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The insular cortex: a comparative perspective

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Abstract The human insular cortex is involved in a variety of viscerosensory, visceromotor, and interoceptive functions, and plays a role in complex processes such as emotions, music, and language. Across mammals, the insula has considerable morphologic variability. We review the structure and connectivity of the insula in laboratory animals (mouse, domestic cat, macaque monkey), and we present original data on the morphology and cytoarchitecture of insular cortex in less common species including a large carnivore (the Atlantic walrus, *Odobenus rosmarus*), two artiodactyls (the pigmy hippopotamus, *Hexaprotodon liberiensis*, and the Western bongo, *Tragelaphus eurycerus*), two cetaceans (the beluga whale, *Delphinapterus leucas*, and the minke whale, *Balaenoptera acutorostrata*), and a sirenian (the Florida manatee, *Trichechus manatus latirostris*). The insula shows substantial variability in shape, extent, and gyral and sulcal patterns, as well as differences in laminar organization, cellular specialization, and structural association with the claustrum. Our observations reveal that the insular cortex is extremely variable among mammals. These differences could be related to the role exerted by specific and selective pressures on cortical structure during evolution. We conclude that it is not possible to identify a general model of organization for the mammalian insular cortex.

Keywords Insular cortex · Cytoarchitecture · Cetaceans · Artiodactyls · Carnivores · Sirenians

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Introduction

Experimental evidence from studies of the brain in humans and laboratory animals, including non-human primates, reveals patterns of neural connectivity and functional contributions to behavior that apparently can be generalized to all mammals. However, studies on a large comparative scale that include species adapted to different ecologic environments are essential to reveal fundamental differences in the organization of cortical regions that might have evolved in response to taxon-specific adaptive processes. Such comparative descriptions of morphology and cytoarchitecture are hindered by the enormous difficulty in obtaining brain specimens from a wide range of mammals. In this article, we focus on the insular cortex. Following a brief summary of its structure in humans and laboratory mammals, we present new comparative data from several species of marine and terrestrial mammals, including cetaceans, sirenians, artiodactyls, and carnivores, which together provide a significantly broader comparative view of the insula. Pictures of the species studied are shown in Fig. 1.

Sample photomicrographs showing the cytoarchitecture of the insular cortex in humans and in laboratory animals (macaque monkey, domestic cat, and C57BL6 mouse) are presented in Fig. 2. In most primates, the insula is a distinct lobe lying in the depth of the sylvian fissure, enclosed by the frontal, parietal, and temporal opercula. In lissencephalic species, such as most rodents, it is an indistinct region of cortex exposed on the lateral surface of the hemisphere, above the rhinal fissure, that is bounded anteromedially by the orbital cortex and dorsally and caudally by sensory cortices. The insular cortex of rodents consists of an agranular portion that includes anterior and posterior fields, and a granular portion that includes dorsal

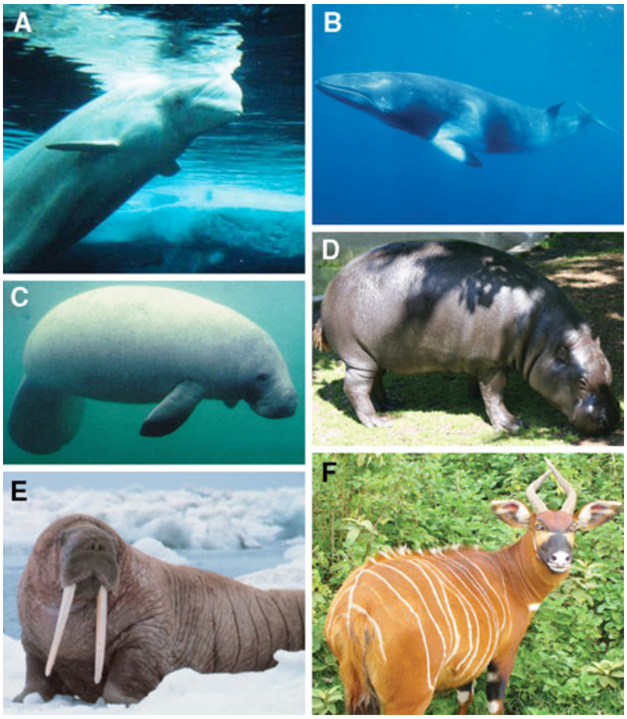

Fig. 1 Species available in the present study. Beluga whale (**a**), minke whale (**b**), Florida manatee (**c**), pigmy hippopotamus (**d**), Atlantic walrus (**e**), and Western bongo (**f**). Images **a**, and **c**–**f** are from http://www.Wikipedia.org; image **b** is taken from http://www.reefteach.com.au

and ventral fields (Rose 1928; Aleksandrov and Fedorova 2003). In macaque monkeys, it is divided into three major sectors: an agranular anterior sector (Iag), a dysgranular middle sector (Idg), and a granular posterior sector (Ig) (Rose 1928; Mesulam and Mufson 1982a, 1985; Johnson et al. 2009). In hominids (i.e., great apes including humans), an additional field is constituted by a cortical belt that lies in front of the anterior insula (AI) at its junction with the posterior part of the orbitofrontal cortex, the so-called frontoinsular cortex (FI; von Economo and Koskinas 1925; Allman et al. 2010). The insular cortex of the cat similarly has an anterior agranular area that includes the dorsal and ventral banks of the anterior rhinal sulcus (the dorsal agranular area, AId, and ventral agranular area, AIv, respectively), a dysgranular area (DI) between the caudal part of the presylvian sulcus and the anterior rhinal sulcus, extending to the rostroventral orbital gyrus, and then most posteriorly a granular area (GI) in the caudodorsal portion of the orbital gyrus (Clasca et al. 1997).

The primate insula is interconnected with several cortical regions, including the anterior cingulate cortex (ACC), the frontal pole and the dorsolateral prefrontal cortex (particularly area 46), the parietal and temporal lobes, as well as the entorhinal cortex. In addition, there are abundant intrainsular connections (Pandya et al. 1981; Mesulam and Mufson 1982b, 1985; Mufson and Mesulam 1982; Insausti et al. 1987; Augustine 1996) and extensive reciprocal interconnections with the amygdala (Turner et al. 1980; Aggleton et al. 1980; Mufson et al. 1981; Mufson and Mesulam 1982; Mesulam and Mufson 1982b, 1985; Augustine 1985; Friedman et al. 1986; Höistad and Barbas 2008), and the dorsal thalamus (Pritchard et al. 1986).

