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The relationship between the claustrum and endopiriform nucleus: a perspective towards consensus on cross-species homology

Jared B. Smith^{1,*}, Kevin D. Alloway², Patrick R. Hof³, Rena Orman⁴, David H. Reser^{5,6}, Akiya Watakabe⁷, and Glenn D. R. Watson⁸

¹Molecular Neurobiology Laboratory, Salk Institute for Biological Studies, La Jolla, CA 92037, USA

²Neural and Behavioral Sciences, Center for Neural Engineering, Pennsylvania State University, University Park, PA 16802, USA

³Fishberg Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, NY 10029, USA

⁴Department of Physiology and Pharmacology, State University of New York Downstate Medical Center, Brooklyn, NY, 11203 USA.

⁵Graduate Entry Medicine Program, Monash Rural Health Churchill, Monash University, Churchill, Victoria 3842, Australia

⁶Department of Physiology, Monash University, Clayton 3800, Victoria, Australia

⁷RIKEN Brain Science Institute, Wako, Saitama, Japan.

⁸Department of Psychology and Neuroscience, Duke University, Durham, NC 27708, USA.

Abstract

With the emergence of interest in studying the claustrum, a recent special issue of the *Journal of Comparative Neurology* dedicated to the claustrum (Volume 525, Issue 6, pp. 1313–1513) brought to light questions concerning the relationship between the claustrum (CLA) and a region immediately ventral known as the endopiriform nucleus (En). These structures have been identified as separate entities in rodents but appear as a single continuous structure in primates. During the recent Society for Claustrum Research meeting, a panel of experts presented data pertaining to the relationship of these regions and held a discussion on whether CLA and En should be considered (1) separate unrelated structures, (2) separate nuclei within the same formation, or (3) subregions of a continuous structure. This review article summarizes that discussion, presenting comparisons of the cytoarchitecture, neurochemical profiles, genetic

*Corresponding author: Molecular Neurobiology Laboratory, Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, jasmith@salk.edu.

Note: This review represents a consensus statement arising from a panel discussion at the 4th Annual Symposium of the Society for Claustrum Research. As such, all authors should be considered to have made equal intellectual contributions to this report.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the content of this article.

markers, and anatomical connectivity of the CLA and En across several mammalian species. In rodents, we conclude that the CLA and the dorsal endopiriform nucleus (DEn) are subregions of a larger complex, which likely performs analogous computations and exert similar effects on their respective cortical targets (e.g. sensorimotor versus limbic). Moving forward, we recommend that the field retain the nomenclature currently employed for this region but should continue to examine the delineation of these structures across different species. Using thorough descriptions of a variety of anatomical features, this review offers a clear definition of the CLA and En in rodents, which provides a framework for identifying homologous structures in primates.

Graphical Abstract

Uncertainty exists regarding the relationship between the claustrum and endopiriform nucleus, with discrepancies in how these nuclei are delineated across mammalian species. In this review, Smith et al. summarize recent findings that have identified anatomical markers for these regions in rodents to guide a reappraisal of these boundaries in primates.

Keywords

claustrum; endopiriform; rodent; primate; human; homology

1. Introduction

Located in the basolateral forebrain of most mammals (Kowia ski, Dziewiatkowski, Kowia ska, & Mory , 1999; for evidence of an avian homolog see Puellas et al., 2016), the claustrum (CLA) has been the topic of a growing body of research because it has been implicated in a number of important cognitive functions including consciousness (Koubeissi, Barolomei, Beltagy, & Picard, 2014; Smith, Liang, Watson, Alloway, & Zhang, 2017), attention (Mathur, 2014; Goll, Atlan, & Citri, 2015; White et al., 2018), salience detection (Smythies, Edelstein, & Ramachandran, 2012), multisensory integration (Edelstein & Denaro, 2004), cross-modal transfer (Hadjikhani & Roland, 1998), coordination of cortical areas involved in sensorimotor processing (Smith & Alloway, 2010; Smith, Radhakrishnan, & Alloway, 2012), and the binding of sensory information across both hemispheres to form globally-unified perceptions (Crick & Koch, 2005; Smith & Alloway, 2014).

In primates, the CLA is easily delineated, as it is encased within the white matter of the external capsule (medially) and the extreme capsule (laterally). However, in mammals such as rodents, in which the white matter of the external capsule is less developed compared to primates, the CLA appears as a dense, nuclear cluster nestled between the external capsule (medially) and the insular cortex (laterally). Some authors have even suggested that the rodent CLA is completely surrounded by the deep layers of the insular cortex (Mathur, Caprioli, & Deutch, 2009; Mathur, 2014). As such, identifying the precise boundaries of the CLA in rodents has been challenging, but has been greatly informed by cytoarchitectural, neurochemical, ontogenetic, and connectivity studies (for previous reviews, see Edelstein & Denaro, 2004; Crick & Koch, 2005; Druga, 2014; Mathur, 2014; Goll, Atlan, & Citri, 2015).

In a recent special issue of the *Journal of Comparative Neurology* dedicated to the claustrum (Volume 525, Issue 6, pp. 1313–1513), several articles considered the relationship of the CLA to the endopiriform nucleus (En), which is located immediately ventral to it (Orman, Kollmar, & Stewart, 2017; Watakabe, 2017; Watson & Puelles, 2017; Watson, Smith, & Alloway, 2017; Wullimann, 2017). As shown in Table 1, the CLA and En (dorsal and ventral subdivisions) are classically thought to comprise the CLA-En complex, which is a subgroup of the greater claustrum-amygdalar complex. The relationship between the CLA and En was recently taken up at the 4th annual Society for Claustrum Research symposium, where a panel of experts discussed whether they should be considered: (1) separate unrelated structures, (2) separate nuclei within the same formation, or (3) subregions of a continuous structure that should be simply called the “claustrum”.

In the discussion, several issues emerged regarding the nomenclature and boundaries for these nuclei in rodents, fruit bats, New World primates, Old World primates, and humans. As shown in Figure 1, major discrepancies exist among the published atlases that delineate the En in non-human primates, with no such structure even identified in humans. This issue has remained unresolved even though it was noted almost 20 years ago that “the ventrally situated paraamygdalar part of the human claustrum may correspond to the endopiriform nucleus or ventral part of the claustrum of other mammals, because of its morphological characteristics and connections with the limbic system” (Kowia ski, Dziewiatkowski, Kowia ska, & Mory , 1999).

The purpose of this review is to outline the cytoarchitectural characteristics, neurochemical make-up, genetic marker specificity, embryological origins, and connectivity of the CLA and En across mammalian species to elucidate the relationship of these two brain regions. Initially, we present a comprehensive description of CLA and En in rodents to identify characters that can be used to delineate the homologous regions in the primate brain. We then discuss the historical confusion in the primate literature regarding the definition of these nuclei and propose a path forward to resolve these issues. Finally, we look to fruit bats to determine if the same anatomical features observed in rodents and other Euarchontoglires are present in the CLA and En of this member of Laurasiatheria; indicating whether claustrum traits are well-conserved or divergent across the mammalian phylogeny. We conclude by summarizing our findings and making recommendations for how to move forward with cross-species comparisons of CLA and En to identify common features as well

as investigate the possibility of genuine differences in anatomy, connectivity, and physiology across-species (Sherk, 1986).

2. Re-assessing the relationship of the CLA and En in rodents

Though the CLA and En have been distinguished for nearly 100 years, their precise anatomical boundaries and classification are still being debated. In rodent studies, there is a wide range of descriptions for what constitutes the CLA, especially with respect to its compartmentalization and its relationship to insular cortex and the underlying En. A fervent controversy exists regarding whether CLA and En should be considered separately or as subdivisions of the same structure, with different investigators falling into camps of “lumpers” and “splitters”. The primary issue is that these regions are characterized by both similarities and differences, and this problem is exacerbated by technical challenges in precisely targeting them for study. In view of new data on genetic markers, developmental origins, and connectivity of the CLA and En, we provide a clear anatomical definition of these nuclei and their subregions. We conclude that the CLA (composed of dorsal and ventral compartments) and the dorsal endopiriform nucleus (DEn) are highly similar and likely related, whereas the intermediate and ventral subregions of the En (IEn and VEn) have few genetic or anatomical features in common with CLA and DEn, and therefore should be classified to a separate group.

2.1 Cytoarchitecture, neurochemistry, and ontogenetics of the CLA and En in rodents

The CLA in rodents (specifically murids) appears as a dense, nuclear cluster located between the external capsule and the insula with the En being located directly ventral. As shown in Figure 2a, Nissl staining of a coronal section of rat brain reveals two compartments, the CLA with very dense cell packing, whereas cell packing in the En is comparatively less dense. This organization was first noted in Nissl-stained sections of opossum (Loo, 1931), and has been subsequently applied to some, but not all, mammalian species as we show in Figure 1.

Some authors and atlases (Paxinos et al., 2012; Scalia, Rasweiler, Scalia, Orman, & Stewart, 2013; Paxinos & Watson, 2007) make distinctions between dorsal and ventral components of the CLA (dCLA and vCLA, respectively) and dorsal, intermediate, and ventral subregions of En (DEn, IEn, and VEn, respectively) as shown in Figure 1. As shown in Figure 2b, these distinctions have largely been made based on cytoarchitecture and neurochemical differences between these subdivisions (for a comprehensive review of the history, see Edelstein & Denaro, 2004 or Druga, 2014). Not all authors accept these distinctions, and there is substantial disagreement concerning the precise boundaries and compartmentalization of the CLA and En. Notably, what we show in Figure 2 as vCLA has been suggested by some authors to be the “claustrum proper”, owing to its striking density of certain CLA-specific markers, such as Gng2 (Mathur, Caprioli, & Deutch, 2009; Mathur, 2014) or its dense, oval-shaped expression of parvalbumin (PV) relative to the PV weak surrounding tissue (Kim, Matney, Roth, & Brown, 2016). Furthermore, a number of gene markers (e.g. NF-160, Crym, Cplx2) seem to avoid this oval shaped region of the vCLA while other gene markers (e.g. Ntng2, Gnb4, Gng2) seem to be “stronger” in the vCLA

when compared to DEn and dCLA. We believe these gene expression data indicate distinct sub compartments of a larger CLA-DEn complex that is comprised of dCLA, vCLA, and DEn, but not IEn or VEn, as shown in Fig. 2.

