substantia Innominata	+
Paraventricular Thalamic Nucleus	+
Parafascicular Thalamic Nucleus	+/-
Ventromedial Thalamic Nucleus	+/-
Subparafascicular Thalamic Nucleus	+/-
Ventral Anterior Thalamic Nucleus	+/-
Paraventricular Thalamic Nucleus	+
Submedial Thalamic Nucleus	+/-
Reuniens Thalamic Nucleus	+
Reticular Thalamic Nucleus	+/-
Central Medial Thalamic Nucleus	+/-
Anterior Hypothalamus	++
Preoptic Nuclei	+++
Parastrial Nucleus	+
Anteroventral Periventricular Nuc	+++
Arcuate Nucleus	++++
Dorsomedial Hypothalamus	+++
Lateral Hypothalamus	++
Paraventricular Nucleus	+++
Premammillary nucleus, ventral	++++

FOREBRAIN	EGFP
Ventromedial Hypothalamus	++
median eminence	++++
Posterior hypothalamus	++
Perifornical area	++
Suprachiasmatic nucleus	++
Medial Tuberal Nucleus	+/-
Lateral Habenula	+/-
Zona Incerta	+/-
Medial Mammillary	++
ventral tuberomammillary nucleus	+/-
MIDBRAIN	EGFP
nucleus of brachium of inferior colliculus	+/-
commissure of inferior colliculus	+
intermed geniculate nuc/superior colliculus	++
Ventral Tegmental Area	+++
Substantia Nigra	+++
Edinger-Westphal Nucleus	+++
Linear Raphe	+++
Dorsal Raphe	++++
A8 Dopamine Cells	+
Median Raphe	+/-
Periaqueductal Gray	++++
Lateral Dorsal Tegmental Nucleus	+/-
red nucleus	+/-
raphe cap	++
Precommissural Nucleus	+/-
Cuneiform Nucleus	+
medial terminal nuc of acc optic tract	+/-
interfascicular nucleus	+++
interpeduncular nucleus	++
Deep Mesencephalic Nucleus	++
Supraoculomotor Cap	+
Supraoculomotor Central Gray	+
nucleus posterior commissure	+/-
parvocellular oculomotor nucleus	+
HINDBRAIN	EGFP
Pontine Reticular Formation, Oral	+/-
Medullary Reticular Nucleus, dorsal	+/-
Nucleus of the Solitary Tract	+++

FOREBRAIN	EGFP
area postrema	+/-
Dorsal Motor Nucleus of Vagus Nerve	+
Hypoglossal Nucleus	+
Parabrachial Nucleus	++
Parvicellular Reticular Nucleus	+
Spinal Trigeminal Nucleus	+/-
nucleus ambiguus	++
cuneate nucleus	+/-
Intermediate Reticular Nucleus	+
Subcoeruleus Nucleus, Dorsal Part	+/-
paralemniscal nucleus	+/-
nucleus intercalatus	+
rostromedial reticular nucleus	+/-
prepositus hypoglossal nucleus	+

++++, highest; +++, high; ++, medium; +, low; +/-, sparse/absent.