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Abundant collateralization of temporal lobe projections to the accumbens, bed nucleus of stria terminalis, central amygdala and lateral septum

Rhett A. Reichard, Suriya Subramanian, Mikiyas T. Desta, Tej Sura, Mary L. Becker, Comeron W. Ghobadi, Kenneth P. Parsley, and Daniel S. Zahm*

Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, 1402 S. Grand Blvd., Saint Louis, Missouri 63104

Abstract

Behavioral flexibility is subserved in part by outputs from the cerebral cortex to telencephalic subcortical structures. In our earlier evaluation of the organization of the cortical-subcortical output system (*J. Neurosci.* 25:11757-67, 2005), retrograde double-labeling was evaluated in the prefrontal cortex following tracer injections into pairs of the following subcortical telencephalic structures: caudate-putamen, core and shell of the accumbens (Acb), bed nucleus of stria terminalis (BST) and central nucleus of the amygdala (CeA). The present study was done to assess patterns of retrograde labeling in the temporal lobe after similar paired tracer injections into most of the same telencephalic structures plus the lateral septum (LS). In contrast to the modest double-labeling observed in the prefrontal cortex in the previous study, up to 60-80% of neurons in the basal and accessory basal amygdaloid nuclei and amygdalopiriform transition area exhibited double-labeling in the present study. The most abundant double-labeling was generated by paired injections into structures affiliated with the extended amygdala, including the CeA, BST and Acb shell. Injections pairing the Acb core with the BST or CeA produced significantly fewer double-labeled neurons. The ventral subiculum exhibited modest amounts of double-labeling associated with paired injections into the Acb, BST, CeA and LS. The results raise the issue of how an extraordinarily collateralized output from the temporal lobe may contribute to behavioral flexibility.

Keywords

dopamine; motivation; emotion; locomotion; motor; movement

The adaptive success of mammalian foragers and predators, mainly in the primate, carnivore and rodent orders, owes in large part to their deft ability to modify behavior in relation to changing contingencies (Aquili et al., 2014, Hamilton and Brigman, 2015). This capacity, behavioral flexibility, is subserved by the interconnections of the telencephalon with structures in the hypothalamus, brainstem and spinal cord (Ferrier, 1876/1966; Cannon and Britton, 1927; Bard, 1928; Bard and Macht, 1958; Harris, 1958; Woods, 1964; MacLean,

*Corresponding author: Tel. (314) 977-8003, Fax (314) 977-6411, zahmds@slu.edu.

1989; Napier et al., 1991; Kalivas and Barnes, 1993; Holstege et al., 1996; 2004) and important additional intra-telencephalic cortico-subcortical connectional relationships (e.g., Alheid and Heimer, 1988; Holstege, 1991; 1992; Saper, 1996; McGinty, 1999; Swanson, 2000; 2003; Alheid 2003; Heimer, 2003). Heimer and colleagues (Alheid and Heimer, 1988; Heimer and Alheid, 1991; Heimer et al., 1991; Heimer et al., 1997b) emphasized cortical outputs to a collection of basal forebrain functional-anatomical “macrosystems”, including dorsal and ventral striatopallidum (Heimer, 1972; Heimer and Wilson, 1975), extended amygdala (Alheid and Heimer, 1988; Martin et al., 1991; Cassell, 1998; Swanson and Petrovich, 1998; Cassell et al., 1999; McDonald, 2003; Oler et al., 2016) and the lateral septal-preoptic system (Alheid and Heimer, 1988; McDonald, 1991b; Jakab and Lanthorn, 1995; Risold and Swanson, 1997a,b; Swanson, 2000; Heimer and Van Hoesen, 2006; Zahm, 2006; 2008a). Macrosystems share a common basic cortico-striato-pallidal framework reflected in similar neuronal types and connections, but also distinct chemical neuroanatomical differentiations, kinds of interneurons and patterns of local connectivity (e.g., Alheid and Heimer, 1988; Zahm et al., 2003), such that what one (or a sector of one) macrosystem imparts to the overall cortical-subcortical interaction may in some ways be similar and in others distinct from what is imparted to the interaction by other macrosystems.

Cortical and basal amygdaloid (BA)¹ outputs to the subcortical telencephalon have a complex organization, projecting to striatum and other medium spiny neuron-enriched structures (which variously are also regarded as striatum), such as the lateral septum (LS), bed nucleus of stria terminalis (BST) and central nucleus of the amygdala (CeA), both with strict topography and extra-topographically, i.e., to sites outside the predicted topography (Yeterian and Van Hoesen, 1978; McGeorge and Faull, 1989; Berendse et al., 1992; Brog et al., 1993; McIntyre et al., 1996; Reynolds and Zahm, 2005). Of particular interest is a ‘triadic’ organization in which strongly interconnected sites in the cortex and BA project convergently to the same subcortical locus (Yeterian and Van Hoesen, 1978; Groenewegen et al., 1990; 1997; McDonald et al., 1991b). Conversely, cortical and BA sites may also project to more than one macrosystem (McDonald et al., 1991b; 1996; 1999; Shammah-Lagnado and Santiago, 1999; Shi and Cassell, 1998a; McDonald, 1998; Zahm, 1998; 2000; 2006; 2008a,b; Reynolds and Zahm, 2005). The overlap of these different patterns of organization fosters a couple possibilities. Divergent outputs from a particular cortical network representing some aspect of an organism's ongoing circumstances could be subject to subcortical processing executed by more than one macrosystem (Zahm, 2006; 2008a,b). Alternately, different macrosystems might receive outputs from interdigitating but distinct subpopulations of neurons representing functionally or anatomically defined cortical networks (e.g., Mesulam, 1990; Carmichael and Price, 1995; 1996; Öngür and Price, 2000; Jones et al., 2005). In an earlier paper, Reynolds and Zahm (2005) speculated that a “divergence” model of cortico-macrosystem projections predicts greater likelihood that individual cortical output neurons project to more than one macrosystem, and, accordingly, they made ipsilateral pairs of injections using two different retrograde tracers, either into

¹Consistent with ample considerations described elsewhere (Alheid and Heimer, 1988; Carlsson and Heimer, 1988), basal amygdala will be treated in this paper as a cortical-like structure.

extended amygdala (EA) and dorsal or ventral striatum (heterotypic pairs), or into different parts of the same macrosystem (homotypic pairs). They reported that homotypic injection pairs produced extensive overlap of retrogradely labeled neurons in the medial prefrontal (mPFC) and insular (IC) cortex, and modest double-labeling, suggesting that distinct cortical projections spread broadly within single macrosystems. Heterotypic injection pairs also produced substantially overlapping distributions of retrograde labeling in the mPFC and IC, but little double-labeling, suggesting that different macrosystems get inputs mainly from separate subpopulations of cortical neurons, possibly representing separate networks. The data suggested that the distinct identities of striatopallidum and extended amygdala reflect a corresponding segregation of cortical output networks.

Insofar as Reynolds and Zahm (2005) evaluated only mPFC and IC, however, nothing is known regarding collateralization of temporal lobe projections to intra-telencephalic subcortical structures. The possibility of such collateralization was strengthened by the observations of McDonald and others, who showed that the BA, accessory basal amygdaloid nucleus (ABA), amygdalohippocampal transition area (AHip) and amygdalopiriform transition area (APir) all project robustly to the Acb, BST and CeA (McDonald, 1991b; McDonald et al., 1999; Shammah-Lagnado and Santiago, 1999; Santiago and Shammah-Lagnado, 2005). Here, we reveal that outputs to basal forebrain macrosystems from the subiculum, amygdala and some additional temporal lobe cortical areas exhibit exuberant homotypic and modest heterotypic collateralization.

Materials and Methods

Animals

All experiments were carried out in accordance with guidelines published in the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the animal care committee of Saint Louis University. Male Sprague Dawley rats (Harlan, Indianapolis, IN) were housed under a 12-hour light-dark cycle in groups of 4 – 6 until the tracers were injected, after which they were housed individually. Food and water were provided *ad libitum*.

Chemicals and reagents

Unless otherwise specified, all were from Sigma Chemical Co., St. Louis, MO.

Injections

Fifty-three male Sprague Dawley rats (Harlan, Indianapolis, IN) weighing between 240 and 320g 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 subjected to intracranial stereotaxic injections of the retrograde axonal tracers, Fluoro-Gold (FG, Fluorochrome, LLC., Denver, CO) and cholera toxin subunit β (Ct β , List Biological Laboratories, Inc., Campbell, CA), both dissolved at a concentration of 1%, in 0.067 M cacodylate buffer (FG) and 0.01 M sodium phosphate buffer (SPB), respectively. The tracers were delivered by iontophoresis

with positive pulses (7 s on and 7 s off for 15 min) at 1 (FG) and 3 (Ct β) μ A through 1.0 mm pipettes pulled to tip diameters of 15-20 μ m. Ipsilateral pairs of basal forebrain structures (Table 1) were injected such that one of the counterparts received FG and the other Ct β . For each pair, which members of an injection site pair received FG and Ct β was alternated in successive cases.

Three to five days after surgery, rats were re-anesthetized and killed by vascular perfusion, first with a brief rinse with sodium phosphate (0.01 M) buffered saline containing 0.5% procaine HCl and 2.5% sucrose, followed for 20 minutes with 0.1 M Sorenson's phosphate buffer (SPB, pH 7.4) containing 4% paraformaldehyde and 2.5% sucrose. The brains were immediately removed, stored overnight in fresh fixative, and then transferred to vials containing a solution of 25% sucrose in SPB until they sank to the bottom. Five adjacent series of 50 μ m frozen sections were cut in the coronal plane with a sliding microtome and stored at -20°C in 0.1 M SPB containing 30% ethylene glycol and 30% sucrose.

Immunoperoxidase processing

Sections were retrieved from the freezer, rinsed in SPB, immersed for 15 min in an aqueous solution containing 1% sodium borohydride and then rinsed repeatedly in SPB. The sections then were immersed overnight at room temperature with gentle agitation in 0.1 M SPB containing 0.2% triton X-100 (SPB-triton) and a 1:5000 dilution of rabbit anti-FG antibody (Chemicon, Temecula, CA), after which they were rinsed in SPB-triton and immersed for 1 hr in SPB-triton containing a 1:200 dilution of biotinylated donkey anti-rabbit IgG (Jackson ImmunoResearch Labs, Inc., West Grove, PA). Another series of rinses preceded immersion of the sections for 1 hr in SPB-triton containing a 1:200 dilution of avidin-biotin-peroxidase complex (ABC; Vector Laboratories, Burlingame, CA), after which the sections were rinsed in SPB (no triton) and then reacted for 10 min in a solution containing 0.015% diaminobenzidine (DAB), 0.4% nickel ammonium sulfate, and 0.006% H₂O₂ in 0.025 M Tris-HCl buffer (pH 8.0). The sections were rinsed and then immersed overnight with agitation in SPB-triton containing a 1:5000 dilution of goat anti-Ct β antibody (List). Following more rinses, the sections were exposed for 1 hr in SPB-triton with intervening rinses to a 1:100 dilution of donkey anti-goat IgG (Jackson) and a 1:3000 dilution of goat peroxidase-antiperoxidase (Cappel, ICN Biomedicals, Irvine, CA) or a 1:200 dilution of biotinylated donkey anti-goat followed by ABC at 1:200. After rinsing in SPB without triton, the sections then were placed for 30 minutes in 0.05 M SPB (pH 7.4) containing 0.05% DAB, and 0.006% H₂O₂ in 0.01 M phosphate buffer (pH 7.4) and thereafter rinsed again in SPB. The sections were then mounted in rostrocaudal sequence on glass slides, air dried and coverslipped under Permount (Fisher Chemical Co., St. Louis, MO).

Immunofluorescence processing

Series of sections were immersed in succession at room temperature, with gentle agitation and intervening rinses in SPB-triton containing: [1] a 1:8000 dilution of anti-FG antibody overnight, [2] a 1:200 dilution of biotinylated donkey anti-rabbit IgG for 1 hr, [3] a 1:200 dilution of avidin-Texas Red (Jackson, excitation: 596 nm; emission: 620 nm) or DyLight 594 (Jackson, excitation: 593 nm emission: 618 nm) conjugated to anti-rabbit IGg made in donkey for 1 hr, [4] a 1:5000 dilution of goat anti-Ct β antibody overnight, and [5] a 1:100

dilution of donkey anti-goat-Cy2 conjugate (Jackson; excitation: 492 nm; emission: 510 nm) or DyLight 488 (Jackson, excitation: 493 nm, emission: 519 nm) conjugated to donkey anti-goat IgG made in donkey for 1 hr. Some series of sections were reacted such that the anti-FG antibody was followed directly by an anti-rabbit IgG-CY2 (or DyLight 488) conjugate used at a dilution of 1:100 and anti-Ct β was labeled with biotinylated anti-goat from donkey followed by avidin-TR (or DyLight 594) used at a dilution of 1:200. Then the sections were rinsed thoroughly in SPB, mounted on glass slides and coverslipped with DPX (Fluka, Sigma-Aldrich, Steinheim, Germany) or ProLong Gold antifade reagent (Invitrogen).

Microscopy

The sections were visualized with a Nikon Eclipse E600 microscope equipped for bright field and fluorescence microscopy. Immunofluorescence images were generated with a dual-band fluorescence filter set (Chroma Technology Corp, Brattleboro, VT) with excitation bands at 480-505 and 560-590 nm, and emission bands at 505-545 and 600-650 nm, for green (Cy2, DyLight 488) and red (Texas Red, DyLight 594) fluorescence, respectively.

Injection Sites

Tracings of immunoperoxidase-reacted injection sites were referenced against archived sections immunoreacted with antibodies against substance P, glutamate decarboxylase and nitric oxide synthase, as described in several previous papers (e.g., Zhou et al., 2009; Zahm et al., 2014; Yetnikoff et al., 2014a;b; 2015).

Analysis of retrograde labeling

Of 53 cases prepared in the present study, 32 (Table 1) were used to acquire data as follows. Retrogradely labeled neurons exhibiting Cy2 (or DyLight 488), Texas Red (or DyLight 594) and both fluorophores, i.e., double-labeled neurons, were visualized. In order to circumvent the problem of fading of immunofluorescence due to prolonged illumination, digital images were captured using the 20 $\times$ objective and assembled into montages encompassing the area of each section containing retrogradely labeled neurons. The photomicrographic maps were then superimposed on outlines of sections traced with the aid of NeuroLucida software (MicroBrightfield, Inc., Williston, VT) and the labeled neurons were plotted. Evaluated sections from each of generally three or four, but at least two, cases for each injection pair were analyzed. Single and double-labeled neurons were tabulated with the aid of NeuroExplorer software (MicroBrightfield). A criterion was set that a structure should contain at least 30 retrogradely labeled neurons from each injection in order for double-labeling data to be accepted. For illustration of red and green fluorescence microscopic images in micrographs, red was converted to magenta with the aid of Photoshop software.

Immunofluorescence controls

In order to confirm the fidelity of yellow fluorescence generated by the dual-band filter as an indicator of double-labeling, samples of images acquired with it were compared with merged digital images generated using single-band filter blocks. This was done both with the Nikon E600 microscope and a Bio-Rad MRC 1024 confocal microscope. Images were merged using Image J software (National Institutes of Health). In illustrations, red was

replaced with magenta in the immunofluorescence images with the aid of Adobe Photoshop software and in confocal images with the aid of Image J software.

Statistics

Data from the amygdala and adjacent temporal lobe cortex were organized by brain site and injection pairs and tested using a repeated measures ANOVA. A one-way ANOVA was used to distinguish double-labeling produced in the ventral subiculum by different injection pairs. Fisher's LSD post hoc test was used.

Results

Injection sites

FG and Ct β injected into the Acb, CeA and LS produced injection sites about 0.5 - 0.8 mm in diameter comprising varyingly dense immunoperoxidase reaction product, with a denser core (Figs. 1A and B; 2I, insets; 3A and B; 5A and B), which was typically smaller for Ct β than FG injections (Figs. 1B, 3B and 5A). Injections into the BST tended to conform to the pyramidal shape that structure typically displays in frontal sections (Fig 1B and 3A). Numerous FG and Ct β injections involved the Acb core or shell to the relative exclusion of the other (Figs. 1A and 5A). Others that involved both Acb subterritories equivalently or nearly so were not mapped or further included in the analysis. Small injections centered in the medial or lateral divisions of the BST and CEA also were obtained but the spread of tracer from injections of normal size tended more in the BST and CeA than in the Acb to involve more than one subdivision of the structure (Figs. 1B and 3B). Thus, we did not specify whether injections preferentially involved the medial and lateral divisions of the BST and CEA.

Retrograde labeling

Perikarya, except where occupied by the nucleus, and proximal dendrites were filled with densely packed FG- or Ct β -immunoreactive granules and diffuse immunoreactivity (Fig. 1C-H). Exposure of FG and Ct β immunoreacted sections first to DAB in the presence of nickel and then to DAB alone generated, respectively, black and brown reaction product (Fig. 1C and D), of which both are permanent and distinguishable in bright field optics, allowing both the injection sites and retrograde labeling patterns to be evaluated, mapped and archived. However, because double-labeling is shown inadequately in immunoperoxidase material, additional sections were processed for immunofluorescence, which showed single labeled neurons as brilliant red or green and double-labeled neurons in bright yellows and oranges (Fig. 1E). Laser scanning confocal images from the same case (15238) and brain area (BA) confirmed the fidelity of immunofluorescence labeling (Fig. 1F-H).

Double-labeling in the amygdala, AHip and APir

Representative cases illustrating the distribution of labeled neurons in the amygdala, AHip and APir are illustrated in Figures 2 and 3 and quantitation of all of the cases is graphed in Figure 4. Labeling in case 15238, also illustrated in Figure 1, is mapped and further illustrated in Figure 2. Abundant labeling, including double-labeling was observed in the

amygdala, AHip and APir following injections of tracers into AcbS&BST (Fig. 2), AcbS&CeA, and CeA&BST (Fig. 3) injection pairs. Very dense double-labeling stretching from rostral parts of the amygdala into caudal APir (e.g., Fig. 2B, 2D-G and 2I and Fig. 3D-E and H-J) was separated by intervals with lesser double-labeling (Figs. 2C and 3F and G). Dense clusters of double-labeled neurons centered in the BA (Fig. 2D), ABA (Figs. 2E and 3F and C) and APir (Figs. 2F-G and 3H-J) spread at their peripheries into adjacent structures such as the LA (Fig. 3F and G) and cortical amygdaloid nuclei (Figs 2F and G and 3A-H). In contrast, AcbC&CeA and AcbC&BST pairs produced only modest double-labeling in all structures (Fig. 4A [blue symbols] and C [blue text]).

Insofar as the raw numbers of double-labeled neurons correlated strongly with the density of retrograde labeling, the *tendency* for neurons to be double-labeled was assessed by expressing double-labeling as percent of total retrograde labeling. Markedly less retrograde labeling by FG as compared to Ct β (Table 2) required that this be calculated using the formula % double-labeling = double-labeled/(FG-labeled + double-labeled) $\times$ 100, which exploits the maximal double-labeling of collateralized, FG-labeled neurons by efficacious uptake and transport of Ct β . Expressed as percent of total, double-labeling was relatively uniform across the APir, ABA, BA, AHip and differed significantly (Fig. 4B; $F_{5,15}=6.044$, $p=0.003$) only between APir and LA ($p=0.005$). Percent double-labeling for injection pairs that included the AcbC was significantly less than for pairs that did not include the AcbC (Fig. 4B [blue symbols] and D [blue text]).

Double-labeling in vSub

Moderate numbers of double-labeled neurons were observed in vSub following injections of tracers into AcbS&BST, AcbS&CeA, AcbC&BST and AcbC&CeA pairs (Figs. 2F-H; 3H-J, 4A and C and 5). As total numbers of double-labeled neurons (Fig. 4A) these values were exceeded only by those in APir ($p=0.026$), whereas, expressed as percent of retrograde labeling (Fig. 4B), double-labeling in vSub was significantly less than APir ($p<0.001$), ABA ($p=0.014$) and AHip ($p=0.035$). Injection pairs that included the AcbC produced the least double-labeling in vSub whether measured as raw numbers (Fig. 4C [blue text]) or percent total (Fig. 4D [blue text])

Double-labeling in vSub resulting from injections of tracers into pairs of sites of which one was the LS is reported in Table 3. In this regard it is important that fewer vSub neurons project to BST and CeA than to LS (Table 3B and C) and Acb (Table 3A and C), so even if all vSub projections to the BST or CeA were collaterals of Acb or LS-projecting neurons, they would comprise only a small percentage of Acb- or LS-projecting neurons. Consequently, double-labeling as percent total calculated from numbers of LS-projecting vSub neurons was low (Table 3, 2nd to last column, sections B and C), whereas double-labeling calculated as percent of total from numbers of BST- and CeA-projecting vSub neurons gave values in the 20-25% range (Table 3, last column, sections B and C). LS&AcbS pairs gave slightly greater values of 16-35% for double-labeling calculated as percent of total (Table 3, last column, section A)

Double-labeling in mPFC and IC

We did not repeat with the new cases the entire analysis of PFC and IC done our earlier paper (Reynolds and Zahm, 2005), but we did evaluate the mPFC and IC in several cases (15104, 15105, 15151 and 15152), observing double-labeling expressed as percent of total ranging between 4.9% and 13%, which is consistent with the relatively low values previously reported.

Discussion

Prominent retrograde double-labeling of temporal lobe neurons was observed in the present study following paired injections of different retrograde tracers into combinations of AcbS, AcbC, BST, CeA and LS. Double-labeling of up to 60-80% of retrogradely labeled neurons occupied the BA, ABA and APir following injections into the AcbS, BST and CeA, whereas injections pairing the AcbC with the BST or CeA produced not only significantly fewer double-labeled neurons, but also less double-labeling expressed as percent of total. Alternatively, nominally heterotypic AcbS&CeA and AcbS&BST injection pairs produced very abundant double-labeling in amygdaloid structures and APir. The caudomedial AcbS, which is the part of the Acb injected in this study, has long been regarded as a transitional structure combining features of striatopallidum and extended amygdala (Alheid and Heimer, 1988; Heimer et al., 1997a;b; Koob et al., 1998; Zahm, 1998), so the high proportion of double-labeled amygdaloid and APir neurons produced by injections that paired the AcbS with the CeA or BST is consistent, and, indeed, further supports affiliation of the caudomedial AcbS with extended amygdala. Apropos the high level of double-labeling in the temporal lobe following tracer injections in basal forebrain nuclei, Calderazzo et al. (1996) reported that neurons in the basal amygdaloid nucleus project to both the septum and entorhinal cortex and McDonald (1991a) demonstrated abundant collateralization of basal amygdaloid projections to ventral striatopallidum and prefrontal cortex. Thus, the branching of axonal projections from the basal amygdala is even more widespread than shown here.

Neurons projecting from vSub to distinct output targets were reported by Naber and Witter (1998) to be segregated and relatively uncollateralized, but also, by others, to be overlapping and moderately collateralized. That is, 30% of subicular neurons may project to the ventromedial nucleus of the hypothalamus and entorhinal cortex (Donovan and Wyss, 1983) and 30% (Swanson et al., 1981) or 50% (Calderazzo et al., 1996) of those projecting to the lateral septum may also project to the entorhinal cortex. Our results are consistent with moderate collateralization of vSub outputs and raise an interesting question as to whether vSub or amygdaloid neurons might project via axon collaterals both to macrosystem input structures and medial hypothalamus or entorhinal cortex.

Methodological considerations

Whereas standard measures taken in the present study to minimize uptake of tracers by fibers of passage - injecting by iontophoresis with small tip diameters and low ejection currents (Brog et al., 1993) – do not entirely exclude the possibility of fiber-of-passage artifact, it is unlikely that fibers-of-passage caused the abundant double-labeling we observed. The present injections into the BST may have inadvertently involved some stria

terminalis or ventral amygdalofugal fibers enroute to the Acb, but it is difficult to conceive that the abundant double-labeling associated with CeA&BST and CeA&Acb injection pairs is attributable to fibers-of-passage, inasmuch as these structures lie at opposite ends of these pathways. Furthermore, all of the injection pairs, except ones that included the AcbC, produced abundant double-labeling and it is unlikely that a fibers-of-passage artifact involved all equivalently. It also seems inappropriate for two reasons to attribute the more efficacious labeling by Ct β observed in the present study to greater uptake of Ct β by fibers of passage (Chen and Aston-Jones, 1995). [1] FG has a similar fiber-of-passage liability (Dado et al., 1990). [2] All analyzed structures exhibited many more Ct β -labeled as compared to FG-labeled neurons (Table 2) and, again, it is unlikely that fibers-of-passage would similarly affect all.

We were confronted in this study with the complication that FG injections produced on average just over one-third as many labeled neurons as Ct β injections (Table 2). To offset this source of error, we calculated percent of total labeling exclusively as a function of numbers of neurons labeled by the less efficacious tracer (i.e., % double-labeled = double-labeled/[FG-labeled + double-labeled] $\times$ 100), which excludes the possibility of misidentifying branched Ct β -labeled neurons that could have been double-labeled, but, due to suboptimal FG transport, were not. Having been compelled to calculate double-labeling in this way in the present material, we came to realize that there is likely no better way to do it. Data calculated in this way were consistently more orderly than data derived with Ct β -labeling in the denominator, by the averaging method used by Reynolds and Zahm (2005) or with total labeled neurons (both tracers) in the denominator (Naber and Witter, 1998), which both artificially about halves percent double-labeled and magnifies errors related to deficient labeling by one of the tracers. While calculations of proportions of collateralized neurons provide an important dimension to the study, the main conclusion of the study, that temporal lobe outputs collateralize abundantly among basal forebrain macrosystem input structures, excepting the AcbC, is adequately supported just by the raw counts of double-labeled neurons.

Functional implications

It is well known that the amygdaloid and cortical projections evaluated in this study are dense, glutamatergic outputs that terminate in relation to medium densely spiny neurons occupying input structures of basal forebrain macrosystems (Krettek and Price, 1978a; Groenewegen et al., 1987; Brog et al., 1993; Sun and Cassell, 1994; McDonald et al., 1999; Shammah-Lagnado and Santiago, 1999; Canteras and Swanson, 1992; Jolkkonen et al., 2001; deCampo and Fudge, 2013). As noted in the Introduction, highly differentiated patterns of organization characterize some of these projections, not the least being the 'triadic' organization in which strongly interconnected cortical loci tend to have convergent corticostriatal projections (Yeterian and Van Hoesen, 1978; Groenewegen et al., 1990; 1997; McDonald et al., 1991b). The specificity of such highly differentiated connectional relationships, if not degraded, is certainly complicated, by abundant collateralization of cortical outputs. That is, the functional impact of convergence of cortico-subcortical outputs (e.g., McDonald, 1991b) must in some way be modulated if neurons that give rise to it also project via collaterals to other intra-telencephalic sites, perhaps in other macrosystems.

Collateralized outputs may reflect a more primitive, less specific pattern of cortical-subcortical connectivity that diminishes with cortical evolution, resulting in a greater proportional representation of the more specific 'triadic' organization in areas with greater connectional specificity, such as the mPFC and IC.

To the extent that collateralization may degrade the specificity of inputs to macrosystems, it should be noted that the functions of the different macrosystems may be less specific than once had been entertained. Despite abundant evidence that the EA is most concerned with detection and response to threat (Pascoe and Kapp, 1985; Campeau and Davis, 1995; LeDoux, 1995; 2000; Rogan and LeDoux, 1996; Killcross et al., 1997; Nader and LeDoux, 1997; Davis and Shi, 1999; Fendt and Faneslow, 1999; Guarraci et al., 1999a; 1999b; 2000; Amorapanth et al., 2000; Jolkkonen et al., 2001; Walker et al., 2003; 2009; Paré et al., 2004; Maren, 2005a; b; Phelps and LeDoux, 2005; Santiago and Shammah-Lagnado, 2005; Wilensky et al., 2006; Miller and Urcelay, 2007; Walker and Davis, 2008; Duvarci et al., 2009; Skorzewska et al., 2009; Ciocchi et al., 2010; Davis et al., 2010; Haubensak et al., 2010; McDannald, 2010; Poulos et al., 2010; Crestani et al., 2013; Davis and Walker, 2013; Elharrar et al., 2013; Haufler et al., 2013; Ide et al., 2013; Li et al., 2013; Nagai et al., 2013; Silberman and Winder, 2013; Shackman and Fox, 2016; Gungor and Paré, 2016) and ventral striatum with approach (Kelly et al., 1975; Yokel and Wise, 1975; 1976; Roberts et al., 1980; Wise, 1985; 2004; 2008; Petit et al., 1984; Taylor and Robbins, 1984; 1986; Wickens and Kötter, 1995; Berridge and Robinson, 1998; Cannon and Palmiter, 2003; Hnasko et al., 2005; Robinson et al., 2005; Berridge, 2007; Berridge et al., 2009; Schultz et al., 1997), much additional evidence indicates that the EA and ventral striatum are in many ways *functionally redundant*. Thus, the CeA is reported to modulate responses to appetitive stimuli (Gallagher et al., 1990; Gallagher and Holland, 1994; Will et al., 2004; Lee et al., 2005; 2010; Difeliceantonio and Berridge, 2012; Mahler and Berridge, 2012; Knapska et al., 2013), just as the Acb modulates responses to threatening ones (Riedel et al., 1997; Parkinson et al., 1999; Haralambous and Westbrook, 1999; Reynolds and Berridge, 2001; 2008; Levita et al., 2002; Richard and Berridge, 2011a;b; McCutcheon et al., 2012; Richard et al., 2013). The expression of cyclic AMP response element binding protein (CREB) in Acb medium spiny neurons is regulated bidirectionally in relation to anxiety and reward (Carlezon et al., 1998; Barrot et al., 2002; 2005; Carlezon et al., 2005; Nestler and Carlezon, 2006; Dong et al., 2006; Wallace et al., 2009). EA and ventral striatum both respond vigorously to novelty (Garris et al., 1997; Rebec et al., 1997; Lee et al., 2006; 2008; Horvitz, 2000) and both also modulate reactions to stress, pain and depression (Altier and Stewart, 1998; 1999; Neugebauer et al., 2004; Leknes and Tracey, 2008; Rouwette et al., 2012; Chen et al., 2012; Kim et al., 2016). An integrated response to reward, threat and novelty thus reflects considerable overlap among the functions of the EA and ventral striatum, and it shouldn't be neglected that the LS also contributes to numerous and diverse associative and affective functions shared by the EA and ventral striatum (Jakab and Leranthe, 1995; Risold and Swanson, 1997a; b; Sheehan et al., 2003).

Substantial functional differences do appear to characterize parts of the macrosystem complex that receive exuberantly versus sparsely collateralized inputs. Earlier results reported by Reynolds and Zahm (2005) and counts from 4 cases in the present study support a generally low level of collateralization of prefrontocortical projections to basal forebrain

macrosystems, consistent with the finely discriminative control that the frontal lobe is thought to exert on behavioral flexibility (e.g., de Bruin et al., 1994; Seamans et al., 1995; Ragozzino et al., 1999; Dias et al., 2000; Lanser et al., 2001; Ragozzino, 2002; 2007; Floresco et al., 2006; 2009; Block et al., 2007; Boulougouris et al., 2007; Ragozzino and Rozman, 2007; Stainaker et al., 2007; Tait and Brown, 2007; Churchwell et al., 2009; Clarke et al., 2008; Kosaki and Watanabe, 2012; Cholvin et al., 2013; Mala et al. 2015). Our present observation of relatively few collateralized temporal lobe projections to the AcbC is consonant with strong AcbC connections to the dorsal prelimbic and dorsal aganular insular cortices (Brog et al., 1993; Zahm, 2000; 2008a;b) and the key AcbC role in the acquisition and execution of conditioned responses (Kelley et al., 1997; Smith-Roe and Kelley, 2000), critical elements in behavioral flexibility. Alternatively, the abundance of collateralized temporal lobe projections from the amygdala and APir, and, to a lesser extent, vSub, to the AcbS fits with the reported role of that ventral striatal subterritory in modulating the vigor of conditioned responding (Taylor and Robbins, 1984; 1986; Parkinson et al., 1999), which, while not contributing specifically to decision-making, can nonetheless also be conceived as an important aspect of behavioral flexibility.

In summary, generously collateralized temporal lobe cortical and amygdaloid projections to the macrosystems could act in a variety of ways. They may serve to activate functionally similar circuitries in multiple input structures, possibly to facilitate more coherent subcortical responses to particular kinds of stimuli. Responding of parts of the input structure complex that are pertinent to specific sensory modalities could be enhanced by such an arrangement. For example, the afference of APir, a highly collateralized input to EA and AcbS, and, to a lesser extent, LS, is more or less dominated by converging inputs from the olfactory system (Santiago and Shammah-Lagnado, 2005). Alternatively, the impact of axon collaterals may truly generalize so as to decrease response threshold in parts of the macrosystem complex that receive highly collateralized inputs, rendering those parts of the complex more sensitive to inputs from the prefrontal cortex, which, as referenced, likely exerts granular, discriminative effects within the subcortical forebrain. The tremendous associative capacity of forebrain macrosystems might thus be more sensitively brought to bear in the service of behavioral flexibility. Yet another possibility is that the target relationships of the collaterals are flexible and change with an organism's sensory-motor experiences. These speculations aside, figuring out a mechanistic basis underlying any benefits conferred by abundant collateralization of inputs to the LS, Acb, BST and CeA is a tantalizing goal.

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Literature Cited

- Alheid GF. Extended amygdala and basal forebrain. *Ann N Y Acad Sci.* 2003; 985:185–205. [PubMed: 12724159]
- 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]

- Altier N, Stewart J. Dopamine receptor antagonists in the nucleus accumbens attenuate analgesia induced by ventral tegmental area substance P or morphine and by nucleus accumbens amphetamine. *J Pharmacol Exp Ther*. 1998; 285:208–215. [PubMed: 9536012]
- Altier N, Stewart J. The role of dopamine in the nucleus accumbens in analgesia. *Life sciences*. 1999; 65:2269–2287. [PubMed: 10597883]
- Amorapanth P, LeDoux JE, Nader K. Different lateral amygdala outputs mediate reactions and actions elicited by a fear-arousing stimulus. *Nat Neurosci*. 2000; 3:74–79. [PubMed: 10607398]
- Aquili L, Liu AW, Shindou M, Shindou T, Wickens JR. Behavioral flexibility is increased by optogenetic inhibition of neurons in the nucleus accumbens shell during specific time segments. *Learning & memory (Cold Spring Harbor, NY)*. 2014; 21:223–231.
- Bard P. A diencephalic mechanism for the expression of rage with special reference to the sympathetic nervous system. *Am J Physiol*. 1928; 84:490–515.
- Bard, P., Macht, MB. The behaviour of chronically decerebrate cats. In: Wolstenholme, GEW., O'Connor, CM., editors. *Ciba Foundation Symposium on the Neurological Basis of Behavior*. J & A Churchill, LTD; London: 1958. p. 55-75.
- Barrot M, Olivier JD, Perrotti LI, DiLeone RJ, Berton O, Eisch AJ, Impey S, Storm DR, Neve RL, Yin JC, Zachariou V, Nestler EJ. CREB activity in the nucleus accumbens shell controls gating of behavioral responses to emotional stimuli. *Proc Natl Acad Sci USA*. 2002; 99:11435–11440. [PubMed: 12165570]
- Barrot M, Wallace DL, Bolanos CA, Graham DL, Perrotti LI, Neve RL, Chambliss H, Yin JC, Nestler EJ. Regulation of anxiety and initiation of sexual behavior by CREB in the nucleus accumbens. *Proc Natl Acad Sci USA*. 2005; 102:8357–8362. [PubMed: 15923261]
- 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]
- Berridge KC. The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacol*. 2007; 191:391–431.
- Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Rev*. 1998; 28:309–369. [PubMed: 9858756]
- Berridge KC, Robinson TE, Aldridge JW. Dissecting components of reward: 'liking', 'wanting', and learning. *Curr Opin Pharmacol*. 2009; 9:65–73. [PubMed: 19162544]
- Block AE, Dhanji H, Thompson-Tardif SF, Floresco SB. Thalamic-prefrontal cortical-ventral striatal circuitry mediates dissociable components of strategy set shifting. *Cerebral cortex (New York, NY : 1991)*. 2007; 17:1625–1636.
- Boulougouris V, Dalley JW, Robbins TW. Effects of orbitofrontal, infralimbic and prelimbic cortical lesions on serial spatial reversal learning in the rat. *Behav Brain Res*. 2007; 179:219–228. [PubMed: 17337305]
- 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]
- Calderazzo L, Cavalheiro EA, Macchi G, Molinari M, Bentivoglio M. Branched connections to the septum and to the entorhinal cortex from the hippocampus, amygdala, and diencephalon in the rat. *Brain Res Bull*. 1996; 40:245–251. [PubMed: 8842407]
- Campeau S, Davis M. Involvement of the central nucleus and basolateral complex of the amygdala in fear conditioning measured with fear-potentiated startle in rats trained concurrently with auditory and visual conditioned stimuli. *J Neurosci*. 1995; 15:2301–11. [PubMed: 7891168]
- Cannon CM, Palmiter RD. Reward without dopamine. *J Neurosci*. 2003; 23:10827–10831. [PubMed: 14645475]
- Cannon WB, Britton SW. Pseudoaffective meduloadrenal secretion. *Am J Physiol*. 1927; 79:4333–465.
- Canteras NS, Swanson LW. Projections of the ventral subiculum to the amygdala, septum, and hypothalamus: a PHAL anterograde tract-tracing study in the rat. *J Comp Neurol*. 1992; 324:180–194. [PubMed: 1430328]

- Carlezon WA Jr, Duman RS, Nestler EJ. The many faces of CREB. *Trends in neurosciences*. 2005; 28:436–445. [PubMed: 15982754]
- Carlezon WA Jr, Thome J, Olson VG, Lane-Ladd SB, Brodtkin ES, Hiroi N, Duman RS, Neve RL, Nestler EJ. Regulation of cocaine reward by CREB. *Science (New York, NY)*. 1998; 282:2272–2275.
- Carlssen J, Heimer L. The basolateral amygdaloid complex as a cortical-like structure. *Brain Res*. 1988; 441:377–380. [PubMed: 2451985]
- Carmichael ST, Price JL. Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. *J Comp Neurol*. 1995; 363:615–641. [PubMed: 8847421]
- Carmichael ST, Price JL. Connectional networks within the orbital and medial prefrontal cortex of macaque monkeys. *J Comp Neurol*. 1996; 371:179–207. [PubMed: 8835726]
- Cassell MD. The amygdala: myth or monolith? *Trends Neurosci*. 1998; 21:200–201. [PubMed: 9610882]
- Cassell MD, Freedman LJ, Shi C. The intrinsic organization of the central extended amygdala. *Ann N Y Acad Sci*. 1999; 877:217–241. [PubMed: 10415652]
- Chen S, Aston-Jones G. Evidence that cholera toxin B subunit (CTb) can be avidly taken up and transported by fibers of passage. *Brain Res*. 1995; 674:107–111. [PubMed: 7773677]
- Chen YW, Rada PV, Bützler BP, Leibowitz SF, Hoebel BG. Corticotropin-releasing factor in the nucleus accumbens shell induces swim depression, anxiety, and anhedonia along with changes in local dopamine/acetylcholine balance. *Neuroscience*. 2012; 206:155–166. [PubMed: 22245501]
- Cholvin T, Loureiro M, Cassel R, Cosquer B, Geiger K, De Sa Nogueira D, Raingard H, Robelin L, Kelche C, Pereira de Vasconcelos A, Cassel JC. The ventral midline thalamus contributes to strategy shifting in a memory task requiring both prefrontal cortical and hippocampal functions. *J Neurosci*. 2013; 33:8772–8783. [PubMed: 23678120]
- Churchwell JC, Morris AM, Heurtelou NM, Kesner RP. Interactions between the prefrontal cortex and amygdala during delay discounting and reversal. *Behav Neurosci*. 2009; 123:1185–1196. [PubMed: 20001103]
- Ciocchi S, Herry C, Grenier F, Wolff SBE, Letzkus JJ, Vlachos I, Ehrlich I, Sprengel R, Deisseroth K, Stadler MB, Müller C, Luthi A. Encoding of conditioned fear in central amygdala inhibitory circuits. *Nature*. 2010; 468:277–282. [PubMed: 21068837]
- Clarke HF, Robbins TW, Roberts AC. Lesions of the medial striatum in monkeys produce perseverative impairments during reversal learning similar to those produced by lesions of the orbitofrontal cortex. *J Neurosci*. 2008; 28:10972–10982. [PubMed: 18945905]
- Crestani CC, Alves FH, Gomes FV, Resstel LB, Correa FM, Herman JP. Mechanisms in the bed nucleus of the stria terminalis involved in control of autonomic and neuroendocrine functions: a review. *Curr Neuropharmacol*. 2013; 11:141–159. [PubMed: 23997750]
- Dado RJ, Burstein R, Cliffer KD, Giesler GJ Jr. Evidence that Fluoro-Gold can be transported avidly through fibers of passage. *Brain Res*. 1990; 533:329–333. [PubMed: 1705157]
- Davis M, Walker DL. Role of bed nucleus of the stria terminalis and amygdala AMPA receptors in the development and expression of context conditioning and sensitization of startle by prior shock. *Brain Struct Funct*. 2013; 219:1969–82. [PubMed: 23934654]
- Davis M, Walker DL, Miles L, Grillon C. Phasic vs sustained fear in rats and humans: Role of the extended amygdala in fear vs anxiety. *Neuropsychopharmacol*. 2010; 35:105–135.
- 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]
- de Bruin JP, Sanchez-Santed F, Heinsbroek RP, Donker A, Postmes P. A behavioural analysis of rats with damage to the medial prefrontal cortex using the Morris water maze: evidence for behavioural flexibility, but not for impaired spatial navigation. *Brain Res*. 1994; 652:323–333. [PubMed: 7953746]
- deCampo DM, Fudge JL. Amygdala projections to the lateral bed nucleus of the stria terminalis in the macaque: comparison with ventral striatal afferents. *The Journal of comparative neurology*. 2013; 521:3191–3216. [PubMed: 23696521]

- Dias R, Aggleton JP. Effects of selective excitotoxic prefrontal lesions on acquisition of nonmatching- and matching-to-place in the T-maze in the rat: differential involvement of the prelimbic-infralimbic and anterior cingulate cortices in providing behavioural flexibility. *Eur J Neurosci*. 2000; 12:4457–4466. [PubMed: 11122356]
- Difeliceantonio AG, Berridge KC. Which cue to ‘want’? Opioid stimulation of central amygdala makes goal-trackers show stronger goal-tracking, just as sign-trackers show stronger sign-tracking. *Behav Brain Res*. 2012; 230:399–408. [PubMed: 22391118]
- Dong Y, Green T, Saal D, Marie H, Neve R, Nestler EJ, Malenka RC. CREB modulates excitability of nucleus accumbens neurons. *Nature neuroscience*. 2006; 9:475–477. [PubMed: 16520736]
- Donovan MK, Wyss JM. Evidence for some collateralization between cortical and diencephalic efferent axons of the rat subicular cortex. *Brain Res*. 1983; 259:181–192. [PubMed: 6402268]
- Duvarci S, Bauer EP, Paré D. The Bed Nucleus of the Stria Terminalis Mediates Inter-individual Variations in Anxiety and Fear. *J Neurosci*. 2009; 29:10357–10361. [PubMed: 19692610]
- Elharrar E, Warhaftig G, Issler O, Sztainberg Y, Dikshtein Y, Zahut R, Redlus L, Chen A, Yadid G. Overexpression of corticotropin-releasing factor receptor type 2 in the bed nucleus of stria terminalis improves posttraumatic stress disorder-like symptoms in a model of incubation of fear. *Biol Psychiat*. 2013; 74:827–836. [PubMed: 23871471]
- Fendt M, Fanselow MS. The neuroanatomical and neurochemical basis of conditioned fear. *Neurosci Biobehav Rev*. 1999; 23:743–760.
- Ferrier, D. The Functions of the Brain. Smith Elder; London: 1876/1966. 1876; reprinted in 1966 by Dawsons of Pall Mall, London
- Floresco SB, Magyar O, Ghods-Sharifi S, Vexelman C, Tse MT. Multiple dopamine receptor subtypes in the medial prefrontal cortex of the rat regulate set-shifting. *Neuropsychopharmacol*. 2006; 31:297–309.
- Floresco SB, Zhang Y, Enomoto T. Neural circuits subserving behavioral flexibility and their relevance to schizophrenia. *Behav Brain Res*. 2009; 204:396–409. [PubMed: 19110006]
- Gallagher M, Graham PW, Holland PC. The amygdala central nucleus and appetitive Pavlovian conditioning: lesions impair one class of conditioned behavior. *J Neurosci*. 1990; 10:1906–1911. [PubMed: 2355257]
- Gallagher M, Holland PC. The amygdala complex: multiple roles in associative learning and attention. *Proc Natl Acad Sci USA*. 1994; 91:11771–11776. [PubMed: 7991534]
- Garris PA, Christensen JR, Rebec GV, Wightman RM. Real-time measurement of electrically evoked extracellular dopamine in the striatum of freely moving rats. *Journal of neurochemistry*. 1997; 68:152–161. [PubMed: 8978721]
- Gungor NZ, Paré D. Functional heterogeneity in the bed nucleus of the stria terminalis. *J Neurosci*. 2016; 36:8038–8049. [PubMed: 27488624]
- Groenewegen HJ, Berendse HW, Wolters JG, Lohman AH. The anatomical relationship of the prefrontal cortex with the striatopallidal system, the thalamus and the amygdala: evidence for a parallel organization. *Prog Brain Res*. 1990; 85:95–116. discussion 116–118. [PubMed: 2094917]
- 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]
- Groenewegen HJ, Wright CI, Uylings HB. The anatomical relationships of the prefrontal cortex with limbic structures and the basal ganglia. *J Psychopharmacol*. 1997; 11:99–106. [PubMed: 9208373]
- Guarraci FA, Frohardt RJ, Falls WA, Kapp BS. The effects of intra-amygdaloid infusions of a D2 dopamine receptor antagonist on Pavlovian fear conditioning. *Behav Neurosci*. 2000; 114:647–651. [PubMed: 10883814]
- Guarraci FA, Frohardt RJ, Young SL, Kapp BS. A functional role for dopamine transmission in the amygdala during conditioned fear. *Ann N Y Acad Sci*. 1999; 877:732–736. [PubMed: 10415694]
- Guarraci FA, Frohardt RJ, Kapp BS. Amygdaloid D-1 dopamine receptor involvement in Pavlovian fear conditioning. *Brain Research*. 1999; 827:28–40. [PubMed: 10320690]
- Hamilton DA, Brigman JL. Behavioral flexibility in rats and mice: contributions of distinct frontocortical regions. *Genes, brain, and behav*. 2015; 14:4–21.

- Haralambous T, Westbrook RF. An infusion of bupivacaine into the nucleus accumbens disrupts the acquisition, but not the expression, of contextual fear conditioning. *Behav Neurosci.* 1999; 113:925–940. [PubMed: 10571476]
- Harris, GW. Chairman's opening remarks. In: Wolstenholme, GEW., O'Connor, CM., editors. *Ciba Foundation Symposium on the Neurological Basis of Behavior.* J & A Churchill, LTD; London: 1958. p. 1-3.
- Haubensak W, Kunwar PS, Cai HJ, Cioocchi S, Wall NR, Ponnusamy R, Biag J, Dong HW, Deisseroth K, Callaway EM, Fanselow MS, Luthi A, Anderson DJ. Genetic dissection of an amygdala microcircuit that gates conditioned fear. *Nature.* 2010; 468:270–U230. [PubMed: 21068836]
- Haufler D, Nagy FZ, Paré D. Neuronal correlates of fear conditioning in the bed nucleus of the stria terminalis. *Learning & memory (Cold Spring Harbor, NY).* 2013; 20(11):633–641.
- 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 and Evol.* 1972; 6:484–523.
- Heimer L. A new anatomical framework for neuropsychiatric disorders and drug abuse. *Am Journal Psychiat.* 2003; 160:1726–1739. [PubMed: 14514480]
- 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.* New York, NY: Plenum Press; 1991. p. 1-42. 1991
- Heimer L, Alheid GF, de Olmos JS, Groenewegen HJ, Haber SN, Harlan RE, Zahm DS. The accumbens: beyond the core-shell dichotomy. *J Neuropsych Clin Neurosci.* 1997a; 9:354–381.
- Heimer L, de Olmos JS, Alheid GF, Zaborszky L. “Perestroika” in the basal forebrain: opening the border between neurology and psychiatry. *Prog Brain Res.* 1991; 87:109–165. [PubMed: 1866444]
- Heimer L, Harlan RE, Alheid GF, Garcia MM, de Olmos JS. Substantia innominata: a notion which impedes clinical-anatomical correlations in neuropsychiatric disorders. *Neuroscience.* 1997b; 76:957–1006. [PubMed: 9027863]
- Heimer L, Van Hoesen GW. The limbic lobe and its output channels: implications for emotional functions and adaptive behavior. *Neurosci Biobehav Rev.* 2006; 30:126–147. [PubMed: 16183121]
- 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.* New York, NY: Raven Press; 1975. p. 173-193.
- Hnasko TS, Sotak BN, Palmiter RD. Morphine reward in dopamine-deficient mice. *Nature.* 2005; 438:854–857. [PubMed: 16341013]
- Holstege G. Descending motor pathways and the spinal motor system: Limbic and non-limbic components. *Prog Brain Res.* 1991; 87:307–421. [PubMed: 1678191]
- Holstege G. The emotional motor system. *Eur J Morphol.* 1992; 30:67–79. [PubMed: 1642954]
- Holstege, G., Bandler, R., Saper, CB. *Progress in Brain Research.* Vol. 107. Amsterdam: Elsevier; 1996. *The Emotional Motor System*; p. 3-6.
- Holstege, GG., Mouton, LJ., Gerrits, MN. Emotional motor system. In: Paxinos, G., Mai, JK., editors. *The Human Nervous System.* Elsevier; Amsterdam: 2004. p. 1306-1324.
- Horvitz JC. Mesolimbocortical and nigrostriatal dopamine responses to salient non-reward events. *Neuroscience.* 2000; 96:651–656. [PubMed: 10727783]
- Ide S, Hara T, Ohno A, Tamano R, Koseki K, Naka T, Maruyama C, Kaneda K, Yoshioka M, Minami M. Opposing roles of corticotropin-releasing factor and neuropeptide Y within the dorsolateral bed nucleus of the stria terminalis in the negative affective component of pain in rats. *J Neurosci.* 2013; 33:5881–5894. [PubMed: 23554470]
- Jakab, RL., Lanthorn, C. Septum. In: Paxinos, G., editor. *The Rat Nervous System.* San Diego: Academic Press; 1995. p. 405-442.
- Jhou TC, Geisler S, Marinelli M, Degarmo BA, Zahm DS. The mesopontine rostromedial tegmental nucleus: A structure targeted by the lateral habenula that projects to the ventral tegmental area of Tsai and substantia nigra compacta. *J Comp Neurol.* 2009; 513:566–596. [PubMed: 19235216]
- Jolkkonen E, Miettinen R, Pitkanen A. Projections from the amygdalo-piriform transition area to the amygdaloid complex: a PHA-I study in rat. *J Comp Neurol.* 2001; 432:440–465. [PubMed: 11268008]

- Jones BF, Groenewegen HJ, Witter MP. Intrinsic connections of the cingulate cortex in the rat suggest the existence of multiple functionally segregated networks. *Neuroscience*. 2005; 133:193–207. [PubMed: 15893643]
- Kalivas, PW., Barnes, CD., editors. *Limbic Motor Circuits and Neuropsychiatry*. CRC Press; Boca Raton: 1993.
- Kelley AE, Smith-Roe SL, Holahan MR. Response-reinforcement learning is dependent on N-methyl-D-aspartate receptor activation in the nucleus accumbens core. *Proc Natl Acad Sci U S A*. 1997; 94:12174–9. [PubMed: 9342382]
- 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]
- Killcross S, Robbins TW, Everitt BJ. Different types of fear-conditioned behaviour mediated by separate nuclei within amygdala. *Nature*. 1997; 388:377–380. [PubMed: 9237754]
- Kim HD, Hesterman J, Call T, Magazu S, Keeley E, Armenta K, Kronman H, Neve RL, Nestler EJ, Ferguson D. SIRT1 mediates depression-like behaviors in the nucleus accumbens. *J Neurosci*. 2016; 36:8441–8452. [PubMed: 27511015]
- Knapka E, Lioudyno V, Kiryk A, Mikosz M, Gorkiewicz T, Michaluk P, Gawlak M, Chaturvedi M, Mochol G, Balcerzyk M, Wojcik DK, Wilczynski GM, Kaczmarek L. Reward learning requires activity of matrix metalloproteinase-9 in the central amygdala. *J Neurosci*. 2013; 33:14591–14600. [PubMed: 24005309]
- Koob GF, Sanna PP, Bloom FE. Neuroscience of addiction. *Neuron*. 1998; 21:467–476. [PubMed: 9768834]
- Kosaki Y, Watanabe S. Dissociable roles of the medial prefrontal cortex, the anterior cingulate cortex, and the hippocampus in behavioural flexibility revealed by serial reversal of three-choice discrimination in rats. *Behav Brain Res*. 2012; 227:81–90. [PubMed: 22061799]
- Krettek JE, Price JL. Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. *J Comp Neurol*. 1978a; 178:225–254. [PubMed: 627625]
- Lanser MG, Ellenbroek BA, Zitman FG, Heeren DJ, Cools AR. The role of medial prefrontal cortical dopamine in spontaneous flexibility in the rat. *Behav Pharmacol*. 2001; 12:163–171. [PubMed: 11485053]
- LeDoux JE. Emotion: clues from the brain. *Annual Rev Psychol*. 1995; 46:209–235. [PubMed: 7872730]
- LeDoux JE. Emotion circuits in the brain. *Annu Rev Neurosci*. 2000; 23:155–184. [PubMed: 10845062]
- Lee HJ, Gallagher M, Holland PC. The central amygdala projection to the substantia nigra reflects prediction error information in appetitive conditioning. *Learn Mem (Cold Spring Harbor, NY)*. 2010; 17:531–538.
- Lee HJ, Groshek F, Petrovich GD, Cantalini JP, Gallagher M, Holland PC. Role of amygdalo-nigral circuitry in conditioning of a visual stimulus paired with food. *J Neurosci*. 2005; 25:3881–3888. [PubMed: 15829640]
- Lee HJ, Youn JM, Gallagher M, Holland PC. Temporally limited role of substantia nigra-central amygdala connections in surprise-induced enhancement of learning. *Eur J Neurosci*. 2008; 27(11):3043–3049. [PubMed: 18588542]
- Lee HJ, Youn JM, O MJ, Gallagher M, Holland PC. Role of substantia nigra-amygdala connections in surprise-induced enhancement of attention. *J Neurosci*. 2006; 26:6077–6081. [PubMed: 16738251]
- Leknes S, Tracey I. Science & society - A common neurobiology for pain and pleasure. *Nature Rev Neurosci*. 2008; 9:314–320. [PubMed: 18354400]
- Levita L, Dalley JW, Robbins TW. Disruption of Pavlovian contextual conditioning by excitotoxic lesions of the nucleus accumbens core. *Behav Neurosci*. 2002; 116:539–552. [PubMed: 12148922]
- Li HH, Penzo MA, Taniguchi H, Kopec CD, Huang ZJ, Li B. Experience-dependent modification of a central amygdala fear circuit. *Nature Neurosci*. 2013; 16:332–339. [PubMed: 23354330]