Functional studies in primates suggest that the insula participates in a variety of viscerosensory, visceromotor, somatosensory, and interoceptive functions (Penfield and Faulk 1955; Showers and Lauer 1961; Augustine 1985, 1996; Mesulam and Mufson 1985; Yaxley et al. 1990; Schneider et al. 1993; Burton et al. 1993; Platel et al. 1997; Pritchard et al. 1999; Craig et al. 2000; Craig 2002, 2010; Bamiou et al. 2003; Stephan et al. 2003; Critchley et al. 2004; Shelley and Trimble 2004; Critchley 2005; Mutschler et al. 2007, 2009; Naqvi and Bechara 2010; Paulus and Stein 2010). Studies in humans suggest that the insula is also involved in bodily awareness and self-recognition (Craig 2003; Karnath et al. 2005; Devue et al. 2007; Karnath and Baier 2010), emotional awareness (Seeley et al. 2007; Craig 2009a), time perception (Craig 2009b; Kosillo and Smith 2010), integration of interoceptive information with emotions (Taylor et al. 2009), subjective feelings such as empathy and uncertainty (Singer et al. 2009; Gu et al. 2010; Lamm and Singer 2010), as well as language and music perception (Platel et al. 1997; Bamiou et al. 2003; Mutschler et al. 2007; Ackermann and Riecker 2010).

As such, a common pattern of cytoarchitectural organization of the insular cortex among mammals, including the three major divisions of different granularity, is generally recognized. However, the morphology and cytoarchitecture of the insular cortex of mammals other than commonly used laboratory animals provide examples of exception to this general pattern of organization. Early anatomists provided descriptions of the cytoarchitecture of the insula of rarely studied species of rodents (red squirrel, *Sciurus vulgaris*; rabbit, *Lepus cuniculus;* Rose 1928), chiropterans (parti-colored bat or rearmouse, *Vespertilius murinus;* Rose 1928), insectivores (European hedgehog, *Erinaceus europaeus;* Rose 1928), primates (ring-tailed lemur, *Lemur catta*; Hamadryas baboon, *Papio cynocephalus hamadryas;* Rose 1928), and artiodactyls (sheep, *Ovis aries*; Rose 1942), as well as gross anatomical descriptions of the insula of cetaceans (bottlenose dolphin, *Tursiops truncatus*; common porpoise, *Phocoena phocoena*; Langworthy 1932; Ries and Langworthy 1937; see also Morgane et al. 1980). Modern cytoarchitectural studies of the cellular and laminar organization of the insular cortex include the dog (*Canis lupus familiaris*; Salazar et al. 1988), the bottlenose dolphin (Jacobs et al. 1984; Manger et al. 1998), the humpback whale

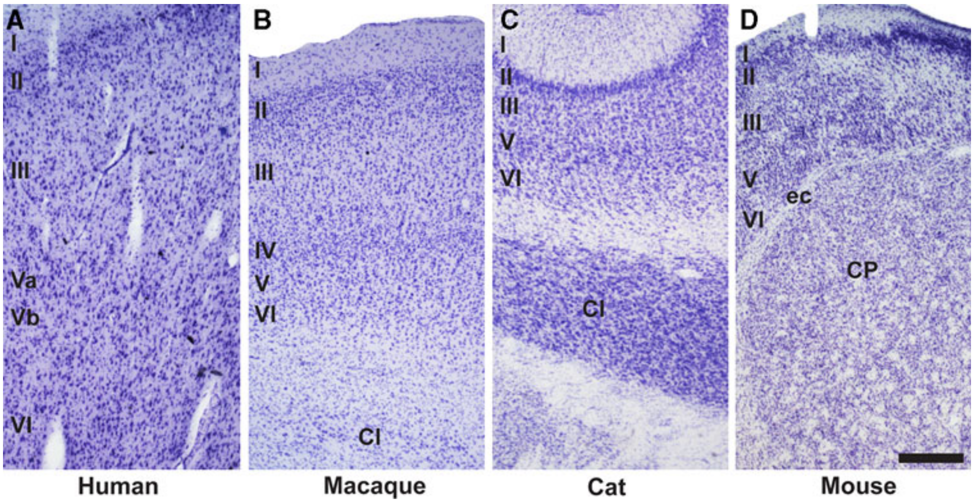

Fig. 2 Photomicrographs showing examples of cytoarchitecture of the insular cortex in human (**a**), macaque (**b**), cat (**c**), and mouse (**d**). **a** Frontoinsular cortex, FI; **b** posterior insular cortex, PI; **c**, **d** anterior insular cortex, AI. Cortical layers are indicated by Roman numerals. *Cl* claustrum, *CP* caudate-putamen, *ec* external capsule. *Scale bar* 300 µm

(*Megaptera novaeangliae*; Hof and Van der Gucht 2007), and the bull (*Bos taurus*; Russo et al. 2008). Altogether, these studies show a remarkable variability in shape, extent, complexity of the gyral and sulcal patterns, and degree of infolding of the insular cortex among mammals. Dogs, bats, hedgehogs, cetaceans, and artiodactyls seem to represent exceptions to the generally recognized pattern of cytoarchitectural organization that includes three major divisions of different granularity, their insular cortex being entirely (or almost) agranular. In dogs, the insular cortex is divided into a "dysgranular" and an "agranular" field (Salazar et al. 1988); in bats it is characterized by a unique agranular field (Rose 1928); in hedgehogs it is composed of two agranular (dorsal and ventral) fields (Rose 1928); and in the cetaceans it is completely agranular and presents a large number of radial gyri (up to 20 depending on the species; Morgane et al. 1980; Jacobs et al. 1984; Hof and Van der Gucht 2007) that are organized around the sylvian cleft in a nearly vertical axis. Moreover, an additional field that has a distinct cytoarchitecture lies at the junction between the anterior insula (AI) and the caudal portion of the frontopolar cortex, which may correspond to the human FI (Hof and Van der Gucht 2007). Finally, the insular cortex of artiodactyls is entirely agranular, with a number of subregions along the rostrocaudal extent of the sylvian gyrus in the bull (Russo et al. 2008). A dorsoanterior granular and a ventral agranular fields have been described in the sheep (Rose 1942).

