Feline Islands of Calleja Complex:  
I. Cytoarchitectural Organization and  
Comparative Anatomy

KONRAD TALBOT, NANCY J. WOOLF, AND LARRY L. BUTCHER  
Department of Psychology and Brain Research Institute, University of California, Los Angeles, California 90024-1563

ABSTRACT
Cytoarchitectural analyses demonstrated that the islands of Calleja complex (ICC) is highly developed and discretely organized in the cat. The feline complex is clearly divided into morphological units, each containing a granular Callejal island and a population of satellite neurons. These ICC units change progressively in cytoarchitecture from the lateral to the medial edge of the olfactory tubercle. In particular, the islands flatten, sink into the tubercular molecular layer, and increase in cell density, while their satellite neurons increase in number and decrease in size. The lateromedial transformation was judged to take place in five stages, resulting in the successive appearance of lateral, lateral transitional, central, medial transitional, and medial ICC units. The first two unit types display prominently two additional components of the feline ICC—namely, clusters of dwarf cells and small pyramidal-like neurons constituting the densocellular layer cupping the base of lateral Callejal islands.

All of the various types of ICC units contact the tubercular molecular layer via their dwarf and/or granule cell components, raising the possibility of direct olfactory input to the entire Callejal complex (apart from the isla magna). Output from the complex is presumed to arise from the satellite neurons, which are distinguished from adjoining cell populations by their close association with Callejal islands, typical chromophilic character, and relatively large size (15–42 μm in soma length). In the tubercular ICC, these neurons are most numerous immediately above Callejal islands in a fiber-rich zone continuous with the supratubercular zone and hence with the ventral pallidum. In the accumbal ICC, satellite neurons are most conspicuous in granule-cell-poor spaces within the isla magna, where many non-granular neurons are uncharacteristically small and chromophobic. The isla magna itself is unusual not only for its large size but for lateral extensions encircling a group of accumbal neurons far caudally. Such extensions are one of several indications that the isla magna is intimately associated with the nucleus accumbens.

A comparative anatomical survey of the ICC in rats, cats, and macaque monkeys demonstrated a number of species differences. Of particular interest is the finding that the complex is unambiguously divided into discrete island-satellite cell units only in cats and macaques. In these species, the complex is also distinguished by a predominance of superficial islands and an especially prominent isla magna. ICC units, however, were most conspicuous in cats.

Key words: basal forebrain, nucleus accumbens, olfactory tubercle, striatopallidal systems

Among the most conspicuous, yet enigmatic, components of the mammalian basal forebrain are the dense clusters of granule cells commonly known today as the islands of Calleja. These clusters dot the entire width of the olfactory

Accepted April 7, 1988.
Address reprint requests to Larry L. Butcher, Department of Psychology, University of California, Los Angeles, CA 90024-1563.

© 1988 ALAN R. LISS, INC.
tubercle, along whose medial border one of the granular clusters extends dorsally to form the isla magna partially embedded in the paramedian wall of the nucleus accumbens (Fox, '40; Crosby and Humphrey, '41; Lauer, '45; Sanides, '58; Stephan, '75, pp. 311, 314, 326, 327; Fallon et al., '78; '83; Chronister et al., '81; Berman and Jones, '82, p. 75; Mesulam et al., '84). Both here and in the tubercle, the islands are accompanied by a more-or-less diffuse population of medium- to large-size satellite neurons, often located in or near depressions of the granule cell masses (cf. Ramón y Cajal, '01, '02, pp. 729, 730; Gurdjian, '25; Fox, '40; Lauer, '45; Sanides, '58; Fallon et al., '78, '83; Chronister et al., '81; Hedreen, '81; Mesulam et al., '84; Meyer and Wahle, '86; Millhouse, '87). Golgi studies indicate that at least a subset of the satellite cells extends dendrites into neighboring Callejal islands, where the processes usually ramify (Fallon et al., '78; Chronister et al., '81; Meyer and Wahle, '86; see also Millhouse and Heimer, '84, Fig. 11) and may receive input from granule cell axons (Meyer and Wahle, '86; see also Chronister et al., '81). Primarily because of their spatial proximity and overlapping dendritic fields, the ensemble of granular clusters and associated satellite neurons has been called the islands of Calleja complex (ICC) by Fallon and his colleagues (Ribak and Fallon, '82; Fallon, '83; Fallon et al., '83).

While clearly present in other species (see Fox, '40; Mesulam et al., '84; Ellison et al., '87), the ICC has been identified and characterized only in the rat (Ribak and Fallon, '82; Fallon, '83; Fallon et al., '83). Yet our cytoarchitectural studies indicate that the Calleja complex is differently and more discretely organized in the cat. We report the results of those studies here, along with a brief comparison of the ICC found in the rat, cat, and macaque monkey. Attention will be focused on the conspicuous lateromedial differentiation of the feline complex into distinct anatomical units. As explained in a subsequent paper (Talbot et al., '88), this organizational feature provides the context in which the cholinergic features of the cat ICC are best understood. A historical perspective on the islands of Calleja is likewise presented to supply the context necessary for a clear discussion of various anatomical features of the ICC, specifically its cellular composition, topographic differentiation, and comparative anatomy.

**Materials and Methods**

**Animals and tissue processing**

Data were obtained from 12 cats (2.5-4.0 kg), five Sprague-Dawley rats (180-350 g), and four Macaca nemestrina monkeys (3.5-4.0 kg). All animals were killed under pentobarbital anesthesia by transcardial perfusion with 0.9% saline followed by 10% neutral-buffered formalin (pH 7.0-7.2). After removal from the cranial cavity, each brain was stored under cold (-4°C) conditions for 24-48 hours in the perfusion fixative and then transferred to cold 30% sucrose. Following sucrose saturation, the brains were sectioned coronally on a freezing microtome at 40 μm, except for sets of 80-μm slices taken from four cat brains for various cytoarchitectural analyses. In one feline case, all 40-μm sections through the olfactory tubercle were retained. In the remaining cases, every fourth or fifth tubercular section was kept for further processing.

After being mounted on slides and air-dried, all brain sections from the four macaques and from four cats (including the continuous feline series) were stained for Nissl substance according to the thionin method specified by Skinner ('71). Nissl staining was likewise performed on every third section from eight additional cats and on alternate sections from five rats. The remaining material from these 13 cases was processed for acetylcholinesterase (AChE) neuropil as described elsewhere (Talbot et al., '88). Half of the resulting cholinesterase material from the eight feline cases was counterstained by using the cresyl violet technique given by Woolf and Butcher ('81). After rinsing, both stained and counterstained AChE sections were dehydrated in successively more concentrated alcohol solutions and transferred to a 3:1 chloroform-alcohol mixture. The material was then cleared in toluene or xylene and coverslipped under Permount (Fischer Scientific Co., Fairlawn, NJ).

**Data analysis**

The form, distribution, and dimensions of the ICC were studied in macaque monkeys, cats, and rats. Data analysis was nevertheless focused on the feline material because of the unexpected finding that the Calleja complex in cats is more conspicuous than in macaques and more discretely organized than in rats. Pilot studies demonstrated that the Calleja island-satellite cell ensembles constituting most of the feline ICC change progressively in structure and AChE histochemistry from the lateral to the medial edge of the olfactory tubercle. The changes seen in Nissl material were such that five types of island-satellite cell ensembles (called ICC units in the present report) could be identified at successively more medial tubercular areas. To test the validity of this parcellation scheme and simultaneously clarify the lateromedial changes occurring in the cat ICC, the five pro-
posed unit types were characterized in terms of the topographical and cytoarchitectural features specified below.

Rostrrocaudal topography. The number of each proposed type of ICC unit was counted at eight coronal levels of the olfactory tubercle in nine Nissl series. These data were used to calculate the mean number of any given unit type per hemisphere at each of the eight tubercular levels. All of these levels were sampled in the following cytoarchitectural analyses.

Characteristics of Callejal islands. Several features of these granule cell masses were tabulated in three Nissl series. This entailed study of 32–85 samples (i.e., coronal transections) of Callejal islands belonging to each proposed type of ICC unit to determine their coronal length, granule cell density, displacement of the underlying tubercular densocellular layer, and penetration of the tubercular molecular layer. Several of these features require explanation. 1) The coronal length of an island designates its maximum extent (in width or height) in 40-μm frontal sections. 2) Callejal island displacement of the tubercular densocellular layer refers to the apparent thinning of that layer by the granular mass of the island. Displacement ratings were made by comparing the thickness of the densocellular layer alongside each island with that found beneath it. These ratings were made on an ordinal scale recording five different conditions, from no displacement (−) to complete replacement (+ + + + +) of the densocellular layer. 3) Callejal island penetration of the tubercular molecular layer indicates the extent to which the island sinks below the adjoining densocellular layer. If the main mass of the island (as opposed to its ventrally scattered microcellular neurons) reached one-fourth, one-half, three-fourths, or all of the islands, however, their penetrations of the molecular layer indicated maximum extent in 40-μm frontal sections. These ratings were made on an ordinal scale recording five different conditions, from no displacement (−) to complete replacement (+ + + + +) of the densocellular layer. Consequently, individual ratings were often intermediate between those just explained (e.g., + +/+ + +), accounting for much of the variability recorded in Table 1.

Number of Nissl-rich satellite neurons. In 32–85 samples of each proposed type of ICC unit, cell counts were made on chromophic neurons in the fiber-rich area immediately overlying tubercular Callejal islands and in the cell-poor spaces of the isla magna. Samples of all the proposed unit types were drawn equally from each of three different Nissl series. Because of its poorly delineated nature, we operationally defined the suprainsular zone as a columnar area equal in width to the subjacent granular island, rising from that cell mass up to the ventral border of the tubercular white matter (i.e., a distance of 250–450 μm in most cases). The columnar area was assumed to rise perpendicularly to the underlying brain surface, except in cases where the suprainsular zone clearly reached one side (e.g., Fig. 3b) and was treated as such.

Since ratings of various ICC features on ordinal scales (to + + + + +) were determined subjectively, it is important to add that the vast majority of the values recorded (≥85%) were confirmed on retests of each feature. It should also be noted that what are called "typical" ratings in Table 1 are those applying to at least 75% of all studied samples of the ICC units involved. For example, 83.5% of all analyzed transections of central units were judged to have high to maximal granule cell densities, hence the listing + + + / + + + + +.

RESULTS

The tubercular environment of the feline ICC

An accurate description of the cat ICC requires clarification of the laminar and areal divisions of the tubercular region engulfing much of the Callejal complex. Previous accounts of these divisions (Fox, '40; Berman and Jones, '82; Meyer and Wahle, '86) are insufficiently detailed for our purposes. We distinguished three strata in the olfactory tubercle proper (Fig. 1): a superficial molecular layer, an intermediate densocellular (or pyramidal) layer, and a deep parvicellular layer of relatively dispersed neurons structurally similar to those in the pyramidal stratum. The parvicellular layer should not be confused with the polymorphic cell field deep to the olfactory tubercle proper (described below as the supratubercular zone). It corresponds instead to the β layer of the dog tubercle (Brockhaus, '42) and to the sub-α margin (Popoff and Popoff, '29) or third layer (Luskin and Price, '83) of the rat tubercle.

Although the densocellular layer is most conspicuous for its compact and undulating form, its discontinuities and cellular heterogeneity are of greater relevance here. Only simple breaks occur laterally, but the remainder of the layer is interrupted periodically by granule cell islands near or at the brain surface (Fig. 1). Such islands also occur laterally, but do not substantially displace the densocellular layer there (see below). As this layer approaches the tubercular islands, it bends downward to reach the islands and in so doing it changes progressively in cellular composition, especially in the lateral tubercle. According to the Golgi analyses of Meyer and Wahle ('86), the compositional changes are such that medium to large spiny neurons are replaced by small spiny cells, which are in turn supplanted by dwarf neurons adjacent to the granule cell masses. We treat both dwarf and granule cells as microcellular neurons, but designate only clusters of the latter, slightly smaller cells as Callejal islands (see Discussion for explanation of this nomenclature).

As implied in our description of the densocellular layer, the three areal sectors of the feline olfactory tubercle are arranged interomedially (Fox, '40). Since they are not sharply delimited, it is worth specifying their distinguishing features as seen in coronal Nissl sections. While the densocellular layer is more or less corrugated throughout the tubercle, it is least consistently so in the lateral sector. This area is also marked by a thick molecular layer, an especially compact densocellular stratum surmounted at intervals by relatively small Callejal islands, and a group of large, Nissl-rich cells in the overlying white matter. The wide intermediate sector is linked by cell bridges with the basal putamen and nucleus accumbens, but is most notable for the depth and regularity of its densocellular layer corrugations, each interrupted at its base by a substantial Callejal island. By contrast, the medial sector (along the hemispheric wall facing the midline) is poorly laminated. It has only a very thin molecular layer, a rudimentary densocellular stratum largely supplanted by extensive Callejal islands, and a parvicellular layer frequently fused with nucleus accumbens. Figure 1 indicates the approximate boundaries of these tubercular divisions and illustrates some of their differential properties (see also Fox, '40; Berman and Jones, '82; Meyer and Wahle, '86).

Immediately above the olfactory tubercle proper is an area we term the supratubercular zone (SZ in Fig. 1). This is a relatively cell-poor, fiber-rich area largely devoid of...
Fig. 1. Coronal mapping of ICC units at eight rostrocaudal levels of the feline olfactory tubercle. Five different unit types are indicated: lateral, lateral transitional, central, medial transitional, and medial (see Table 1 for the features differentiating these units). The solid black masses are Callejal islands, and the dotted areas above them are suprainsular zones. Note that the latter are frequently continuous with the lightly shaded supratubercular zone. The darkly shaded structures are anterior commiss
sure components. Satellite neurons alongside or beneath the islands are not shown. None of the rare Callejal islands deep to the caudal olfactory tubercle occurred in the relevant sections (g,h) mapped here. The dividing line between tubercular and accumhal segments of the medial ICC unit in d is shown in Figure 3c. Arrowheads mark the approximate lateral and medial limits of the intermediate olfactory tubercle. The full scale bar in h is 3.0 mm.
Fig. 2. Cytarhitectural differentiation of major ICC units in the telencephalic olfactory tubercle. Each row of photomicrographs displays four to six examples of the same type of ICC unit, all shown in 40-μm coronal Nissl material. Note that from the most lateral (a-f) to the most medial (t-w) unit type, the Calleja islands not only flatten, but increase in density, size, and basilar location (see also Table 1). Arrows point to the small, relatively diffuse islands of lateral units (a-f). These essentially remain on top of the densocellular layer. In lateral transitional units, however, the granular islands partially displace that layer (g-k; see also Fig. 7), and in all the more medial ICC units, the islands entirely supplant the densocellular stratum (l-w). Clustered Nissl-rich neurons in the suprainsular area are most common in lateral transitional and central units (g-o), though the cells are larger in the former units (identified by arrowheads in g-k; see also Fig. 6). Double arrowheads indicate ectopic groups or islets of microcellular neurons partially (r) or completely (p, s) detached from the Calleja islands of the more medial ICC units. The open arrow in w identifies the granular link between tubercular and accumbal segments of the medial ICC unit (see also Figs. 1 and 3). Scale bars in k and w are 200 μm.
Fig. 3. Anterior (a,b) and central (c-f) levels of the medial ICC unit as seen in 40-μm coronal Nissl sections from the cat. This unit has distinct tubercular and accumbal segments. The one or two tubercular segments are first seen alone at far rostral levels (Figs. 1c and 2t-w), but shortly thereafter they extend beyond the dorsomedial edge of the olfactory tubercle (marked by arrowheads in a-d). At successively more caudal levels, this extension (essentially the isla magna) rises along and within the medial edge of nucleus accumbens (c,d). At central levels of this nucleus, the isla magna forms a clublike structure (c,d; see also Fig. 1d,e) enclosing narrow labyrinthine spaces (arrowed in d and e) containing small satellite neurons. The spaces appear to be hilar since they open onto neighboring structures at various points (not illustrated). Broader hilar spaces containing satellite neurons occur in the tubercular segment of the medial ICC unit at or near its juncture with the isla magna (a-d,f). Such spaces appear entirely enclosed in some sections (d,f). The scale bar in d (applying to a-d) is 500 μm; that in f (applying to e,f) is 200 μm.
Fig. 4. Posterior levels of the medial ICC unit seen in a rostrocaudal series of 80-μm coronal Nissl sections from the cat. At these levels, only the accumbal segment of the unit is present. Its internal labyrinthine spaces are better developed more rostrally (Fig. 3d,e), but are still evident in a and b (e.g., arrowed areas). Further caudally, two ectopic granular protrusions arch laterally in pincer fashion to encircle a striatal cell field near the dorsal extremity of nucleus accumbens (marked by arrowheads in c–e). A remnant of the resultant granular ring persists in f (indicated by arrowhead) but disappears at the caudal pole of the accumbal ICC (not illustrated). The scale bar in f is 500 μm.
Fig. 5. Deep Calleja islands (a,b) and minor ICC units (c,d) viewed in coronal Nissl sections through the feline olfactory tubercle. The deep islands indicated by arrowheads are located in the caudal supratubercular zone (a) and in the paratubercular nucleus (b). The minor ICC units illustrated in c and d are, respectively, elliptical and intrapyramidal forms of such units (see also Fig. 1c,h); the latter form is shown at higher magnification. The scale bar in c (applying to a–c) is 200 μm; that in d is 100 μm.

with the tubercular end of the medial forebrain bundle (see Figs. 3–5 of Nieuwenhuys et al., '82). The same area has previously been considered the polymorphic (or multiform) layer of the olfactory tubercle (e.g., Calleja, 1893, pp. 22–24; Fox, '40; Stephan, '75, pp. 310, 326; Millhouse and Heimer, '84; Meyer and Wahle, '86; Millhouse, '87). Yet polymorph layer neurons clearly differ from underlying tubercular cells. They are more diverse in size and morphology (Stephan, '75; Millhouse and Heimer, '84; present report) and probably receive little, if any, direct olfactory input since their dendrites are rarely, if ever, long enough to enter the tubercular molecular layer (Calleja, 1893, p. 22; see also Millhouse and Heimer, '84). Moreover, polymorph layer neurons, but not subjacent tubercular cells, project to brain structures other than the ventral pallidum (Heimer et al., '87; see also Luskin and Price, '83, and below).

Observations such as these lead us to regard the deep tubercular region as separate from the olfactory tubercle proper. This view is consistent with increasing evidence that the present supratubercular zone is actually the rostral confluence of the basal nuclear complex and ventral pallidum (cf. Brockhaus, '42; Haberly and Price, '78, p. 732; Switzer et al., '82; Saper, '84; Young et al., '84; Zaborszky et al., '86; Heimer et al., '87). Like both of these structures, the supratubercular zone projects to the mediadorsal thalamus (Benjamin et al., '82; Price and Slotnick, '83; Young et al., '84; Velasos and Reinoso-Suárez, '85; Russchen et al., '87) and ventral tegmental area of the midbrain (Troiano and Siegel, '78, case 6; Phillipson, '79; Fallon, '83). Like adjoining basal nuclear cell groups (magnocellular preoptic area and ventral diagonal band nucleus), the supratubercular zone also projects to the olfactory bulb (Broadwell and Jacobowitz, '76; de Olmos et al., '78; Dennis and Kerr, '76; Woolf et al., '84; Zaborszky et al., '86). Finally, like the ventral pallidum, it is filled with neuropil rich in ferric iron (Switzer et al., '82), leu- and/or met-enkephalin (Haber and Nauta, '83; Groenewegen and Russchen, '84; McLean et al.,
Fig. 6. Decreasing size of Nissl-rich satellite neurons from lateral to medial ICC units in the cat. Like ChAT and AChE cells in the same areas (see Talbot et al., '88, Figs. 5–7), Nissl-rich neurons in suprainsular ICC sectors progressively diminish in average soma length toward the midline. While the decrement appears minor from lateral to lateral transitional units (cf. a and b), it is pronounced from the latter to central units (cf. b and c) and from these to medial transitional units (cf. c and d). In the last case, the Nissl-rich satellites are especially small (e.g., those marked by arrows) and are not often clustered (see also Fig. 2t–w). The scale bar in d is 50 μm.
Fig. 7. A typical lateral transitional ICC unit viewed in a rostrocaudal series of coronal Nissl sections from the cat. Its Calleja island shows marked variability in displacing the adjoining tubular densocellular layer, ranging from what is common in lateral units (a) to what is typical in central units (c). At most levels, the densocellular layer is substantially, but incompletely, displaced (b–d,f). In some places, this layer is only broken by small spaces invaded by a few granule cells of the overlying Calleja island (arrowed in a). In other places, larger interruptions of the densocellular layer are invaded by more substantial granule cell populations (areas between arrowheads in b–f). Note also that where the Calleja island is most massive, it is surmounted by a cluster of Nissl-intensive satellite neurons (indicated by small arrows in c and d). The numbers 1–3 in b, respectively label the molecular, densocellular, and parvicellular layers of the feline olfactory tubercle. The scale bar in f is 200 μm.

As documented by the studies just cited, none of these various connectional and histochemical features is shared by the olfactory tubercle proper.

The supratubercular zone is separated from the diagonal band by the paratubercular nucleus. To date, this structure has been identified only in primates and prosimians (Brockhaus, ’42), but it occurs in all the species we examined, judging from the consistent presence of a similarly located cell group virtually identical in cytoarchitecture. Moreover, the same structure in the rat, cat, and macaque displays many choline acetyltransferase (ChAT) neurons (Talbot, Woolf, and Butcher, unpublished observations), at least a subset of which innervates the olfactory bulb (cf. Mesulam...
vestigators have treated the paratubercular cell group as
simply a ventrolateral extension of the diagonal band nu-
eral nucleus is actually a rostromedial extension of the cau-
dal adjoining magnocellular preoptic area. This view is
critically distinguished from cells in the densocellular
layer at the lateral extremity of the olfactory tubercle.

Due to its location and ill-defined boundaries, many in-
vestigators have treated the paratubercular cell group as
simply a ventrolateral extension of the diagonal band nu-
erus (e.g., Fox, '40; Dennis and Kerr, '76; Krayniak et al.,
'80). Such treatment is unwarranted given that neurons in
the paratubercular domain are distinctly smaller than most
diagonal band nerve cells (current study) and rarely, if ever,
project to the hippocampal formation (see Amaral and
Cowen, '80, Fig. 3A; DeVito, '80, Fig. 2; Krayniak et al.,
'80; Amaral and Kurz, '85, Figs. 7-11). The fact that an
appreciable number of its neurons do project to the olfactory
bulb (see Rye et al., '84, Fig. 4B; Woolf et al., '84, Fig. 4;
Zaborszky et al., '86, Fig. 4) suggests that the paratubercu-
lar nucleus is actually a rostromedial extension of the cau-
dally adjoining magnocellular preoptic area. This view is
consistent with evidence that these two cell groups have
highly similar cerebrocortical projection patterns (see Saper,
'84, Figs. 7, 9, 10, 12).

Cytoarchitecture of the feline ICC

Callejal islands were found to be distributed throughout
the mediolateral extent of the olfactory tubercle in close
association with depressions or corrugations in its densocellu-
lar layer (Figs. 1, 2). An additional microcellular cluster
extended dorsolaterally from the most medial tubercular
island into and along the medial edge of nucleus accumbens
(Figs. 1d,e, 3c,d, 4). The roughly vertical orientation of this
accumbal cluster explains its prominence in coronal sec-
tions (Figs. 3, 4) and hence its common designation as the
insula (or insula) magna. A number of the tubercular islands
were noted to be comparably expansive, but their sagittal
orientation (see also Fox, '40, Fig. 10) makes them less
conspicuous in frontal sections.

The accumbal and tubercular islands were each accom-
panied by a more-or-less diffuse population of satellite neu-
rons cytoarchitecturally distinguished from cells in
neighboring structures (see below). Since these neuronal
populations did not overlap with one another, the feline
Callejal complex was divided into separate island-satellite
cell ensembles (i.e., ICC units). Only minor components
of the complex failed to show such unitary organization, spe-
cifically the deep Callejal islands sporadically encountered
in the caudal supratubercular zone (Fig. 5a), the paratuber-
cular nucleus (Fig. 5b), and occasionally in adjoining parts
of the diagonal band nucleus and magnocellular preoptic
area. These generally small, relatively diffuse granular
clusters were not accompanied by distinctive satellite cell
populations. Due to their rarity, the deep islands will not
be considered further.

Structure of individual ICC units. Each ICC unit was
divisible into a central insular component and several pe-
ripheral cell zones. In units confined to the olfactory tuber-
cle, the peripheral zones consisted of supra-, para-, and
subinsular sectors (see also Fig. 1 in Talbot et al., '88). The
suprainsular sectors of medial tubercular units lay par-
tially within insular depressions (Figs. 1, 2p-w) and could
thus be regarded as hilar areas. In the accumbal extension
of the most medial ICC unit, the cytoarchitectural equiva-
tent of tubercular suprainsular sectors is entirely hilar,
lying within narrow, labyrinthine spaces apparently formed
by invaginations of the isla magna (Figs. 3d,e, 4a,b). Due to
its form and to its separation from the tubercular molecular
layer, the accumbal ICC lacks a subinsular sector compa-
rable to that observed in other parts of the Callejal complex.

The insular component of an ICC unit was its dense mass
of chomophlic, microcellular neurons. In most cases, this
consisted of a Callejal island composed of granule cells 6–
10 μm in soma size, flanked by a small group of slightly
more voluminous dwarf cells 9–13 μm in soma length and
continuous with the tubercular densocellular layer. In the
lateral olfactory tubercle, however, the insular component
of each ICC unit contained a large number of both granule
and dwarf cells, the latter aggregated just below the Calle-
jal island. The spatial segregation of the two types of micro-
cellular neurons proved critical in differentiating such cells
in Nissl material.

As their name implies, satellite neurons lay primarily in
the peripheral ICC sectors named above. A few, however,
appeared to be ectopically located within Callejal islands.
Regardless of location, such cells were medium to large
neurons (15–42 μm in soma length) with oval, fusiform, or
occasionally triangular somata. In the tubercular ICC, they
were most common (though not necessarily numerous) in
suprainsular zones, where at least a subset of the cells
would be the aspiny and spine-poor cells of Meyer and
Wahle ('86). In the accumbal ICC, satellite cells were most
common in hilar spaces within the isla magna. Hilar these
neurons could be differentiated into Nissl-rich and Nissl-
poor varieties. The former were conspicuous throughout
the ICC, but the latter were not readily identified in strictly
tubercular units, where it was difficult to differentiate Nissl-
poor satellite cells from partially intermixed parvicellular
tubercular neurons. In general, only the smallest satellite
neurons tended to be chromophobic.
The suprainsular zone was distinguished from other peripheral ICC sectors not only by its greater number of satellite neurons, but also by its continuity with the overlying supratubercular zone (Fig. 1) and hence with the rostral extremity of the ventral pallidum (see above). The density of oligodendroglia and diffractive properties of the tissue matrix suggested that ventral pallidal fibers swept down into the suprainsular zone (see also Meyer and Wahle, '86; and Wahle and Meyer, '86). How clearly this zone was visualized in Nissl material depended on the density of Nissl-rich satellite cells and oligodendroglia, as well as on the degree to which parvicellular tubercular neurons were excluded from the suprainsular domain.

In contrast to that domain, the subinsular zone was marked by an extreme paucity of satellite neurons and by the presence of olfactory system fibers filling the tubercular molecular layer. Nissl material was sufficient to delineate the upper and lower limits of this zone, but not its external boundaries. These were evident only in the ChAT and AChE material described in a subsequent paper (Talbot et al., '88).

The parainsular zone contained very few Nissl-rich satellite cells and merged peripherally with tubercular or accumbal neuronal populations. Its width was consequently nuclear in Nissl material, but was judged in our AChE preparations to be 40–150 μm alongside tubercular islands and 80–170 μm around the isla magna (see also Talbot et al., '88). Parainsular zones in the tubercular ICC appear to coincide with the groups of small pyramidal-like cells observed by Meyer and Wahle ('86) alongside feline Callejal islands. Such cell groups consist of relatively chromophobic neurons 12–18 μm in soma length. They constitute transitional segments of the densocellular layer linking its purely tubercular stretches with the dwarf cells of ICC units. We will explain later the connectional and histochemical reasons for including the small pyramidal-like cells as components of the Callejal complex (see Discussion).

A number of sporadically encountered ICC units were diminutive, having very small microcellular islands and very scarce Nissl-rich satellite cells. Serial section analysis demonstrated that these minor ICC units were not fragments of larger units. Two kinds were observed. One had an elliptical island oriented more or less vertically (Fig. 5c) and occurred almost exclusively at central levels of the olfactory tubercle (Fig. 1c). The other kind of minor unit had a smaller, less compact island inserted like a peg into the dorsal surface of the densocellular (i.e., pyramidal) layer (Fig. 5d) and occurred with few exceptions at caudal levels of the olfactory tubercle (Fig. 1h). At many different tuber-

Fig. 9. Comparative anatomy of the ICC in Macaca nemestrina monkeys (a), cats (b), and albino rats (c). Each of the three coronal sections shows the complex at or near its broadest mediolateral and dorsoventral extent, as attested in the feline case by Figure 1. Single arrows point to Callejal islands within the olfactory tubercle proper. Double arrows point to the isla magna in the medial wall of nucleus accumbens. Dots mark the locus of Nissl-rich satellite cells closely associated with the tubercular islands (i.e., within about 400 μm of those islands). Due to spatial constraints, such neurons are individually mapped only in the rat (c). Because of their proportional representation in the monkey and cat, rare satellite cells alongside and/or beneath the tubercular islands in a and b are not indicated. The supratubercular zone overlying the olfactory tubercle is lightly shaded. A dashed line traces the tubercular densocellular layer in the cat and rat sections but not in the monkey section since no such layer was clear there. The apparent discontinuity between the monkey ICC units and the supratubercular zone contrasts with their fusion in adjoining sections. The full lengths of scale bars in a, b, and c span 5.0, 5.0, and 2.0 mm, respectively.
cultural levels, we also noted what may be a larger, more heterogeneous version of the peglike islands—namely, clusters of microcellular and small neurons in the densocellular layer adjoining the piriform cortex (not illustrated; see also Berman and Jones, '82, p. 75).

**Lateromedial differentiation of major ICC units.** Feline Callejal islands differed in size, form, and basilar locus from lateral to medial sectors of the olfactory tubercle. In particular, they flattened, widened, and sank into the molecular layer, while their granule cell densities steadily increased (cf. Figs. 1, 2, and Table 1). Since these modifications were often accompanied by rising numbers (Table 1) and diminishing size (Fig. 6) of suprainsular satellite cells, the cytoarchitecture of ICC units as a whole changed lateromedially. The transformation occurred in stages, so that even cursory inspection disclosed at least three distinct sets of ICC units—namely, lateral, intermediate, and medial units.

Further study demonstrated that the cytoarchitectural transformation was more gradual, requiring recognition of three different forms of the intermediate island-satellite cell ensembles. Those at the mediolateral heart of the ICC were designated as central units. Flanking them were lateral and medial transitional units. Altogether, then, we distinguished five different types of island-satellite cell ensembles in the cat ICC. Figure 1 maps their locations, while Figure 2 contrasts their cytoarchitectural features. Those features may be summarized as follows.

1. **Lateral units** had small, relatively diffuse, granular islands atop the tubercular densocellular layer (Fig. 2a–d), here composed mainly of diminutive neurons (i.e., dwarf cells and small pyramidal-like neurons: see also Meyer and Wahle, '86). The sagittal extent of the granular islands was short in many cases, judging from the rather small number of coronal sections through which they could be followed. The suprainsular areas above these islands were typically rudimentary, containing a small to moderate number of large, Nissl-rich satellite cells poorly segregated from both the parvicellular tubercle and the supratubercular zone. Callejal islands in the following three forms of intermediate ICC units were flatter, denser, and significantly broader ($P < .05$ in all cases, Newman-Keuls analysis) than in lateral units (see Table 1). They were also generally longer in the sagittal plane and surrounded by a significantly larger number of Nissl-rich satellite neurons ($P < .01$ in all cases, Newman-Keuls analysis) than in lateral units (see Table 1). With respect to this last feature, however, the three types of intermediate units were not significantly different from one another.

2. **Lateral transitional units** contained moderately dense, partially flattened, granular islands incompletely (albeit substantially) displacing the adjoining densocellular layer of small neurons (Figs. $2g–k, 7$). Their suprainsular zones had clusters of relatively large, often Nissl-intensive satellite neurons (Figs. $2g–k, 6b, 7$). These zones lacked the ectopic islets of microcellular neurons rising from, or suspended above, some Callejal islands (see Fig. 2/m.p.r). Such ectopic islets were not seen in lateral or lateral transitional ICC units.

3. **Central units** were marked by islands flatter, denser, and significantly wider ($P < .01$, Newman-Keuls analysis) than in lateral transitional ensembles (Fig. 2l–o, Table 1). In further contrast to those ensembles, the islands here virtually supplanted the tubercular densocellular layer and appreciably penetrated the underlying molecular layer (Fig. 2l–o, Table 1). The form of the islands tended to be concave in frontal sections, due to a tendency for the granular mass to curve upward toward its medial and lateral edges (Figs. 1, 2). The adjoining suprainsular zone had smaller (Figs. 2, 6c), often less chromophilic satellite neurons than in lateral

<table>
<thead>
<tr>
<th>Feature</th>
<th>Lateral</th>
<th>Lateral transitional</th>
<th>Central</th>
<th>Medial transitional</th>
<th>Medial</th>
<th>Tubercular</th>
<th>Accumbal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location (see also Fig. 1)</td>
<td>Lateral OT$^2$</td>
<td>Intermediate OT, lateral sector</td>
<td>Intermediate OT, medial sector</td>
<td>Medial OT, ventral sector</td>
<td>Medial OT, dorsal sector</td>
<td>Dorosmedial edge of NA</td>
<td></td>
</tr>
<tr>
<td>Insular sector</td>
<td>185.5 ± 76.5</td>
<td>383.9 ± 112.1</td>
<td>583.6 ± 171.7</td>
<td>727.6 ± 297.0</td>
<td>761.4 ± 251.1</td>
<td>1322.1 ± 566.3</td>
<td></td>
</tr>
<tr>
<td>Coronal length ($\pm SD$, μm)$^2$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typical granule cell density</td>
<td>++/++</td>
<td>++/++</td>
<td>++/++</td>
<td>++/++</td>
<td>++/++</td>
<td>++/++</td>
<td></td>
</tr>
<tr>
<td>Typical displacement of OT pyramidal layer</td>
<td>--/++</td>
<td>++/++</td>
<td>++/++</td>
<td>++/++</td>
<td>++/++</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Typical penetration of OT molecular layer$^2$</td>
<td>--</td>
<td>--</td>
<td>++/++</td>
<td>++/++</td>
<td>++/++</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>Suprainsular (or hilar) sector</td>
<td>+</td>
<td>++</td>
<td>++++</td>
<td>++/++</td>
<td>++/++</td>
<td>++/++</td>
<td></td>
</tr>
<tr>
<td>Prominence of ectopic granular islets$^2$</td>
<td>--</td>
<td>--</td>
<td>++</td>
<td>++/++</td>
<td>++/++</td>
<td>++/++</td>
<td></td>
</tr>
<tr>
<td>No. of Nissl-rich cells (μm$^2$)</td>
<td>7.89 ± 4.5</td>
<td>15.60 ± 9.2</td>
<td>18.42 ± 11.5</td>
<td>20.97 ± 12.8</td>
<td>14.63 ± 7.9</td>
<td>14.39 ± 7.9</td>
<td></td>
</tr>
</tbody>
</table>

$^1$Based on data specified in Materials and Methods. Absence of a listed feature is specified by a minus sign. One to four plus signs designate low to maximum degrees of a property relative to the highest ICC levels observed. A slash between symbols indicates that the feature involved varies in degree between the values given, whether within single transected ICC units or within the population of such units studied. The "typical" values given for relative properties (e.g., granule cell density) apply to at least 75% of the cases examined for any given type of ICC unit. NA = nucleus accumbentes OT = olfactory tubercle.

$^2$This feature is defined in the data analysis section of Materials and Methods.

$^3$This feature refers to the relative frequency, cell density and cross-sectional area of the typically small clusters of microcellular neurons rising from, or suspended above, some Callejal islands (see Fig. 2/m.p.r). Such ectopic islets were not seen in lateral or lateral transitional ICC units.

$^4$This feature is the relative frequency, cell density and cross-sectional area of the typically small clusters of microcellular neurons rising from, or suspended above, some Callejal islands (see Fig. 2/m.p.r). Such ectopic islets were not seen in lateral or lateral transitional ICC units.

$^5$The ectopic islets in this case are the granular protrusions from the caudal segment of the isla magna (see Fig. 4).
transitional units. These neurons tended to form loose clusters (Fig. 2l-o) and were occasionally accompanied by diminutive clumps or islets of microcellular neurons.

4. The medial transitional unit contained an island similar in form to that of central ICC ensembles, yet differing from the latter by its slightly higher cell density, significantly greater width \((P < .05,\) Newman-Keuls analysis), and typically more complete penetration of the tubercular molecular layer (Table 1 and Fig. 2p-s). Its suprainsular zone was distinguished from that of central units by unusually small, commonly scattered satellite neurons (Figs. 2p-s, 6d) and often by relatively prominent ectopic islets of microcellular neurons (Fig. 2p,r,s). It is worth adding that this unit was always found in the oblique (never the vertical) part of the medial hemisphere wall.

5. The medial unit differed strikingly from all other ICC ensembles by its vertical extension into the medial edge of nucleus accumbens (Figs. 1, 3, 6). It was thus divisible into tubercular and accumbal segments (Figs. 1, 3). Though consistently restricted to the vertical part of the medial hemisphere wall, the tubercular segments varied in shape (Fig. 2t-w) and number across animals. In cases where two such segments were present, they were connected by a granule cell bridge (Fig. 3b). The insular component of the accumbal segment (i.e., the isla magna) also displayed some unusual features. Especially at central levels of the tubercle, it enclosed the previously mentioned hilar spaces containing satellite cells (Fig. 3d,e). Caudally, it extended two granular "arms" about a small population of ventral striatal neurons near the apex of nucleus accumbens (Fig. 4). In virtually all the cat brains studied, these protrusions were seen immediately rostral to the caudal pole of the isla magna. We should add that in some brains these protrusions were tenuous and did not fully encircle an accumbal population in the sections retained for analysis.

The lateral and medial transitional units characterized above were so named because their cytoarchitectural features were intermediate between those of more external and more internal types of ICC ensembles. Thus, for example, the size, shape, density, and dendrocellular layer displacement of lateral transitional islands were intermediate between those of lateral and central units (Table 1, Figs. 2, 7). Analogously, the size and molecular layer penetration of medial transitional islands were intermediate between those of central and far medial units (Table 1, Fig. 2), as was the frequency of microcellular islets and density of chromophic satellite cells above such islands (not clearly illustrated). The transitional character of these medial Callejcal clusters was further demonstrated by the finding that despite their resemblance to central unit islands, they approached and in some cases merged with the far medial island for a few consecutive sections. Slender granular trails could even be traced in some brains from the medial transitional unit to the base of the isla magna. The inconsistent spatial relationships just noted between medial transitional and far medial islands reflect the variable form of the ICC along the medial hemisphere wall from animal to animal (see also Meyer and Wahle, '86).

Each of the five types of island-satellite cell ensembles recognized here can be identified reliably in coronal Nissl material with the aid of Table 1 and Figures 1-3. Considerations simplify this task. First, three of the ICC unit types are easily distinguished by idiosyncratic properties worth reiterating: lateral units by small, relatively diffuse, unflattened islands remaining on top of the densocellular layer; central units by compact, more-or-less concave islands completely replacing the densocellular layer without obliterating the underlying molecular layer; and the medial unit by its prominent extension into the medial edge of nucleus accumbens from one or two tubercular islands in the vertical part of the medial hemisphere wall. Second, the island nearest the medial unit on the oblique part of the medial hemisphere wall is invariably part of the single medial transitional unit in each hemisphere. Third, accurate identification of lateral transitional units is achieved by a multisection analysis of granular Callejcal islands near the junction between the lateral and intermediate sectors of the olfactory tubercle, searching for islands substantially, but incompletely, displacing the underlying densocellular layer. The multisectin survey is important, because lateral transitional islands may superficially resemble lateral or central islands in some sections (see Fig. 7).

Rostrocaudal distribution of ICC units. In every coronal series studied, the various ICC unit types characterized above were initially encountered and ultimately passed in a fairly strict rostrocaudal order. This sequence defined eight successive levels of the olfactory tubercle (a-h in Fig. 1). No ICC elements occurred at the rostral pole of the tubercle, but just behind that area lateral and lateral transitional units were present (level a). Somewhat more caudally, central and medial transitional units were added (level b), after which the medial unit was introduced, usually without its accumbal extension (level c). This was typically first encountered attached to its tubercular root (level d), yet thereafter appeared separately near the apex of nucleus accumbens (level e). Past that level, the olfactory tubercle and the ICC were gradually replaced by the laterally expanding diagonal band nucleus. The tubercular segment of the medial unit consequently disappeared first (level f), followed by the medial transitional unit (level g) and then by the central units (level h). Lateral and lateral transitional units (along with the isla magna in some cases) were still seen at level h, but not at the caudal pole of the tubercle. In that area, the only remaining ICC elements were minor units (Figs. 1h, 5d) and sporadic deep islands (Fig. 5a).

Figure 8 graphs the distribution of ICC unit types across the eight tubercular levels just identified. It should not be taken as evidence that lateral and lateral transitional units have a greater sagittal length than more medial units. As noted earlier, the reverse is true. The relatively short lateral and lateral transitional units were simply present at a wider range of tubercular levels, in some cases lined up one behind the other with short, but definite, breaks between them.

Comparative anatomy of the ICC

We identified three general classes of microcellular clusters in tubercular Nissl material from rats, cats, and macaque monkeys: 1) superficial islands closely associated with the densocellular layer and/or the pial surface of the olfactory tubercle, 2) deep islands located more dorsally in the tubercular region, and 3) vertically oriented islands having both superficial and deep components. Included in this last class of islands is the most conspicuous and consistent microcellular cluster observed in the three species studied. From a superficial site near the medial edge of the olfactory tubercle, it penetrated deeply along and within the medial edge of nucleus accumbens. The accumbal extension or isla
magna was consequently prominent in frontal sections from all three species, much more so than its tubercular root, which expanded appreciably only in the sagittal plane.

The remaining microcellular clusters of the ICC were confined to the olfactory tubercle. Deep clusters here, like the vertical island described above (see also Beccari, '10, pp. 198–200; Fallon et al., '78; Meyer and Wahle, '86), seemed to be simple granule cell masses containing a few nonmicrocellular neurons (see also Millhouse, '87). In contrast, superficial clusters proved to be more heterogeneous masses displaying greater species differences. Such clusters in the rat were integrated into depressions or ruffles of the tubercular densocellular layer, in which basally aggregated dwarf cells merged with more deeply placed nonmicrocellular neurons (see also Millhouse, '87). As noted previously, however, superficial islands in the cat were more complex, consisting of a granule cell cluster resting on (or displacing) a bed of dwarf cells at the base of densocellular layer depressions (see also Meyer and Wahle, '86). Corresponding microcellular clusters in the macaque seemed to be composed primarily of granule cells, but Golgi studies are needed to evaluate this impression.

The various classes of microcellular clusters identified were not equally represented or similarly distributed in the olfactory tubercle of the three species examined. We have already described the predominance of superficial islands throughout the cat tubercle, deep islands being rare and confined to the supratubercular zone, paratabercular nucleus, and adjacent basal nuclear structures. The macaque also displayed a preponderance of superficial islands (primarily in area TOL 2 of Rose, '27; see our Fig. 9a), but contained in addition a sparse population of variably sized deep islands at central and especially caudal tubercular levels. In the rat, however, superficial islands dominated only the rostral sector of the olfactory tubercle, where they lay at the bottom of folds or depressions in the densocellular layer as noted above. At intermediate levels of the rat tubercle, both superficial and deep islands were common, the former situated as at rostral levels. Further caudally in the same species, deep islands were nearly the only microcellular masses observed, usually lying at or near the interface between the parvicellular layer of the tubercle and the supratubercular zone (Fig. 9c). Given the great extent of the caudal tubercular sector in rats, deep islands could be said to dominate the olfactory tubercle in this species. Rats also displayed some small, vertically oriented islands with both superficial and deep components at intermediate rostrocaudal levels of the tubercle. Such islands were occasionally observed in macaques, but never in cats.

Several progressive changes occurred in the ICC from rats to cats to macaques, most of which are illustrated in Figure 9. First, the isla magna became more prominent. It increased not only in sagittal extent, but also in width and especially in height (x = 0.45 mm in rats, 1.32 mm in cats, and 3.11 mm in macaques). Second, the tubercular islands generally flattened and sank from the dorsal to the ventral border of the olfactory tubercle, especially medially. As expected from progressive flattening, these islands also expanded in width (x = 0.31 mm in rats, 0.50 mm in cats, and 0.96 mm in macaques), but the increments were not as impressive as the phylogenetic enlargement of the isla magna. Third, the basal shift of the tubercular islands drew them away from the main body of the supratubercular zone, so that they appeared to be isolated from that zone in some areas of the cat (Fig. 9d,g) and macaque (Fig. 9a) tubercle. Yet even in those areas, the tissue above Callejal islands proved to be continuous with supratubercular white matter when followed across closely spaced sections, consistent with the rostral extension of ventral pallidal neuropil visualized with substance P immunohistochemistry (see Beach and McGeer, '84, Figs. 6A, 15C; Wahle and Meyer, '86). Fourth, the Nissl-rich satellite cells of the ICC became less evenly distributed among the tubercular islands, tending instead to aggregate consistently about individual islands in cats and macaques.

As in cats, satellite cell aggregation in macaques was seen to divide the ICC into relatively distinct units, each composed of a microcellular island and a set of satellite cells preferentially located in a suprainsular (or hilar) area. Nevertheless, such units were more prominent in cats. At central coronal levels of the olfactory tubercle, at least 5–7 granular islands were regularly seen in cats (as in rats), whereas no more than 3–5 such islands were commonly noted in macaques. The cat islands were also more sharply defined due to a higher density of peripheral granule cells. Moreover, Nissl-rich satellite cells in feline cases were more often conspicuously clustered and usually more fully segregated from supratubercular neurons.

The progressive lateromedial changes in ICC cytoarchitecture detailed earlier were also most obvious in the cat. Such changes were not apparent in the rat, although the most medial Callejal island in this species was similarly unusual in form, orientation, and subpial contact. Some changes did occur in the macaque ICC from the lateral to the medial edge of the olfactory tubercle (e.g., increasing superficiality and widening of individual islands), but these were not distinctive enough to allow identification of the five different types of island-satellite cell ensembles observed in the cat.

In addition to these ensembles, we identified two minor types of feline ICC units, each containing a distinctive microcellular cluster with no apparent counterpart in the rat or macaque. A variant of the peglike island in one of these units (Fig. 5d) might account for the unusually heterogeneous cluster of microcellular and small neurons at the lateral extremity of the tubercular densocellular layer (see also Herman and Jones, '82, p. 75). This cluster does not appear to be uniquely feline. A similar cell group was found in the rat, but situated more laterally in the adjoining pyramidal layer of the piriform cortex, which is heavily innervated by the lateral olfactory tract nucleus (Luskin and Price, '83). Such input may indicate the presence of dwarf cells, because the lateral olfactory tract nucleus preferentially innervates segments of the olfactory tubercle containing those cells (i.e., densocellular layer folds rich in microcellular neurons; cf. Luskin and Price, '83, esp. Fig. 15; and Millhouse, '87).

**DISCUSSION**

The species differences just described explain why we cannot characterize the cat ICC simply by reference to the pioneering studies of Fallon and his colleagues (Fallon et al., '78, '83; Ribak and Fallon, '82; Fallon, '83) on the Callejal complex of the rat. To establish the cytoarchitectural organization of the feline ICC, we have had to resolve several basic issues—namely, what cell types should be included, what nomenclature is appropriate, and what parcellation scheme accurately reflects the topographic differentiation observed. These are complex topics not explicitly discussed in the literature. We address them here with the
aid of an older literature no longer familiar to most neuro-
scientists yet important in understanding the roots and
rationale of the ICC as we conceive it. Since this literature
is exceptionally difficult, frequently misrepresented, and
not yet the subject of a critical review, we begin our discus-
sion with a historical perspective on the essential studies
involved, including as well recent Golgi reports relevant to
our findings. In this context, we then discuss 1) the cellular
composition and nomenclature of the ICC, 2) the concept
and basic features of ICC units, and 3) the lateral-medial
differentiation of feline ICC units.

The islands of Calleja complex in historical
perspective

A review of the literature related to the ICC is particu-
larly valuable in reintroducing significant but largely for-
gotten findings of early investigators. Their observations
called attention to several basic features of the complex not
widely appreciated today, such as the existence of both deep
and superficial tubercular islands, species differences in
those islands, and an exceptionally dense afferent plexus in
the superficial islands. We deal below only with major dis-
coveries such as these, relating our findings to them in each
case.

Ganser’s discovery. On the basis of carmine prepara-
tions from an unspecified species of mole, Ganser (1882)
gave the first account of cell clusters in the olfactory tuber-
cle. These were not the solid tubercular islands found in
rats, cats, and macaques, but rather hollow cell masses
enclosing prominent internal cavities. To quote Ganser
(1882, pp. 645, 646):

The cortex along the head of the striatum [i.e., the olfac-
tory tubercle: see Stephan, ’75, p. 310] ... contains ... clus-
ters of the small cells which I have described as the major
constituent of the fourth layer of the olfactory bulb [i.e.,
“Körner” or granule cells: see Ganser, 1882, pp. 644–645].
The clusters themselves penetrate from the surface into the
deep layer of the cortex. It produces the impression as if
they were invaginated from without, so that they extend into
the deep layer like a flask with a wide neck; they
thus appear in frontal sections partly as wreaths, partly as
pegs, partly in a flask-shaped figure [translated from the
original German; see also Ganser’s Fig. 9].

Hollow tubercular clusters of microcellular neurons were
also found by Becacci (’10, p. 220 and Fig. 23) in the mole
Talpa europaea, though apparently not by other investiga-
tors (see Johnston, ’13, Figs. 48–50; Johnson, ’57, p. 390,
Fig. 4) in a different genus (Scalopus) of the same insect-
vore. Variants of Ganser’s highly invaginated cell clusters
are displayed by at least two prosimians—namely, Galago
demidowii (see Stephan, ’75, Figs. 208, 221, 257) and Tupaia
glis (see Skeen and Hall, ’77, Fig. 20). To our knowledge,
however, such cell islands are at best rare in the olfactory
tubercle of marsupials, rodents, lagomorphs, carnivores,
and primates. The potential significance of these species
differences is that satellite neurons within the deep cavity
of Ganser’s clusters or their variants are probably isolated
dendritically from the rest of the olfactory tubercle. In
mammals other than insectivores and prosimians, such iso-
lation seems assured only for satellite neurons in the nar-
row cavities of the isla magna and for similar cells (see
Millhouse, ’87) embedded within granule cell clusters.

Calleja’s islands. Without referring to Ganser’s find-
ings, Calleja (1893, pp. 14–24) reported cell islands in the
olfactory tubercle, as well as in the immediately adjoin-
ing striatum. He observed them in rabbits, guinea pigs, and
mice by using carmine, Weigert-Pal, and especially Golgi
techniques. Since his report is not widely available and is
often misunderstood, we need to consider it in more detail
than given elsewhere.

The least well known of Calleja’s islands are those called
islotes del cuerpo estriado (1893, pp. 22–24), which were
described as very dense, variably shaped cell clusters situ-
ated in the corpus striatum close to the white matter of the
olfactory tubercle. Like the most common form of striatal
neuron (see Pasik et al., ’79), their constituent cells were
noted to be small to medium stellate neurons with spine-
rich dendrites and axons aborizing extensively near the
parent cell bodies. Given, then, their location, cell density,
and neuronal morphology, the islotes del cuerpo estriado
probably correspond to the clusters of relatively small, spiny
neurons located along the ventral border of nucleus accum-
bens (Herkenham et al., ’84). There is consequently some
justification for the occasional practice of extending the
name “islands of Calleja” to cell clusters in and/or near the
ventral striatum (Loo, ’31; Sanides, ’57; Takimoto et al.,
’62; Berman and Jones, ’82, p. 75). Such extended terminol-
ogy may ultimately prove anatomically significant, because
the ventral accumbal clusters involved are frequently
aligned with the microcellular islands today attributed to
Calleja in the olfactory tubercle and in the paramedian
accumbens (see Herkenham et al., ’84).

While Calleja did not actually identify the large cell clus-
ter in the latter location (i.e., the isla magna), he did focus
attention on tubercular islands, which he called islotes ol-
fativos (1893, pp. 14–21). Three such cell clusters were noted
in his introductory remarks: 1) superficial islands of semi-
lunar shape situated very close to the brain surface, 2)
deeper islands of round or oval shape, which were some-
times seen in isolation from superficial cell clusters, and 3)
extended islands with both superficial and deep compo-
nents. This categorization scheme is very similar to the one
adopted in the present study (see Comparative Anatomy of
the ICC above).

As expected from the fact that Calleja’s study was
prompted by Ramón y Cajal, the islands were characterized
primarily as seen in Golgi material. Unfortunately, he only
describes superficial islands in this manner. One of these is
described at length and is eventually identified as an “or-
dinary island” (see Calleja’s Fig. 3). Another is given a
relatively cursory treatment and receives no unique name—
hence called an “innominate island” by us (see Calleja’s
Fig. 4). Both island forms were regarded as specialized
portions of the tubercular pyramidal layer: they are each
characterized and at least once identified as islotes de pir-
ámides, although the cell forms are globular rather than
pyramidal. In both cases, the basal aspect of the islands is
occupied by a conspicuous group of small neurons, which
are surmounted by larger, elongated nerve cells. What dif-
ferentiates the two island forms according to Calleja (1893,
p. 17) is the larger size and more irregular form of the
islotes ordinarios. They also appear to differ with respect to
their small stellate cells. Judging from Calleja’s illustra-
tions, the dendritic field of these cells resembles that of
the tubercular dwarf cells in the ordinary islands and that of
tubercular densocellular neurons in the innominate islands
(see Millhouse and Heimer, ’84; Millhouse, ’87). This may
be one reason why only the former islands are noted by
Calleja to have exceptionally small stellate cells.
On the basis of his own extensive Golgi observations in rats, Millhouse ('87) has recently clarified the identity of Calleja's ordinary and innominate tubercular islands. Consistent with the summary description given above, Millhouse equates the ordinary islands with cell clusters constituting parts or all of the descending ruffles in the tubercular densocellular layer. Such clusters consist of two fused layers: a superficial stratum of dwarf cells and a deep stratum of medium spiny neurons. In contrast, Millhouse equates what we call the innominate islands (ambiguously identified by him as islotes de pirámides) with clusters lacking microcellular neurons, containing instead only typical densocellular layer neurons (i.e., spiny cells of variable, but most often medium, size). These more homogeneous cell clusters are said to account for the second of Calleja's Golgi-defined island types in unruflled parts of the tubercular densocellular layer, in the overlying multiform layer, and in the still deeper striatal cell bridges. It should be noted, however, that Calleja gives no indication that he observed such islands anywhere but in superficial aspects of the tubercle.

We cannot agree with the further assertion of Millhouse ('87) that Calleja did not identify the deep islands of granule cells so prominent in the rodent olfactory tubercle. We noted above that deep islands are mentioned by Calleja, though not described in his Golgi material. Calleja also states that the islotes olfativos are clearly demonstrated in carmine preparations (1893, pp. 14, 18), with which Ganser (1882, pp. 645, 646) had discovered prominent granule cell clusters in the olfactory tubercle (see above). It thus seems implausible that Calleja did not include deep granular clusters among his islotes olfativos, especially given the form of his deep islands. His cursory description of them may simply reflect failure in impregnating granule cell groups. Even Ramón y Cajal ('11, pp. 765–772) gives no indication of finding deep granular islands in Golgi preparations from the mouse olfactory tubercle. Material unusually refractory to impregnation of island neurons nevertheless allowed Calleja to discover an exceptionally dense terminal plexus within superficial islotes olfativos (1893, pp. 19, 20). This consisted of varicose axonal arborizations of extraordinary fineness filling the intercellular spaces and outer limits of the superficial islands. It derived from fine fibers descending from the olfactory tract, which focuses its tubercular projections on the microcellular neurons found in the olfactory tubercle. Material unusually refractory to impregnation of island neurons nevertheless allowed Calleja to discover an exceptionally dense terminal plexus within superficial islotes olfativos (1893, pp. 19, 20). This consisted of varicose axonal arborizations of extraordinary fineness filling the intercellular spaces and outer limits of the superficial islands. It derived from fine fibers descending from the olfactory tract, which focuses its tubercular projections on tissue known to contain dawrf cell clusters—namely, descending ruffles of the densocellular layer (see Luskin and Price, '83, Fig. 15).

Ramon y Cajal's contributions. While acknowledging the occurrence of Calleja's islotes ordinarios, Ramón y Cajal reported an entirely different set of tubercular islands. His observations were based on Nissl and Golgi material from mice, guinea pigs, rabbits, dogs, humans, and especially cats. The results were published first in Spanish ('01/'02, pp. 79–85) and later in a revised French translation incorporated into the widely cited Histologie du Système Nerveux (Ramón y Cajal, '11, pp. 727–732). In both publications, three different types of cell clusters are described as seen in Golgi preparations. One of these is a superficial, submeningeal island illustrated in the cat. It consists of microcellular neurons, though both smaller and larger variants of such cells are present. The other two island types reported are deep cell clusters illustrated in the mouse. They are respectively composed of small and medium-size neurons in the form of globular pyramids. Given the spine-rich dendrites and long, dorsally directed axons of their globular neurons, the deep islands probably correspond to the clusters of spiny cells observed by Millhouse ('87) in the multiform layer of the rat olfactory tubercle.

In describing the three island types, Ramón y Cajal refers to Calleja only with respect to the submeningeal island, which he implicitly regarded as one of that investigator's islotes ordinarios. Yet even in this case, closer inspection leads to a different conclusion, one independent of Calleja's observations. In all the species he studied, Ramón y Cajal found the submeningeal island to be especially massive, particularly in carnivores. In the cat, he describes the cell cluster in some detail and illustrates it in a sagittal Nissl section ('01/'02, Fig. 38; '11, Fig. 465). This shows clearly that the submeningeal island truly rests on the brain surface (see also Ramón y Cajal, '01/'02, Fig. 39; '11, Fig. 466) and that it lies medially in the olfactory tubercle, as attested by simultaneous transection of the diagonal band nucleus (i.e., the diagonal band area that Ramón y Cajal called the "perichiasmatic [or caudal] region of the olfactory tubercle": see Stephan, '75, p. 313). Consistent with the accompanying text, the same figure also illustrates the prominent, dorsally directed edges of the island, creating a markedly concave hiliar space loosely filled with small to medium-size neurons. Ramón y Cajal ('01/'02, p. 81; '11, p. 730) goes on to state that the same cell cluster in the dog "descends to deep zones and emits ramifying and anastomosing cords and bands in which larger cells are enmeshed...." Considering, then, that only one such cell cluster was observed per hemisphere, the various features of the submeningeal island just enumerated collectively identify it as the tubercular segment of the far medial island described earlier in the present report. The accumbal segment of this island was discovered almost simultaneously by Martinotti ('02), though its continuity with the underlying submeningeal cluster was only later established by Becanci ('10, pp. 198–199, Fig. 22; see also Humphrey, '36, Fig. 3; Fox, '40, Fig. 8; and Comparative Anatomy of the ICC above).

In addition to its dorsal extensions, the feline submeningeal island described by Ramón y Cajal differs from the islotes ordinarios of Calleja in cellular composition. While the former are heterogeneous masses of microcellular and larger neurons (see above), the latter is characterized and portrayed as a purely microcellular cluster (see Ramón y Cajal, '01/'02, pp. 81–83; '11, pp. 728–731). Furthermore, the microcellular neurons found in islotes ordinarios are specifically dwarf cells (Millhouse, '87; see also Hosoya and Hirata, '74), whereas those dominating the submeningeal island of the cat are granule cells, given that such cells dominate all medially situated islands in the feline olfactory tubercle (Meyer and Wahle, '86). Ramón y Cajal ('01/'02, pp. 81–83) does in fact refer to the island under discussion as a great submeningeal cluster of granules ("gran pláyade submeningea de granos") and as a peripheral cap of minute cells or granules ("un casquete de células menudísimas ó granos"), some of which were said to be no larger than 5 μm in Golgi material. It is true that the same neuronal cluster is subsequently identified as a large (or immense) island of dwarf cells (Ramón y Cajal, '01/'02, p. 83 and Figs.

---

1This should not be confused with the dwarf cell caps of Hosoya and Hirata ('74), which are simply Calleja's islotes ordinarios (see also W. Fox, '87).
38, 39), but it is clear upon comparing the original Spanish article ('01/'02, pp. 81–83) with its revised translation into French ('11, pp. 728–731) that Ramón y Cajal used "dwarf (or dwarfish)" as a synonym for "granule (or granular)," as he did explicitly in describing microcellular neurons in olfactory glomeruli ('01/'02, cf. pp. 4 and 5), the fascia dentata ('01/'02, p. 89), and the cerebellar cortex ('11, pp. 33, 34). Only in recent years has a clear distinction been made between granule and dwarf neurons in tubercular cell islands (see Millhouse and Heimer, '84; Meyer and Wahle, '86; Millhouse, '87; and below).

We may summarize Ramón y Cajal's contributions to the present topic by reiterating that he discovered several cell islands not reported by Calleja. Most notable of these is the submeningeal cluster at the medial edge of the olfactory tubercle. Ramón y Cajal recognized its constant occurrence across mammalian species, accurately described its unusual form, specified its dominant granule cell population, and identified its associated satellite cell population. Due to the last two accomplishments, he may be credited with discovering two basic elements of the ICC.

**Beccari's account of Calleja's islands.** Apart from Köelliker's (1896, p. 725) passing reference to the *isoles ordinarios* as the olfactory islands ("Riechinseln") of Calleja, the first investigator to name tubercular cell clusters after the Spanish anatomist was Beccari ('10, pp. 197, 198, Figs. 21–24, 26, 27). He referred to the islands formally as nests of Calleja and Cajal but labelled them in his figures simply as nests of Calleja. Beccari characterized these cell clusters in the course of an extensive comparative anatomical report on the mammalian olfactory tubercle studied in Nissl, Cajal, and Weigert material. In nearly all the many species he investigated, including marsupials, edentates, insectivores, rodents, carnivores, and primates, Beccari noted three basic types of Callejalan nests:

*Type 1 nests* were identified as cell accumulations at the base of densocellular ruffles in the more lateral part of the olfactory tubercle. Beccari states that these nests only represent thickening of the second tubercular layer, but he illustrates their cells as being distinctly smaller than typical densocellular neurons (see his Fig. 24) and comments later that they are only somewhat larger than the smallest cells he observed in tubercular islands (i.e., those in his type 2 nests: see Beccari, '10, p. 199; cf. also his Figs. 24 and 26). This naturally suggests that the neurons dominating type 1 nests are dwarf cells, which are in fact clustered in ruffles of the tubercular densocellular layer (see Hosoya and Hirata, '74; Meyer and Wahle, '86; Millhouse '87). Moreover, like dwarf (but not granule) cells, neurons in superficial islands of the lateral olfactory tubercle (i.e., those primarily within type 1 nests) were found to project into the supratubercular zone (Beccari, '10, cf. p. 206 and Figs. 24, 27, 30). Thus, contrary to Beccari's impression, his type 1 nests probably correspond to the *isoles ordinarios* of Calleja. As such, they would also be the densocellular curls or "Papillen" of Popoff and Popoff ('29, pp. 278–281, Pls. 23, 24) and the dwarf cell caps of Hosoya and Hirata ('74).

*Type 2 nests* were described by Beccari ('10, pp. 199, 200, Figs. 22, 23, 26) as clusters of very small neurons located near, or extending from, the brain surface. They lay preferentially in the medial part of the olfactory tubercle. Several further observations by Beccari establish that these clusters are specifically granule cell islands. First, as noted above, their constituent neurons were reported to be somewhat smaller than those of type 1 nests. Second, on the basis of both cell arrangement and magnitude, Beccari included among type 2 clusters his septal nest (our isla magna), which he agreed with Martinotti ('02) was composed of neurons the size of cerebellar granules. Third, Beccari noted another conspicuous type 2 nest along the base of the medial hemisphere wall, which undoubtably corresponds to the granular submeningeal island of Ramón y Cajal discussed earlier. Fourth, the hollow granule cell clusters found in the mole by Ganser (1882, see above) were also classified as type 2 nests by Beccari ('10, p. 200). Finally, a smaller form of type 2 cluster found near the brain surface was illustrated clearly as an aggregation of exceptionally diminutive neurons (Beccari, '10, Fig. 26). This particular type of cell nest seems to encompass many of the tubercular islands we observed in the cat olfactory tubercle, because it also displays a hilar depression connected to the overlying supratubercular zone by a fiber column (Beccari, '10, pp. 199, 200, Fig. 26). The constituent fibers were considered efferents from the underlying cell nest, but this is unlikely since tubercular granule cells apparently lack long axons (see Fallon et al., '78; Meyer and Wahle, '86; Millhouse, '87).

*Type 3 nests* were poorly defined by Beccari ('10, p. 200, Fig. 21), who refers to them only as deep tubercular accumulations lacking continuity with superficial cell nests. Nevertheless, the additional observation that they are numerous in rats is sufficient to identify them as granule cell islands, which are the only common form of cell clusters deep within the rodent olfactory tubercle (see also Popoff and Popoff, '29, pp. 278–281, Pls. 23–25; Rose, '29, p. 35 and Pls. 3–8; Fallon et al., '78). This conclusion is consistent with the high cell density of the only type 3 nests illustrated by Beccari ('10, Fig. 21), specifically in the armadillo. As his classification scheme attests, Beccari ('10) was the first to distinguish between what we know today as dwarf and granule cell clusters in the olfactory tubercle. In noting the differential distribution of these clusters (specifically type 1 vs. type 2 nests), he was likewise the first to uncover one of the features differentiating lateral and medial parts of the ICC, later clarified by Popoff and Popoff ('29, p. 281 and text Fig. 12) in the rat and by Meyer and Wahle ('86) in the cat.

Given the comparative focus of his study, Beccari ('10) also discovered a number of species differences in tuberculacell clusters. We noted earlier the variant of his type 2 nests peculiar to the mole, though present in modified forms in some prosimians (see Stephan, '75, Figs. 208, 221, 257; Sken and Hall, '77, Fig. 20). Two more species differences reported by Beccari ('10, pp. 199, 200) are worth mentioning here. First, apart from the isla magna and its tubercular root, almost no type 2 nests were found in the rat (or armadillo), consistent with our observation that superficial granule cell islands are rare in the rat compared to the cat. Second, marked differences were noted across species in the form of type 1 nests, which appear in some animals (e.g., the armadillo) as only a slight thickening of the densocellular layer and in others (e.g., the mouse) as thick curls in that layer. The former kind of type 1 nest is very similar to the microcellular islands we observed in the far lateral olfactory tubercle of the cat. In both cases, a modest thickening at the base of a densocellular layer ruffle (presumably due to an aggregation of dwarf cells: see Meyer and
FELINE ICC: CYTOARCHITECTURE, COMPARATIVE ANATOMY

In contrast to the species differences observed in tubercular cell islands, Beccari ('10, p. 198) found the isla magna to be a constant mammalian feature. Calling it the "nest(s) of the septum," he clearly described the island's location, form, and granule cell composition, as well as its invariant continuity with another type 2 nest at the medial edge of the olfactory tubercle. This last feature was said to be more prominent in animals with well-developed olfactory tubercles, but we found the isla magna more securely anchored to the tubercule in the microsmatic monkey than in the macrosmatic rat. Our finding is not surprising in view of the exceptionally large isla magna present in primates (see Crosby and Humphrey, '41, Figs. 5-7; Lauer, '45, Fig. 6; Sanides, '58, Figs. 9, 13-16).

Recent Golgi studies. Since the early 1960s, many advances have been made in our understanding of the histochemistry and connections of tubercular cell islands (see literature cited in Stepman, '75, pp. 327-329; Fallon et al., '83; Talbot et al., '88). Only more recently, however, have we gained detailed information on the full set of neuron types in and around these islands. Despite Ramón y Cajal's ('01/02, pp. 82, 83) belief that Calleja had reached the limit of discoveries possible with the Golgi method, much more has been found with that procedure in the last 14 years (cf. Hosoya and Hirata, '74; Fallon et al., '78; Chronister et al., '81; Millhouse and Heimer, '84; Záborszky et al., '85; Meyer and Wahle, '86). It is clear from the literature just cited and from brain atlases in current use (e.g., Paxinos and Watson, '86, PIs. 9-18) that this narrow definition of Callejal islands has been adopted almost universally in recent decades. Indeed, the few apparent exceptions in the last 10 years are cases where the term has merely been extended to designate both granule and dwarf cell clusters (i.e., superficial, as well as deep, islands: Ribak and Fallon, '82; Luskin and Price, '83).

Wahle, '86) is surmounted by a loose, vertically oriented cluster of what are undoubtedly granule cells (cf. Beccari, '10, Fig. 21 and our Fig. 2a-f).

In the 50 years from 1913 to 1963, virtually all publications dealing with tubercular (and related accumbal) cell islands were cytoarchitectural reports. Some of these offered detailed information on the distribution, form, cellular composition, and/or cytology of the islands (Popoff and Popoff, '29, pp. 279-281, Figs. 23-25; Fox, '40, pp. 18-22; Sanides, '57, '58; Takimoto et al., '62). Most, however, simply gave brief descriptions of the cell clusters and/or cytology of the islands (Popoff and Popoff, '29, pp. 389, 390, 125, Figs. 27, 42, 45-50, 60, 61, 67, 73; Obenchain, '25, p. 197, Figs. 28-32; Gurdjian, '25, pp. 143, 144; Rose, '29, p. 35, Figs. 3-8; Loo, '31, pp. 19, 20; Humphrey, '36, pp. 611, 612, Figs. 2, 3, 6, 8; Young, '36, p. 310, Figs. 7, 8, 17; Crosby and Humphrey, '41, pp. 321-325; Lauer, '45; Breathnach, '53; Johnson, '57).

The two other island types presumably included the clusters of small and medium-size spiny neurons described earlier—namely, Calleja's ('13, pp. 22-24) islotes del cuerpo estrido along the base of nucleus accumbens and Ramón y Cajal's ('01/02, p. 84; '11, pp. 730-732) islands of small and medium pyramids deep within the olfactory tubercle.

A second group of investigators more conservative than Beccari ('10) applied the term "islands of Calleja" only to microcellular clusters. This phenomenon remains puzzling but may be related to the fact that none of the anatomists fostering such nomenclature cited Calleja's work (see Johnston, '13; Obenchain, '25; Gurdjian, '25; Popoff and Popoff, '29; Crosby and Humphrey, '41; Lauer, '45; Johnson, '57). It may also be significant that Johnston ('13), who apparently introduced the restrictive terminology (see especially his Figs. 48-50, 61), does cite Ramón y Cajal's authoritative Histologie du Système Nerveux ('11). In this work, Calleja is mentioned with respect to only one type of tubercular island—namely, that composed of microcellular neurons. This is also the only island type illustrated by Ramón y Cajal ('11, Fig. 465) in a Nissel section through the olfactory tubercle.

Johnston ('13) does not specifically state that his islands of Calleja are microcellular clusters, but this is evident from his limitation of the term to deep tubercular and parparamedian accumbal cell clusters in the mole (see his Figs. 48-50), where such clusters are known to be granule cell masses (cf. Gasner, 1882, pp. 645-646; Beccari, '10, pp. 198, 200). Perhaps under Johnston's influence, Obenchain ('25, p. 197) explicitly defined the islands of Calleja in the marsupial Caenolestes as dense masses of extremely small cells, which she accordingly illustrated as lying exclusively in the deep layer of the olfactory tubercle and along the medial wall of nucleus accumbens (see her Figs. 28-32). Gurdjian ('25, pp. 143, 144), who cites a preprint of Obenchain's paper, adopted the same restrictive terminology in the rat.

Most, however, have simply equated granule cell clusters with Callejal islands, which they distinguished from the microcell-rich ruffles of the tubercular demicellular layer (i.e., their "Papilen" or "curls").
microcellular neurons in granule cell islands are smaller and have thinner, more varicose dendrites with fewer branches and often far fewer spines (see Millhouse and Heimer, '84; Meyer and Wahle, '86; Millhouse, '87). Second, with rare exceptions (see Fallon et al., '78), granule cell axons are short and do not appear to leave their parent cell clusters (see Millhouse and Heimer, '84; Meyer and Wahle, '86; Millhouse, '87), whereas dwarf cell axons are long and ascend into the supratubercular zone (see Hosoya and Hirata, '74; Meyer and Wahle, '86; Millhouse, '87). Third, granule cell islands are the special targets of an unusually thick type of afferent fiber (Millhouse, '87), while dwarf cell islands (i.e., *isoles ordinarios* of Calleja) are targeted by distinctly finer, more profusely terminating afferents (cf. Calleja, 1893, pp. 19, 20; Millhouse, '87). Our earlier suggestion that the latter afferents may derive from the lateral olfactory tract nucleus is consequently reinforced, because this nucleus projects heavily to dwarf cell-rich segments of the tubercular densocellular layer while only lightly innervating more deeply situated granule cell islands (see Luskin and Price, '83, Fig. 15).

As evident in Nissl material, the microcellular masses of both granule and dwarf cell islands are intimately associated with larger neurons (cf. Ramón y Cajal, '01/02, p. 82, '11, p. 730; Obenchain, '25, p. 197; Gurdjian, '25, p. 143; Popoff and Popoff, '29, p. 280; Fox, '40, pp. 18, 20; Lauer, '45; Somides, '58; Jacobs et al., '71; Van Hoesen et al., '76; Fallon et al., '78; Chronister et al., '81). The more recent Golgi studies demonstrate, however, that the identity and spatial arrangement of the larger neurons is different in the two cases. Consistent with cytoarchitectural observations, they show that each granule cell island is accompanied by a sparse population of medium- to large-size isodendritic neurons falling into two broad categories: 1) cells inside or alongside the island with dendritic trees essentially buried within the microcellular mass (i.e., the special hilar cells of Millhouse, '87; see also Meyer and Wahle, '86, Fig. 7) and 2) cells alongside or distal to the island with only part of their dendritic trees deeply implanted in the microcellular cluster (see Fallon et al., '78, Figs. 4–6; Chronister et al., '81, Fig. 53; Millhouse and Heimer, '84, Fig. 11; Meyer and Wahle, '86, Figs. 4, 7). The outlying cells in both of these groups are more-or-less evenly distributed around granule cell islands in rats (see Fallon et al., '78) but are concentrated above such cell clusters in cats (see Meyer and Wahle, '86). This species difference is clearly reflected in Nissl material (current study) as well as in preparations visualizing AChE and ChAT neurons (Talbot et al., '88).

Unlike the case with granule cell islands, the nonmicrocellular neurons associated with dwarf cell clusters are primarily isodendritic cells linking such clusters to the tubercular densocellular layer. These are specifically small versions of the spiny cells elsewhere dominating the densocellular layer. Calleja (1893, pp. 15–17) had observed such cells within the deeper part of his *isoles ordinarios*, the superficial segment of which contained mainly dwarf cells (see Millhouse, '87). He found that the two cell groups were always contiguous (and sometimes interdigitated) in the cat olfactory tubercle (see Meyer and Wahle, '86). Such cells in turn merge gradually with the more dorsal, unruflled portion of the densocellular layer. In more medial sectors of the feline olfactory tubercle, the dwarf cell clusters (and associated small pyramidal-like neurons) are reduced and displaced to the periphery of the densocellular layer depressions by the descent of prominent granule cell islands (Meyer and Wahle, '86).

Not all the nonmicrocellular neurons associated with dwarf cell islands are small isodendritic cells. Such islands contain at least a few small isodendritic neurons of variable size (cf. Calleja, 1893, p. 17; Meyer and Wahle, '86, Fig. 4). These neurons presumably account for the occasional AChE and ChAT cells observed in the more lateral densocellular depressions of the feline olfactory tubercle (see Talbot et al., '88).

**Cellular composition and nomenclature of the ICC**

As conceived here, the ICC is composed of both granule and dwarf cell islands, along with the nonmicrocellular neurons closely associated with those cell clusters. This is more inclusive than the ICC of Fallon, who excluded the dwarf cell clusters of the rat olfactory tubercle by omitting the superficial microcellular islands in all but the most medial densocellular layer depressions (cf. Fallon, '83, p. 777; Fallon et al., '83, pp. 94, 95; Fallon and Leslie, '86, Figs. 4–6). While recognizing the previously specified differences between the two types of microcellular islands, there are several reasons to include both within the same anatomical complex. First, granule and dwarf cell clusters are always contiguous (and sometimes interdigitated) in the cat olfactory tubercle (see Meyer and Wahle, '86). Such a close spatial relationship is not consistently observed in rodents, but at intermediate rostrocaudal levels of the rat olfactory tubercle, deep granule cell clusters extend ventrally to approach and reach the dwarf cell-rich bases of densocellular layer ruffles (see also Popoff and Popoff, '29, pp. 280, 281; Herkenham et al., '84, Fig. 2; Millhouse, '87, Fig. 1). Second, the microcellular neurons in these ruffles establish specialized junctions between their somata, as do granular neurons in deep cell clusters (Riba and Fallon, '82; see also Hosoya, '73, Fig. 1). Third, satellite neurons of the granular islands appear to receive input not only from short granule cell axons, but also from long dwarf cell efferents (see Meyer and Wahle, '86). Fourth, the neuropil in both types of microcellular islands is exceptionally rich in AChE (Talbot et al., '88). Finally, the dwarf cells and at least one population of granule cells in these islands contain substance P (see Beckstead and Kersey, '85; Wahle and Meyer, '86).

The nonmicrocellular neurons of the ICC are simply those small to large cells extending one or more dendrites far into the microcellular clusters of the complex. As discussed earlier, such cells are small isodendritic neurons in the case of dwarf cell islands and medium to large isodendritic neu-
rons in the case of granule cell islands. Published illustrations of these isodendritic neurons indicate that they are 15 μm or larger in soma length (Fallon et al., '78, Figs. 4–6; Chronister et al., '81, Fig. 53; Millhouse and Heimer, '84, Fig. 11; Meyer and Wahle, '86, Figs. 4, 7), a conclusion reinforced by the apparent absence of tubercular or accum- bul isodendritic cells less than 15 μm in soma size (cf. Chronister et al., '81, Figs. 19–22, 53; Millhouse and Heimer, '84, Figs. 9[b], 11[a,b], 17, 18[1], 19–22; Meyer and Wahle, '86, Figs. 2, 4, 7; Millhouse, '87, Figs. 8, 12–14). For this reason, we do not include in the ICC many of the 10–20-μm cells Fallon and his colleagues (Fallon, '83; Fallon et al., '83) consider to be closely associated with tubercular and accumbal granule cell islands. Another reason to ex- clude the smaller elements in this cell population is that such elements would include 10–16-μm spiny neurons whose dendrites avoid granule cell clusters (see Millhouse and Heimer, '84; Millhouse, '87).

We may assume that the isodendritic neurons whose den- drites do not avoid granular islands are a subset of the satellite cells identified in the present report. Such cells are distinguished in Nissl material by their preferential distribu- tion in the vicinity of granule cell clusters (i.e., within about 400 μm of those islands). They prove to be 15–42-μm neurons (no more than 35 μm in rets), but all the smallest of which tend to be Nissi-rich. Such chromophobia differentiates the vast majority of these cells from the relatively chromophoric medium spiny neurons common in adjoining tissue, most of which are also smaller than the satellite cells (i.e., 10–16 μm in the rat [cf. Herkenham et al., '84; and Millhouse and Heimer, '84], 14–25 μm in the cat [pre- sent study; see also Meyer and Wahle, '86]). Taking this into account, the observed size range of the satellite neurons indicates that most, if not all, of these cells are specifically isodendritic neurons, which as noted above are never less than 15 μm in soma length either in the olfactory tubercle or in nucleus accumbens. Since isodendritic cells in these structures have dendrites up to 500 μm in length (cf. Chron- ister et al., '81; Millhouse and Heimer, '84), all satellite neurons are potentially capable of extending such processes far into the granular islands they accompany. Not all of them do so, however (Millhouse, '87; see also Millhouse and Heimer, '84, Fig. 21). Consequently, only a subset of isoden- dritic satellite cells is dendritically integrated with granu- lar islands. We regard this subset alone as part of the ICC. It includes the medium- to large-size cells aggregated in supragranular sectors of the cat ICC (see Meyer and Wahle, '86), but cannot be identified selectively in rat Nissi mate- rial, with the possible exception of the few satellite cells occupying invaginated niches of granular islands (Fallon et al., '76; Millhouse, '87).

Within the microcellular mass of these islands is an ad- ditional, sparse population of isodendritic neurons, which are thus intimately associated with granule cells. Millhouse ('87) discovered several varieties of these "special hilar cells," which he considered to be unique to the granu- lar islands and their immediately surrounding environ- ment. One variety may nevertheless be ectopic AChE and ChAT neurons more commonly found throughout the sat- ellite cell zones around granular islands (Taibo et al., '88). Ectopic neurons of this type could account not only for rare cholinergic neurons in such islands (Armstrong et al., '84), but also for the occasional presence of those neurons in dwarf cluster cells (Taibo et al., '88).

The superficially situated dwarf cell clusters merge on both their lateral and medial ends with numerous idioden- dritic neurons (i.e., the small pyramidal-like cells of Meyer and Wahle, '86). Such cells complicate delineation of the ICC, because they are part of the tubercular densocellular layer and not only resemble the larger, medium spiny neu-rons elsewhere dominating that layer, but also share with these larger cells a major projection to the supratubercular zone (cf. Millhouse and Heimer, '84; Meyer and Wahle, '86; Heimer et al., '87; Millhouse, '87). In other respects, how- ever, the small pyramidal-like neurons are more closely related to dwarf cells, which are both differentiated from the rest of the densocellular layer by three features: 1) an unusually dense cholinergic neuropil typical of ICC compo- nents (Taibo et al., '88), 2) somatal immunoreactivity to opioid and substance P antibodies (Wahle and Meyer, '86), and 3) axonal projections to the suprainular satellite cell zones of the ICC (Meyer and Wahle, '86; see also Hosoya and Hirata, '74). These projections are strong and do not spread to the adjoining olfactory tubercle (Meyer and Wahle, '86). Considering all the information currently available, we regard the small pyramidal-like cells adjoining dwarf cell clusters as ICC, rather than tubercular, elements.

Fallon's term "islands of Calleja complex" (Fallon, '83; Fallon et al., '83) remains an appropriate name for the cell groups encompassed by the present ICC. As argued earlier, Calleja's isoles olfattivos undoubtedly included both types of microcellular clusters around which the current complex is built. Only one of these clusters is described in detail by Calleja (1893, pp. 15–17), but here too we find an important precedent for our conception, because the isoles ordinarios were noted to contain microcellular neurons equivalent to dwarf cells (see Millhouse, '87), as well as an intimately associated group of nonmicrocellular neurons correspond- ing to the small pyramidal-like cells of Meyer and Wahle ('86). Since by contrast Calleja (1893, pp. 14, 15) says very little about the islands representing granule cell clusters, it may seem odd that we continue to restrict the term "islands of Calleja" to these specific clusters. Our reasons are ones of custom and economy. As explained previously, the term has long since become synonymous with granule cell clusters in the olfactory tubercle and paramedian accumbens, so that any different usage now would further complicate an already complex area of study. Millhouse ('87) favors dropping the term altogether, but the alterna- tive expressions are not necessarily succinct, because "granule cell clusters" without qualification could refer to structures in the olfactory tubercle, paramedian accumbens, or even the amygdala, though the microcellular masses in this last area are more properly classed as dwarf cell clusters (cf. Millhouse, '86, '87). Nor do any alternative expressions have the immediate recognition value of the current term "islands of Calleja." To avoid any remaining ambiguity, it must be stressed that the present ICC excludes cell clusters not dendritically integrated with microcellular islands. Accordingly, the complex does not include any of the following cell groups: 1) the relatively loose aggregates of small to medium-size neurons in the tubercular multiform layer or in neighboring striatal cell bridges (cf. Ramón y Cajal, '02, p. 84, '11, pp. 724–726; Field, '81, p. 20, Sanides, '66, '70; Takimoto et al., '82; Millhouse, '87), 2) the innominate islands of Calleja (1893, pp. 17, 18; see also above), which appear to be nothing more than sporadic compressions of the tubercular densocellular layer (see Millhouse, '87), and 3) the ventral...
striatal islands of Calleja (1893, pp. 22–24), presumably corresponding to basal accumbal cell clusters (Herkenham et al., '84; see also Berman and Jones, '82, p. 75, and above). All three of these diverse cell masses are spatially segregated from microcellular islands, though the accumbal clusters are often aligned with such islands in the rat (see Herkenham et al., '84). Furthermore, they all differ considerably in neuronal morphology, connectivity, and histochemistry from the ICC cell groups specified above (cf. Ramón y Cajal, '01/02, p. 84, '11, pp. 730–732; Herkenham et al., '84; Millhouse and Heimer, '84; Meyer and Wahle, '86; Wahle and Meyer, '86; Millhouse, '87).

The concept and basic features of ICC units

Our comparative anatomical observations emphasize that the ICC is differently structured in the rat, cat, and macaque monkey. Of the various species differences found, the most obvious concerned the predominant location of the tubercular microcellular clusters and the distribution of non microcellular neurons capable of dendritic integration with those clusters (i.e., chromophilic satellite neurons within a radius of about 400 μm). With respect to these factors, the complex in rats is readily distinguished from that in cats and macaques. In rats, deep islands dominate the ICC, with satellite cells more-or-less evenly distributed among the microcellular clusters, despite occasional, loose aggregations near some islands. In macaques and especially cats, superficial islands prevail, with satellite cells essentially gathered about individual microcellular masses. Of the three species examined, then, only the macaque and cat possess an ICC which is clearly and consistently divided into separate units, each containing a granular island and its satellite neurons.2 The feline complex is further distinguished by an extreme paucity of deep islands and by the prominence and regularity of its island-satellite cell ensembles.

As reported elsewhere (Talbot et al., '88), these ensembles are unified by a pervasive cholinergic neuropil even denser than in most adjoining ventral striatal areas. This is not the only reason to call them ICC units, however. Their structural cohesiveness is apparent in several Golgi observations of Meyer and Wahle ('86), who refer to most of the present ICC units as cap/hilus regions of the cat olfactory tubercle. They find that feline Callejal islands are penetrated by dendrites of large and medium-size suprainsular neurons (see also Ramón y Cajal, '11, Fig. 466), consistent with previous observations on ICC satellite neurons in other species (see Calleja, 1893, Fig. 6; Fallon et al., '78, Figs. 4–6; Chronister et al., '81, Fig. 53; Millhouse and Heimer, '84, Fig. 11). Some of these penetrant dendrites appeared to be contacted by granule cell axons. More proximal dendrites of large suprainsular neurons were judged to receive multiple contacts from terminal varicosities of dwarf cells and small pyramidal-like neurons, which lie along the periphery of the underlying Callejal island. At least in some cases, these various axonal connections were found to be reciprocated by medium-size satellite neurons with axons ramifying within the island and among its associated dwarf cells.

The various sectors of feline ICC units recognized here are justified largely by the different cell types or fiber systems dominating them. The insular sector has been defined as the combined mass of granule and dwarf cells, all of which we regard as microcellular neurons. Other cell types are generally rare in the islands (see also Meyer and Wahle, '86; Millhouse, '87). In contrast to the microcellular clusters, the parainsular zones flanking the islands are composed of somewhat more substantial neurons—namely, the previously mentioned small pyramidal-like cells seen in Golgi studies to have spine-rich dendrites (see Meyer and Wahle, '86). Suprainsular or equivalent hilar areas are in turn differentiated from other ICC sectors by their relatively diffuse population of medium- and large-size neurons observed in Golgi material to have aspiny or spine-poor dendrites (see Ramón y Cajal, '11, Fig. 466; Meyer and Wahle, '86). Given the size, shape, and isodendritic form of these neurons, they probably include the ChAT and AChE cells we found to be common in suprainsular and hilar sectors but relatively rare elsewhere in the feline Callejal complex (Talbot et al., '88). The last of the four sectors in this complex (i.e., the subinsular zone) is distinguished by the presence of fiber systems traversing the tubercular molecular layer and by a subpial band of what appears to be terminal cholinergic neuropil (see Talbot et al., '88). Microcellular neurons and/or their dendrites may well be targets of olfactory bulb fibers in the subinsular zone, judging from the preferential termination of such fibers immediately below superficial Callejal islands reported by Skenes and Hall ('77) in the tree shrew (see also Hosoya and Hirata, '74; Ribak and Fallon, '82; Fallon et al., '83).

A number of factors hamper attempts to equate the ICC sectors observed in the cat with those identified by Fallon and his colleagues ('83) in the rat. Their studies naturally focused on the deep islands so common in the rat Callejal complex, while ours necessarily concentrated on the superficial islands virtually monopolizing the cat ICC. This would explain why Fallon et al. ('83) do not mention a subinsular sector of the ICC traversed by fibers of the tubercular molecular layer, which obviously applies only to superficial parts of the complex. Both superficial and deep Callejal islands in rats are cupped by dopamine-rich rim regions (cf. Fallon et al., '78, '83; Vroom et al., '86), but it is uncertain whether this occurs in cats due to the absence of published histochemical studies on the catecholamine distribution in the feline olfactory tubercle. For a similar reason, it is unknown whether the cat ICC contains patches of intense GAD activity equivalent to those defining “cap” regions above rat Callejal islands (Fallon et al., '83; see also Pérez de la Mora et al., '81; Young et al., '84; Mugnaini and Oertel, '85). The invaginated spaces in these islands constitute the enkephalin- and substance P-rich core regions of the rat ICC (Fallon et al., '83). These indentations do not correspond to the more-or-less hilar sectors of the cat ICC (i.e., its suprainsular and invaginated zones), because the center of such sectors is conspicuously poor in both enkephalins and substance P (Wahle and Meyer, '86). The feline hilar zones are more likely to correspond to the ill-defined region of the rat ICC between Callejal islands (i.e., lying outside the rim, cap, and core areas noted above). Like the feline suprainsular and hilar zones, is relatively poor in enkephalins and substance P (Fallon et al., '83) and contains the bulk of AChE and ChAT cells in the rat ICC (Talbot et al., '88).

2Although Fallon and his colleagues (Fallon, '83; Fallon et al., '82) consider the Callejal complex of the rat to be a composite of small ICAs, each consisting of a granular island and its associated neurons, it is not clear if they regard these as separate morphological units of the complex. Our observations call for caution in accepting such a conclusion.
The species difference just touched upon explains why the cat provides an opportunity not afforded by the rat to study relatively pure populations of ICC satellite neurons, specifically those known to be dendritically integrated with Callejal islands. The coalescence of these cells (including AChE and ChAT neurons; see Talbot et al., '88) about individual islands helps segregate the satellite population from adjoining structures. The fact that such aggregation only occurs sporadically in the rat may thus explain why the ICC in this species is reported to contain relatively large, AChE-intensive neurons characteristic of the olfactory tubercle and supratubercular zone (see Fallon et al., '83). Such neurons are not found in the cat ICC, where AChE cells never demonstrate intensive cholinesterase activity and are typically small or medium size (Taibolt et al., '88). Even in the feline case, however, complete segregation of satellite neurons from non-ICC cells is attained only in the hilus of the isla magna. Elsewhere, ICC satellite cells are intermixed to a variable degree with accumbal or parvicellular tubercular neurons.

Lateromedial differentiation of feline ICC units

Apart from its striking division into separate units, the feline ICC was most notable in the present study for its progressive lateromedial changes in cytoarchitecture. These topographic changes were numerous, allowing us to differentiate five types of island-satellite cell ensembles at successively more medial portions of the olfactory tuber cle. Such a parcellation scheme reflects the gradually shifting topographic properties of the ICC more accurately than the simple lateral-medial division of the feline Callejal complex described by Meyer and Wahle ('86).

Although much further study is needed to determine the usefulness of our parcellation scheme for the cat ICC, there are already a number of findings consistent with its emphasis on a lateromedial differentiation. At successively more medial portions of the Callejal complex, we found a progressive increase in cholinergic parameters (i.e., number of satellite cells and density of neuropil reactive for AChE and ChAT; Talbot et al., '88). With respect to cellular composition, it has been reported that dwarf cells and small pyramidal-like neurons are much more common in the lateral half of the cat ICC (Meyer and Wahle, '86). Conversely, granule cells containing substance P are more numerous in the medial third of the feline Callejal complex (see Wahle and Meyer, '86). Similar topographic differences are found with respect to afferents of the complex. The infralimbic cortex of the cat projects to Callejal islands only in the medial half of the ICC (see Room et al., '85, Fig. 8). The ventral tegmental area of the rat also innervates primarily the medial Callejal complex, while the adjoining substantia nigra supplies mainly intermediate and lateral parts of that complex (Fallon and Moore, '78; Fallon et al., '78). Finally, ICC afferents containing gonadotropin-releasing hormone (GnRH) are restricted to the medial half of the rat Callejal complex (Fallon et al., '83; Merchenthaler et al., '84).

Our treatment of the far medial ICC as a separate division of the complex recognizes its many unusual characteristics. It is the only part of the ICC extending beyond the olfactory tubercle to invade the medial edge of nucleus accumbens, a part of the complex whose prominence is species dependent. Both its tubercular and accumbal segments differ from most of the remaining ICC by the presence of GnRH fibers (Fallon et al., '83; Merchenthaler et al., '84), a parainsular concentration of neurotensin neurons (Köhler and Eriksson, '84), and exceptionally dense ChAT and AChE neuropil among granule cells (cf. Parent et al., '77; Herkenham et al., '84; Mesulam et al., '84; Smith and Parent, '84; Talbot et al., '88). Other unusual features are displayed by the accumbal segment alone. This is the sole ICC area which 1) lacks input from inferotemporal cortex (Van Hoesen et al., '76, '81), 2) contains atriopeptin cells (Standaert et al., '86) and 3) exhibits neuropil rich in atriopeptin (Standaert et al., '86) and vasoactive intestinal polypeptide (Abrams et al., '85). The accumbal island (i.e., isla magna) is further distinguished from other Callejal masses by its enclosure of satellite cells in labyrinthine spaces and by the presence of occasional granule cells with extrinsic projections (Fallon et al., '78). In cats, the isla magna exhibits yet another unique feature far caudally, where it extends slender granular "arms" about a small set of accumbal neurons. Detached offshoots of these microcellular protrusions may account for the small aggregations of granule cells noted by Meyer and Wahle ('86) within the body of the feline nucleus accumbens.

These last observations strengthen the view that the isla magna is more closely associated with nucleus accumbens than with the medially adjacent septum (see also White, '81). As noted earlier, the isla magna is largely embedded in the medial wall of nucleus accumbens, with which it shares a strong cholinergic neuropil (see Herkenham et al., '84; Mesulam et al., '84; Smith and Parent, '84; Talbot et al., '88). Clusters of accumbal neurons are also found to aggregate near the isla magna in diverse species (see Sandés, '88; Fallon et al., '78; Herkenham et al., '84). Despite such close associations, the two structures remain separate entities. In addition to marked cytoarchitectural differences, the isla magna and nucleus accumbens display sharply contrasting neuronal morphologies (Chronister et al., '81), opioid receptor densities (Herkenham et al., '84), and different afferent sources (see Groenewegen et al., '82; Kelley and Domesick, '82; Kelley et al., '82; Herkenham et al., '84; Witter and Groenewegen, '86).

In a subsequent paper on the cholinergic and cholinesterase features of the feline ICC (Talbot et al., '88), we discuss further the relationship of the Callejal complex to other basal forebrain structures and consider its potential functions.

ACKNOWLEDGMENTS

This research was supported by USPHS grant NS 10928 to L.L.B. We gratefully acknowledge the dedicated assistance of Michael S. Gold in word processing, table construction, and bibliographic services.

LITERATURE CITED


FELINE ICC: CYTOARCHITECTURE, COMPARATIVE ANATOMY