As indicated by Tables 2 and 3, numerous neurochemical similarities and differences between CLA and DEn indicate that neurochemistry alone is not conclusive for determining what should be associated with the CLA (for additional thorough discussions of neurochemical profiles of CLA-DEn see Druga, 2014 and Baizer, 2014). The most striking difference concerns the expression of PV, which is strongly expressed in vCLA in rodent but is almost non-existent in dCLA and DEn (Figure 2b). This pattern matches the descriptions of PV immunoreactivity in marmoset and macaque monkeys, with insular parts of CLA having strong PV expression, whereas ventral CLA (presumably the primate homolog of DEn, see discussion below) expression is very weak (Hardman & Ashwell, 2012; Saleem & Logothetis, 2012). Similar patterns have been observed for other calcium-binding proteins, such as calretinin (CR) and calbindin (CB) (Druga, 2014). However, caution should be made in interpreting neurochemical differences, as they may represent compartmentalization within a structure rather than separate nuclei. For example, the dorsal striatum is a single structure but has neurochemically distinct regions (identified as the patch/striosome and matrix compartments) that represent heterogeneous compartments within the greater-whole of the dorsal striatum (Smith et al., 2016). In summary, neurochemical profiling indicates many similarities with some dramatic differences between CLA and DEn, which suggests they should be considered separate nuclei within a larger complex.

Genetic marker expression, as revealed by *in situ* hybridization, is another method for assessing the relationship between CLA and DEn. As shown in Figure 2c–l, a number of genetic markers are shared in the dCLA, vCLA, and DEn, but not in the underlying VEn. For a complete summary of the gene expression profiling of the CLA-DEn complex, see Table 4 and its pictorial visualization in Figure 2m. Of the 47 genes analyzed, 80% of them were expressed in all three compartments (dCLA, vCLA, and DEn), while a small subset of these genes was selective for vCLA (12%), and another subset showed no expression in vCLA (8%). Thus, the data indicate that dCLA, vCLA, and DEn are highly similar in terms of genetic markers and should be considered part of the same complex (that excludes VEn which shared only a single gene). Nonetheless, based on neurochemical profiling, there is some specialization of vCLA that indicates functional compartmentalization within the CLA-DEn complex.

Developmental studies provide another lens for elucidating the relationship between CLA and DEn. Canonically, the CLA and En are thought to have originated from the dorsal and lateral pallium (as defined by new nomenclature), respectively, based on studies employing the 3H thymidine cell birthdating method (Bayer & Altman, 1991; Wulliman, 2017). However, an article in the recent claustrum special issue of the *Journal of Comparative Neurology* contradicts this view (Watson & Puelles, 2017, for more comprehensive discussion see Puelles, 2014). As shown in Figure 3b, analysis of Nr4a2 expression revealed that CLA and DEn originate from the same part of the pallium, but as the Nr4a2-expressing neurons migrate to the ventrolateral dorsal pallium to become the CLA, a subset of Nr4a2-expressing neurons continue migrating more ventrally to become the DEn. By contrast, cells

in VEn are Nr4a2-negative and originate from a completely separate population within the lateral pallium. These data provide the strongest evidence yet that dCLA, vCLA and DEn should be considered subnuclei that are part of the same complex, whereas IEn and VEn are separate structures.

2.2 Topographic organization of CLA and DEn connections with cortex

Beyond the cytoarchitectural, neurochemical, and developmental descriptions of the CLA and DEn, anatomical tracing studies have revealed functional specialization of these nuclei based on their connections with different parts of the neocortex.

Most of the early literature mapping the anatomical connectivity of the rodent CLA employed retrograde tracer injections into various parts of the cortex, which revealed general principles of the topography of connections between CLA and neocortex (Minciacchi, Molinari, Bentivoglio, & Macchi, 1985; Li, Takada, & Hattori, 1986; Sloniewski, Usunoff, & Pilgrim, 1986; Sadowski, Morys, Jakubowska-Sadowska, & Narkiewicz, 1997; Kowiański et al., 2001). These studies identified a loose topographical arrangement shown in Figure 4a, such that somatomotor circuits are more dorsally situated in the CLA, whereas projections to auditory and visual cortices are located more ventrally in the CLA (Li, Takada, & Hattori, 1986; Sloniewski, Usunoff, & Pilgrim, 1986; Sadowski, Morys, Jakubowska-Sadowska, & Narkiewicz, 1997; Kowiański et al., 2001). This dorsoventral topography was recently confirmed in mice by a study employing virus based anterograde tracing from different cortical regions in mice (Atlan, Terem, Peretz-Rivlin, Groysman, & Citri, 2017), where they also observed projections from the olfactory bulbs to the most ventral part of the CLA complex, which likely represent the DEn. This finding suggests that the sensorimotor topography of the CLA transitions into a limbic-related zone in the DEn.

Many of these same principles in the topographical organization between cortex and CLA are conserved in Old World monkeys. As shown in Figure 4b, the classic study by Pearson et al. compiled the projections from horseradish peroxidase injections in multiple cortices across a number of different macaque monkeys to reveal the topography within the CLA (Pearson, Brodal, Gatter, & Powell, 1982). This study observed the same principles of organization observed in rodents; a dorsoventral topography with somatomotor regions being dorsal in CLA, associative in central CLA, and audiovisual in ventral CLA.

The anatomical topography showing separate processing areas within the CLA strongly corresponds to functional divisions in the CLA identified by electrophysiology. As shown in Figure 4c, electrophysiological recordings in monkey CLA have revealed only unimodal responses, with forelimb motor responses found dorsally (Shima, Hoshi, & Tanji, 1996), whereas auditory and visual responses are found more ventrally in CLA, but not in overlapping areas (Remedios, Logothetis, & Kayser, 2010). Together these findings demonstrate that the principles guiding claustrorocortical interactions are largely conserved in both rodents and primates.

More recently, anatomical tracing from electrophysiologically identified regions of motor cortex to the CLA have revealed another functional topographic organization, in this case between different body representations in motor cortex and the CLA. As shown in Figure 5a,

lateral regions of motor cortex controlling the forelimb (i.e. lateral agranular cortex) receive projections from the most dorsal part of CLA, more medial regions of motor cortex controlling the whiskers (i.e. medial agranular cortex) receive projections from the intermediate parts of CLA in the dorsoventral axis, and regions on the midline of motor cortex that control eye movements (i.e. cingulate cortex) are innervated by the most ventral part of the rodent CLA (Smith & Alloway, 2010, 2014). Thus, beyond the original dorsoventral topographic arrangement identified by early studies linking CLA to cortex, these recent studies have revealed a medial-to-lateral gradient in motor cortex that maps ventral-to-dorsal in CLA, respectively.

Whereas the CLA appears to have topography involving connections with sensorimotor cortex, the DEn is connected with limbic areas of cortex. As shown in Figure 5a, tracing studies in rodents have identified connections of the DEn with the infralimbic, entorhinal, and piriform cortices, and other limbic areas (Behan & Haberly, 1999; Hoover & Vertes, 2007; Watson, Smith, & Alloway, 2017). This continues the gradient of connectivity observed between CLA and motor cortex as infralimbic cortex, which is located deeper in the medial bank of mPFC than cingulate cortex, receives inputs from the most ventral part of the CLA-DEn complex, i.e. DEn. These common themes in connectivity suggest that CLA and DEn perform similar functions and are part of the same complex.

2.3 Intranuclear long-range connectivity of CLA and DEn in rodents

One unique feature of CLA anatomy is the presence of long-range, intranuclear connections that run along the anterior-posterior axis of the CLA. As proposed by Crick and Koch, these projections could allow information to be shared and integrated across different modalities within the CLA (Crick & Koch, 2005). Evidence in rats from retrograde tracer injections in the central region of CLA showed retrogradely labeled cells throughout its entire anterior-posterior extent (Smith & Alloway, 2010). Similarly, both anterograde and retrograde tracer injections in the rostral, middle, and caudal regions of DEn reveal connectivity to every other part of DEn, but these long range fibers stay within DEn and do not send projections dorsally into the CLA proper (Behan & Haberly, 1999; Watson, Smith, & Alloway, 2017). As shown in Figure 5b, these data suggest there is an “all-to-all” connection along the rostrocaudal axis of the CLA-DEn complex, with a dorsoventral specificity of CLA-to-CLA and Den-to-DEn, respectively.

Such anatomical segregation of integrative intranuclear projections within isolated processing streams within CLA is supported by physiological recordings in rodent (Orman, 2015) and explains the segregation of sensory modalities observed in macaque monkeys (as shown in Figure 4c). Interestingly, another prominent electrophysiological study in slice preparation found very little connection between excitatory cells (Kim, Matney, Roth, & Brown, 2016). However, these brains were sliced coronally, likely severing the longitudinal connections between excitatory neurons. The finding of intrinsic, longitudinal connections for both CLA and DEn also provide additional evidence that these nuclei represent different components of the same complex.

2.4 Interhemispheric connectivity of CLA and DEn in rodents

Recent anatomical tracing studies have demonstrated a unique hemispheric imbalance in the strength of the connections between CLA and areas of motor cortex (Alloway, Smith, Beauchemin, & Olson, 2009; Smith & Alloway, 2010, 2014; Smith, Radhakrishnan, & Alloway, 2012). Specifically, these studies placed anterograde and retrograde tracers in homotopic regions of motor cortex of each hemisphere in the same animal (i.e. anterograde tracer in left motor cortex and retrograde tracer in right motor cortex) to reveal an additional pathway by which motor cortex in one hemisphere communicates with corresponding motor areas in the opposite hemisphere via the CLA. As indicated by the schematic diagram in Figure 5c, the motor cortex projects much more strongly to the contralateral than to the ipsilateral CLA. This contralateral projection provides dense innervation of claustral neurons that, in turn, project within that hemisphere to the motor cortex, thereby creating an interhemispheric cortico-claustral-cortical circuit.

A similar set of interhemispheric circuit connections was observed linking DEn with infralimbic cortex (Figure 5c), which represents a limbic area of medial prefrontal cortex (Watson, Smith, & Alloway, 2017), suggesting similar rules of interhemispheric connectivity between frontal cortex regions and the CLA-DEn complex.

2.5 Intrahemispheric connectivity of CLA and DEn in rodents

Several studies show that claustral neurons targeting a specific representation of motor cortex also send collateral projections to the corresponding modality-specific region of sensory cortex (Smith, Radhakrishnan, & Alloway, 2012). For example, populations of claustral neurons that innervate the frontal eye fields also project to the visual areas of sensory cortex (Smith & Alloway, 2014). This finding is aligned with previous work showing that claustral neurons have branched axonal projections that innervate cortical areas that are widely separated (Minciacchi, Molinari, Bentivoglio, & Macchi, 1985). It also supports data obtained from monkeys demonstrating that overlapping domains within the CLA project to both frontal and parietal-temporal-occipital cortical regions that are interconnected (Pearson, Brodal, Gatter, & Powell, 1982). Together, these studies provide strong evidence that activation of the claustrum by motor cortex could enable coordination of motor and sensory cortical regions that process modality-related information.

Similar circuit connections have also been observed more rostrally in the medial prefrontal cortex (mPFC). The mPFC projects most strongly to the contralateral CLA-DEn complex which, in turn, innervates the homotopic region of mPFC in that hemisphere and sends a collateral projection to other limbic associated cortical areas (Smith, Liang, Watson, Alloway, & Zhang, 2017; Watson, Smith, & Alloway, 2017). Furthermore, a dorsoventral topography was observed such that dorsal mPFC (i.e. prelimbic cortex) has stronger connections with the CLA, whereas ventral mPFC (i.e. infralimbic cortex) innervates the DEn, a finding confirmed by other tracing studies (Behan & Haberly, 1999; Hoover & Vertes, 2007). In addition, just as neurons in the CLA have collateral projections that innervate modality-related regions of sensory and motor cortex that share corticocortical connections (Smith et al., 2012), the DEn sends collateral projections to infralimbic and entorhinal cortices that share corticocortical connectivity (Watson et al., 2017). Thus, the

DEn appears to have similar patterns of intrahemispheric connectivity to the CLA, indicating they subserve similar computation functions, but interact with limbic cortices as opposed to sensorimotor.

2.6 Laminar organization of connectivity between CLA-DEn and cortex in rodents

There is some ambiguity regarding the laminar specificity of the projections between CLA and cortex. Early tracing data indicated that corticoclaustral projections originate from layer 6 (LeVay & Sherk, 1981), an idea that has been generalized to the entirety of the corticoclaustral projection largely on the basis of cat studies (Goll, Atlan, & Citri, 2015). Corticoclaustral projections from layer 6 have been observed in rodents, but only in primary sensory cortices (Smith & Alloway, 2014). The corticoclaustral projections from frontal cortex, however, have an organization that differs substantially from what is seen in sensory cortex (Figure 5d). Retrograde tracer injections in the CLA of rats indicate that corticoclaustral projections from frontal cortex originate in layers 2 to 5 (Smith & Alloway, 2010), whereas layer 6 is completely lacking in labeled neurons. Interestingly, this same pattern of corticoclaustral output arising from layers 2 to 5 (but not layer 6) holds true for higher-order cortical areas like area V2 in rodents (Carey & Neal, 1985; Smith & Alloway, 2014).

Comparatively, the output of the CLA seems to target all layers of the neocortex, with a very sparse distribution of small terminals (Figure 5d); a pattern observed in a number of mammalian species including mouse (Zingg et al., 2015; Wang et al., 2017), rat (Smith, Liang, Watson, Alloway, & Zhang, 2017), and cat (LeVay and Sherk, 1981; da Costa et al., 2010; Fursinger & Martin, 2010). Based on analysis of cat primary visual cortex, the CLA sends denser inputs to layers 2, 3, and 6 (da Costa et al., 2010; Fursinger & Martin, 2010), but in rodents the CLA sends projections across the entire cortical mantle with wide variation in laminar specificity (Zingg et al., 2014; Wang et al., 2017). Furthermore, there is a wide variation in the density of innervation across different areas of cortex (see Zingg et al., 2014 and Wang et al., 2017), with primary sensory areas receiving minimal CLA input, whereas frontal regions (including cingulate cortex and mPFC) have considerably denser input from the CLA.

The terminal boutons of claustrorocortical projections are very small with a morphology that has been suggested to resemble “modulator” type synapses. As shown in the photomicrograph in Figure 5d, these terminals appear as small ($<0.5\ \mu\text{m}$ diameter), “mushroom-like” boutons that extend off the axon (da Costa et al., 2010; Fursinger & Martin, 2010; Smith, Liang, Watson, Alloway, & Zhang, 2017), which is consistent with “modulator” type synapses that exhibit paired-pulse facilitation on their post-synaptic targets (Sherman & Guillery, 1998). However, there is some controversy surrounding the “driver/modulator” categorization for claustral synapses, especially for corticoclaustral synapses (Day-Brown et al., 2017; Brown et al., 2017). Specifically, these authors measured the size of corticoclaustral synapses ($0.34 \pm 0.02\ \mu\text{m}^2$) and compared them to the size of corticothalamic modulators ($0.24 \pm 0.01\ \mu\text{m}^2$) and corticothalamic drivers ($0.94 \pm 0.08\ \mu\text{m}^2$). They found that corticoclaustral terminals were significantly larger than corticothalamic modulators and significantly smaller than corticothalamic drivers. However, corticoclaustral

synapses and corticothalamic modulators are still relatively close in size, both being 3–4 times smaller than corticothalamic drivers. Furthermore, beyond sheer size, corticoclaustral and corticothalamic modulators have similar morphological structure, exhibiting “mushroom-like” stalks, in contrast to driver-type corticothalamic synapses. This suggests that claustral terminals represent a “modulator” type synapse. Future electrophysiology studies, however, are needed to determine if claustracortical synapses exhibit paired-pulse facilitation, which is a functional marker of a “modulatory” effect. Indeed, corticoclaustral synapses need to be assessed for short-term synaptic plasticity and the presence of metabotropic glutamate receptors to determine further how these synapses relate to the driver/modulator framework (Brown et al., 2017).

So how do the projections from DEn compare to these observations on the CLA’s connection with cortex? Dual anterograde/retrograde injections into DEn show retrogradely labeled cells in layers 2 to 5 of the infralimbic cortex, whereas the terminal boutons from the anterograde projections appeared throughout all layers of cortex (Behan & Haberly, 1999; Watson, Smith, & Alloway, 2017). Furthermore, the same terminal morphology of small en passant boutons as well as “mushroom-like” terminals extending from the axon have been observed for DEn projections to infralimbic cortex (Behan & Haberly, 1999; Watson, Smith, & Alloway, 2017). These similarities of DEn and CLA summarized in Figure 5d provide additional evidence that CLA and DEn should be classified as being subnuclei within a greater CLA-DEn complex.

2.7 Conclusions on the relationship of CLA and En in rodents

Our analyses indicate that the CLA and DEn are parts of the same complex, whereas there is mounting evidence that the VEn is a completely separate structure. This view is largely based on a high degree of overlap of neurochemical and genetic markers between CLA and DEn, which are absent from VEn. However, there are also genetic, neurochemical, and architectural differences between CLA and DEn, indicating they are functionally separate nuclei within the same complex.

The differential, yet parallel, circuit connections of CLA and DEn suggest a functional delineation between these two nuclei, in which sensorimotor functions are ascribed to the CLA whereas the DEn processes limbic information. Though connecting to different parts of cortex, CLA and DEn have highly similar patterns of connectivity including intra-nuclear connectivity, interhemispheric loops between frontal cortical regions, intrahemispheric branching of projections to cortical areas that share direct corticocortical connections, and the same input-output organization with the different layers of cortex. Because of these common themes in connectivity, we believe the CLA and DEn operate similar computational mechanisms on different functional circuits (i.e. sensorimotor vs. limbic). Therefore, these should be considered separate regions within a greater CLA-En complex, similar to the way the in which the basal ganglia have distinct input nuclei (e.g. accumbens, caudate, and putamen) that perform similar functions on different sets of cortical inputs.

In contrast to the wealth of connectivity known about CLA and DEn, the literature on IEn and VEn is sparse. Classic anatomical tracing studies indicate that VEn is connected to medial prefrontal cortex, posterior insular cortex, and the hypothalamus (MacDonald, 1987;

MacDonald & Jackson, 1987; Price, Slotnick, & Revial, 1991). These findings suggest that VEn is connected with brain regions that process visceral, emotional, and other limbic-related information, but it is unclear whether VEn follows the same principles of connectivity with cortex that we have outlined for CLA and DEn. Future work should revisit the connectivity of IEn and VEn with cortex to look for patterns that match or contrast with those observed with CLA and DEn.

3. Re-assessing the identity of the CLA and En in anthropoid primates

The CLA in primates is a very conspicuous structure, situated between the striatum and the insular cortex. By comparison, the definition of En is rather ambiguous. In some studies, En is considered to be identical to the “ventral claustrum” (Kowia ski et al., 1999; Buchanan & Johnson, 2011), whereas in other studies it is considered part of the amygdaloid complex (Kritzer, Innis, & Goldman-Rakic, 1988; Kordower, Le, & Mufson, 1992; Ding et al., 2016). Based on morphological appearance, the same structure appears to be annotated differently across atlases and peer-reviewed reports (see below for more details). This confusion may partly stem from the difficulty in delineating the dorsal and ventral divisions of the En, which are quite different (see Table 4). In adult rodent brains, the DEn is differentiated from the VEn by its position (Swanson & Petrovich, 1998), embryonic origins (Watson & Puelles, 2017, see Figure 3b) and expression of several molecular markers (Figure 2). Using the framework of anatomical markers identified in rodents, this next section will examine the same anatomical features in marmosets, macaque monkeys, and humans to appropriately delineate the homologous boundaries for CLA, DEn, and VEn in these primate species.