The variability in morphology and cytoarchitectonic organization across these species, along with the large spectrum of functions that the insula subserves, highlights the need for comprehensive comparative studies across a broad range of mammals. Here we present original data on the morphology and cytoarchitecture of the insular cortex in several uncommon mammals, for comparison with the available data.

Materials and methods

Brain specimens and histological preparation

Six brains including two cetaceans (the beluga whale, *Delphinapterus leucas* and the minke whale, *Balaenoptera acutorostrata*), two artiodactyls (the pigmy hippopotamus, *Hexaprotodon liberiensis*, and the Western bongo, *Tragelaphus eurycerus*), a large carnivore (the Atlantic walrus, *Odobenus rosmarus rosmarus*) and a sirenian (the Florida manatee, *Trichechus manatus latirostris*) were included in the present study (Fig. 1). All brains were obtained from strandings, marine parks, and zoos where the animal died of natural causes and the brains were available for prompt postfixation in 4% paraformaldehyde. Whole hemispheres or blocks containing the insula were sampled from each brain, cryoprotected in graded sucrose solutions up to 30% in phosphate buffer saline (PBS), frozen in dry ice and cut into 80 µm-thick coronal sections using a sliding microtome (Leica Biosystems, Nussloch, Germany). The brain of the beluga whale was sampled into blocks of the regions of interest which were consequently dehydrated in graded alcohol solutions, embedded in celloidin, and cut into 35 µm-thick coronal sections. Fifty micrometers-thick coronal sections processed using a Vibratome (Leica Microsystems, Wetzlar, Germany) for previous investigations, and containing the insula from a human brain and from macaque, cat, and mouse brains, were used for comparative purposes. The human brain was fixed postmortem in 4% paraformaldehyde and the perfusions of macaque, cat, and mouse were performed using 4% paraformaldehyde with 0.125% glutaraldehyde. Every 10th, 20th or 50th section were mounted on glass slides, stained with a solution of 0.2% cresyl violet and coverslipped in 100% DPX in xylene or clarite for examination. Photomicrographs were acquired on a Zeiss Axiophot photomicroscope equipped with an

Optronics MicroFire digital camera (Optronics, Goleta, CA, USA) using a 2.5× Plan-Neofluar, 20× Plan-Apochromat or a 40× oil Plan-Neofluar objectives. Maps were made outlining the relevant sections of the brain using Stereo-Investigator (MBF Bioscience, Williston, VT, USA) and edited using Adobe Illustrator. Final adjustments to contrast and luminosity were made using Adobe Photoshop.

Localization of the insular cortex

Drawings of the location of the insular cortex in coronal sections at different levels in the species for which at least one hemisphere was available are shown in Fig. 3.

Boundaries of the insular cortex were based on descriptions of the neuroanatomy of the bottlenose dolphin brain (Jacobs et al. 1971, 1979, 1984; Morgane et al. 1980, 1982; Manger et al. 1998; Hof et al. 2005) as a reference for the beluga whale and on the description of the structure of the cortex of the humpback whale brain (Hof and Van der Gucht 2007) as a reference for the minke whale. Moreover, in cetaceans, the FI was identified as the cortical domain that forms an extension from the most rostral part of the insular cortex and merges with the posterior part of the frontopolar cortex (FP) as described by Hof and Van der Gucht (2007). The insular cortex of the Florida manatee was identified as part of the cluster cortex described by Reep et al. (1989). For the pigmy hippopotamus, Western bongo, and Atlantic walrus, for which literature is not available, the insular cortex was identified as the comparable topographical domain distributed on the medial wall of the pocket formed by the pseudosylvian cleft. The insular cortex of human, macaque, cat, and mouse was identified as described in von Economo (1927), Mesulam and Mufson (1982a), Clasca et al. (1997), and Hof et al. (2000), respectively.

Results

Cetaceans

The cytoarchitecture of the insular cortex of a mysticete, the minke whale, and of an odontocete, the beluga whale, is shown in Figs. 4 and 5, respectively. Like in other cetaceans (Jacobs et al. 1984; Hof and Van der Gucht 2007), the insula of these two species is lodged under an extensive frontoparietal operculum, consists of a large and variable number of gyri, and contains a relatively thin cortex. As typical for the cetacean neocortex, the insular cortex of the minke whale is agranular in its entire rostrocaudal extent. The only available part of the insular cortex of the beluga whale was the most anterior sector, that was, as well, agranular. The insula of the minke whale can be divided

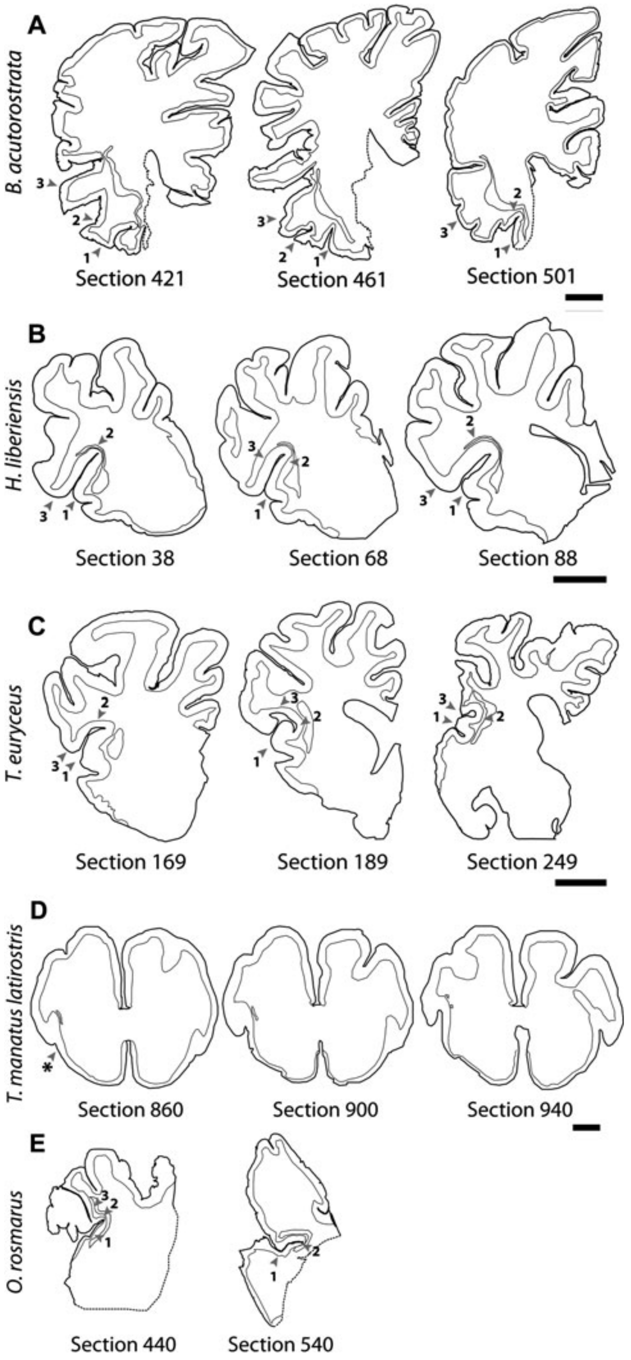

Fig. 3 Drawings of coronal sections, in a rostrocaudal order, throughout the insular cortex of the minke whale (**a**), pigmy hippopotamus (**b**), Western bongo (**c**), Florida manatee (**d**), and Atlantic walrus (**e**). The numbers in the map refer to the cytoarchitectonic location shown in Figs. 4, 6, 7, 8 and 9. The location numbers do not refer to any previous cortical classification but are purely indicative of the location sampled. The *asterisk* (**d**) indicates the approximate area of sampling of the photomicrographs in Fig. 9. The additional two sections of the brain of the Florida manatee were not available for sampling and are shown exclusively as a reference for the rostrocaudal extension of the insula in this species. These two sections were drawn using as a reference the Atlas of the brain of the manatee from the Comparative Mammalian Brain Collection of the National Museum of Health and Medicine (http://www.brainmuseum.org). *Scale bar* 1 cm

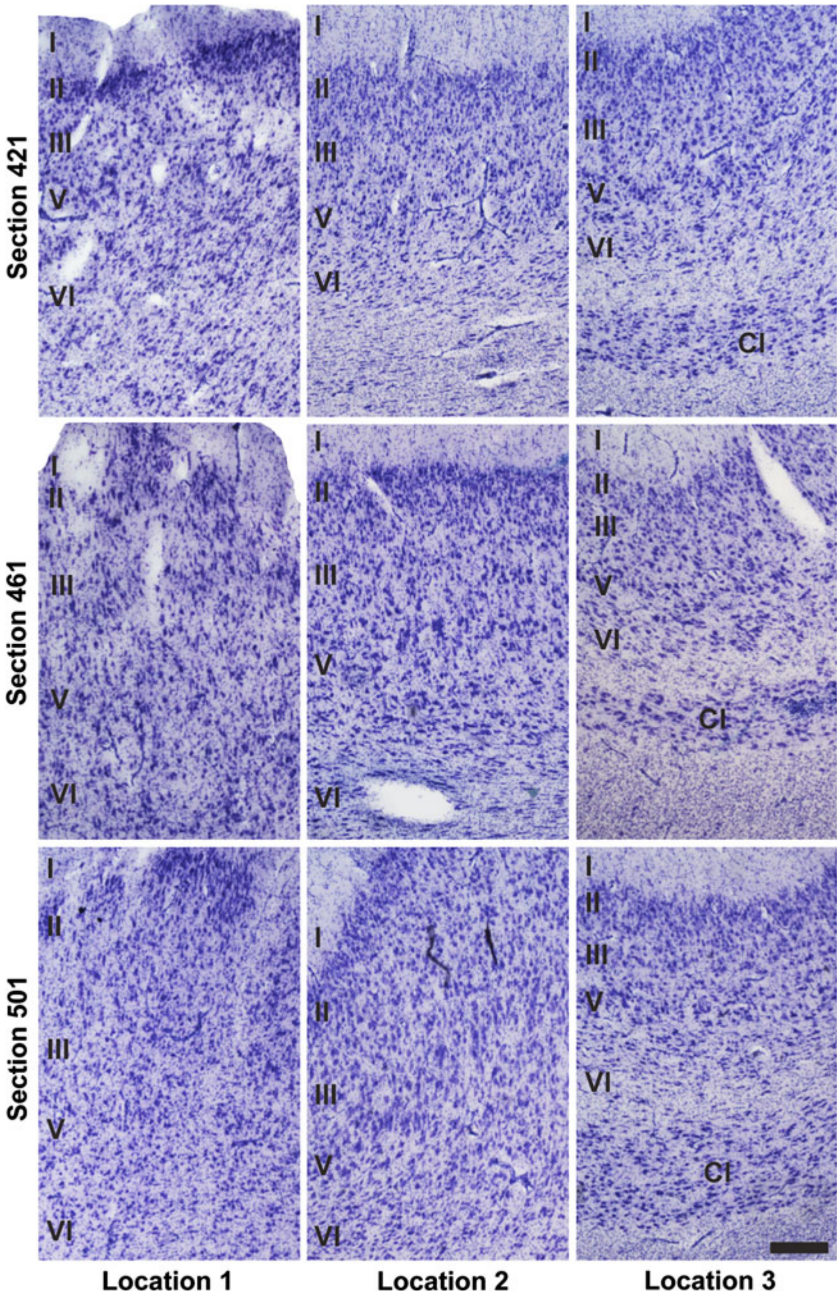

Fig. 4 Photomicrographs showing the cytoarchitecture of the insular cortex of the minke whale. The field number refers to the sampling areas shown in Fig. 3. Note the evident clustering of layer II and the columnar appearance of the upper layers in the most rostroventral extent of the insula (Location 1), the rostrocaudal thinning of the cortex (Locations 1 through 3) and the extensive claustrum that reaches the most posterior aspect of the insula (Location 3). Cortical layers are indicated by Roman numerals. *Cl* claustrum. *Scale bar* 300 µm