- MacLean, PD. *The Triune Brain in Evolution: Role in Paleocerebral Functions*. Plenum Press; New York: 1989.
- Mahler SV, Berridge KC. What and when to “want”? Amygdala-based focusing of incentive salience upon sugar and sex. *Psychopharmacol.* 2012; 221:407–426.
- Mala H, Andersen LG, Christensen RF, Felbinger A, Hagstrom J, Meder D, Pearce H, Mogensen J. Prefrontal cortex and hippocampus in behavioural flexibility and posttraumatic functional recovery: reversal learning and set-shifting in rats. *Brain Res Bull.* 2015; 116:34–44. [PubMed: 26033702]
- Maren S. Building and burying fear memories in the brain. *Neuroscientist.* 2005a; 11:89–99. [PubMed: 15632281]
- Maren S. Synaptic mechanisms of associative memory in the amygdala. *Neuron.* 2005b; 47(6):783–786. [PubMed: 16157273]
- Martin LJ, Powers RE, Dellovade TL, Price DL. The bed nucleus-amygdala continuum in human and monkey. *J Comp Neurol.* 1991; 309:445–485. [PubMed: 1918444]
- McCutcheon JE, Ebner SR, Loriaux AL, Roitman MF. Encoding of aversion by dopamine and the nucleus accumbens. *Front Neurosci.* 2012; 6:137. [PubMed: 23055953]
- McDannald MA. Contributions of the amygdala central nucleus and ventrolateral periaqueductal grey to freezing and instrumental suppression in Pavlovian fear conditioning. *Behav Brain Res.* 2010; 211:111–117. [PubMed: 20298722]
- McDonald AJ. Organization of amygdaloid projections to the prefrontal cortex and associated striatum in the rat. *Neuroscience.* 1991a; 44:1–14. [PubMed: 1722886]
- McDonald AJ. Topographical organization of amygdaloid projections to the caudatoputamen, nucleus accumbens, and related striatal-like areas of the rat brain. *Neuroscience.* 1991b; 44:15–33. [PubMed: 1722890]
- McDonald AJ. Cortical pathways to the mammalian amygdala. *Prog Neurobiol.* 1998; 55:257–332. [PubMed: 9643556]
- McDonald AJ. Is there an amygdala and how far does it extend? *Ann NY Acad Sci.* 2003; 985:1–21. [PubMed: 12724144]
- McDonald AJ, Mascagni F, Guo L. Projections of the medial and lateral prefrontal cortices to the amygdala: a Phaseolus vulgaris leucoagglutinin study in the rat. *Neuroscience.* 1996; 71:55–75. [PubMed: 8834392]
- McDonald AJ, Shammah-Lagnado SJ, Shi C, Davis M. Cortical afferents to the extended amygdala. *Ann N Y Acad Sci.* 1999; 877:309–338. [PubMed: 10415657]
- McGeorge AJ, Faull RLM. The organization of the projection from the cerebral cortex to the striatum in the rat. *Neuroscience.* 1989; 29:503–537. [PubMed: 2472578]
- McGinty, JF., editor. *Ann NY Acad Sci. Vol. 877. New York: 1999. Advancing from the Ventral Striatum to the Extended Amygdala.*
- McIntyre DC, Kelly ME, Staines WA. Efferent projections of the anterior perirhinal cortex in the rat. *J Comp Neurol.* 1996; 369:302–318. [PubMed: 8727002]
- Mesulam MM. Large-scale neurocognitive networks and distributed processing for attention, language and memory. *Ann Neurol.* 1990; 28:597–613. [PubMed: 2260847]
- Miller SS, Urcelay GP. The central amygdala joins the lateral amygdala in the fear memory party. *J Neurosci.* 2007; 27:2151–2152. [PubMed: 17329410]
- Naber PA, Witter MP. Subicular efferents are organized mostly as parallel projections: a double-labeling, retrograde-tracing study in the rat. *J Comp Neurol.* 1998; 393:284–297. [PubMed: 9548550]
- Nader K, Ledoux JE. Is it time to invoke multiple fear learning systems in the amygdala? *Trends Cogn Sci.* 1997; 1:241–244. [PubMed: 21223916]
- Nagai MM, Gomes FV, Crestani CC, Resstel LB, Joca SR. Noradrenergic neurotransmission within the bed nucleus of the stria terminalis modulates the retention of immobility in the rat forced swimming test. *Behav Pharmacol.* 2013; 24:214–221. [PubMed: 23625378]
- Napier, TC, Kalivas, PW., Hanin, I., editors. *Adv Exp Med Biol. Vol. 295. Plenum Press; New York: 1991. The Basal Forebrain: Anatomy to Function.*

- Nestler EJ, Carlezon WA Jr. The mesolimbic dopamine reward circuit in depression. *Biological psychiatry*. 2006; 59:1151–1159. [PubMed: 16566899]
- Neugebauer V, Li W, Bird GC, Han JS. The amygdala and persistent pain. *Neuroscientist*. 2004; 10:221–234. [PubMed: 15155061]
- Oler JA, Tromp DP, Fox AS, Kovner R, Davidson RJ, Alexander AL, McFarlin DR, Birn RM, EB B, deCampo DM, Kalin NH, Fudge JL. Connectivity between the central nucleus of the amygdala and the bed nucleus of the stria terminalis in the non-human primate: neuronal tract tracing and developmental neuroimaging studies. *Brain Struct Funct*. 2016 Feb 23. 2016. Epub ahead of print.
- Öngür D, Price JL. The organization of networks within the orbital and medial prefrontal cortex of rats, monkeys and humans. *Cereb Cortex*. 2000; 10:206, 219. [PubMed: 10731217]
- Paré D, Quirk GJ, Ledoux JE. New vistas on amygdala networks in conditioned fear. *J Neurophysiol*. 2004; 92:1–9. [PubMed: 15212433]
- Parkinson JA, Robbins TW, Everitt BJ. Selective excitotoxic lesions of the nucleus accumbens core and shell differentially affect aversive Pavlovian conditioning to discrete and contextual cues. *Psychobiol*. 1999; 27:256–266.
- Pascoe JP, Kapp BS. Electrophysiological characteristics of amygdaloid central nucleus neurons during Pavlovian fear conditioning in the rabbit. *Behav Brain Res*. 1985; 16:117–133. [PubMed: 4041212]
- Pettit HO, Ettenberg A, Bloom FE, Koob GF. Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. *Psychopharmacol*. 1984; 84:167–173.
- Phelps EA, LeDoux JE. Contributions of the amygdala to emotion processing: from animal models to human behavior. *Neuron*. 2005; 48:175–187. [PubMed: 16242399]
- Poulos AM, Li V, Sterlace SS, Tokushige F, Ponnusamy R, Fanselow MS. Persistence of fear memory across time requires the basolateral amygdala complex. *PNAS USA*. 2009; 106:11737–11741. [PubMed: 19567836]
- Ragozzino ME. The effects of dopamine D(1) receptor blockade in the prelimbic-infralimbic areas on behavioral flexibility. *Learning & memory (Cold Spring Harbor, NY)*. 2002; 9:18–28.
- Ragozzino ME. The contribution of the medial prefrontal cortex, orbitofrontal cortex, and dorsomedial striatum to behavioral flexibility. *Ann N Y Acad Sci*. 2007; 1121:355–375. [PubMed: 17698989]
- Ragozzino ME, Detrick S, Kesner RP. Involvement of the prelimbic-infralimbic areas of the rodent prefrontal cortex in behavioral flexibility for place and response learning. *J Neurosci*. 1999; 19:4585–4594. [PubMed: 10341256]
- Ragozzino ME, Rozman S. The effect of rat anterior cingulate inactivation on cognitive flexibility. *Behav Neurosci*. 2007; 121:698–706. [PubMed: 17663595]
- Rebec GV, Christensen JR, Guerra C, Bardo MT. Regional and temporal differences in real-time dopamine efflux in the nucleus accumbens during free-choice novelty. *Brain research*. 1997; 776:61–67. [PubMed: 9439796]
- 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. Emotional environments retune the valence of appetitive versus fearful functions in nucleus accumbens. *Nat Neurosci*. 2008; 11:423–425. [PubMed: 18344996]
- Reynolds SM, Zahm DS. Specificity in the projections of prefrontal and insular cortex to ventral striatopallidum and the extended amygdala. *J Neurosci*. 2005; 25:11757–11767. [PubMed: 16354934]
- Richard JM, Berridge KC. Metabotropic glutamate receptor blockade in nucleus accumbens shell shifts affective valence towards fear and disgust. *Eur J Neurosci*. 2011a; 33:736–747. [PubMed: 21198990]
- Richard JM, Berridge KC. Nucleus accumbens dopamine/glutamate interaction switches modes to generate desire versus dread: D1 alone for appetitive eating but D1 and D2 together for fear. *J Neurosci*. 2011b; 31:12866–12879. [PubMed: 21900565]

- Richard JM, Castro DC, Difeliceantonio AG, Robinson MJ, Berridge KC. Mapping brain circuits of reward and motivation: in the footsteps of Ann Kelley. *Neurosci Biobehav Rev.* 2013; 37:1919–31. [PubMed: 23261404]
- Riedel G, Harrington NR, Hall G, Macphail EM. Nucleus accumbens lesions impair context, but not cue, conditioning in rats. *NeuroReport.* 1997; 8:2477–2481. [PubMed: 9261812]
- Risold PY, Swanson LW. Chemoarchitecture of the rat lateral septal nucleus. *Brain Res Rev.* 1997a; 24:91–113. [PubMed: 9385453]
- Risold PY, Swanson LW. Connections of the rat lateral septal complex. *Brain Res Rev.* 1997b; 24:115–195. [PubMed: 9385454]
- Roberts DCS, Koob GF, Klonoff P, Fibiger HC. Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. *Pharmacol Biochem Behav.* 1980; 12:781–787. [PubMed: 7393973]
- Robinson S, Sandstrom SM, Denenberg VH, Palmiter RD. Distinguishing whether dopamine regulates liking, wanting, and/or learning about rewards. *Behav Neurosci.* 2005; 119:5–15. [PubMed: 15727507]
- Rogan MT, LeDoux JE. Emotion: systems, cells, synaptic plasticity. *Cell.* 1996; 85(4):469–475. [PubMed: 8653782]
- Rouvette T, Vanelderen P, Roubos EW, Kozicz T, Vissers K. The amygdala, a relay station for switching on and off pain. *Eur J Pain (London, England).* 2012; 16:782–792.
- Santiago AC, Shammah-Lagnado SJ. Afferent connections of the amygdalopiriform transition area in the rat. *J Comp Neurol.* 2005; 489:349–371. [PubMed: 16025448]
- Saper CB. Role of the cerebral cortex and striatum in emotional motor response. *Prog Brain Res.* 1996; 107:537–550. [PubMed: 8782541]
- Schultz W, Dayan P, Montague PR. A neural substrate of prediction and reward. *Science.* 1997; 275:1593–1599. [PubMed: 9054347]
- Seamans JK, Floresco SB, Phillips AG. Functional differences between the prelimbic and anterior cingulate regions of the rat prefrontal cortex. *Behav Neurosci.* 1995; 109:1063–1073. [PubMed: 8748957]
- Shackman AJ, Fox AS. Contributions of the central extended amygdala to fear and anxiety. *J Neurosci.* 2016; 36:8050–8063. [PubMed: 27488625]
- Shammah-Lagnado SJ, Santiago AC. Projections of the amygdalopiriform transition area (APir). A PHA-L study in the rat. *Ann N York Acad Sci.* 1999; 877:655–660.
- 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]
- Shi CJ, Cassell MD. Cortical, thalamic, and amygdaloid connections of the anterior and posterior insular cortices. *J Comp Neurol.* 1998; 399:440–468. [PubMed: 9741477]
- Silberman Y, Winder DG. Emerging role for corticotropin releasing factor signaling in the bed nucleus of the stria terminalis at the intersection of stress and reward. *Front Psychiat.* 2013; 4:42.
- Skorzevska A, Bidzinski A, Hamed A, Lehner M, Turzynska D, Sobolewska A, Szyndler J, Maciejak P, Wislowska-Stanek A, Plaznik A. The effect of CRF and alpha-helical CRF(9-41) on rat fear responses and amino acids release in the central nucleus of the amygdala. *Neuropharmacol.* 2009; 57:148–156.
- Smith-Roe SL, Kelley AE. Coincident activation of NMDA and dopamine D1 receptors within the nucleus accumbens core is required for appetitive instrumental learning. *J Neurosci.* 2000; 20:7737–7742. [PubMed: 11027236]
- Stalnaker TA, Franz TM, Singh T, Schoenbaum G. Basolateral amygdala lesions abolish orbitofrontal-dependent reversal impairments. *Neuron.* 2007; 54:51–58. [PubMed: 17408577]
- Sun N, Yi H, Cassell MD. Evidence for a GABAergic interface between cortical afferents and brainstem projection neurons in the rat central extended amygdala. *The Journal of comparative neurology.* 1994; 340:43–64. [PubMed: 7513719]
- Swanson LW. Cerebral hemisphere regulation of motivated behavior. *Brain Res.* 2000; 886:113–164. [PubMed: 11119693]

- Swanson LW. The amygdala and its place in the cerebral hemisphere. *Ann N Y Acad Sci.* 2003; 985:174–184. [PubMed: 12724158]
- Swanson LW, Petrovich GD. What is the amygdala? *Trends Neurosci.* 1998; 21:323–331. [PubMed: 9720596]
- Swanson LW, Sawchenko PE, Cowan WM. Evidence for collateral projections by neurons in Ammon's horn, the dentate gyrus, and the subiculum: a multiple retrograde labeling study in the rat. *J Neurosci.* 1981; 1:548–559. [PubMed: 6180146]
- Tait DS, Brown VJ. Difficulty overcoming learned non-reward during reversal learning in rats with ibotenic acid lesions of orbital prefrontal cortex. *Ann N Y Acad Sci.* 2007; 1121:407–420. [PubMed: 17698991]
- Taylor JR, Robbins TW. Enhanced behavioural control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. *Psychopharmacol.* 1984; 84:405–412.
- Taylor JR, Robbins TW. 6-hydroxydopamine lesions of the nucleus accumbens, but not of the caudate nucleus, attenuate enhanced responding with reward-related stimuli produced by intra-accumbens d-amphetamine. *Psychopharmacol.* 1986; 90:390–397.
- Walker DL, Davis M. Role of the extended amygdala in short-duration versus sustained fear: a tribute to Dr. Lennart Heimer. *Brain Struct Funct.* 2008; 213:29–42. [PubMed: 18528706]
- Walker DL, Miles LA, Davis M. Selective participation of the bed nucleus of the stria terminalis and CRF in sustained anxiety-like versus phasic fear-like responses. *Prog Neuropsychopharmacol Biological Psych.* 2009; 33:1291–1308.
- Walker DL, Toufexis DJ, Davis M. Role of the bed nucleus of the stria terminalis versus the amygdala in fear, stress, and anxiety. *Eur J Pharmacol.* 2003; 463:199–216. [PubMed: 12600711]
- Wallace DL, Han MH, Graham DL, Green TA, Vialou V, Iniguez SD, Cao JL, Kirk A, Chakravarty S, Kumar A, Krishnan V, Neve RL, Cooper DC, Bolanos CA, Barrot M, McClung CA, Nestler EJ. CREB regulation of nucleus accumbens excitability mediates social isolation-induced behavioral deficits. *Nature Neurosci.* 2009; 12:200–209. [PubMed: 19151710]
- Wickens, J., Kotter, R. Cellular models of reinforcement. In: Houk, JC, Davis, JL., Beiser, DG., editors. *Models of Information Processing in the Basal Ganglia.* MIT Press; Cambridge MA: 1995. p. 187–214.
- Wilensky AE, Schafe GE, Kristensen MP, LeDoux JE. Rethinking the fear circuit: the central nucleus of the amygdala is required for the acquisition, consolidation, and expression of Pavlovian fear conditioning. *J Neurosci.* 2006; 26:12387–12396. [PubMed: 17135400]
- Will MJ, Franzblau EB, Kelley AE. The amygdala is critical for opioid-mediated binge eating of fat. *Neuroreport.* 2004; 15:1857–1860. [PubMed: 15305124]
- Wise RA. The anhedonia hypothesis: Mark III. *Behav Brain Sci.* 1985; 8:178–186.
- Wise RA. Dopamine, learning and motivation. *Nat Rev Neurosci.* 2004; 5:483–494. [PubMed: 15152198]
- Wise RA. Dopamine and reward: the anhedonia hypothesis 30 years on. *Neurotox Res.* 2008; 14:169–183. [PubMed: 19073424]
- Woods JW. Behavior of chronic decerebrate rats. *J Neurophysiol.* 1964; 27:635–44. [PubMed: 14194963]
- Yeterian EH, Van Hoesen GW. Cortico-striate projections in the rhesus monkey: the organization of certain cortico-caudate connections. *Brain Res.* 1978; 139:43–63. [PubMed: 413609]
- Yetnikoff L, Cheng AY, Lavezzi HN, Parsley KP, Zahm DS. Sources of input to the rostromedial tegmental nucleus, ventral tegmental area, and lateral habenula compared: A study in rat. *The Journal of comparative neurology.* 2015; 523:2426–2456. [PubMed: 25940654]
- Yetnikoff L, Lavezzi HN, Reichard RA, Zahm DS. An update on the connections of the ventral mesencephalic dopaminergic complex. *Neuroscience.* 2014a; 282C:23–48.
- Yetnikoff L, Reichard RA, Schwartz ZM, Parsley KP, Zahm DS. Protracted maturation of forebrain afferent connections of the ventral tegmental area in the rat. *J Comp Neurol.* 2014b; 522:1031–1047. [PubMed: 23983069]
- Yokel RA, Wise RA. Increased lever pressing for amphetamine after pimozide in rats: implications for a dopamine theory of reward. *Science.* 1975; 187:547–549. [PubMed: 1114313]

- Yokel RA, Wise RA. Attenuation of intravenous amphetamine reinforcement by central dopamine blockade in rats. *Psychopharmacol.* 1976; 48:311–318.
- 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(2):148–172. [PubMed: 16125239]
- Zahm, DS. Chapter 5: Cooperation and competition of macrosystem outputs. In: Heimer, L. Van Hoesen, GW. Trimble, M., Zahm, DS., editors. *Anatomy of Neuropsychiatry: The New Anatomy of the Basal Forebrain and its Implications for Neuropsychiatric Disease.* Elsevier; Amsterdam-New York - San Diego: 2008a. p. 101-139. 2008
- Zahm, DS. Accumbens in a functional-anatomical systems context. In: David, H., editor. *The nucleus accumbens: neurotransmitters and related behaviours.* Transworld Research Network-Research Signpost; Kerala, India: 2008b. p. 1-37.
- Zahm DS, Grosu S, Irving JC, Williams EA. Discrimination of striatopallidum and extended amygdala in the rat: a role for parvalbumin immunoreactive neurons? *Brain Res.* 2003; 978:141–154. [PubMed: 12834908]
- Zahm DS, Schwartz ZM, Lavezzi HN, Yetnikoff L, Parsley KP. Comparison of the locomotor-activating effects of bicuculline infusions into the preoptic area and ventral pallidum. *Brain structure & function.* 2014; 219:511–526. [PubMed: 23423460]

List of Abbreviations

ABA	accessory basal nucleus of the amygdala
ABC	avidin-biotin-peroxidase complex
Acb	nucleus accumbens
AcbC	Acb core
AcbS	Acb shell
AHip	amygdalohippocampal transition area
APir	amygdalopiriform transition area
BA	basal nucleus of the amygdala
BST	bed nucleus of the stria terminalis
CeA	central nucleus of the amygdala
CPu	caudate-putamen
Ctβ	cholera toxin, β subunit
DAB	diaminobenzidine
FG	FluoroGold
IC	insular cortex

LS	lateral septum
mPFC	medial prefrontal cortex
SPB	Sorenson's phosphate buffer
vSub	ventral subiculum

Figure 1.

Photomicrographs of case 15238 showing sections of rat forebrain cut in the frontal plane. A shows an FG injection into the AcbS, which is demarcated by the broken line. B is a Ctβ injection into the BST. C-H are photomicrographs of immunoperoxidase (C and D) and immunofluorescence (E-H) labeling of neurons. C shows the boxed area in its inset, which gives an overview of retrograde labeling produced in the medial temporal lobe by these injections. The box in C is enlarged in D. Black and brown immunoperoxidase labeling can be distinguished but it was generally uncertain with immunoperoxidase whether neurons were double-labeled. E shows immunofluorescence labeling. Ctβ-, FG- and double-labeled neurons are magenta; green and yellow-orange, respectively. F-H show laser scanning confocal images of single and double-labeling (white in H). Abbreviations: ABA - accessory basal nucleus of the amygdala; AcbS - accumbens shell; APir - amygdalopiriform transition area; BA - basal nucleus of the amygdala; BSTl and m – medial and lateral divisions of the bed nucleus of the stria terminalis; CeAm and l - medial and lateral divisions of the central nucleus of the amygdala; Ctβ - cholera toxin β subunit; FG - Fluorogold; ot - optic tract. Scale bar: A and B - 1 mm; C - 100μm; D-H – 50 μm.

Figure 2.

Further illustration of case 15238. Neurons labeled by injection of FG into the AcbS (green dots) and Ct β into the BST (magenta dots) and double-labeled neurons (yellow) were mapped in frontal sections of the temporal lobe of ordered in rostrocaudal sequence (A to H). Insets in A-H are provided for orientation. Note densely clustered double-labeled neurons in panels B and D-G. I is a photomicrograph showing green (Acb injection), magenta (BST injection) and yellow (double) labeling of neurons diagrammed in the box in panel F. Insets in I show the immunofluorescence images of the injection site in the Acb (above) and BST. The immunoperoxidase renderings of the injections are shown in Figs. 1A and B, respectively. Abbreviations: ABA - accessory basal nucleus of the amygdala; AHip - amygdalohippocampal transition area; APir - amygdalopiriform transition area; BA - basal nucleus of the amygdala BSTl - bed nucleus of stria terminalis, lateral division; CAa, CApl, CApm - anterior, posterolateral and posteromedial divisions of the cortical nucleus of the amygdala; CeAl - central nucleus of amygdala, lateral division; LA - lateral nucleus of the amygdala; vSub - ventral subiculum. Scale bar: 100 μ m

Figure 3.

Sites of tracer injections of FG into the BST (A) and Ct β into the CeA (B) and distributions of neurons labeled by BST (green dots) and CeA (magenta dots) injections. Double-labeled neurons are shown in (yellow). The case (16115) is mapped in the temporal lobe in frontal sections ordered in rostrocaudal sequence from C to J. Insets in C-J are provided for orientation. Note densely clustered double-labeled neurons in panels D and E and H-J.

Abbreviations: ABA - accessory basal nucleus of the amygdala; AHip - amygdalohippocampal transition area; APir - amygdalopiriform transition area; BA - basal nucleus of the amygdala; BSTl - bed nucleus of stria terminalis, lateral division; CAa, CApl, CApm - anterior, posterolateral and posteromedial divisions of the cortical nucleus of the amygdala; CeAl - central nucleus of amygdala, lateral division; LA - lateral nucleus of the amygdala; vSub - ventral subiculum. Scale bar: 1 mm in A and B

Figure 4.

Scatterplots showing double-labeled neurons expressed as raw numbers (A and C) and percent total of labeled neurons (B and D) and organized by brain structure (A and B) and injection pair (B and D). Symbols reflect averaged data from 2-4 cases as indicated in Table 1. Arrows indicate means of the means. In A and B green and blue symbols indicate homotypic and heterotypic injection pairs, respectively, and magenta symbols indicate pairs that include the caudomedial AcbS. In C and D green and blue text indicates homotypic and heterotypic injection pairs, respectively, and magenta text indicates pairs that include the caudomedial AcbS. Data were tested by repeated measure ANOVA. Brackets indicate differences revealed using the Fishers LSD post hoc test. Missing values reflect trials in which a minimum of 30 retrogradely labeled neurons for each tracer was not reached, which affected mainly the LA. Abbreviations: ABA – accessory basal amygdaloid nucleus; AHip – amygdalohippocampal transition area; APir – amygdalopiriform transition area; BA – basal amygdaloid nucleus; LA – lateral amygdaloid nucleus; vSub – ventral subiculum.

Figure 5.

Photomicrographs showing injection sites in the AcbS (A) and LS (B) and a laser scanning confocal microscopic image of the resulting retrograde labeling in the vSub, shown as green and magenta, respectively. White indicates double-labeling. Abbreviations: ac – anterior commissure; AcbC – accumbens core; AcbS – accumbens shell; CPu – caudate-putamen; LS – lateral septum; MS – medial septum. Scale bar: A and B - 1 mm; C - 40 μm.

Table 1

List of the cases, injection site pairs and injected tracers evaluated in the present study.

	case	Inj 1 -Tracer/Site	Inj 2 -Tracer/Site
AcbS&BST	09062 *	Ctβ/AcbS	FG/BST
	09065 *	Ctβ/AcbS	FG/BST
	09066	FG/AcbS	Ctβ/BST
	15236	Ctβ/AcbS	FG/BST
	15237	Ctβ/AcbS	FG/BST
	15238	FG/Acb	Ctβ/BST
	15239	FG/Acb	Ctβ/BST
AcbS&CeA	15240	FG/AcbS	Ctβ/CeA
	16118	FG/AcbS	Ctβ/CeA
	16119	FG/AcbS	Ctβ/CeA
	16120	FG/AcbS	Ctβ/CeA
AcbC&BST	15155	FG/AcbC	Ctβ/BST
	15195	FG/AcbC	Ctβ/BST
	15196	FG/AcbC	Ctβ/BST
	15197	Ctβ/AcbC	FG/BST
AcbC&CeA	15104	FG/AcbC	Ctβ/CeA
	15105	FG/AcbC	Ctβ/CeA
BST&CEA	15151	FG/BST	Ctβ/CeA
	15152	FG/BST	Ctβ/CeA
	16090	Ctβ/BST	FG/CeA
	16115	FG/BST	Ctβ/CeA
LS&Acb	09011 *	Ctβ/LS	FG/AcbS
	09019 *	FG/LS	Ctβ/AcbS
	09028 *	FG/LS	Ctβ/AcbS
	09056 *	FG/LS	Ctβ/AcbS
LS&BST	10021 *	FG/LS	Ctβ/BST
	10024 *	Ctβ/LS	FG/BST
	10025 *	FG/LS	CtβBST
	10027 *	FG/LS	Ctβ/BST
LS&CeA	10008 *	Ctβ/LS	FG/CeA
	16015	FG/LS	Ctβ/CeA
	16016	FG/LS	Ctβ/CeA

* Ventral subiculum only

Abbreviations: AcbC - accumbens core; AcbS - accumbens shell; BST - bed nucleus of the stria terminalis; CeA - central nucleus of the amygdala; Ctβ - cholera toxin β subunit tracer; FG - Fluorogold tracer; l - lateral division; LS - lateral septum; m - medial division.

Table 2

Numbers of retrogradely labeled neurons in evaluated structures following injections of Ctb and FG into the accumbens shell (AcbS) and core (AcbC), bed nucleus of stria terminalis (BST) and central nucleus of the amygdala (CeA).

	<u>Ctb</u>				<u>FG</u>										
	vSub	LA	BA	ABA	AHip	APir	Sum	vSub	LA	BA	ABA	AHip	APir	Sum	%
AcbS	1672	40	594	452	310	874	4255	774	11	204	201	71	372	1499	35.2
AcbC	2228	73	399	57	162	540	3459	510	51	310	92	245	88	1296	37.5
BST	800	131	919	751	743	2280	5676	406	40	313	471	272	742	2130	37.5
CeA	364	147	646	1553	270	1753	7394	100	8	304	310	36	863	1621	36.9

Each value is the mean of the following numbers of cases: AcbS/Ctb - 7 (vSub), 2 (others); AcbS/FG - 8 (vSub), 6 (others); AcbC/Ctb - 1; AcbC/FG - 5; BST/Ctb - 9 (vSub), 6 (others); BST/FG - 8 (vSub), 6 (others); CeA/Ctb - 12 (vSub), 9 (others); CeA/FG - 1. Sum is the sum of all structures for each Ctb and FG injection site (AcbS, AcbC, BST, CeA). % = sum (FG)/sum (Ctb)×100. Additional abbreviations: ABA - accessory basal amygdaloid nucleus; AHip - amygdalohippocampal transition area; APir - amygdalopiriform transition area; BA - basal amygdaloid nucleus, LA - lateral amygdaloid nucleus; vSub - ventral subiculum.

Table 3

Double labeling in the ventral subiculum from paired injections into the lateral septum (LS) and the accumbens shell (AcbS), bed nucleus of stria terminalis (BST) or central nucleus of the amygdala (CeA).

Injection pair	case	number of sections	number of single labeled, Inj 1 (LS)	number of single labeled, Inj 2 (AcbS, BST, CeA)	number double-labeled	double as percent total, Inj 1	double as percent total, Inj 2
A.							
LS&AcbS	09011	3	589	581	308	34.3	34.6
LS&AcbS	09019	8	2474	1551	556	18.3	26.4
LS&AcbS	09028	8	1236	2383	491	28.4	17.1
LS&AcbS	09056	6	1480	520	103	6.5 22±6* # †	16.5 24±4**
B.							
LS&BST	10021	5	1986	435	141	6.6	24.5
LS&BST	10024	6	3536	678	136	3.7	16.7
LS&BST	10025	5	2047	1266	205	9.1	13.9
LS&BST	10027	7	2796	421	156	5.3 6±1##	27.0 20±3
C.							
LS&CeA	10008	6	864	222	39	4.3	14.9
LS&CeA	16015	5	584	353	40	6.4	10.2
LS&CeA	16016	2	380	107	25	6.2 6±1###	18.9 15±3

Computed values, last 2 columns, are means±SEM

* ($F_{2,8}=5.580$, $p=0.030$), group A vs B - $p=0.019$, A vs C - $p=0.023$, group B vs C - NS, one way ANOVA

** ($F_{2,8}=1.481$, $p=0.284$) groups A vs B vs C - NS, one way ANOVA

vs last column, group A - NS, t test

vs last column, group B - $p=0.005$, t test

vs last column, group C - $p=0.026$, t test

† ($F_{3,11}=0.664$, $p=0.591$) 2nd last column, group A vs last column, group B vs last column, group C - NS, one way ANOVA

Additional abbreviations: NS - not significant.