3.1 Delineation of CLA and En in marmosets based on molecular markers

The common marmoset (*Callithrix jacchus*) is a New World monkey that separated from Old World monkeys about 35–40 million years ago (Mitchell & Leopold, 2015). Recently, comparative gene expression analyses have revealed a panel of genes enriched in the mouse CLA that are also highly enriched in the marmoset CLA (Watakabe, 2017). How these genes relate to En in marmosets, however, is unclear. As shown in Figure 6, Nurr1, a well-conserved gene marker for CLA (Arimatsu et al., 2003; Watakabe, 2017; Watson & Puelles, 2017) is strongly expressed in the “insular claustrum” as well as its ventral extension, which corresponds to the dorsal, intermediate and ventral endopiriform nuclei (DEn, IEn, and VEn) as shown in a recent marmoset brain atlas (Paxinos et al., 2012a,b). In a different marmoset atlas, a similar region is annotated simply as “DEn” (Hardman & Ashwell, 2012). Several other genes exhibit a similar expression pattern, although latexin showed very low scattered expression in the regions that correspond to IEn and VEn of the Paxinos atlas (Watakabe, 2017). In rodents, these CLA-enriched genes are strongly expressed in DEn but not VEn (Figure 2). As such, either the gene expression is different between marmosets and rodents or IEn and VEn of the Paxinos atlas are actually subdivisions of the DEn. As discussed below, the CLA-En complex of the marmoset can be subdivided into several compartments by myelinated fibers (Figure 9), as well as by variations in the strength of gene expression within this complex (Figure 6, Watakabe, 2017). Because similar variations in gene expression are also observed in mice (Figure 2), it is likely that claustral gene expression is

well-conserved across primates and rodents, and that the marmoset homologue of the VEn lies outside of the Nurr1-expressing area.

3.2 Identification of the CLA and En in macaque monkeys by molecular markers

In several studies, “endopiriform nucleus” in macaque monkeys is explained as the homologue of the rat DEn (Amaral & Price, 1984; Insausti, Amaral, & Cowan, 1987; Kritzer et al., 1988; Carmichael, Clugnet, & Price, 1994). However, the VEn is not documented in these studies. By contrast, the dorsal/ventral distinction of En is present in an atlas for rhesus monkeys (Paxinos et al., 2000). Comparisons of this atlas and these articles revealed discrepancies in the definition of the dorsal and ventral subdivisions of En in macaques. Generally speaking, the position of the “endopiriform nucleus” corresponds to a region inside the piriform cortex, which is readily distinguished by the presence of dense Nissl staining of its granular layer and its proximity to the CLA. This location precisely matches that of the “dorsal endopiriform nucleus” of the rhesus monkey atlas (Paxinos et al., 2000), but the “endopiriform nucleus” in some studies appears to also include the “ventral endopiriform nucleus” of the Paxinos atlas (e.g., Insausti et al., 1987; Kritzer et al., 1988; Carmichael, Clugnet, & Price, 1994). To clarify the cross-species definition of DEn, we examined the expression of CLA-specific genes relative to other anatomical markers in the macaque monkey.

Serial sections of a Japanese monkey (*Macaca fuscata*) brain stained for various histological and molecular markers, including the CLA marker Nurr1 (or Nr4A2) gene, are shown in Figures 7 and 8. Nurr1 is expressed in a subset of layer 6 neurons across the entire cerebral cortex, including the adjacent insular and entorhinal cortices (Watakabe et al., 2007, 2014). Therefore, it is necessary to carefully delineate the surrounding structures using other markers. In this series of sections, *in situ* hybridization for the VGluT1 gene was performed on the adjacent sections of the Nurr1-stained sections. VGluT1 is the major glutamate transporter gene in the cerebral cortex (Fujiyama, Furuta, & Kaneko, 2001; Nakamura et al., 2007) and is expressed both in the CLA and in subregions of the amygdala (lateral, basolateral and basomedial amygdala) but not in the striatum, preoptic area or certain subregions of the amygdala including its medial and central nuclei, and the anterior amygdala area (Figure 8k–o). This series also contains classic histological stains for cytochrome oxidase (CO), acetylcholine esterase (AChE), and Nissl material.

As shown in Figure 8, the strong Nurr1-expressing areas are considered to correspond to the CLA-DEn complex. The specificity of Nurr1 gene expression for the CLA is evident from its lack of expression in any amygdala subnuclei (Barbas & De Olmos, 1990), as is obviously evident in the Nurr1 avoidance of PLBL in Figure 8q. Furthermore, the large nuclear cluster within the temporal lobe white matter also showed high Nurr1 gene expression, further confirming the specificity of “claustral” expression (Figure 8p, q and their traces in panel 038T and 063T). In section #059 shown in Figure 8r, the Nurr1-expressing area splits into the less stained medial part and the strongly stained lateral part that continues up to the dorsally positioned CLA. By location, the medial part is considered to be the posterior end of the DEn, and the lateral part is considered to extend further posteriorly (sections #084 and beyond), where it shows the typically elongated morphology

of the CLA. Most strikingly, there exists a clear cut border between the Nurr1-expressing “CLA” and its mediolaterally adjacent VGluT1-enriched areas in sections #083 and #084 (denoted by asterisks). This border is visible in other anatomical stains as shown in sections #076–#088, including CO and AChE (red arrows), and suggests a physical discontinuity of the CLA-DEN complex from the surrounding brain regions (i.e. amygdala).

By comparing the morphology and gene expression patterns between the marmoset and macaque, it is parsimonious to conclude that the Nurr1-positive areas represent the CLA-DEN complex in both species. It implies that the VEn, if it exists, is out of this Nurr1-expressing region. When compared with the Macaque monkey atlas (Paxinos et al., 2000), one candidate for VEn is the island of VGluT1-positive/Nurr1-negative cells in sections #083–#088 mentioned above (denoted by the asterisks in Fig. 8) and/or its anterior extension. Alternatively, this region could be a part of the lateral amygdala. At present, there are no firm criteria for identifying VEn across-species. However, both in rodents (Swanson & Petrovich, 1998; Paxinos & Watson, 2007) and in macaques (Paxinos et al., 2000), what is designated as the “ventral endopiriform nucleus” is a structure that lies closer to the amygdala than to the DEN. It is likely that the “endopiriform nucleus” has been classified into the amygdaloid complex in some studies (Kritzer et al., 1988; Kordower, Le, & Mufson, 1992; Ding et al., 2016), because it contained the VEn, which should be regarded as entirely different from the CLA-DEN complex.

It is also important to consider distinctions between the CLA and DEN. Although the Nurr1-expressing areas in the caudal sections are all labeled as “CLA” in the bottom panels in Figure 8, which is consistent with most of the monkey literature, it is possible that the “root of the claustrum” (Buchanan & Johnson, 2011) actually corresponds to DEN, as in the marmoset (Figure 6). In this regard, retrograde tracer injections into the macaque entorhinal cortex results in labeling of the ventral part of the CLA (Insausti et al., 1987), which suggests that it may be the homologue of the rodent DEN. Conversely, the region labeled as DEN in sections #009 and #013, is denoted as the CLA in some studies (Insausti et al., 1987; Paxinos et al., 2000) or left unannotated (Saleem & Logothetis, 2012). Again, retrograde tracing shows its projection to the entorhinal cortex (Insausti et al., 1987), which supports its identity as DEN. This region also sends projections to the caudal orbitofrontal area, Iam (Carmichael, Clugnet, & Price, 1994). It is thus likely that a part of what has been designated as the “claustrum” may well correspond to the homologue of the “dorsal endopiriform nucleus” of the rodents, although such homology still needs to be further explored.

3.3 Identification of the En in humans

The atlas by Mai et al. (Mai, Majtanik, & Paxinos, 2016) and the human reference atlas by the Allen Institute for Brain Science (Ding et al., 2016) provide the anatomical framework for the definition of the CLA and En in human. The comparison of the two atlases illustrates the difficulty of identifying the “same structure” across different samples. One characteristic feature of the human CLA-En complex common to both atlases, however, is that its ventral aspect is subdivided into many cell islands by myelinated passing fibers. In the Allen Institute atlas (Ding et al., 2016, see also <http://atlas.brain-map.org/>), this part is designated

as the temporal CLA altogether, while the Mai atlas is subdivided into many groups, such as “diffuse insular claustrum”, “ventral claustrum”, “periamygdaloid claustrum”, “limitans claustrum”, and “temporal claustrum”. In reference to the position of the anterior commissure, and of a distinct white matter region medial to these cell islands, it is highly likely that these claustral subdivisions correspond to the Nurr1-expressing “CLA” and/or “DEn” in Figure 6. The combined morphology of the CLA as a whole in humans is strikingly similar to the Nurr1-enriched CLA-DEn complex of the macaque. Unfortunately, the “endopiriform nucleus” does not exist in the Mai atlas, but is present in the Allen Institute atlas, being classified as a part of the amygdaloid complex. In comparison to the macaque monkey atlas (Paxinos et al., 2000), the position of the “endopiriform nucleus” in the Allen Institute atlas appears to correspond mainly to VEn, although part of it could be DEn. As such, if we establish the DEn as a part of the “claustrum” and the VEn as a part of the “amygdala”, we could clearly distinguish them using CLA marker genes, which would resolve the apparent inconsistencies. Presently, we need to bear in mind that the CLA, DEn, VEn, and possibly unidentified amygdala subareas may be intermixed in the primate literature, and as a field we must conduct the appropriate studies to clear up these discrepancies and adopt a precise definition of each structure to guide future studies.

3.4 Delineating CLA and En in primates by anatomical connectivity and cytoarchitecture

The CLA complex has largely been overlooked in cyto-, myelo-, and chemoarchitectural studies of nonhuman primates. Recent reports (Pirone et al., 2012; Watakabe, Ohsawa, Ichinohe, Rockland, & Yamamori, 2014; Reser et al., 2014; 2017) have added to the sparse classical literature (Brand, 1981; Braak & Braak, 1982; Miyashita, Nishimura-Akiyoshi, Itohara, & Rockland, 2005) surrounding the cell types and neurochemical markers associated with the primate CLA. However, there is no current agreement regarding such fundamental questions as to whether there are internal subdivisions of the CLA, or what the morphological or functional determinants of such compartments might be. Similarly, recent anatomical connectivity studies have built upon past descriptions of cortical connectivity, mainly from macaque monkeys (LeVay & Shirk, 1981; Tanne-Gariepy et al., 2002), and these studies have expanded into new primate families to enable more detailed comparative analyses (Burman et al., 2011; Gattass, Soares, Desimone, & Ungerleider, 2014). A key feature of these newer studies is the combination of detailed connectional analysis of CLA projections with cytoarchitectural and topographical organizational analysis of the cortical origins of these projections. Thus, we are now aware of a topographical organization of projections to the CLA complex originating from various levels of the parallel distributed hierarchy of visual cortex areas of the macaque (Baizer, Desimone, & Ungerleider, 1993; Baizer, Lock, & Youakim 1997; Gattass, Soares, Desimone, & Ungerleider, 2014), between adjacent areas of the mPFC and anterior cingulate cortex of the marmoset (Reser et al, 2017), and between the closely related parietal areas PE and P_{Ec} in the macaque (Gamberini et al., 2017). On a more granular level, differential connectivity to the CLA has been described for connections arising within the medial and lateral portions of a single cytoarchitecturally-defined area of prefrontal cortex, the frontal polar Area 10 (Burman et al., 2011; Reser et al., 2014). This latter observation is particularly interesting, as it has been reported in two separate New World monkey species, the marmoset (*Callithrix jacchus*) and the capuchin (*Cebus apella*), suggesting that differential CLA connectivity may have arisen

early in the evolutionary and/or ontological processes through which cytoarchitectural areas of cortex appeared. Together, these connectivity studies lead to an obvious question regarding the internal organization of the CLA complex in the primate brain: does the connectivity of CLA complex divisions with the cerebral cortex follow a pattern by which we might infer the functional distinctions, if any, between subdivisions of the CLA complex?

To address this question, it is necessary to briefly review the cytoarchitecture and myeloarchitecture of the primate CLA complex, to relate the locations of tracer-labeled neurons arising from connectional studies. This is complicated by the relative paucity of primate species for which detailed information is available, as well as by the gross morphological variation in the CLA complex across primates. In humans, in particular, the En has been described as “vestigial” (Buchanan & Johnson, 2011), and is evident as a series of fragmented cell clusters or “islands” ventral to the insular portion of the CLA. This fragmentation is much less prominent in Old World monkeys, and is only marginally evident in New World species, as seen in occasional cell clusters lying outside the CLA complex in Nissl-stained specimens (Reser et al., 2014).

The CLA-En boundary in primate brains is somewhat loosely defined. Using only the marmoset as an example, there are substantial regions of uncertainty which could lead to confusion and may hamper experimental progress by making it difficult to selectively target CLA vs. En for injection of viral constructs, as have been employed in rodent studies (White et al., 2018). In the marmoset stereotaxic atlas of Paxinos et al. (2012), the En is identified on the basis of increased cell density in the Nissl-stained preparation, accompanied by a relatively high density of CR-immunoreactive neurons compared to the CLA. Another marmoset atlas shows the En in much the same position over approximately the middle third of the CLA, but there are some notable differences (Hardman & Ashwell, 2012). Specifically, Hardman and Ashwell do not identify an En region caudal to the posterior end of the anterior commissure, whereas the En of Paxinos et al. extend to the caudal end of the entire CLA complex, and indeed the last remaining coronal section in which the CLA complex appears is identified in its entirety as DEn.

A second prominent difference between the atlases for the marmoset lies in the relationship between the CLA complex and the amygdaloid complex. In the parcellation of Hardman and Ashwell, the En occupies the entire region between the CLA and amygdaloid complex, with the external and extreme capsules terminating more dorsally. Thus, in this representation, the CLA-En complex is contiguous with the dorsolateral amygdala. This is not in accordance with our observations, and may result from the heavy reliance upon cyto- and chemoarchitecture, as opposed to myeloarchitecture, for resolution of both internal and external CLA complex boundaries.

In both New World (Reser et al, 2014; 2017) and Old World monkey species (Baizer, 2014), the CLA complex is particularly well resolved by staining for myelin, which is present at much lower levels in the CLA than in the adjacent white matter tracts. The myeloarchitecture in the marmoset indicates that the CLA complex is isolated from the dorsolateral amygdala, as seen in Figure 9. Furthermore, close examination of the

myeloarchitecture shows that internal compartments in the CLA complex can be demarcated in the marmoset. Identifiable borders include the CLA-DEn border, as well as transitions between the dorsal, intermediate, and ventral subdivisions of DEn (Figure 9). No clear homologue of the rodent IEn or VEn has yet been identified in the marmoset, as detailed in the previous section.

We propose that the regions identified by relative density of myelin staining and the directionality of the stained axons, represent subdivisions of the DEn (Figure 9). Corroborating regions of varying stain intensity are evident in Nissl-stained specimens (not shown), and give us the following working model for our preferred model species, the common marmoset: the dorsal segment of the CLA, corresponding approximately from the dorsal terminus, represents the classical “insular claustrum”, or simply CLA (Figure 9). This structure is contiguous with a myelin-poor, cell body-dense region occupying approximately the middle third of the complex along the dorsoventral axis, and running from the rostral end to approximately the caudal extent of the anterior commissure. We regard this as the DEnD, and in the marmoset it is the most reliably identifiable component of the DEn. Ventral to this region, intermediate (DEnI) and ventral subdivisions (DEnV) of the DEn are visible as myelin-poor regions with varying proportions of axons traveling either transversely between the external and extreme capsules (DEnI), or along the dorsoventral axis or longitudinally along the rostral-caudal axis of the CLA complex (DEnV). At all levels along the dorsoventral axis in the marmoset, the CLA complex can be resolved from the medially adjacent amygdaloid complex in myelin stained sections (arrowheads in Fig. 9), and this is corroborated by chemoarchitectural evidence as well (e.g. in sections immunostained for the neurofilament M and H subunit proteins in the Paxinos et al., 2012 atlas).

With this framework in mind, we now turn our attention to the question of connectivity, and especially whether the internal compartmentalization of the CLA as described above corresponds to a pattern of cortical connections, which could shed light on the function or functions of the primate CLA. Nearly forty years ago, Pearson and colleagues showed that for a wide range of cortical regions, areas of high corticocortical connectivity exhibit overlapping projections to and from the CLA (see Figure 4b), in both primate and non-primate species (Pearson, Brodal, Gatter, & Powell, 1982). Additional work has shown that there are some modality-specific characteristics of CLA connectivity, including localization of visual connections to the ventral and caudal aspects of the CLA, with somatomotor projections concentrated in the dorsal regions (Pearson, Brodal, Gatter, & Powell, 1982; Baizer, Lock, & Youakim, 1997). Regionalization of CLA connections is also evident following tracer injections in prefrontal (Selemon & Goldman-Rakic, 1988; Burman et al., 2011; Reser et al., 2014; 2017), inferior temporal (Baizer, Desimone, & Ungerleider, 1993), posterior parietal (Selemon & Goldman-Rakic, 1988; Baizer, Desimone, & Ungerleider, 1993; Gamberini et al., 2017), and orbitofrontal cortex (Morecraft, Geula, & Mesulam, 1992; also shown by fiber degeneration staining, Leichnetz & Astruc, 1975). Finally, there has been a long-standing assertion that the En components of the CLA complex project selectively to the “limbic” areas of cortex, especially the orbitofrontal, ventromedial prefrontal, and anterior cingulate areas, while the insular CLA projects more to higher-order sensory and association areas.

Based upon the proposed myeloarchitecture-based parcellation of the CLA complex described above, it is clear that there is no simple division of connectivity between different cortical areas and either the CLA or En components of the CLA. The frontal pole, for instance, is now recognized as a highly developed cognitive area that has undergone significant expansion over the course of primate evolution (Burman et al., 2011; Mansouri, Koechline, Rosa, & Buckley, 2017). This area is essential for cognitive tasks such as comparative evaluation of rewards under variable task conditions yet receives CLA input predominantly from a region corresponding to the DEn and DEnI (Burman et al., 2011; Reser et al., 2014). In area 9 of the dorsal prefrontal cortex in *Cebus*, the predominant input is from a region corresponding to DEnI and DEnV, even though area 9 is recognized as a prefrontal area involved in attention in New World monkeys (Dias, Robbins, & Roberts, 1996). Similarly, prestriate (prelunate gyrus) and posterior parietal injections in macaque monkeys label regions that are likely to include all subdivisions of DEn, based on their morphological similarity to the structure of the CLA complex in New World species (Baizer, Lock, & Youakim, 1997; Gamberini et al., 2017). However, that similarity must be viewed with some skepticism, as the equivalent myeloarchitecture-based parcellation has not yet been performed on macaques. Interestingly, in the marmoset anterior cingulate cortex, there is a clear segregation of CLA projections from the rostral to caudal extent of the anterior cingulate, such that tracer injections into the supragenual areas 24a and 24b have extensive connectivity with the region corresponding to the DEn, while the somewhat more caudal areas 24c and 24d receive strong projections from the “insular claustrum”, which also has notably stronger projections to secondary somatosensory areas and parietal areas implicated in control of reaching and grasping movements (e.g. area 2 of the macaque monkey, Gamberini et al., 2017).

Although a large amount of work remains to be done with respect to the anatomical framework for the primate CLA proposed above, a key component of both future tracer studies and retrospective analyses will be localization of claustralcortical projections within the proposed subdivisions of the CLA complex. This approach may yield new insight into the differential functions of the various CLA compartments and may also lead to a clearer understanding of the overall function of the CLA complex itself.

4. Anatomical delineation of CLA and En in a fruit bat

The Seba's short tailed fruit bat, *Carollia perspicillata*, is a mammalian species outside the Euarchontoglires superorder, which offers a unique perspective for determining if CLA and En show enough structural and functional independence to preserve their unique identifiers. First, as an evolutionary clade, which some authors argue is closer to whales and horses than to rats and mice (Buckley, 2015; Gunnell & Simmons, 2005; Meredith et al., 2011; Welker et al., 2015), bats show remarkable definition of individual structures in the forebrain that allows for much easier delineation of different regions, such as the CLA (Orman, Kollmar, & Stewart, 2017; Scalia, Rasweiler, Scalia, Orman, & Stewart, 2013). Second, compared to rodents, the fruit bat has impressively large subcortical regions, particularly CLA and amygdala (Orman, Kollmar, & Stewart, 2017; Scalia, Rasweiler, Scalia, Orman, & Stewart, 2013), which permit anatomical and functional access to these structures that can be difficult to accomplish in other species.

Anatomical analyses in fruit bats of non-specific neuronal markers, such as NeuN, reveals that the sizes, shapes, density, and orientation of cells clearly differentiate the CLA from En (Figure 10a). In NeuN-immunostained material, the boundary between the CLA and En is evident as an abrupt shift from densely packed claustral cells located dorsally (Figure 10f) to the distinctly lower density packing of En neurons located ventrally (Figure 10k). Dorsomedial and ventrolateral subregions based on size, shape, and cell density can be visualized in En. In fact, both CLA and En appear to be subdivided, especially visible using markers like latexin or Gng2 (Mathur, Caprioli, & Deutch, 2009).

A strong argument that CLA and En in fruit bat are closely related enough to be considered as subregions of a parent structure comes from immunohistochemistry for latexin (Figure 10b), which, as presented in the rodent discussion above, is an excellent marker for CLA (Arimatsu & Ishida, 1998; Arimatsu, Nihonmatsu, & Hatanaka, 2009; Kunzle & Radtke-Schuller, 2000; Orman, 2015; Orman, Kollmar, & Stewart, 2017; Pirone et al., 2012; Watakabe, Ohsawa, Ichinohe, Rockland, & Yamamori, 2014). Latexin immunoreactivity selects both CLA and En, but CLA and En subregions are apparent based on dense or light staining (Figure 10g, l). The latexin-rich subregions mirror those visible based on cell size, shape, and density observed with NeuN.

The most impressive difference between CLA and En in *Carollia* brains is observed from staining for calcium-binding proteins (Figure 10e). Calcium-binding proteins (CBP) such as PV, CR, and CB are commonly used to subdivide classes of neurons and have been known to label excitatory and inhibitory neurons, as well as local interneurons and long-range projection neurons. Staining for CBPs has also been used to parcel out subregions within a structure, such as the patch/matrix compartments of striatum (Gerfen, 1992; Smith et al., 2016). As shown in Table 2 and 3, immunoreactivity for these calcium-binding proteins has shown differences between CLA and En in rodents, which has been similarly shown for other species (for comprehensive review see Baizer, 2014; Davila et al., 2005; Druga, Chen, & Bentivoglio, 1993; Hinova-Palova et al., 2007; 2014; Kowia ski, Mory , Wojcik, Dziwiatkowski, & Mory , 2003; Rahman & Baizer, 2007; Real, Davila, & Guirado, 2003; Reynhout & Baizer, 1999; Wojcik et al., 2004). In adult *Carollia*, PV-expressing neurons are found in the CLA, but not in the En (Figure 10c). In CLA, PV-immunoreactive (ir) and CB-ir neurons are the most common of calcium-binding protein-expressing neurons (among PV, CR, CB), and account for close to 10% of all cells based on DAPI labeling of cell nuclei. The PV-ir neurons of CLA are concentrated in the core region of the structure based on latexin immunoreactivity density. PV-ir processes (axons and/or dendrites) could be found in both CLA and En. By contrast, CR-ir neurons are absent from CLA (Figure 10d). Labeled neurons occur in considerable numbers along the ventral edge of En, forming a partial ring around the ventrolateral portion of En. CR-ir processes (axons and/or dendrites) are directed into both CLA and En. The strikingly segregated distributions of PV-ir and CR-ir neuronal classes provide a compelling argument in favor of retaining the separate identities of CLA and En. CB, however, does not appear to differentiate between the two regions, displaying equal presence in both CLA and En.

The greater density of PV-ir neurons and terminals in CLA compared with En is consistent with three additional findings that indicate functional distinctions between these two

structures. First, the density of the microvasculature is greater in CLA than in En (Figure 10e, j, o), which indicates a higher energy demand likely owing to increased neural activity in CLA compared to En (Buzsaki, Kaila, & Raichle, 2007). Second, the increased PV staining in CLA compared to En in *Carollia* is similar to observations in rodents (see Figure 2), which have been shown to have PV-positive interneurons that provide strong inhibition onto claustrum projection neurons through highly active GABAergic projections (Orman, 2015; Kim, Matney, Roth, & Brown, 2016). Finally, the En is notably more epileptogenic than CLA (Hoffman & Haberly, 1991; 1993; 1996; Orman, 2015). Our tracing in rodents has indicated strong interhemispheric circuitry with cortex for both CLA and En, which may increase these regions susceptibility to kindling (Mohapel et al., 2001). Our observation of lower PV staining in En compared to CLA could suggest weaker inhibitory control in En that make it less resilient to tamping down epileptogenic activity, though this view is speculative but demands future work on the relation of these regions to epilepsy. Furthermore, these functional differences support separate identities of CLA and En, despite the wealth of commonalities outlined above in rodents and primates.

Much of the connectivity data available from other species is not yet available for the bat, however, but based on the histological and physiological data that are available we believe the bat brain offers a strong case for retaining the current nomenclature, and more importantly, an opportunity based on their large sizes to explore both regions in novel ways.

5. Concluding remarks, open questions, and future directions

In summary, we provide the following conclusions regarding the relationship between CLA and DEn, as well as recommendations on future studies to provide further clarity on the boundaries of the CLA-DEn complex:

1. Retain the nomenclature of “claustrum” and “dorsal endopiriform nucleus” for all species but revisit the delineation of these structures in non-human primates and identify the En in humans based on the cytoarchitecture, neurochemical profiling, genetic markers, and ontogenic definitions of CLA and DEn in rodents outlined in this review.
2. CLA and DEn should be considered subregions of the same formation (see Table 5), whereas based on gene expression and ontogenetic studies the IEn and VEn should be classified as a part of amygdala (although future studies should revisit the gene expression and connectivity of the subregions before committing to a classification).
3. Future studies should revisit the rostrocaudal limits of the CLA-DEn complex, particularly with respect to the anterior horn of the neostriatum, which has been proposed as the rostral limit of the CLA (Mathur, Caprioli, & Deutch, 2009; Mathur, 2014). Do gene marker analyses indicate an “anterior claustrum” that can be identified rostral to coronal sections containing the striatum and how does it relate to CLA cytoarchitecture, neurochemistry, ontogenetics, and connectivity?

4. What is the functional significance of the “core-shell” phenomenon of vCLA and is it apparent in species other than murid rodents and fruit bats? Is there any compartmentalization of CLA in primates that matches that of rodents (i.e. dCLA vs. vCLA)?
5. Future tracing studies in primates are crucially needed to demonstrate how the projections from emotional, cognitive, associative, premotor, motor and sensory cortical areas relate to CLA and DEn compartments. These studies will provide insight into the evolution of the subregions of the CLA-DEn complex. It seems parsimonious that as cortex expanded in primates compared to rodents, the simple sensorimotor-CLA/limbic-DEn division may have become less clear in favor of a more complex topography.
6. Investigate bats and other species outside of Euarchontoglires to assess whether gene markers distribution and anatomical descriptions are homologous across mammalian species and how these compartments relate to anatomical connectivity as well as how they may relate to each species’ behavioral niche (e.g. vCLA in rodent to whisking; CLA-DEn core region to echolocation in some bats and some cetaceans; enlarged visual claustrum in primates),
7. Use CLA-associated genes identified in rodents to investigate homologs/precursors of the claustrum in avian or reptilian species or in prototherian mammals (or verify that the claustrum is uniquely mammalian).

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ABBREVIATIONS

ac	anterior commissure
Amy	amygdala
BL	basolateral nucleus of amygdala
BLa	basolateral nucleus of amygdala, anterior part
BM	basomedial nucleus of amygdala
CLA	claustrum
dCLA	dorsal claustrum
DEn	dorsal endopiriform nucleus
DEnD	dorsal subdivision of the DEn (marmoset)
DEnI	intermediate subdivision of the DEn (marmoset)
DEnV	ventral subdivision of the DEn (marmoset)

DMS	dorsal migratory stream
ec	external capsule
En	endopiriform nucleus
ex	extreme capsule
IEn	intermediate endopiriform nucleus
Ins	insular cortex
L	lateral nucleus of amygdala
LGE	lateral ganglionic eminence
MGE	medial ganglionic eminence
Pir	piriform cortex
PLBL	paralamellar basolateral nucleus of amygdala
Pu	putamen
rf	rhinal fissure
vCLA	ventral claustrum
VEN	ventral endopiriform nucleus
VMS	ventral migratory stream

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FIGURE 1.

Coronal sections from brain atlases for human (Mai et al., 2016), macaque (Paxinos et al., 2000), marmoset (Paxinos et al., 2012), bat (Scalia, Rasweiler, Scalia, Orman, & Stewart, 2013), and rat (Paxinos & Watson, 2007) showing the delineation of the claustrum (purple), dorsal endopiriform nucleus (beige), ventral endopiriform nuclei (brown), amygdala (cyan), caudate-putamen (white), cortex (white) and anterior commissure (grey). Note the lack of endopiriform designation in human and the obscure location of endopiriform nucleus in macaque compared to other species.

FIGURE 2.

Cytoarchitecture, neurochemical topography, and gene expression profile of CLA and En in rat. (a) Nissl-stained coronal section of rat brain showing the dorsal and ventral parts of the claustrum (dCLA, vCLA, arrows) and dorsal endopiriform nucleus (DEn, arrowheads), note the decreased cell packing density in DEn. (b) Patterns of neurochemical labeling for parvalbumin, cytochrome oxidase, and acetylcholine esterase (AChE) throughout CLA and DEn in rat. (c-l) Coronal sections revealing the expression of different gene markers using *in situ* hybridization for *nurr1* (c, h), *latexin* (d, i), *Ntng2* (e, j), *GNB4* (f, k) relative to the location of the dCLA, vCLA, DEn, and ventral endopiriform nucleus (VEn) as identified by Nissl (g, l). These data show strong expression of each marker in dCLA, vCLA and DEn, whereas VEn shows no expression for any of these markers. (m) Color plot of gene expression for 47 genes listed in Table 4 (from Wang et al., 2017). Note that almost all genes are expressed in dCLA, vCLA, and DEn, but not in VEn. Panels (a) and (b) modified from

Watson, Smith, & Alloway, 2017. Panels (c-l) modified from Watakabe, 2017. ac: anterior commissure; dCLA: dorsal claustrum; DEn: dorsal endopiriform nucleus; Pir: piriform cortex; rf: rhinal fissure; vCLA: ventral claustrum; VEn: ventral endopiriform nucleus. Scale bars = 500 μ m in (a); 1 mm in (c-g); 250 μ m in (h-l).

FIGURE 3.

Embryological expression of CLA-specific genes indicates a common origin for CLA and DEn in rodent. (a) Divisions of the mammalian embryo, with subdivisions of the pallium identified by gene expression (note the updated nomenclature from Wullimann, 2017). (b) Developmental studies of *Nr4a2* (a Nurr family gene) indicate a common origin of CLA and DEn in the ventrolateral dorsal pallium, the same subregion that later gives rise to the insular cortex. During development, DEn cells migrate ventrally into the lateral pallium to take their final position in the adult brain next to piriform cortex, which itself originated in the lateral pallium. Also note the separate embryological origin of the VEn in the lateral pallium, indicating it is not related to the CLA-DEn complex. Modified from Watson & Puelles, 2017 and Wullimann, 2017. CLA: claustrum; DEn: dorsal endopiriform nucleus; DMS: dorsal migratory stream; MGE: medial ganglionic eminence; LGE: lateral ganglionic eminence; VEn: ventral endopiriform nucleus; VMS: ventral migratory stream.

FIGURE 4.

Topography of connectivity between cortex and CLA in rodents and primates. (a) Top panel shows the general topographic organization of CLA connections with cortex in rat, as identified by traditional retrograde tracing from cortex, which reveals a dorsal somatomotor area and a ventral visuo-auditory area (modified from Sadowski, Mory, Jakubowska-Sadowska, & Narkiewicz, 1997). A similar topography is shown in mouse in the bottom panel, as mapped by modern viral tracing techniques, revealing olfactory bulb (allocortex) inputs to a more ventral region of CLA, likely DEn (modified from Atlan et al., 2017). (b) Highly conserved dorsoventral topography in the primate CLA with somatomotor areas represent in dorsal CLA, associative areas in central CLA, and visuo-auditory areas located in ventral regions of CLA, as revealed by the horseradish peroxidase tracing method (modified from Pearson, Brodal, Gatter, & Powell, 1982). Note that each area in primate goes to a region of motor and sensory cortex. Numbers in cortical injection sites indicate Brodmann area. (c) Topography from two electrophysiology studies in primates, which functionally confirm unimodal processing in compartment specific regions of CLA (compiled from Shima et al., 1991 and Remedios, Logothetis, & Kayser, 2010). Note the mismatch in location of auditory regions of CLA between the anatomical tracing (b) and electrophysiology data (c). CLA: claustrum; DEn: dorsal endopiriform nucleus.

FIGURE 5.

Similarities in anatomical connectivity of CLA and DEn in rat. (a) Diagram illustrating the dorsoventral topographic relationship of CLA to somatomotor regions dorsally and visuomotor regions ventrally, whereas DEn connects to limbic cortical areas. (b) Both CLA and DEn have extensive intranuclear longitudinal connectivity that retain separate dorsal (purple arrows) and ventral (orange arrows) domains within their respective nuclei. (c) Circuit diagrams showing the same pattern of intra- and interhemispheric sensorimotor connections of the rodent CLA (purple arrows) compared to the limbic connections of the rodent endopiriform nucleus (orange arrows). (d) Both CLA and DEn have the same pattern of input-output connectivity with the different layers of cortex. Specifically, inputs to the CLA-DEn complex originate from layers 2-5 of frontal and higher-order sensory cortical areas, but from layer 6 of primary sensory cortical areas. The CLA-DEn complex in turn innervates all layers of cortex, with a much stronger innervation of frontal cortices. Inset shows photomicrograph of anterogradely labeled claustrorocortical terminals that appear as small “mushroom-like” terminal boutons that extend from the axon. Based on Alloway, Smith, Beauchemin, & Olson, 2009; Smith & Alloway, 2010, 2014; Smith, Radharikistan, & Alloway, 2012; Watson, Smith, & Alloway 2017. Image of claustrorocortical terminals modified from Smith, Liang, Watson, Alloway, & Zhang, 2017. AGl: agranular lateral cortex; AGm: agranular medial cortex; Cg: cingulate cortex; CLA: claustrum; DEn: dorsal

endopiriform nucleus; M1: primary motor cortex; M2: secondary motor cortex; mPFC: medial prefrontal cortex; S1: primary somatosensory cortex. Scale bar = 2 μ m.

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FIGURE 6.

Delineation of the CLA-En complex in marmosets. Series of coronal sections of a marmoset brain stained for Nissl (panels a-d) or for Nurr1 gene expression by *in situ* hybridization (panels e-h). The red dotted line indicates the location of the CLA-DEN complex, a cell-dense nucleus with strong Nurr1 signals. The positions of the IEn and VEn are based on a marmoset atlas and may not represent the true homologues of their rodent counterparts (Paxinos et al., 2000). ac: anterior commissure; Amy: amygdala; CLA: claustrum; DEn: dorsal endopiriform nucleus; IEn: intermediate endopiriform nucleus; VEn: ventral endopiriform nucleus; Pir: piriform cortex. Scale bars = 1 mm.

FIGURE 7.

Identification CLA-DEn complex in a primate with gene marker expression and histological staining. (a-y) Images of serial coronal sections of a Japanese monkey brain stained for cytochrome oxidase (CO; a-e), acetylcholine esterase (AChE; f-j), VGLUT1 *in situ* hybridization (ISH; k-o) Nurr1-ISH (p-t), and Nissl staining (u-y). Modified from Watakabe, Ichinohe, & Noritaka, 2007. Coronal sections were sliced at 40 μ m intervals and stained periodically (one every 2 mm). Numbers in the bottom right corner of each panel indicate the serial section numbers starting from the top left panel. Scale bars = 10 mm.

FIGURE 8:

Gene expression and white matter tracts clearly delineate the border between CLA-DEn complex and amygdaloid complex in primates. (a-y) Higher magnification images of serial coronal sections of a Japanese monkey brain from Figure 7 showing the CLA-DEn amygdala border by staining for cytochrome oxidase (CO; a-e), acetylcholine esterase (AChE; f-j), VGluT1-*in situ* hybridization (ISH; k-o) Nurr1 ISH (p-t), and Nissl staining (u-y). For the bottom panels denoted as “###T”, the tracing was manually done based on Nissl staining and annotated according to the staining patterns and morphological features from the rhesus monkey atlas (Paxinos et al., 2000). The red arrows indicate the putative border between the CLA-DEn complex and the amygdala-related complex. The asterisks indicate the putative brain area for the VEn (which could actually be part of amygdalar complex). ac: anterior commissure; BL: basolateral nucleus of amygdala; BM: basomedial nucleus of amygdala; CLA: claustrum; DEn: dorsal endopiriform nucleus; L: lateral nucleus of

amygdala; Pir: piriform cortex; PLBL: paralamellar basolateral nucleus of amygdala. Scale bars = 2.5 mm.

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FIGURE 9.

Preliminary myeloarchitecture-based parcellation of the marmoset claustrum complex. Gallyas stained section, approximate A-P coordinate +9.5 mm. The CLA portion of the claustrum complex is outlined in blue, while the DEn is marked in yellow. Note increasing myelin density along the dorsoventral axis. Numerous cut myelinated fibers in DEnV indicate axons running longitudinally along the coronal plane of section. Red arrowheads indicate the myelin boundary between the CLA and dorsolateral extent of the amygdaloid complex. Nomenclature and labeling follow conventions of Paxinos et al. 2012, with the exception of newly identified subdivisions of DEn. ac: anterior commissure; CLA: claustrum; DEnD: dorsal subdivision of the DEn; DEnI: intermediate subdivision of the DEn; DEnV: ventral subdivision of the DEn; ec: external capsule; ex: extreme capsule; Ins: insular cortex; L: lateral amygdala; Pu: putamen. Scale bar = 1mm.

FIGURE 10.

Neurochemical identification of the CLA-DEN complex in a fruit bat. (a-e) Low magnification comparison of CLA and DEN in coronal sections. Immunohistochemistry in each panel is labeled showing NeuN-(a), latexin (b), parvalbumin (c), calretinin (d), and vascularization with rhodamine (e). Red arrows mark the lateral border of CLA. Yellow arrows mark the lateral and ventrolateral border of DEN. The medial border of both structures is demarcated by the white matter of the external capsule. The dense packing of cells in CLA is best seen in NeuN-(a) and latexin-immunolabeled sections (b), but these antibodies do not differentiate CLA from DEN. However, antibodies for the calcium-binding proteins parvalbumin (c) and calretinin (d) clearly show different patterns for these nuclei. Panel e shows the bat brain vasculature via transcardially perfusions with rhodamine-conjugated gelatin before sectioning (images obtained in collaboration with T. Ragan and M. Marek, TissueVision, Somerville, MA). (f-j) High magnification of CLA from each panel in the top row. (k-o) High magnification of DEN from each panel in the top row. The presence of PV-ir neurons in claustrum (h) and calretinin-ir neurons in DEN (n) are also highlighted in these higher magnification images. Scale bar = 2 mm.

Table 1.

Classification scheme of amygdala, claustrum and endopiriform nuclei (modified from Butler & Hodos, 2005)

Clausro-amygdalar complex (pallial)	Frontotemporal amygdala	BLa (basolateral nucleus of the amygdala, anterior part)
		L (lateral nucleus of the amygdala)
	Claustrum-endopiriform formation	DEn (endopiriform nucleus, dorsal part)
		VEN (endopiriform nucleus, ventral part)
		CLA (claustrum, i.e., claustrum proper)

Table 2.

Summary of neurochemical similarities between rodent CLA and DEn (modified from Watson, Smith, & Alloway, 2017)

Substance	Type	Reference
Carboxypeptidase E	enzyme	MacCumber, Snyder, & Ross, 1990
Nitrous oxide synthase	enzyme	Kowiański et al., 2001; 2004; Eiden, Mezey, Eskay, Beinfeld, & Palkovits, 1990; Guirado, Real, Olmos, & Dávila, 2003; Rodrigo et al., 1994; Vincent, Leung, & Watanabe, 1992
Cholecystokinin (CCK)	peptide	Kowiański et al., 2001; 2004; Eiden, Mezey, Eskay, Beinfeld, & Palkovits, 1990; Schiffmann & Vanderhaeghen, 1991; Ingram, Krause, Baldino, Skeen, & Lewis, 1989; Sirinathsinghi, Mayer, Sirinathsinghi, & Dunnnett, 1993; Savasta, Palacios, & Mengod, 1988
Neuropeptide Y	peptide	Kowiański et al., 2001; 2004; Reuss, Hurlbut, Speh, & Moore, 1990
Nociceptin	peptide	Florin, Meunier, & Costentin, 2000; Neal et al., 1999
Somatostatin	peptide	Kowiański et al., 2001; 2004; Eiden, Mezey, Eskay, Beinfeld, & Palkovits, 1990
Urotensin II receptor (GPR14)	peptide	Jégou et al., 2006
Brain-derived neurotrophic factor	protein	Conner, Lauterborn, Yan, Gall, & Varon, 1997
Calbindin D28K	protein	Celio, 1990; Druga, Chen, & Bentivoglio, 1993; Real, Davila, & Guirado, 2003; Wojcik et al., 2004
Calretinin	protein	Kowiański et al., 2001; Real et al., 2003; Wojcik et al., 2004; Druga, Salaj, Barinka, Edelstein, & Kubová, 2015
Ectoderm-neural cortex protein 1	protein	Garcia-Calero & Puelles, 2005
Latexin	protein	Arimatsu & Ishida, 1988
Neurogenin 2, semaphorin 5	protein	Medina et al., 2004
Parvalbumin	protein	Druga, Chen, & Bentivoglio, 1993; Celio, 1990; Real, Davila, & Guirado, 2003
Phosphatase inhibitor-1	protein	Sakagami, Ebina, & Kondo, 1994; Gustafson, Girault, Hemmings, Nairn, & Greengard, 1991
VIP vasoactive intestinal protein	protein	Kowiański et al., 2001; 2004; Eiden, Mezey, Eskay, Beinfeld, & Palkovits, 1990
5HT _{2A} and 5HT _{2C} receptors	receptor	Pompeiano, Palacios, & Mengod, 1994; Sijbesma, Schipper, Cornelissen, & de Kloet, 1991
α 2B adrenoceptor	receptor	Winzer-Serhan & Leslie, 1997
Dopamine D1 receptor	receptor	Dubois, Savasta, Curet, & Scatton, 1986; Savasta, Dubois, & Scatton, 1986; Wamsley, Gehlert, Filloux, & Dawson, 1989
Dopamine D2 receptor	receptor	Weiner & Brann, 1989; Weiner et al., 1991
Estrogen receptors	receptor	Mufson et al., 1999; Toran-Allerand, Miranda, Hochberg, & MacLusky, 1992
GFR α -1 receptor	receptor	Burazin & Gundlach, 1999
Inositol receptor	receptor	Rodrigo et al., 1993
Neuropeptide S receptor	receptor	Clark et al., 2011; Xu, Gall, Jackson, Civelli, & Reinscheid, 2007
Neurotensin receptor	receptor	Alexander & Leeman, 1998
NMDA receptor subunit 1	receptor	Sato, Mick, Kiyama, & Tohyama, 1995
κ 1 opioid receptor	receptor	Mansour et al., 1994; Mansour, Fox, Meng, Akil, & Watson, 1994; Mansour, Burke, Pavlic, Akil, & Watson, 1996; DePaoli, Hurley, Yasada, Reisine, & Bell, 1994; Gackenheimer et al., 2005; Unterwald, Knapp, & Zukin, 1991
μ opioid receptor	receptor	Mansour et al., 1994; Mansour, Fox, Thompson, Akil, & Watson, 1995; Kitchen, Slowe, Matthes, & Kieffer, 1997
δ opioid receptor	receptor	Mansour et al., 1994
Opioid receptor-like receptor	receptor	Neal et al., 1999
Relaxin receptor (LGR7)	receptor	Piccenna et al., 2005
Somatostatin receptor subtype 2	receptor	Helboe, Hay-Schmidt, Stidsen, & Møller, 1999; Señarís, Humphrey, & Emson, 1994; Perez et al., 1994; Breder et al., 1992

Table 3.

Summary of neurochemical markers present in DEn, but not CLA of rodents (modified from Watson, Smith, & Alloway, 2017)

Substance	Type	Reference
Argininosuccinate synthetase	enzyme	Nakamura, Saheki, Ichiki, Nakata, & Nakagawa, 1991
Motopsin	enzyme	Gschwend, Krueger, Kozlov, Wolfer, & Sonderegger, 1997; Iijima et al., 1999
c-kit receptor ligand	ligand	Hirota et al., 1992
Opioid receptor antagonist [3H]-LY255582	ligand	Gackenhaimer et al., 2005
Arginine Vasopressin	peptide	Szot, Ferris, & Dorsa, 1990
Atrial and C-type natriuretic peptide	peptide	Langub, Watson, & Herman, 1995
Nesfatin-1	peptide	Goebel, Stengel, Wang, Lambrecht, & Taché, 2009
Neuropeptide B & W	peptide	Jackson, Lin, Wang, Nothacker, & Civelli, 2006
Pancreatic polypeptide	peptide	Whitcomb, Puccio, Vigna, Tayler, & Hoffmann, 1997
Preproatrial natriuretic peptide	peptide	Gundlach & Knobe, 1992
Substance P	peptide	Dam, Martinelli, & Quirion, 1990; Buck, Helke, Burcher, Shults, & O'Donohue, 1986
Vasopressin	peptide	Szot, Ferris, & Dorsa, 1990
ADP-ribosylation factor-related protein 1	protein	Paratore, Parenti, & Cavallaro, 2008
Doublecortin	protein	Nacher, Crespo, & McEwen, 2001
Glial cell-derived neurotrophic factor α -1	protein	Burazin & Gundlach, 1999
Glucose transporter 1 (GLUT1)	protein	Arluison et al., 2004; Schmitt, Asan, Püschel, Jons, & Kugler, 1996
GLUT2	protein	Arluison et al., 2004
GLUT4	protein	Choeiri, Staines, & Messier, 2002; Messari et al., 1998
α -2A adrenoceptor	receptor	Uhlén, Lindblom, Johnson, & Wikberg, 1997
Insulin-like growth factor-1	receptor	Matsuo et al., 1989; Araujo, Lapchak, Collier, Chabot, & Quirion, 1989
Glucocorticoid receptor	receptor	Aronsson et al., 1988
mGluR3	receptor	Ohishi, Shigemoto, Nakanishi, & Mizuno, 1993
Neurokinin-1	receptor	Dam, Martinelli, & Quirion, 1990
Neuropeptide S receptor	receptor	Clark et al., 2011; Meis et al., 2008; Xu, Gall, Jackson, Civelli, & Reinscheid, 2007
Urotensin II receptor	receptor	Jégou et al., 2006

Table 4.

Summary of gene expression for CLA-En in mouse (modified from Wang et al., 2017)

Gene	Striatum	Layer 6	dCLA	vCLA	DEn	VEn
Adamsl2	-	-	-	++	-	-
Bacel	-	+	+	+++	++	-
B3gat2	-	-	+	++	-	-
BCl0045l	-	-	+	++	++	-
Btgl	-	-	-	++	-	-
Cadps2	-	++	++	+++	++	-
Carl2	++	-	+	+++	++	-
Cbln2	-	+++	+	+++	+	-
Chstll	+	+	+	++	+	-
Cntnap3	+	-	-	+	-	-
Coll1a1	-	+	+	++	-	-
Cux2	-	+	+++	+++	+	-
Gadd45g	-	-	-	+	-	-
Galnt14	+	+	++	++	++	-
Gfral	-	-	++	++	+	-
Gnb4	-	+	+	++	+	-
Gng2	-	-	++	+++	+	-
Gnaol	-	+	+	++	+	-
Gpd2	-	+	++	+++	+	-
Gucyla3	++	-	-	++	-	-
Id2	-	+++	+++	+++	++	-
Inpp5a	+	+	+	++	+	-
Itga7	-	+	+	+	-	-
Laptn4b	-	-	-	++	+	-
LOC433093	-	-	-	++	-	-
Lypd6b	-	++	++	+++	++	+
Lxn	-	+	+	++	++	-
Mt3	-	+	++	++	++	-
Nfx1l	+	-	++	+++	+	-
Nr4a2	-	+	++	+++	++	-
Nsdhl	-	+	+	+++	+	-
Ntng2	-	++	+	++	+	-
Oprkl	-	-	+	+	+	-
Pdia5	-	+	+	+	+	-
Plcll	-	+	+	++	+	-
Ppplrla	+	++	++	+++	++	-
Rab3c	+	+	+	+++	++	+
SStr2	-	+	+	+++	+	-
Tmem163	-	+	++	++	++	-

Gene	Striatum	Layer 6	dCLA	vCLA	DEn	VEEn
Zfp804a	-	-	+	+++	+	-
Col23a1	-	+	+	-	+	-
Cplx2	-	++	+	-	+	-
Crym	++	+++	+	-	+	-
Ctgf	-	++	++	-	++	-
Nxph3	+	+++	++	+	++	-
Rasall	-	++	++	+	++	-
Slit1	+	+	++	+	++	-

Table 5.

Updated classification scheme of amygdala, claustrum and endopiriform nuclei (updated from Butler & Hodos, 2005)

Clausro-amygdalar complex (pallial)	Frontotemporal amygdala	BLa (basolateral nucleus of the amygdala, anterior part)
		L (lateral nucleus of the amygdala)
		VEn and IEn (endopiriform nucleus, ventral and intermediate parts)
	Claustrum-endopiriform formation	DEn (endopiriform nucleus, dorsal part)
		vCLA (claustrum, ventral part, i.e., claustrum proper)
		dCLA (claustrum, dorsal part, i.e., claustrum proper)