into anterior, middle, and posterior sectors by deep insular sulci. Although the cytoarchitectonic boundaries of these sectors are not very distinct, rostrocaudal transitions between adjacent sectors are evident as they show different lamination patterns such that the anterior sector presents a densely cellular layer II containing clusters of neurons, a thick layer III, a well-defined layer V with clusters of large pyramidal neurons and a well-developed multiform layer VI. The pattern of clusters in the upper and lower layers gives to the anterior sector a columnar appearance, as neurons of contiguous layers are organized in modules throughout the cortical plate. In the middle sector the neuron clusters in layer II and V become less visible. The cortex of the posterior sector is generally thinner than the most anterior parts of the insula, layer II less densely populated than in the anterior and middle sectors, and the transition between layer III and V is not well defined, giving the cortex a more homogeneous appearance.

The anterior insular cortex of the beluga whale is thin, and exhibits a thick layer I, a layer III characterized by a low neuronal density and consisting mostly of small pyramidal neurons, a thin layer V in which large pyramidal neurons from small groups, and a layer VI variable in thickness, and with a well-visible columnar organization.

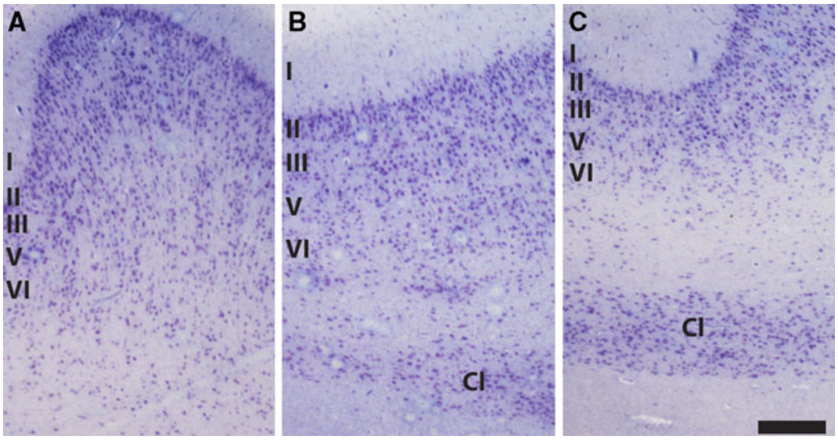

Fig. 5 Photomicrographs showing the cytoarchitecture of the anterior insular cortex of the beluga whale. Note the clusters in layer V (**a**–**c**), the low density of neurons in layer III (**a**–**c**), the extension of the claustrum (**b**, **c**), and the general columnar organization throughout the cortical plate (**a**–**c**). Cortical layers are indicated by Roman numerals. *Cl* claustrum. *Scale bar* 300 μm

Further species-specific differences exist. Notably, while layer III in the mysticete is characterized by a homogeneous neuron distribution, in the beluga whale the small pyramidal neurons are organized in columns that often abut the clusters of layer V. As such, given this peculiar organization, layer III seems, in the beluga whale, less densely populated than in the minke whale. Moreover, as for the clustering of layer II, the large pyramidal neurons in layer V of the minke whale formed groups spanning the whole extent of the layer, which, due to this organization, appeared thicker owing to the irregular vertical distribution of the clusters, whereas layer VI in the beluga whale is much thicker and less densely packed than in the mysticete.

The most remarkable feature of the organization of the insula in the two cetacean species is the pattern of clustering of neurons of layer II, especially in the most anterior portion of the insular cortex, as previously described in the humpback whale (Hof and Van der Gucht 2007), and in the bottlenose dolphin (Jacobs et al. 1984; Manger et al. 1998). However, a different degree of clustering was observed between the two species. While the clusters are very large and extremely separated from each other in the minke whale, a less pronounced pattern of clustering is observed in the beluga whale.

Both cetaceans have a highly developed claustrum, displaying a large number of claustral islands in the white matter underlying the insular cortex, throughout its rostrocaudal extension, that occasionally establish contact with layer VI. The size and distribution of these claustral islands is variable along the insula but very comparable in the two species. Similar islands have been described in the humpback whale and, interestingly, in the African elephant (Hakeem et al. 2009; Hof and Van der Gucht 2007).

Artiodactyls

The cytoarchitecture of the insular cortex of the semi-aquatic pigmy hippopotamus and terrestrial Western bongo is shown in Figs. 6 and 7, respectively. The insula of the pigmy hippopotamus and Western bongo presents only a partial infolding, similar to previous descriptions in another artiodactyl, the bull, by Russo et al. (2008). It consists, in both species, of two main gyri. The insula of the pigmy hippopotamus is agranular throughout its rostrocaudal extent. The anterior sector presents a very thick layer I and a layer II composed of large clusters of neurons that often bulge into layer I. A thick layer III abuts a thick layer V populated by very large pyramidal cells organized in small groups. Layer VI is rather thin and loosely packed. In the most rostral aspect of the anterior sector, layer V presents vertical modules and a patchy distribution of its large pyramidal neurons, giving it a very pronounced columnar appearance. Caudal to the pseudosylvian fissure, the posterior sector of the insula of the pigmy hippopotamus, as in cetaceans, becomes less laminated and the clusters of layer V less evident. Layer II is less densely packed than in the anterior sector and the transition between layer III and V is not well defined.

The insula of Western bongo is agranular in its rostrocaudal extent and, as in the pigmy hippopotamus, can be divided into an anterior and a posterior sector. In the most rostral part of the anterior sector the insular cortex shows a patchy layer II, yet with a lesser degree of clustering than in the pigmy hippopotamus, and well-developed layers III, V, and VI. The transition between layers III and V is not well defined throughout the whole rostrocaudal extent of the insula, and the major difference between the anterior and posterior sectors is that the latter displays a less densely packed layer II.

Finally, the claustrum shows some differences in these two species underlying the ventrodorsal extent of the insular cortex and assuming an elongated shape in the pigmy hippopotamus while being more restricted to the ventral cortex and with a less elongated shape in the Western bongo.

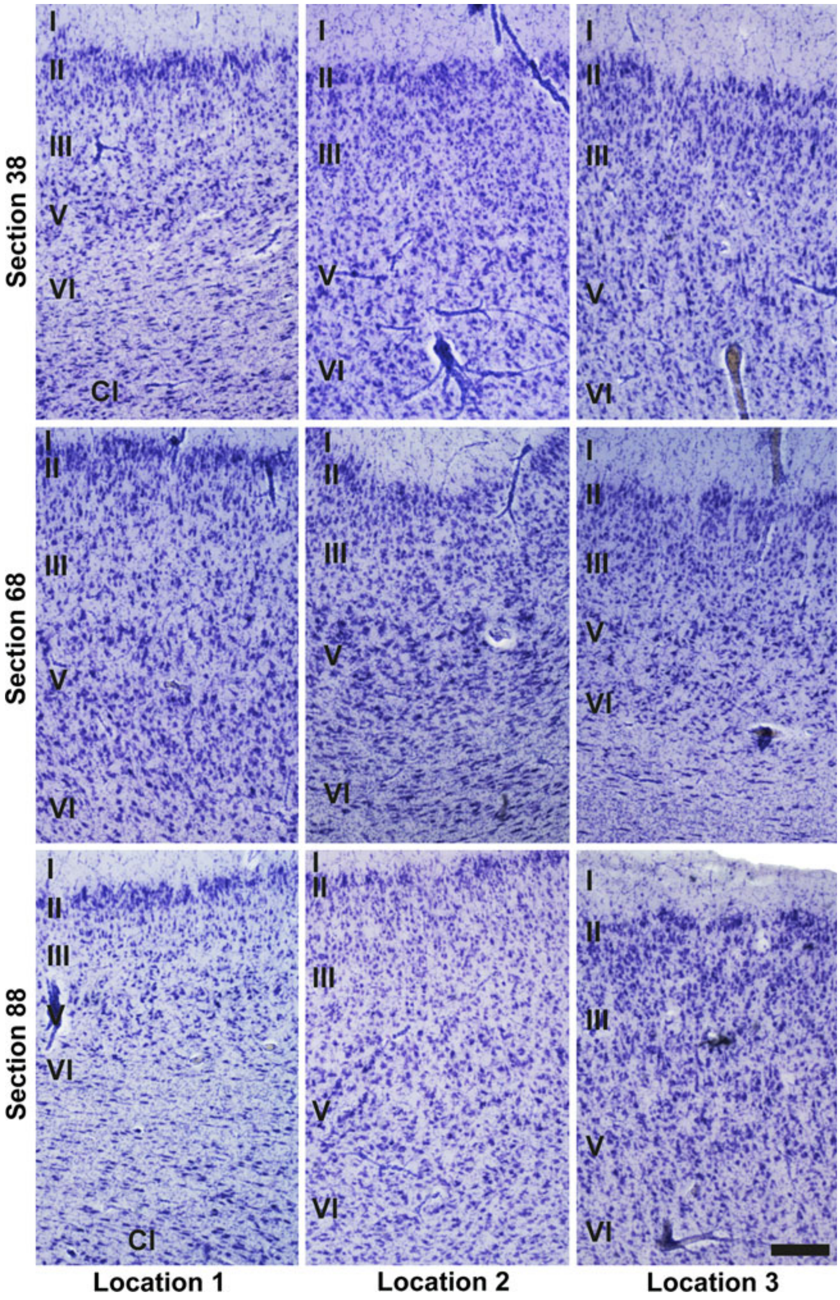

Fig. 6 Photomicrographs showing the cytoarchitecture of the insular cortex of the pigmy hippopotamus. The location number refers to the sampling areas shown in Fig. 3. Note the densely packed and clustered layer II, especially in the dorsal portion of the insula (Location 3), vertical modules of layer V (Location 2), and the rostrocaudal decrease of lamination (Locations 1 through 3). Cortical layers are indicated by Roman numerals. *Cl* claustrum. *Scale bar* 300 μm

Carnivores

The cytoarchitecture of the insular cortex of the Atlantic walrus is shown in Fig. 8. The insula of the Atlantic walrus presents complete infolding. The only portion available of the insula of this species was, unfortunately, the anterior sector. As previously observed in another carnivore, the cat (Clasca et al. 1997), and in contrast to the dog in which a "dysgranular" cortex is reported in the gyrus sylvius rostralis (Salazar et al. 1988), the anterior sector of the insula of the Atlantic walrus is agranular. It presents a relatively thin cortex, with a thick layer I and a thick, loosely packed layer II.

Although layer II is generally characterized by a rather homogeneous distribution of neurons, clusters of neurons in layer II occur in the most rostroventral portion of the walrus anterior insula. A thick layer III containing small pyramidal neurons abuts a thin layer V containing mainly isolated, very large pyramidal neurons extending their axons into the upper layers and that form aggregates of 10–15 neurons. Only a few perikarya were visible between adjacent groups and, as such, this layer is less dense than

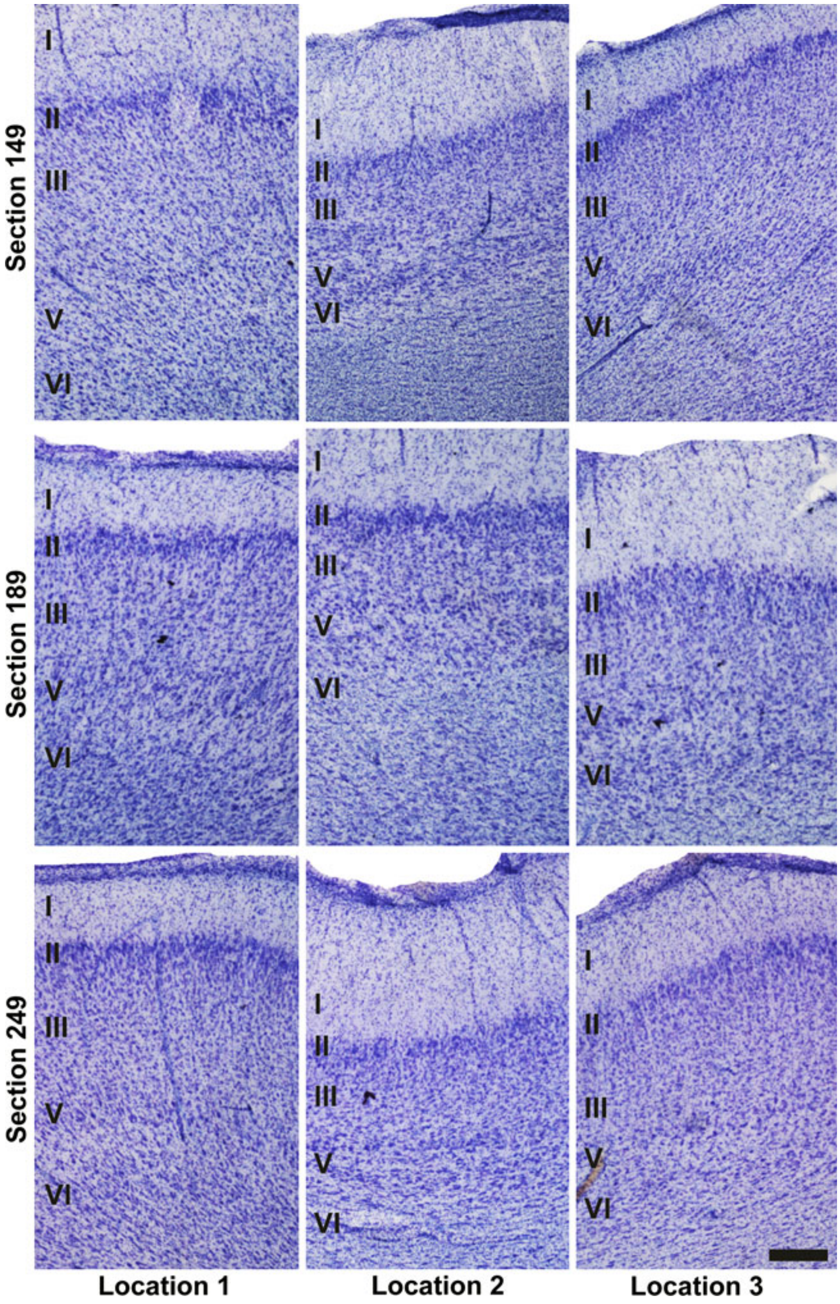

Fig. 7 Photomicrographs showing the cytoarchitecture of the insular cortex of the Western bongo. The location number refers to the sampling areas shown in Fig. 3. Note the weak clustering of cells in layer II (Location 1), the thickness of layer III (Locations 1 through 3) and the rostrocaudal decrease of lamination (Locations 1 through 3). Cortical layers are indicated by Roman numerals. *Scale bar* 300 µm

the upper cortical layers. Layer VI is thick and consists of small multipolar neurons. The claustrum of the Atlantic walrus is well developed and underlies the whole extent of the anterior insular cortex.

Sirenians

The cytoarchitecture of the insular cortex of the Florida manatee is shown in Fig. 9. As the only portion available of the insula of the Florida manatee was the most rostral region, we only investigated the anterior part of the insula in our specimen and refer to Marshall and Reep (1995) for the structure of the posterior insula in this species.

The brain of sirenians represents an important departure from the general *Bauplan* of the mammalian brain as it is characterized by a pronounced lissencephaly except for a deep and extremely wide lateral (pseudosylvian) fissure and two additional fissures originating at its dorsal end and extending caudally (Reep and O'Shea 1990). The width of the lateral fissure results in the lack of hidden cortex that, combined with the lissencephalic condition, makes the

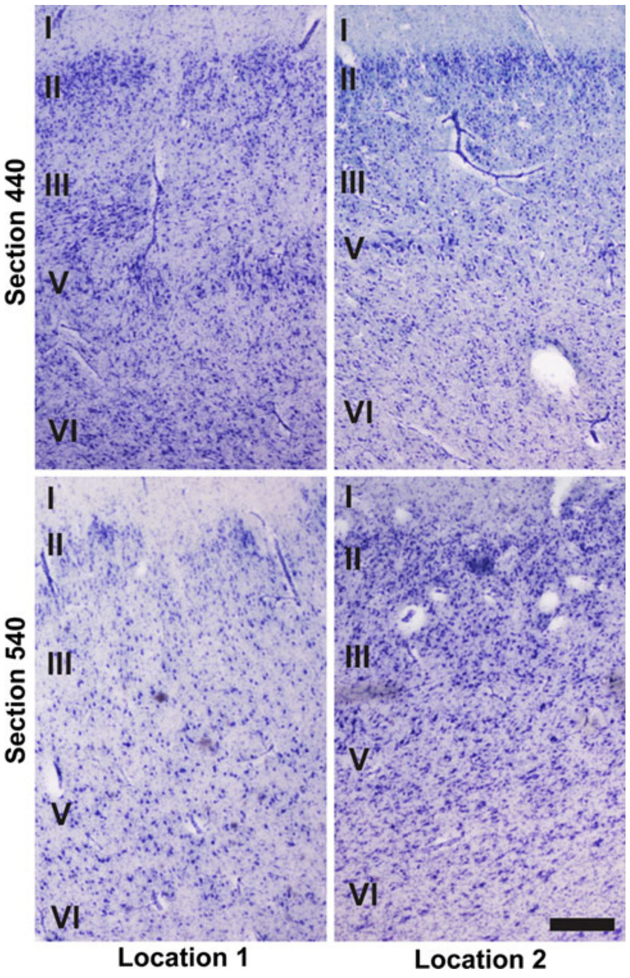

Fig. 8 Photomicrographs showing the cytoarchitecture of the insular cortex of the Atlantic walrus. The location number refers to the sampling areas shown in Fig. 3. Note the large clusters of layer II bulging into layer I (Location 1), the clustering pattern of layer V, and the loosely packed layer II in the most caudodorsal extent of the insula. Cortical layers are indicated by Roman numerals. *Scale bar* 300 μm

insula of the Florida manatee exposed and lacking gyral patterns. Moreover, the extent of the insular cortex appears quite limited within the pseudosylvian fissure.

The anterior sector of the insula is agranular with an obvious clustering of layer II in which, similar to cetaceans, large groups of neurons result in a patchy appearance. However, in this species, the clustering of layer II is more striking given the formation of extremely large columns of cells starting in layer II and extending throughout the whole thickness of the cortex, being most pronounced appearance in layer II and upper layer V. Between layer II and V the columns are obvious owing to the absence of neurons between adjacent columnar structures. In the deeper layers V and VI this columnar pattern is less pronounced as the columnar elements are linked to each other in a more homogeneous manner in both layers. Numerous aggregations of small neurons, previously described in the dugong (*Dugong dugon*) by Dexler (1913), and named “*Rindenkerne*”, are consistently observed in layer VI.

The posterior sector of the insula possibly corresponds to the most anterior part of cluster cortex CL1 described in the rostroventral extent of the caudal half of the brain of the Florida manatee by Marshall and Reep (1995). This cortex, accordingly to the authors, is also agranular, presents a thick layer II, large clusters of cells in the upper part of layer VI, and as such, is similar to what we observed in our sample of the anterior sector of the insula. It therefore appears that the insula of the Florida manatee is agranular in all of its rostrocaudal extent and that its anterior and posterior sectors share a comparable cytoarchitecture. Finally, unlike in other species, the claustrum of the Florida manatee is poorly developed and not consistently identified underlying the insular cortex.

Notes on the presence of von Economo neurons

The morphology of von Economo neurons (VENs) of the insular cortex of beluga whale, minke whale, pigmy hippopotamus, Atlantic walrus, Florida manatee, and of the common zebra (Butti, Sherwood and Hof, unpublished observations) is shown in Fig. 10. VENs are large spindle-shaped projection neurons located in layer V of the ACC

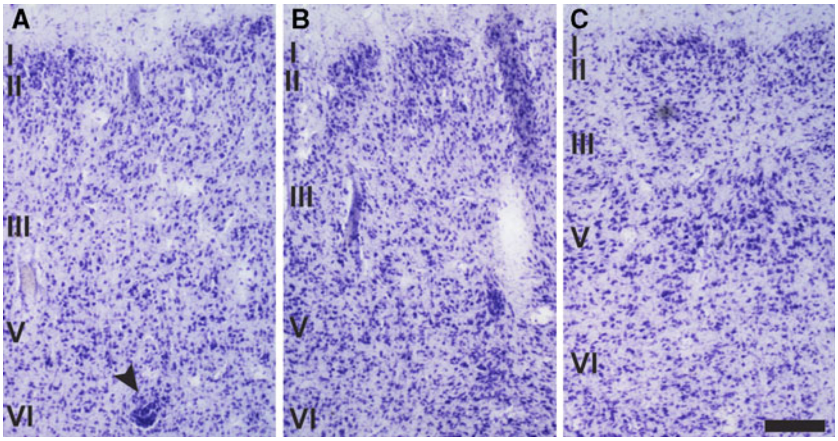

Fig. 9 Photomicrographs showing the cytoarchitecture of the anterior insular cortex of the Florida manatee. Note the pronounced clustering of layer II (**a**–**c**), the general columnarity pattern of layer V (**a**–**c**), the clustering of layer V (**c**), and the typical clusters of layer VI (**a**, **b**). The arrow points to typical aggregations of small neurons in layer VI. Cortical layers are indicated by Roman numerals. *Scale bar* 300 μm

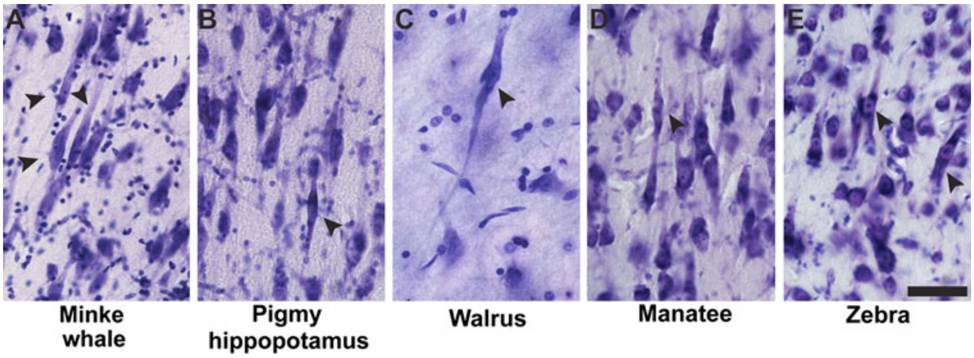

Fig. 10 Photomicrographs showing the morphology of VENs of layer V of the minke whale (**a**), the pigmy hippopotamus (**b**), the Atlantic walrus (**c**), the Florida manatee (**d**) and the common zebra (*E. burchelli*; **e**). Note the characteristic bipolar spindle shape of these large neurons (compare with neighboring pyramidal neurons) that correspond to the morphology originally described in hominids (Nimchinsky et al. 1999; see Allman et al. 2010). *Scale bar* 30 μm (**a**, **b**, **d**, **e**); 50 μm (**c**)

and FI that were first described in human and have subsequently been discovered in great apes (von Economo 1926; Nimchinsky et al. 1995, 1999; Allman et al. 2010). VENs are absent in monkeys and lesser apes, and besides hominids, they have been recently observed with a similar regional distribution in the humpback whale, fin whale (*Balaenoptera physalus*), sperm whale (*Physeter macrocephalus*), and killer whale (*Orcinus orca*) (Hof and Van der Gucht 2007), as well as in the bottlenose dolphin, Risso's dolphin (*Grampus griseus*), and beluga whale (Butti et al. 2009), and in homolog cortical regions of African and Asian elephants (*Loxodonta africana* and *Elephas maximus*, respectively; Hakeem et al. 2009).

Among the species available in the present study, we observed VENs in layer V of the insular cortex of the beluga whale, minke whale, pigmy hippopotamus, Atlantic walrus, and Florida manatee, but not in the insular cortex of the Western bongo. Moreover, it is worth noting that VENs are present in the insular cortex of a Perissodactyl, the common zebra (*Equus burchelli*, Butti, