Published in final edited form as:

J Comp Neurol. 2013 January 1; 521(1): 50–68. doi:10.1002/cne.23157.

On Lateral Septum-Like Characteristics of Outputs From the Accumbal Hedonic “Hotspot” of Peciña and Berridge With Commentary on the Transitional Nature of Basal Forebrain “Boundaries”

Daniel S. Zahm*, Kenneth P. Parsley, Zachary M. Schwartz, and Anita Y. Cheng

Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, St. Louis, Missouri 63104

Abstract

Peciña and Berridge (2005; *J Neurosci* 25:11777–11786) observed that an injection of the μ -opioid receptor agonist DAMGO (D-ala²-N-Me-Phe⁴-Glycol⁵-enkephalin) into the rostromedial part of the accumbens shell (rdAcbSh) enhances expression of hedonic “liking” responses to the taste of an appetitive sucrose solution. Insofar as the connections of this hedonic “hotspot” were not singled out for special attention in the earlier neuroanatomical literature, we undertook to examine them. We observed that the patterns of inputs and outputs of the rdAcbSh are not qualitatively different from those of the rest of the Acb, except that outputs from the rdAcbSh to the lateral preoptic area and anterior and lateral hypothalamic areas are anomalously robust and overlap extensively with those of the lateral septum. We also detected reciprocal interconnections between the rdAcbSh and lateral septum. Whether and how these connections subserve hedonic impact remains to be learned, but these observations lead us to hypothesize that the rdAcbSh represents a basal forebrain transition area, in the sense that it is invaded by neurons of the lateral septum, or possibly transitional neuronal forms sharing properties of both structures. We note that the proposed transition zone between lateral septum and rdAcbSh would be but one of many in the basal forebrain and conclude by reiterating the longstanding argument that the transitional nature of such boundary areas has functional importance, of which the precise nature will remain elusive until the neurophysiological and neuropharmacological implications of such zones of transition are more generally acknowledged and better addressed.

Keywords

accumbens; lateral preoptic area; lateral hypothalamic area; anterior hypothalamic area; reward; feeding; ventral tegmental area

The accumbens (Acb), an input structure of the ventral striatopallidum (Heimer and Wilson, 1975), comprises core, shell (Zaborszky et al., 1985; Heimer et al., 1991b), and rostral pole (Zahm and Brog, 1992; Heimer and Zahm, 1993) subterritories variably concerned with mechanisms of locomotor activation, novelty detection, reward anticipation, and response reinforcement (Kelly et al., 1975; Schultz et al., 1997; Wise, 2004; Zahm, 2000, 2008). The

© 2012 Wiley Periodicals, Inc

*CORRESPONDENCE TO: D. S. Zahm, Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, 1402 S. Grand Blvd., St. Louis, MO 63104. zahmds@slu.edu.

Author contributions

K.P.P. performed the surgeries and processed the tissue, Z.M.S. and A.Y.C. plotted the sections and analyzed the data, D.S.Z. conceived the experiments and, together with K.P.P., Z.M.S., and A.Y.C., wrote the article.

Acb also contributes to hedonic-aversive controls on ingestive behavior. Infusion of μ -opioid receptor agonist into the Acb stimulates vigorous feeding in sated rats (Mucha and Iversen, 1986; Evans and Vaccarino, 1990; Bakshi and Kelley, 1993; Kelley et al., 1996, 2002), which has suggested to some researchers that the release of opioid peptides in the Acb mediates, at least in part, the rewarding or pleasurable attributes of feeding (Cooper and Kirkham, 1990; Evans and Vaccarino, 1990). Consistent with this notion, injection of the μ -opioid receptor agonist DAMGO (D-ala²-N-Me-Phe⁴-Glycol⁵-enkephalin) selectively into the rostradorsal part of the medial shell of the accumbens (rdAcbSh) significantly promotes the expression of hedonic “liking” responses emitted by rats in response to the taste of an appetitive sucrose solution (Peciña and Berridge, 2005). Similarly, a conditioned place preference and stimulated intake preferentially of appetitive food accompanies intra-Acb infusion of muscimol, a γ -aminobutyric acid-A type (GABA_A) receptor agonist, or DNQX (6,7-dinitroquinoxaline-2,3(1H,4H)-dione), an antagonist of the glutamate AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid)-kainate receptor (Reynolds and Berridge, 2001, 2002, 2003). Berridge and colleagues accordingly referred to the effective site, which is coextensive with the rdAcbSh, as a hedonic “hot spot” (Peciña and Berridge, 2005; Peciña et al., 2006; Faure et al., 2010). In contrast, infusion of these compounds into the caudodorsal quadrant of the medial shell of the Acb (cdAcbSh) produced suppression, not only of hedonic “liking” responses to sucrose, but also “disliking” responses to quinine. Injections of muscimol and DNQX into the cdAcbSh also incited rats to fearful posturing and defensive actions such as treading and burying (Reynolds and Berridge, 2001, 2002).

The functional specificity observed by Berridge and colleagues would not readily be predicted from Acb inputs, which, throughout the Acb, including the rdAcbSh, come from the same collection of structures, mainly the basal amygdala, subiculum, prefrontal cortex, midline, and intralaminar thalamic nuclei and midbrain dopaminergic and serotonergic complexes (McGeorge and Faull, 1989; Brog et al., 1993; Voorn et al., 2004). The basic organization of Acb is such that the combination of inputs to any of its parts differs only topographically from that to adjacent parts. This remains true despite the fact that in some parts of the Acb afferents terminate in complicated patterns of overlapping and nonoverlapping patches (Pennartz et al., 1994) of which input–output relationships are but partially worked out (e.g., Berendse et al., 1992). The cdAcbSh is exceptional in receiving some inputs not present in other parts of the Acb that ascend from the caudal brainstem and rostral spinal cord (Cliffer et al., 1991; Brog et al., 1993; Delfs et al., 1998; Brown and Moliver, 2000). In addition, the cdAcbSh merges without perceptible boundary with the bed nucleus of stria terminalis, a part of the extended amygdala (Alheid and Heimer, 1989; Heimer et al., 1991b; Heimer and Alheid, 1991; Zahm, 1998).

Outputs from the Acb likewise are both topographically (Swanson and Cowan, 1975; Conrad and Pfaff, 1976; Powell and Leman, 1976; Williams et al., 1977; Troiano and Seigel, 1978; Nauta et al., 1978; Mogenson et al., 1983; Groenewegen and Russchen, 1984; Haber et al., 1990; Heimer et al., 1991; Zahm and Heimer, 1993; Kirouac and Ganguly, 1995; Usuda et al., 1998) and compartmentally (Berendse et al., 1992) organized. Those originating in dorsal and lateral parts of the Acb, mainly in the core subterritory, project in topographic fashion to the ventromedial globus pallidus, ventral pallidum, subthalamic nucleus, and substantia nigra in a quite basal ganglia-like manner (Zahm and Heimer, 1990, 1993; Heimer et al., 1991b; Usuda et al., 1998). Projections from the Acb medial shell similarly ramify in a very dense, basal ganglia-like fashion within the ventral pallidum, but then, in a manner uncharacteristic of any “classical” basal ganglia structure, descend in the medial forebrain bundle through the lateral preoptic area, lateral hypothalamus, ventral mesencephalon, midbrain paramedian zone, and periaqueductal gray (Nauta et al., 1978; Groenewegen and Russchen, 1984; Heimer et al., 1991b; Heimer and Zahm, 1993; Usuda et

al., 1998). Within all of these structures descending Acb fibers are decorated with moderate numbers of axonal varicosities, signifying likely synaptic function.

Interestingly, the connections of the part of the Acb lately designated as a hedonic “hotspot” were not singled out for special attention in earlier neuroanatomical literature, and thus the striking functional specificity reported by Berridge and colleagues may have a heretofore unrecognized anatomical correlate. Accordingly, we undertook to examine these connections and observed that the rdAcbSh and lateral septum are reciprocally interconnected and outputs from the rdAcbSh converge in the lateral preoptic area with those of the lateral septum. These relationships suggest to us that the rdAcbSh represents a basal forebrain transition area in the sense that it is invaded by lateral septal neurons or transitional neuronal forms sharing properties of both structures. The findings reported herein and how we have interpreted them are quite different from those described in another recently published report on the connections of the rdAcbSh (Thompson and Swanson, 2010).

MATERIALS AND METHODS

Male Sprague–Dawley rats (Harlan, Indianapolis, IN) weighing 225–300 g were used in accordance with guidelines mandated in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. The rats were housed on a 12-hour light/dark cycle in groups of four until surgeries were performed, after which they were singly housed. Access to food and water was provided ad libitum to all rats throughout the study. Unless stated otherwise, chemicals were purchased from Sigma Chemical (St. Louis, MO).

The study comprised an analysis of cases in which rats received injections of anterograde or retrograde axonal tracer. Representative cases were mapped with the aid of a dedicated hardware-software system (NeuroLucida, MBF Bioscience, Williston VT). Anterograde tracing was evaluated mainly in cases that received injections of *Phaseolus vulgaris*-leucoagglutinin (PHA-L, see below) and, in just a few cases, biotinylated dextran amine (BDA, see below). For evaluation of outputs from the rdAcbSh, 22 new cases with injections of PHA-L were prepared, of which nine had injection sites in the rdAcbSh and the remainder served as controls. In addition, 18 archived cases with Acb injections of PHA-L or BDA were studied, of which three and two had injection sites located in the cdAcbSh and rdAcbSh, respectively. Outputs from the lateral septum were studied in 22 archived cases with injections of PHA-L or BDA. Retrograde tracing was done with FluoroGold (FG, see below) and comprised eight new cases in which the tracer was deposited in the dorsomedial lateral preoptic area, 11 new cases in which the tracer was deposited in the ventromedial lateral preoptic area, and 12 archived cases in which the tracer was placed in the lateral septum. Seven archived cases in which FG was deposited in the lateral septum were evaluated.

Tracer injections

Rats were deeply anesthetized by intraperitoneal injections of a mixture of ketamine (72 mg/kg) and xylazine (11.2 mg/kg), administered as a cocktail consisting of 45% ketamine (100 mg/ml), 35% xylazine (20 mg/ml), and 20% physiological saline at a dose of 0.16 ml/100g of body weight. The anesthetized rats were placed in a Kopf stereotaxic instrument, the skulls were exposed, and small bore holes were created to allow the Acb, lateral septum, or lateral preoptic area to be targeted by filament-containing borosilicate glass pipettes (O.D., 1.0 mm). The pipettes were pulled to tip diameters of 10–25 μ m and contained an anterograde tracer, either PHA-L (Vector Laboratories, Burlingame, CA; 2.5% in 0.01 M phosphate buffer, injected with tips of 10 μ m) or BDA (Molecular Probes, Eugene, OR; 10% in 0.01 M phosphate buffer, injected with tips of 20–25 μ m), or the retrograde tracer

FluoroGold (FG; Fluorochrome, Englewood, CO; 1% in 0.1 M cacodylate buffer, pH 7.4, injected with tips of 15–18 μm). A silver wire inserted into the pipettes contacted the solution containing the tracer, which was ejected into the brain substance using positive current pulses (7 sec on, 7 sec off, for 15 min) of 4, 3, and 1 μA (for PHA-L, BDA, and FG, respectively). After surgery the rats were kept warm until they awakened.

Fixation and sectioning of brains

Ten days after PHA-L injections, 5 days after BDA injections, and 3 days after FG injections the rats were deeply anesthetized (as above) and perfused transaortically, first with 0.01 M Sorensen's phosphate buffer (SPB; pH 7.4) containing 0.9% sodium chloride and 2.5% sucrose, followed by 0.1 M SPB (pH 7.4) containing 4% paraformaldehyde (PFA) and 2.5% sucrose. The brains were removed, postfixed, infiltrated with 25% sucrose, and sectioned frozen at 50 μm . Five adjacent series of sections were collected, with each thus reflecting the structure of the entire brain from frontal pole to caudal medulla in sections spaced at intervals of 250 μm . Each series of sections was stored in a separate glass vial at -20°C in a cryoprotectant consisting of SPB containing 30% sucrose (by weight) and 30% ethylene glycol (by volume). Descriptions of the antibodies used in the study are given below and in Table 1.

Descriptions of antibodies

Rabbit polyclonal antibody against FG (hydroxystilbamidine) was purchased as antibody-containing serum without preservative (cat. no. AB153) from Chemicon, now Bioscience Research Reagents, a division of Millipore (Temecula, CA). The vendor states that AB153 also reacts with aminostilbamidine in frozen, 4% PFA-fixed tissues. In our hands, sections from brains that have not received FG injections are devoid of reaction product when processed with AB153 at a range of dilutions and, in brains that have received injections of the tracer, immunostaining is observed only at the injection sites, in retrogradely labeled neurons, and, occasionally, microglial cells, which, however, are easily distinguished by morphology from labeled neurons.

Rabbit polyclonal antibody against nitric oxide synthase (NOS) provided in 0.01 M phosphate-buffered saline, pH 7.4, containing 15 mM sodium azide (cat. no. N7155) was supplied by the Sigma Chemical. According to the vendor, it was raised against a synthetic peptide corresponding to amino acids 251–270 (GDNDRVFNDLWGKDNVPVILC) of NOS of rat brain origin conjugated to KLH (Riveros-Moreno et al., 1993). In our hands, the antibody stains rat brain sections in a manner consistent with literature descriptions of the distribution of brain NOS immunoreactivity (e.g., Rodrigo et al., 1994).

Goat polyclonal antibody raised against *Phaseolus vulgaris* agglutinin (E+L) was purchased (cat. no. AS-2224) from Vector Laboratories. According to the vendor, this antibody is produced by hyperimmunizing goats with the pure lectin. Following conventional purification steps, specific antibody was isolated by affinity chromatography on lectin-agarose columns and supplied lyophilized in buffered saline. Sections from brains that have not received PHA-L injections are devoid of reaction product when immunoprocessed with AS-2224 at a range of dilutions and, in brains that have received PHA-L injections, immunostaining is observed only at the injection sites and in anterogradely labeled axons.

Antibody against substance P (SP) was purchased from ImmunoStar (Hudson, WI) as a rabbit polyclonal antibody generated against a conjugate of synthetic peptide and carbodiimide/keyhole limpet hemocyanin (Cat. no. 20064). According to the supplier, SP immunolabeling was completely abolished by preadsorption with SP, whereas the following peptides resulted in no reduction of staining: neurokinin A, neurokinin B, somatostatin, and

neuropeptide K. In our hands, the antibody stains rat brain sections in a manner consistent with literature descriptions of the distribution of brain SP immunoreactivity (e.g., Cuello et al., 1979).

Antibody against tyrosine hydroxylase (TH) was purchased from the Bioscience Research Reagents (Chemicon) division of Millipore and is a mouse monoclonal antibody purified from PC12 cells (cat. no. MAB318). The antibody is supplied as ascites fluid with 3% bovine serum albumin (BSA) and no preservative. According to the vendor, the antibody recognizes an epitope on the outside of the regulatory N-terminus of TH. In western blots, the antibody recognizes a protein of ≈ 59 –63 kDa. It does not react with the following on western blots: dopamine-beta-hydroxylase, phenylalanine hydroxylase, tryptophan hydroxylase, dehydropteridine reductase, sepiapterin reductase, or phenethanolamine-N-methyl transferase. In our hands, the anti-TH antibody stains rat brain sections in a manner fully consistent with literature descriptions (e.g., Lindvall and Björklund, 1983; Hökfelt et al., 1984).

Immunohistochemistry

Prior to processing with immunohistochemical or ABC (see below) reagents, all series of sections were pretreated by immersion in 1% aqueous sodium borohydride for 15 minutes followed by thorough rinsing. One series of sections from each case was immersed in SPB containing 0.1% Triton X-100 (SPB-t) and polyclonal antibodies raised against PHA-L made in goat and used at a dilution of 1:10,000. The following day, after thorough rinsing in SPB-t, the sections were immersed for an hour in SPB-t containing biotinylated antibodies made in donkey against goat at a dilution of 1:200 (Jackson ImmunoResearch Laboratories, West Grove, PA). Afterward, the sections were rinsed in SPB and then immersed in SPB containing avidin-biotin-peroxidase complex (ABC, Vector Laboratories) at a dilution of 1:200, also for an hour. Cases with BDA injections were placed immediately in SPB-t containing ABC reagents at a dilution of 1:200. For cases with injections of FG the sections were immersed overnight in 0.1 M SPB-t containing anti-FG (Chemicon International; cat. no. AB153), made in rabbit at a dilution of 1:5,000. The following day the sections were rinsed in 0.1 M SPB-t and immersed for 1 hour in 0.1 M SPB containing biotinylated antibody made in donkey against rabbit IgGs at a dilution of 1:200 (Jackson Laboratories). After additional thorough rinsing in SPB, the sections were immersed for 20–30 minutes in 0.05 M SPB (pH 7.4) containing 0.05% DAB (3,3'-diaminobenzidine), 0.04% ammonium chloride, 0.2% β -D-glucose, and 0.0004% glucose oxidase, which generates an insoluble brown reaction product, or, if the sections were destined to be reacted with a second primary antibody, in 0.025 M Tris buffer (pH 8.0) containing 0.015% DAB, 0.4% nickel ammonium sulfate, and 0.003% hydrogen peroxide (DAB-glucose oxidase), which generates an insoluble black reaction product.

Immunoperoxidase counterstains, including against SP, which emphasizes ventral pallidum, and nitric oxide synthase (NOS), which delineates various structures, such as for example the bed nucleus of stria terminalis, subnucleus extended amygdala, and supraoptic nucleus, were utilized to facilitate visualization of forebrain neuroanatomical organization. Thus, sections intended for additional immunocytochemical processing were then further rinsed in SPB and immersed in SPB-t containing anti-NOS or anti-SP made in rabbit or anti-TH made in mouse, used at dilutions of 1:5,000, and 1:15,000, respectively. The following morning the sections were rinsed in SPB-t and immersed for 1 hour in SPB-t containing a donkey antibody against rabbit or mouse IgGs, as appropriate, each used at a dilution of 1:200 (Jackson). Following further rinsing in SPB the sections were immersed for 1 hour in SPB containing, respectively, mouse or rabbit peroxidase-anti-peroxidase (PAP) at a dilution of 1:3,000 (MP Biomedicals, Solon, OH), after which they were again rinsed thoroughly. Then the sections were immersed for 20–30 minutes in DAB-glucose oxidase (brown reaction

product) or 0.025 M Tris buffer (pH 8.0) containing 0.015% DAB and 0.003% hydrogen peroxide (also brown) and, after rinsing, mounted onto gelatin-coated slides, dehydrated through a series of ascending concentrations of ethanol, transferred into xylene, and coverslipped with Permount (Fisher, Pittsburgh, PA).

Maps and photomicrographs

Retrograde and anterograde labeling was plotted in representative frontal sections throughout the central nervous system (CNS; excluding spinal cord) with the 10× or 20× objective under brightfield optics (Nikon Optiphot) with the aid of the NeuroLucida dedicated hardware-software platform. Anterograde tracing was mapped using the NeuroLucida system mainly in a manner such that only axonal varicosities, i.e., puncta, were plotted to the exclusion of nonvaricose, presumably mainly nonsynaptic, parts of labeled axons. Varicosities were recognized as distinct, punctate swellings or dilatations of PHA-L filled axons that often were more intensely immunoreactive than adjacent nonvaricose parts of labeled axons (see Zahm et al., 2011b, for more details). Digital micrographs were captured with a Q Imaging Fast 1394 digital camera and adjusted mainly for brightness and contrast with Adobe Photoshop (CS2; San Jose, CA) software. The plates were constructed using Adobe Illustrator (CS2) software.

RESULTS

Injections of anterograde tracer into the rdAcbSh

Insofar as no markers that we know of objectively identify a ventral boundary of the hedonic “hotspot,” we arbitrarily designated the dorsomedial one-quarter of the Acb shell as rdAcbSh (Fig. 1). Cases with injections of PHA-L in the rdAcbSh (Fig. 1A,B) exhibited robust labeling in the ventral pallidum, lateral preoptic area, and lateral hypothalamus (Fig. 2A–H, left column). Following such injections, the main descending collection of labeled axons occupied the medial forebrain bundle, from which one contingent of labeled fibers could be seen arching into the ventromedial part of the lateral preoptic area and adjacent medial preoptic area (Figs. 2C–E, left column, and 3A–H), whereas another diverged dorsomedially, nearly to the hypothalamic paraventricular nucleus (PVN). The area between these distinct concentrations of labeled fibers contained a less dense, but nonetheless substantial, accumulation of labeled axons, such that the entire lateral preoptic area and an adjacent lateral part of the medial preoptic area was reached by abundant outputs from the rdAcbSh (Figs. 2C–E, left column, and 3C–H). Axons comprising these labeled projections were highly branched and varicose, consistent with synaptic potency (Fig. 3A–H). The numbers of such labeled fibers decreased in the transition from lateral preoptic area to lateral hypothalamus and continued to decrease incrementally through the entire length of the hypothalamus (Fig. 2F–H). The overall pattern of anterograde labeling produced by injections into the rdAcbSh much resembled that observed following injections of anterograde tracer into the intermediate division of the lateral septum (Fig. 2A–G, insets).

By comparison, injections of anterograde tracer into the Acb shell in sites ventral to the rdAcbSh (Fig. 1C) produced robust anterograde labeling in the ventral pallidum (Fig. 2A, right column) and lateral extremity of the lateral preoptic area (Fig. 2B–H, right column), but almost no labeled fibers diverging into the ventromedial and dorsomedial parts of the lateral preoptic area. This point was emphasized by our use of BDA, which typically labels more projections than PHA-L, to prepare the case illustrated by Figure 2A–M, right column. Despite the robust transport properties of BDA, the case exhibits negligible spread of tracer to medial parts of the lateral preoptic / lateral hypothalamic continuum.

In contrast to the anomalously dense projections from the rdAcbSh to the lateral preoptic area and lateral hypothalamus, the pattern of labeled projections to the brainstem was similar irrespective of what part of the Acb shell was injected with tracer, i.e., all injections into the rostromedial shell produced at least moderate numbers of varicose labeled fibers in the midbrain dopaminergic complex (Figs. 2I–M, left and right columns, and 4). All of the injection sites shown in Figure 1 produced labeling that was more dense in the rostral than caudal half of the ventral tegmental area, sparse in the substantia nigra, and near negligible in the retrorubral area (Fig. 4).

Control injections of retrograde tracer in the lateral preoptic area

To further validate the impression that the output of the rdAcbSh substantially overlaps that of the lateral septum, FG was injected into ventromedial (Fig. 5) and dorsomedial (Fig. 6) parts of the lateral preoptic area, where labeling was dense following injections of anterograde tracer into the rdAcbSh. Injections of FG in both sites produced robust labeling of both the lateral septum and rdAcbSh (Figs. 5, 6). These data, in addition, render the possibility that minimized anterograde labeling in medial districts of the preoptic area is due to uptake of PHA-L by lateral septal projections that traverse the rdAcbSH.

Reciprocal connections of the Acb shell and lateral septum

Injection of PHA-L into the rdAcbSh produced moderate anterograde labeling in the lateral septum (Fig. 2A, left column) that was not observed following injections of tracer into other Acb sites. Consistent with this finding, injections of FG into the lateral septum produce retrogradely labeled neurons in the rdAcbSH (archived cases, data not shown).

Alternatively, injections of PHA-L into the lateral septum produced moderate labeling in the accumbens shell, including the rdAcbSh (Fig. 7), together with robust anterograde labeling in a variety of additional basal forebrain structures, including the medial septum–diagonal band complex, ventral pallidum, preoptic area, and lateral hypothalamus (Fig. 2A–G, insets), as has also been reported by others (see Discussion). Injections of FG into the rdAcbSh, but not the Acb core or lateral shell, accordingly produce numerous retrogradely labeled neurons in lateral septum (see Figs. 7, 9, and 11 in Brog et al., 1993).

Caudodorsal Acb shell (cdAcbSh)

Cases with injections into the cdAcbSh were also processed, but these injection sites invariably included the caudal extent of the rdAcbSh. The main projection labeled by such injections much resembled that seen after rdAcbSh injections. The most rostral part of the projection labeled by such injections was a robust input to the ventral pallidum. Beyond this the labeled projection occupied the lateral extremity of the lateral preoptic area from which a few labeled fibers arched ventromedialward and dorsomedialward within the lateral preoptic area. The main bundle of labeled fibers continued its descent through the lateral hypothalamus to not only the rostral ventral tegmental area, but also its caudal half (that has sparse labeled projections following injections into the rostral parts of the Acb shell). After caudomedial shell injections, moderate numbers of labeled fibers occupied the midbrain paramedian zone, from which some entered the periaqueductal gray.

DISCUSSION

The present study was done to detect a morphological correlate of the rdAcbSh hedonic “hotspot” described by Pecina and Berridge (2005). We observed that the rdAcbSh does indeed exhibit connectivity distinguishing it from other parts of the Acb. Specifically, projections from the rdAcbSh are anomalously robust in the lateral preoptic area. Efferents from other parts of the Acb shell innervate the lateral preoptic area moderately and do not reach its medial part at all, being instead constrained within the lateral extremity of the

lateral preoptic area (Heimer et al., 1991b; Usuda et al., 1998; Thompson and Swanson, 2010). In contrast, particularly dense projections from the “hotspot” occupy ventromedial and dorsomedial sectors of the lateral preoptic area and the area between them (to a lesser extent), as well as the lateral part of the medial preoptic area.

Interestingly, the distribution pattern of the “hotspot” projection in the lateral preoptic area almost perfectly overlies that of the lateral septum, although rdAcbSh projections are less dense. Furthermore, the striking overlap of the lateral septal and rdAcbSh projection fields extends into the lateral hypothalamus and continues throughout its length, although at diminishing fiber densities at incrementally more caudal levels. Consistent with the idea that the rdAcbSh, lateral septum, and lateral preoptic area form a connectional network, anatomical evidence was provided nearly 30 years ago for an enkephalinergic (opioid agonist) pathway from the ventromedial extremity of the lateral preoptic area to the lateral septum (Sakanaka et al., 1982) and, nearly 20 years ago, from the lateral preoptic area to the Acb shell (Brog et al., 1993). Convergence of lateral septal and Acb hedonic “hotspot” projections in the lateral preoptic area suggests that both contribute to the control of lateral preoptic area neuronal activation. That they may do this coordinately is consistent with the second main finding of our study, that the Acb hedonic “hotspot” and lateral septum are reciprocally interconnected, which further supports the notion that the lateral septum, rdAcbSh, and lateral preoptic / lateral hypothalamic continuum comprise a connectional network. Interrogation of all of the various components of this proposed network with both anterograde and retrograde tracers and utilization of small pipette tips and low ejection currents (see discussions in Brog et al., 1993; Geisler et al., 2005; Zahm et al., 2011b) renders the possibility negligible that the experimental basis upon which the network is proposed involves fiber of passage artifacts.

Numerous investigators in addition to Berridge and colleagues (Mucha and Iversen, 1986; Evans and Vaccarino, 1990; Bakshi and Kelley, 1993; Kelley et al., 1996, 2002) have reported that widespread Acb sites stimulate feeding when infused with opioid and GABA agonists and AMPA antagonists. Facilitation of feeding produced by infusions of the aforementioned compounds into the Acb depends on disinhibition of lateral hypothalamic feeding centers (Will et al., 2003; Kelley et al., 2005), which, when stimulated, elicit ravenous, autonomous feeding (Bernardis and Bellinger, 1996). The lateral septum has also long been recognized as an important node in the circuitry subserving feeding and energy balance (Fried, 1972; Vasudev et al., 1985; Flynn et al., 1986; Stanley et al., 1989; Wang and Kotz, 2002). Vigorous feeding in sated rats, also attributable to disinhibition of the lateral hypothalamus (Oliveira et al., 1990), has been reported following lesions (Koppell and Sodetz, 1972) and infusions of various compounds, including opioid agonists (Stanley et al., 1988), into the lateral septum (Wang and Kotz, 2002; Scopinho et al., 2008). Although frequently regarded as conceptually archaic, lateral hypothalamic and, by extension, lateral septal and accumbens feeding sites unquestionably exist. However, much remains to be learned about how these sites actually elicit feeding or interact with peripheral feeding signals and manifold other central feeding sites and pathways, such as those in the medial hypothalamus (see, e.g., Saper et al., 2002).

rdAcbSh vs. cdAcbSh

According to Berridge and colleagues, the rostral and caudal quadrants of the dorsomedial Acb shell are functionally distinct. Infusion of the rdAcbSh hedonic “hotspot” with an opioid agonist, GABA agonist, or AMPA antagonist enhances responses to hedonic stimuli (Peciña and Berridge, 2005; Peciña et al., 2006; Faure et al., 2010). In contrast, similar treatments of the cdAcbSh produce behavioral patterns animated by fear and defense posturing (Reynolds and Berridge, 2001, 2002) and suppress affective “liking” responses to

hedonic gustatory stimuli and also, interestingly, “disliking” responses to aversive taste stimuli (Peciña and Berridge, 2005).

Projections from the Acb to the lateral preoptic area arise in the rdAcbSh and cdAcbSh and, thus, although those from the cdAcbSh appear weaker, these projections considered alone do not distinguish the rdAcbSh from the cdAcbSh. What may distinguish these two ventral striatal subterritories is reflected in the interdigitation of and transitional nature of boundaries between distinct basal forebrain functional-anatomical entities (Alheid and Heimer, 1988; Heimer et al., 1991a; Heimer and Alheid, 1991). More specifically, the cdAcbSh directly abuts the rostral part of the extended amygdala, represented by the bed nucleus of stria terminalis (Alheid et al., 1994; Zahm, 1998, 2006, 2008), and, where this occurs, the two structures share afferent connections, are penetrated by local (intrinsic) axonal connections belonging to the other, and harbor projection neurons that either belong to the other or possess transitional properties intermediate between the two. Moreover, the cdAcbSh is a recipient of long ascending projections from the rostral spinal cord and caudal brainstem also present in extended amygdala (e.g., Sofroniew, 1983), but not elsewhere in the Acb (Cliffer et al., 1991; Brog et al., 1993; Delfs et al., 1998; Brown and Moliver, 2000). Together, these features distinguish the connections and neurochemical composition of the cdAcbSh from those of the rdAcbSh.

The transition area between the cdAcbSh of the Acb and bed nucleus of stria terminalis likely plays a significant role in mediating behavioral responses elicited following infusions of compounds into the cdAcbSh. Indeed, the relatively small size of the cdAcbSh makes it difficult to conceive that even small volumes of compounds infused there do not to some extent diffuse into the bed nucleus proper, so that behaviors observed following such infusions would invariably reflect some degree of bed nucleus involvement. Numerous reports implicate the bed nucleus of stria terminalis in behavioral responses to fear-arousing stimuli and anxiety (Onaka and Yagi, 1998; Moreira et al., 2012), including but not limited to light enhancement of startle, freezing to a fear-eliciting context, and suppression of punished behaviors (Walker and Davis, 1997; Davis et al., 1997; Davis and Shi, 1999; Resstel et al., 2008). Suppression of hedonic and aversive taste responses following stimulation of the cdAcbSh by infusion of DAMGO (Peciña and Berridge, 2005) may simply reflect behavioral inhibition that seems to be a cardinal function of the bed nucleus of stria terminalis. It can be noted further that a role for the bed nucleus in defensive burying behavior also has been proposed (Linfoot et al., 2009) and it is reasonable in this regard to speculate that active forms of fearful and defensive posturing (Reynolds and Berridge, 2001) might emerge from infusions of compounds in a manner that influences both the bed nucleus and Acb (see, e.g., Reynolds et al., 2001, 2002), the Acb long being considered to contribute to motivated actions (Mogenson et al., 1980; Mogenson, 1984).

Interconnections between the Acb and lateral septum

Perhaps these also reflect an admixing of elements of two distinct basal forebrain structures, lateral septum and Acb shell, along a transitional boundary (see Figs. 5D–F, 6D–G). In this point of view, neurons of the lateral septum, or transitional forms sharing properties of both structures, occupy the rdAcbSh and the demonstrated interconnections between the two structures reflect accordingly displaced intrinsic connections known to characterize both lateral septum (Jakab and Leranth, 1995) and Acb (Heimer et al., 1991b; van Dongen et al., 2005, 2008). Insofar as the lateral septum itself projects strongly to the lateral preoptic area (Raisman, 1966; Berk and Finkelstein, 1981; Staiger and Wouterlood, 1990; Staiger and Nuernberger, 1991; Risold and Swanson, 1997; Fig. 2A–G, insets), those parts of the Acb shell with efferent projections to the lateral preoptic area would logically be those thought to house lateral septum-like neurons.

Hedonic impact

It remains to consider the site-specificity that characterizes the ability of infusions of appropriate compounds into the rdAcbSh to facilitate hedonic impact of palatable food (Peciña and Berridge, 2005). This capacity, which supersedes reinforcement associated with feeding simply to reduce hunger, may reflect the actions of rdAcbSh projections to the ventral midbrain, either direct or via relays in the lateral preoptic / lateral hypothalamic continuum or other structures, such as, e.g., the rostromedial tegmental nucleus (Jhou et al., 2009; Zahm et al., 2011a). These projections could mediate disinhibition of ventral tegmental area dopamine neurons and thus produce a reinforcing bump in the release of dopamine in the Acb, an effect that could be dampened by recruitment of the cdAcbSh, which contains the aforementioned admixture of aversion-responsive neurons.

Alternatively, rdAcbSh control over hedonic impact may reflect the operation in toto of the interconnected network consisting of the rdAcbSh, lateral septum, lateral preoptic area, and ventral mesencephalon. In view of the reported role of the lateral septum in modulating reward perception (reviewed in Fried, 1972; Sheehan et al., 2004), particularly with regard to the tendency of lateral septum lesions to promote “over-responding to positively motivating stimuli” (Fried, 1972), it seems reasonable that interconnectivity with lateral septum may contribute to the observed capacity of the rostral dorsomedial Acb shell to modulate expressions of hedonic impact. It is also relevant to this line of thought that Aldridge, Berridge, and colleagues (Tindell et al., 2004, 2005, 2006; Smith et al., 2009, 2011, and references cited therein) have studied a second hedonic “hotspot” said to be located in the posterior part of the ventral pallidum that may actually significantly involve the lateral preoptic area or a transitional merging of the ventral pallidum and lateral preoptic area (Becker et al., 2009). This arrangement is consistent with the idea that hedonic impact is modulated by a network constituting the rdAcbSh, lateral septum, and lateral preoptic area.

CONCLUSION

We have presented some ideas in the preceding paragraphs about how certain freshly detected and some well-known neuroanatomical relationships might inform regarding functional parceling in the dorsal tier of the Acb shell. These hypotheses emerge from a new appreciation of rdAcbSh connections described herein and are contingent upon experimental validation. These new data and ideas complement those provided in other reports addressing distinct functional and neuroanatomical features in the rostral and caudal accumbens (e.g., Voorn and Docter, 1992; Rinaldi and Beninger, 1994; Zimmerman et al., 1999; Martin et al., 2002). In addition, other explanations for the segregation of functional specificity in the dorsal Acb shell have been offered in various articles (e.g., Will et al., 2003; Peciña and Berridge, 2005), mainly based on readings of the then extant neuroanatomical literature. Among these, Thompson and Swanson (2010) proffer a reductionist approach to the problem based on what the present and much published data cited herein suggest is a misrepresentation of the relevant connectivity. In brief, Thompson and Swanson (2010) emphasize the density of rdAcbSh projections to the anterior hypothalamic area, rather than the preoptic area, and deny that the rdAcbSh projects to the midbrain dopaminergic complex at all (but see our Figs. 2I–M, left column, and 4). Nor do our published (Brog et al., 1993) and unpublished data agree that midbrain projections to the rdAcbSh arise only in the interfascicular nucleus, as they assert. Also, our unpublished cases indicate that the prefrontocortical input to the rdAcbSh arises in infralimbic, prelimbic, anterior cingulate, and orbital areas, and not predominantly in the infralimbic area, as Thompson and Swanson (2010) indicate. To summarize, our data indicate that, aside from abundant projections to the lateral preoptic area and moderate interconnections with lateral septum, the connections of the rdAcbSh do not otherwise deviate significantly from the well-known, topographic

patterns of input and output present throughout the Acb (e.g., Nauta et al., 1978; Groenewegen and Russchen, 1984; McGeorge and Faull, 1989; Zahm and Heimer, 1990; Heimer et al., 1991b; Heimer and Zahm, 1993; Brog et al., 1993; Usuda et al., 1998; and others cited in the introduction).

In closing, we would like to revisit the idea that the functional specificity of the Acb hedonic “hotspot” may correlate with its conceptualization as a transition area. So designated, the rdAcbSh would in no way be unique. The transitional nature of the cdAcbSh has been noted (see also Alheid et al., 1994). Indeed, transitional areas are pervasive in basal forebrain, intervening between dorsal and ventral striatum (Heimer and Wilson, 1975) and the accumbens and olfactory tubercle (Heimer, 1972). The lateral preoptic-hypothalamic continuum exhibits transitional merging with the ventral pallidum, entopeduncular nucleus, and subthalamic nucleus (Heimer et al., 1985; Becker et al., 2009) and with the bed nucleus of stria terminalis and central nucleus of amygdala (Zahm et al., 2011a). We thus would like to emphasize the general importance of the neuroanatomically transitional nature of boundary areas between adjacent basal forebrain structures (again, see also Alheid et al., 1994). Until the functional natures of such zones of transition are adequately explained in neurophysiological and neuropharmacological terms, understanding will remain elusive regarding how basal forebrain subserves cooperation and competition among various brain structures in the synthesis of adaptive behavior (Zahm, 2006, 2008; Heimer et al., 2008).

Acknowledgments

U.S. Public Health Service (USPHS)

NS-23805

National Institutes of Health (NIH)

The authors thank Willis K. (Rick) Samson for critically reading an earlier version of the article.

LITERATURE CITED

- Alheid GF, Heimer L. New perspectives in basal forebrain organization of special relevance for neuropsychiatric disorders: the striatopallidal, amygdaloid and corticopetal components of substantia innominata. *Neuroscience*. 1988; 27:1–39. [PubMed: 3059226]
- Alheid, GF.; Beltramino, C.; Braun, A.; Miselis, RR.; François, C.; de Olmos, JS. Transition areas of the striatopallidal system and extended amygdala in the rat and primate: observations from histochemistry and experiments with monoand transsynaptic tracer. In: Percheron, G.; McKenzie, JS.; Féger, J., editors. *New ideas and data on structure and function*. Vol. 41. Plenum Press; New York: 1994. p. 95-107. The basal ganglia IV
- Bakshi VP, Kelley AE. Striatal regulation of morphine-induced hyperphagia: an anatomical mapping study. *Psychopharmacology (Berl)*. 1993; 111:207–214. [PubMed: 7870954]
- Becker ML, Parsley KP, Lee TJ, Zahm DS. Dorsal and ventral parts of the lateral septum have the character, respectively, of striatopallidum and extended amygdala medial division. *Soc Neurosci Abstr*. 2009;661.19.
- Berendse HW, Groenewegen HJ. Organization of the thalamostriatal projections in the rat, with special emphasis on the ventral striatum. *J Comp Neurol*. 1990; 299:187–228. [PubMed: 2172326]
- Berendse HW, Galis-de Graaf Y, Groenewegen HJ. Topographical organization and relationship with ventral striatal compartments of prefrontal corticostriatal projections in the rat. *J Comp Neurol*. 1992; 316:314–347. [PubMed: 1577988]
- Berk ML, Finkelstein JA. Afferent projections to the preoptic area and hypothalamic regions in the rat brain. *Neuroscience*. 1981; 6:1601–1624. [PubMed: 7266881]
- Bernardis LL, Bellinger LL. The lateral hypothalamic area revisited: ingestive behavior. *Neurosci Biobehav Rev*. 1996; 20:189–287. [PubMed: 8811717]

- Brog JS, Salyapongse A, Deutch AY, Zahm DS. The patterns of afferent innervation of the core and shell in the “accumbens” part of the rat ventral striatum: immunohistochemical detection of retrogradely transported fluoro-gold. *J Comp Neurol.* 1993; 338:255–278. [PubMed: 8308171]
- Brown P, Molliver ME. Dual serotonin (5-HT) projections to the nucleus accumbens core and shell: relation of the 5-HT transporter to amphetamine-induced neurotoxicity. *J Neurosci.* 2000; 20:1952–1963. [PubMed: 10684896]
- Cliffer KD, Burstein R, Giesler GJ Jr. Distributions of spinothalamic, spinohypothalamic, and spinotelencephalic fibers revealed by anterograde transport of PHA-L in rats. *J Neurosci.* 1991; 11:852–868. [PubMed: 1705972]
- Conrad LC, Pfaff DW. Autoradiographic tracing of nucleus accumbens efferents in the rat. *Brain Res.* 1976; 113:589–596. [PubMed: 953754]
- Cooper, SJ.; Kirkham, RC. Basic mechanisms of ‘opioids’ effect on eating and drinking. In: Reid, L., editor. *Opioids, bulimia and alcohol abuse & alcoholism.* Springer; New York: 1990. p. 91-110.
- Cuello AC, Galfre G, Milstein C. Detection of substance P in the central nervous system by a monoclonal antibody. *Proc Natl Acad Sci U S A.* 1979; 76:3532–3536. [PubMed: 386341]
- Davis M, Shi C. The extended amygdala: are the central nucleus of the amygdala and the bed nucleus of the stria terminalis differentially involved in fear versus anxiety? *Ann N Y Acad Sci.* 1999; 877:281–291. [PubMed: 10415655]
- Davis M, Walker DL, Lee Y. Roles of the amygdala and bed nucleus of the stria terminalis in fear and anxiety measured with the acoustic startle reflex. Possible relevance to PTSD. *Ann N Y Acad Sci.* 1997; 821:305–331. [PubMed: 9238214]
- Delfs JM, Zhu Y, Druhan JP, Aston-Jones GS. Origin of noradrenergic afferents to the shell subregion of the nucleus accumbens: anterograde and retrograde tract-tracing studies in the rat. *Brain Res.* 1998; 806:127–140. [PubMed: 9739125]
- Evans KR, Vaccarino FJ. Amphetamine- and morphine-induced feeding: evidence for involvement of reward mechanisms. *Neurosci Biobehav Rev.* 1990; 14:9–22. [PubMed: 2325945]
- Faure A, Richard JM, Berridge KC. Desire and dread from the nucleus accumbens: cortical glutamate and subcortical GABA differentially generate motivation and hedonic impact in the rat. *PLoS One.* 2010; 5:e11223. [PubMed: 20585461]
- Flynn FW, Evey LA, Steele TL, Mitchell JC. The relation of feeding and activity following septal lesions in rats. *Behav Neurosci.* 1986; 100:416–421. [PubMed: 3730150]
- Fried PA. Septum and behavior: a review. *Psychol Bull.* 1972; 78:292–310. [PubMed: 4628196]
- Geisler S, Zahm DS. Afferents of the ventral tegmental area in the rat-anatomical substratum for integrative functions. *J Comp Neurol.* 2005; 490:270–294. [PubMed: 16082674]
- Groenewegen HJ, Russchen FT. Organization of the efferent projections of the nucleus accumbens to pallidal, hypothalamic, and mesencephalic structures: a tracing and immunohistochemical study in the cat. *J Comp Neurol.* 1984; 223:347–367. [PubMed: 6323552]
- Groenewegen HJ, Vermeulen-Van der Zee E, te Kortschot A, Witter MP. Organization of the projections from the subiculum to the ventral striatum in the rat. A study using anterograde transport of Phaseolus vulgaris leucoagglutinin. *Neuroscience.* 1987; 23:103–120. [PubMed: 3683859]
- Haber SN, Lynd E, Klein C, Groenewegen HJ. Topographic organization of the ventral striatal efferent projections in the rhesus monkey: an anterograde tracing study. *J Comp Neurol.* 1990; 293:282–298. [PubMed: 19189717]
- Heimer L. The olfactory connections of the diencephalon in the rat. An experimental light- and electron-microscopic study with special emphasis on the problem of terminal degeneration. *Brain Behav Evol.* 1972; 6:484–523. [PubMed: 4662205]
- Heimer, L.; Alheid, GF. Piecing together the puzzle of basal forebrain anatomy. In: Napier, TC.; Kalivas, PW.; Hanin, I., editors. *The basal forebrain: anatomy to function.* Plenum Press; New York: 1991. p. 1-42.
- Heimer, L.; Wilson, RD. The subcortical projections of allocortex: similarities in the neuronal associations of the hippocampus, the piriform cortex and the neocortex. In: Santini, M., editor. *Golgi Centennial Symposium Proceedings.* Raven Press; New York: 1975. p. 173-193.

- Heimer, L.; Alheid, GF.; Zaborszky, L. Basal ganglia. In: Paxinos, G., editor. Forebrain and midbrain. Vol. 1. Academic Press; Sydney: 1985. p. 37-86. The rat nervous system
- Heimer L, de Olmos JS, Alheid GF, Zaborszky L. "Perestroika" in the basal forebrain: opening the border between neurology and psychiatry. *Prog Brain Res.* 1991a; 87:109–165. [PubMed: 1866444]
- Heimer L, Zahm DS, Churchill L, Kalivas PW, Wohltmann C. Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience.* 1991b; 41:89–125. [PubMed: 2057066]
- Heimer, L.; Van Hoesen, GW.; Trimble, M.; Zahm, DS. The new anatomy of the basal forebrain and its implications for neuropsychiatric illness. Elsevier; Amsterdam: 2008. Anatomy of neuropsychiatry.
- Hökfelt, T.; Mårtensson, R.; Björklund, A.; Kleinau, S.; Goldstein, M. Distribution maps of tyrosine-hydroxylase-immunoreactive neurons in the rat brain. In: Björklund, A.; Hökfelt, T., editors. Classical transmitters in the CNS. Handbook of chemical neuroanatomy. Vol. 2. Elsevier; Amsterdam: 1984. p. 277-379. Part I
- Jakab, RL.; Leranth, C. Septum. In: Paxinos, G., editor. The rat nervous system. Academic Press; San Diego: 1995. p. 405-442.
- Kelley AE, Domesick VB. The distribution of the projection from the hippocampal formation to the nucleus accumbens in the rat: an anterograde- and retrograde-horseradish peroxidase study. *Neuroscience.* 1982; 7:2321–2335. [PubMed: 6817161]
- Kelly PH, Seviour PW, Iversen SD. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res.* 1975; 94:507–522. [PubMed: 1171714]
- Kelley AE, Domesick VB, Nauta WJ. The amygdalostratial projection in the rat—an anatomical study by anterograde and retrograde tracing methods. *Neuroscience.* 1982; 7:615–630. [PubMed: 7070669]
- Kelley AE, Bless EP, Swanson CJ. Investigation of the effects of opiate antagonists infused into the nucleus accumbens on feeding and sucrose drinking in rats. *J Pharmacol Exp Ther.* 1996; 278:1499–1507. [PubMed: 8819538]
- Kelley AE, Bakshi VP, Haber SN, Steininger TL, Will MJ, Zhang M. Opioid modulation of taste hedonics within the ventral striatum. *Physiol Behav.* 2002; 76:365–377. [PubMed: 12117573]
- Kelley AE, Baldo BA, Pratt WE, Will MJ. Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. *Physiol Behav.* 2005; 86:773–795. [PubMed: 16289609]
- Kirouac GJ, Ganguly PK. Topographical organization in the nucleus accumbens of afferents from the basolateral amygdala and efferents to the lateral hypothalamus. *Neuroscience.* 1995; 67:625–630. [PubMed: 7675191]
- Koppell S, Sodetz FJ. Septal ablation in the rat and bar pressing under appetitive and aversive control. *J Comp Physiol Psychol.* 1972; 81:274–280. [PubMed: 5084444]
- Lindvall, O.; Björklund, A. Dopamine- and norepinephrine-containing neuron systems: Their anatomy in the rat brain. In: Emson, PC., editor. Chemical neuroanatomy. Raven Press; New York: 1983. p. 229-256.
- Linfoot I, Gray M, Bingham B, Williamson M, Pinel JP, Viau V. Naturally occurring variations in defensive burying behavior are associated with differences in vasopressin, oxytocin, and androgen receptors in the male rat. *Prog Neuropsychopharmacol Biol Psychiatry.* 2009; 33:1129–1140. [PubMed: 19540895]
- Martin TJ, Kim SA, Lyupina Y, Smith JE. Differential involvement of mu-opioid receptors in the rostral versus caudal nucleus accumbens in the reinforcing effects of heroin in rats: evidence from focal injections of beta-funaltrexamine. *Psychopharmacology (Berl).* 2002; 161:152–159. [PubMed: 11981595]
- McGeorge AJ, Faull RL. The organization of the projection from the cerebral cortex to the striatum in the rat. *Neuroscience.* 1989; 29:503–537. [PubMed: 2472578]
- Mogenson, GJ. Limbic-motor integration — with emphasis on initiation of exploratory and goal-directed locomotion. In: Bandler, R., editor. Modulation of sensorimotor activity during alterations in behavioral states. Liss; New York: 1984. p. 121-137.

- Mogenson GJ, Jones DL, Yim CY. From motivation to action: functional interface between the limbic system and the motor system. *Prog Neurobiol.* 1980; 14:69–97. [PubMed: 6999537]
- Mogenson GJ, Swanson LW, Wu M. Neural projections from nucleus accumbens to globus pallidus, substantia innominata, and lateral preoptic-lateral hypothalamic area: an anatomical and electrophysiological investigation in the rat. *J Neurosci.* 1983; 3:189–202. [PubMed: 6822855]
- Moreira FA, Aguiar DC, Resstel LB, Lisboa SF, Campos AC, Gomes FV, Guimaraes FS. Neuroanatomical substrates involved in cannabinoid modulation of defensive responses. *J Psychopharmacol.* 2012; 26:40–55. [PubMed: 21616976]
- Mucha RF, Iversen SD. Increased food intake after opioid microinjections into nucleus accumbens and ventral tegmental area of rat. *Brain Res.* 1986; 397:214–224. [PubMed: 3026557]
- Nauta WJ, Smith GP, Faull RL, Domesick VB. Efferent connections and nigral afferents of the nucleus accumbens septi in the rat. *Neuroscience.* 1978; 3:385–401. [PubMed: 683502]
- Oliveira LA, Gentil CG, Covian MR. Role of the septal area in feeding behavior elicited by electrical stimulation of the lateral hypothalamus of the rat. *Braz J Med Biol Res.* 1990; 23:49–58. [PubMed: 2386849]
- Onaka T, Yagi K. Role of noradrenergic projections to the bed nucleus of the stria terminalis in neuroendocrine and behavioral responses to fear-related stimuli in rats. *Brain Res.* 1998; 788:287–293. [PubMed: 9555060]
- Peciña S, Berridge KC. Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? *J Neurosci.* 2005; 25:11777–11786. [PubMed: 16354936]
- Peciña S, Smith KS, Berridge KC. Hedonic hot spots in the brain. *Neuroscientist.* 2006; 12:500–511. [PubMed: 17079516]
- Pennartz CM, Groenewegen HJ, Lopes da Silva FH. The nucleus accumbens as a complex of functionally distinct neuronal ensembles: an integration of behavioural, electrophysiological and anatomical data. *Prog Neurobiol.* 1994; 42:719–761. [PubMed: 7938546]
- Powell EW, Leman RB. Connections of the nucleus accumbens. *Brain Res.* 1976; 105:389–403. [PubMed: 816427]
- Raisman G. The connexions of the septum. *Brain.* 1966; 89:317–348. [PubMed: 5939044]
- Ranaldi R, Beninger RJ. Rostral-caudal differences in effects of nucleus accumbens amphetamine on VTA ICSS. *Brain Res.* 1994; 642:251–258. [PubMed: 8032886]
- Resstel LB, Alves FH, Reis DG, Crestani CC, Correa FM, Guimaraes FS. Anxiolytic-like effects induced by acute reversible inactivation of the bed nucleus of stria terminalis. *Neuroscience.* 2008; 154:869–876. [PubMed: 18479825]
- Reynolds SM, Berridge KC. Fear and feeding in the nucleus accumbens shell: rostrocaudal segregation of GABA-elicited defensive behavior versus eating behavior. *J Neurosci.* 2001; 21:3261–3270. [PubMed: 11312311]
- Reynolds SM, Berridge KC. Positive and negative motivation in nucleus accumbens shell: bivalent rostrocaudal gradients for GABA-elicited eating, taste “liking”/“disliking” reactions, place preference/avoidance, and fear. *J Neurosci.* 2002; 22:7308–7320. [PubMed: 12177226]
- Reynolds SM, Berridge KC. Glutamate motivational ensembles in nucleus accumbens: rostrocaudal shell gradients of fear and feeding. *Eur J Neurosci.* 2003; 17:2187–2200. [PubMed: 12786986]
- Risold PY, Swanson LW. Connections of the rat lateral septal complex. *Brain Res Brain Res Rev.* 1997; 24:115–195. [PubMed: 9385454]
- Riveros-Moreno V, Beddell C, Moncada S. Nitric oxide synthase: structural studies using anti-peptide antibodies. *Eur J Biochem.* 1993; 215:801–808. [PubMed: 7689053]
- Rodrigo J, Springall DR, Uttenthal O, Bentura ML, Abadia-Molina F, Riveros-Moreno V, Martínez-Murillo R, Polak JM, Moncada S. Localization of nitric oxide synthase in the adult rat brain. *Philos Trans R Soc Lond B Biol Sci.* 1994; 345:175–221. [PubMed: 7526408]
- Russchen FT, Price JL. Amygdalostriatal projections in the rat. Topographical organization and fiber morphology shown using the lectin PHA-L as an anterograde tracer. *Neurosci Lett.* 1984; 47:15–22. [PubMed: 6087218]
- Sakanaka M, Senba E, Shiosaka S, Takatsuki K, Inagaki S, Takagi H, Kawai Y, Hara Y, Tohyama M. Evidence for the existence of an enkephalin-containing pathway from the area just ventrolateral to

- the anterior hypothalamic nucleus to the lateral septal area of the rat. *Brain Res.* 1982; 239:240–244. [PubMed: 6178464]
- Saper CB, Chou TC, Elmquist JK. The need to feed: homeostatic and hedonic control of eating. *Neuron.* 2002; 36:199–211. [PubMed: 12383777]
- Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. *Science.* 1997; 275:1593–1599. [PubMed: 9054347]
- Scopinho AA, Resstel LB, Correa FM. alpha(1)-Adrenoceptors in the lateral septal area modulate food intake behaviour in rats. *Br J Pharmacol.* 2008; 155:752–756. [PubMed: 18641672]
- Sheehan TP, Chambers RA, Russell DS. Regulation of affect by the lateral septum: implications for neuropsychiatry. *Brain Res Rev.* 2004; 46:71–117. [PubMed: 15297155]
- Smith KS, Tindell AJ, Aldridge JW, Berridge KC. Ventral pallidum roles in reward and motivation. *Behav Brain Res.* 2009; 196:155–167. [PubMed: 18955088]
- Smith KS, Berridge KC, Aldridge JW. Disentangling pleasure from incentive salience and learning signals in brain reward circuitry. *Proc Natl Acad Sci USA.* 2011; 108:E255–264. [PubMed: 21670308]
- Sofroniew MV. Direct reciprocal connections between the bed nucleus of the stria terminalis and dorsomedial medulla oblongata: evidence from immunohistochemical detection of tracer proteins. *J Comp Neurol.* 1983; 213:399–405. [PubMed: 6833532]
- Staiger JF, Nurnberger F. The efferent connections of the lateral septal nucleus in the guinea pig: projections to the diencephalon and brainstem. *Cell Tissue Res.* 1991; 264:391–413. [PubMed: 1868517]
- Staiger JF, Wouterlood FG. Efferent projections from the lateral septal nucleus to the anterior hypothalamus in the rat: a study combining Phaseolus vulgaris-leucoagglutinin tracing with vasopressin immunocytochemistry. *Cell Tissue Res.* 1990; 261:17–23. [PubMed: 2383883]
- Stanley BG, Lanthier D, Leibowitz SF. Multiple brain sites sensitive to feeding stimulation by opioid agonists: a cannula-mapping study. *Pharmacol Biochem Behav.* 1988; 31:825–832. [PubMed: 3252274]
- Swanson LW, Cowan WM. A note on the connections and development of the nucleus accumbens. *Brain Res.* 1975; 92:324–330. [PubMed: 1236758]
- Thompson RH, Swanson LW. Hypothesis-driven structural connectivity analysis supports network over hierarchical model of brain architecture. *Proc Natl Acad Sci U S A.* 2010; 107:15235–15239. [PubMed: 20696892]
- Tindell AJ, Berridge KC, Aldridge JW. Ventral pallidal representation of pavlovian cues and reward: population and rate codes. *J Neurosci.* 2004; 24:1058–1069. [PubMed: 14762124]
- Tindell AJ, Berridge KC, Zhang J, Pecina S, Aldridge JW. Ventral pallidal neurons code incentive motivation: amplification by mesolimbic sensitization and amphetamine. *Eur J Neurosci.* 2005; 22:2617–2634. [PubMed: 16307604]
- Tindell AJ, Smith KS, Pecina S, Berridge KC, Aldridge JW. Ventral pallidum firing codes hedonic reward: when a bad taste turns good. *J Neurophysiol.* 2006; 96:2399–2409. [PubMed: 16885520]
- Troiano R, Siegel A. Efferent connections of the basal forebrain in the cat: the nucleus accumbens. *Exp Neurol.* 1978; 61:185–197. [PubMed: 308010]
- Usuda I, Tanaka K, Chiba T. Efferent projections of the nucleus accumbens in the rat with special reference to subdivision of the nucleus: biotinylated dextran amine study. *Brain Res.* 1998; 797:73–93. [PubMed: 9630528]
- van Dongen YC, Deniau JM, Pennartz CM, Galis-de Graaf Y, Voorn P, Thierry AM, Groenewegen HJ. Anatomical evidence for direct connections between the shell and core subregions of the rat nucleus accumbens. *Neuroscience.* 2005; 136:1049–1071. [PubMed: 16226842]
- van Dongen YC, Mailly P, Thierry AM, Groenewegen HJ, Deniau JM. Three-dimensional organization of dendrites and local axon collaterals of shell and core medium-sized spiny projection neurons of the rat nucleus accumbens. *Brain Struct Funct.* 2008; 213:129–147. [PubMed: 18239939]
- Vasudev R, Gentil CG, Covian MR. Taste preferences in a free-choice situation following electrical stimulation and lesion of septal area in rats. *Physiol Behav.* 1985; 34:619–624. [PubMed: 4011743]

- Voorn P, Docter GJ. A rostrocaudal gradient in the synthesis of enkephalin in nucleus accumbens. *Neuroreport*. 1992; 3:161–164. [PubMed: 1623166]
- Voorn P, Vanderschuren LJ, Groenewegen HJ, Robbins TW, Pennartz CM. Putting a spin on the dorsal-ventral divide of the striatum. *Trends Neurosci*. 2004; 27:468–474. [PubMed: 15271494]
- Walker DL, Davis M. Double dissociation between the involvement of the bed nucleus of the stria terminalis and the central nucleus of the amygdala in startle increases produced by conditioned versus unconditioned fear. *J Neurosci*. 1997; 17:9375–9383. [PubMed: 9364083]
- Wang C, Kotz CM. Urocortin in the lateral septal area modulates feeding induced by orexin A in the lateral hypothalamus. *Am J Physiol Regul Integr Comp Physiol*. 2002; 283:R358–367. [PubMed: 12121849]
- Will MJ, Franzblau EB, Kelley AE. Nucleus accumbens mu-opioids regulate intake of a high-fat diet via activation of a distributed brain network. *J Neurosci*. 2003; 23:2882–2888. [PubMed: 12684475]
- Williams DJ, Crossman AR, Slater P. The efferent projections of the nucleus accumbens in the rat. *Brain Res*. 1977; 130:217–227. [PubMed: 884524]
- Wise RA. Dopamine, learning and motivation. *Nat Rev Neurosci*. 2004; 5:483–494. [PubMed: 15152198]
- Zaborszky L, Alheid GF, Beinfeld MC, Eiden LE, Heimer L, Palkovits M. Cholecystokinin innervation of the ventral striatum: a morphological and radioimmunological study. *Neuroscience*. 1985; 14:427–453. [PubMed: 3887206]
- Zahm DS. Is the caudomedial shell of the nucleus accumbens part of the extended amygdala? A consideration of connections. *Crit Rev Neurobiol*. 1998; 12:245–265. [PubMed: 9847057]
- Zahm DS. An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. *Neurosci Biobehav Rev*. 2000; 24:85–105. [PubMed: 10654664]
- Zahm DS. The evolving theory of basal forebrain functional-anatomical ‘macrosystems’. *Neurosci Biobehav Rev*. 2006; 30:148–172. [PubMed: 16125239]
- Zahm, DS. Accumbens in a functional-anatomical systems context. In: David, HN., editor. *The nucleus accumbens: neurotransmitters and related behaviours*. Research Signpost; Trivandrum, Kerala, India: 2008. p. 1-36.
- Zahm DS, Brog JS. On the significance of subterritories in the “accumbens” part of the rat ventral striatum. *Neuroscience*. 1992; 50:751–767. [PubMed: 1448200]
- Zahm DS, Heimer L. Specificity in the efferent projections of the nucleus accumbens in the rat: comparison of the rostral pole projection patterns with those of the core and shell. *J Comp Neurol*. 1993; 327:220–232. [PubMed: 8425943]
- Zahm DS, Arends JJA, Parsley KP, Geisler S. Projection to the mesopontine rostromedial tegmental nucleus from the lateral preoptic area. *Soc Neurosci Abstr*. 2011a:79.09.
- Zahm DS, Cheng AY, Lee TJ, Ghobadi CW, Schwartz ZM, Geisler S, Parsley KP, Gruber C, Veh RW. Inputs to the midbrain dopaminergic complex in the rat, with emphasis on extended amygdala-recipient sectors. *J Comp Neurol*. 2011b; 519:3159–3188. [PubMed: 21618227]
- Zimmermann P, Privou C, Huston JP. Differential sensitivity of the caudal and rostral nucleus accumbens to the rewarding effects of a H1-histaminergic receptor blocker as measured with place-preference and self-stimulation behavior. *Neuroscience*. 1999; 94:93–103. [PubMed: 10613500]

Figure 1.

Diagrams illustrating more rostral (front section) and caudal (back section) levels of three injections of anterograde tracer further illustrated in Figures 2-4. Case numbers are provided at the top of each front section. The extent of diffuse spread of the injection sites is encircled, with the densest diffuse immunolabeling represented by the shaded areas. Dots represent tracer-impregnated neurons which are thought to give rise to anterogradely labeled fibers. **A,B:** Representative PHA-L injection sites in the rostradorsal shell of the accumbens (rdAcbSh, red), designated as approximately the dorsomedialmost quarter of the accumbens shell (AcbSh). Anterograde labeling resulting from the injection shown in A is illustrated in Figures 2A–M, left panels, and 3. That for the injection shown in B is illustrated in Figure 4. **C:** A representative biotinylated dextran amine (BDA) injection located in the accumbens shell (AcbSh) beneath the rostradorsal sector. Anterograde labeling resulting from the injection shown in C is illustrated in Fig. 2A–M, right panels. BDA gives rise to a more widespread constellation of impregnated neurons, most of which in C are in the shell.

Figure 2.

Diagrams (A–M) and photomicrographs (A–G, insets) showing a series of sequentially more caudal frontal levels through the rat brain illustrating the distributions of anterogradely labeled fibers in representative cases that received injections of tracer into the rostradorsal Acb shell (A–M, left column, injection site shown in Fig. 1A), accumbens shell beneath the rostradorsal sector (A–M, right column, injection site shown in Fig. 1C) and, for comparison, the intermediate division of the lateral septum (A–G, insets; the injection site is shown in the inset in A, inset). Case numbers and injection site locations are provided at the top of each of the three columns. The diagrams comprise dots that represent exclusively labeled axonal varicosities, which is where synapses tend to be located on axons. Note that anterograde labeling in A–G, left column, but not A–G, right column, spreads medialward from the lateral part of the lateral preoptic area (ILPO) into the ventromedial (vm) and dorsomedial (dm) parts of the LPO (A–D, left column) and anterior hypothalamic area (AHA, E,F, right column) so as to be distributed coextensively with the outputs from the lateral septum (same panels, insets). Immunoperoxidase counterstains done on different series of sections from the same cases and shown in the insets are against substance P (A, inset), which emphasizes ventral pallidum (VP), and nitric oxide synthase (NOS) (B–G, insets), which enhances various structures, such as, e.g., the bed nucleus of stria terminalis (BST), subnucleus of extended amygdala (SLEA), and supraoptic nucleus (SON). Labeling in the VP, ILPO, and lateral extremity of the lateral hypothalamic area (LH) is present after injections of anterograde tracer in the all parts of the Acb shell, including the rostradorsal hedonic hotspot (A–G, both columns). The density of anterograde labeling diminishes somewhat in the caudal hypothalamus (G,H). Note also that both accumbens shell injections give rise to similar patterns of anterograde labeling in the ventral mesencephalon (I–M, left column, and I–M, right column). Boxes 3A in left column A, 3B in left column B, 3C and 3D in left column C, 3E and 3F in left column D, and 3G and 3H in left column E are shown as micrographs of the indicated areas and identified by the same tags in Figure 3. aq,

cerebral aqueduct; cp, cerebral peduncle; IPN, interpeduncular nucleus; MB, mammillary body; ml, medial lemniscus; MPO, medial preoptic area; PAG, periaqueductal gray; RRF, retrorubral field; SNc, substantia nigra compacta; SNr, substantia nigra reticulata; VTA, ventral tegmental area. Scale bars = 1 mm in A inset (applies to insets A–G).

Figure 3.

Photomicrographs showing the actual anterograde labeling illustrated in the boxes in Figure 2A–E. **A–H:** Corresponds to the tags associated with the boxes in Figure 2. Arrows in A,B indicate varicose anterogradely labeled axons, which are also abundant, although not indicated, in columns C–H. As noted in the text, only the varicosities were plotted in Figure 2. Note that the dense terminations of labeled axons in ventral pallidum (VP) in the upper right corner in A,B transition into dense anterograde labeling in the lateral extremity of the lateral preoptic area (ILPO) which occupies the upper right corners in D,F. This labeling is present after injections of anterograde tracer in the all parts of the Acb shell, including the rostradorsal hedonic hotspot. Anterograde labeling of axons in the dorsomedial and ventrolateral lateral preoptic area (dm and vl LPO, respectively) is characteristic of the rostradorsal Acb shell and, perhaps to a lesser extent, the caudodorsal shell. Stained neurons at the bottom of D,F reflect anti-NOS immunoperoxidase counterstain of the suproptic nucleus (SON). AHA, anterior hypothalamic area; f, fornix; ot, optic tract. Scale bar = 250 μ m.

Figure 4.

Anterograde (PHA-L) labeling shown diagrammatically in a rostrocaudally ordered series of sections (A–F) through the ventral mesencephalon in the case in which the PHA-L injection site is illustrated in Figure 1B. The case number (11102) is provided at the top of the rostralmost section (A). The shading in each section illustrates the area of the section rich in tyrosine hydroxylase immunoreactivity, which was traced from a serially adjacent section and added to the current section with the aid of Adobe Illustrator software. Note that nearly all of the labeling occupies the shaded area, confirming that the rostradorsal shell projects to the midbrain dopaminergic complex. This case is representative of 10 others done in the study in which PHA-L injections exclusively involving the rostradorsal Acb shell also gave rise to a pattern of anterograde labeling in the ventral midbrain comparable to that shown in this figure. cp, cerebral peduncle; IPN, interpeduncular nucleus; ml, medial lemniscus; RRF, retrorubral field; SNc, substantia nigra pars compacta; VTA, ventral tegmental area.

Figure 5.

Retrograde labeling in the forebrain following an injection of the retrograde tracer FluoroGold into the ventromedial part of the lateral preoptic area (vmLPO, shaded in **I**). Each dot represents one retrogradely labeled neuron. Note the dense patch of labeling in the rostradorsal Acb shell (rdAcbSh) in **A–D** and dense labeling in the lateral septum (LS, **E–H**). Dense labeling in the ventral division of the lateral septum (LSv) and medial division of the bed nucleus of stria terminalis (BSTm) reflect the involvement of the medial preoptic area in the injection site.

Figure 6.

Retrograde labeling in the forebrain following an injection of FluoroGold into the dorsomedial part of the lateral preoptic area (dmLPO, shaded in **I**). Each dot represents one retrogradely labeled neuron. Note the dense patch of labeling in the rostradorsal Acb shell (rdAcbSh) in **A–F** and dense labeling in the lateral septum (LS, **E–H**). Note that this injection site also produces retrograde labeling in the caudodorsal accumbens shell (cdAcbSh, **G,H**).

Figure 7.

Diagrams of sections through the forebrain arranged in rostrocaudal sequence with **A** most rostral showing a PHA-L injection site in the lateral septum (shading, **B–F**) and anterograde labeling in the rostradorsal and caudodorsal accumbens shell (rdAcbSh and cdAcbSh, respectively). Note also in **F** heavy anterograde labeling in rostral part of the lateral preoptic area (LPO) that extends into the ventral pallidum (VP).

Table 1**Primary Antibodies**

Antiserum	Immunogen	Source (cat. no.)	Working dilution
Rabbit polyclonal anti-Fluorogold (FG)	Fluorogold itself	Bioscience Research Reagents, a division of Millipore, Temecula, CA (AB153)	1:5,000 – 1:10,000
Rabbit polyclonal anti-nitric oxide synthase (Nos)	Amino acids 251–270 of nitric oxide synthase (GDNDRVFNDLWGKDNVPVILC) conjugated to keyhole limpet cyanin	Sigma Chemical, St. Louis, MO (N7155)	1:5,000 – 1:10,000
Rabbit polyclonal anti- <i>Phaseolus vulgaris</i> leucoagglutinin (PHA-L)	<i>Phaseolus vulgaris</i> -leucoagglutinin (E &L) itself	Vector Laboratories, Burlingame, CA (AS-2224)	1:5,000 – 1:10,000
Rabbit polyclonal anti-substance P (SP)	synthetic substance P conjugated to carbodiimide/keyhole limpet hemocyanin	Immunostar, Hudson WI (20064)	1:5,000 – 1:10,000
Mouse monoclonal anti-tyrosine hydroxylase (TH)	Tyrosine hydroxylase from PC12 cells	Bioscience Research Reagents, a division of Millipore, Temecula, CA (MAB318)	1:5,000 – 1:15,000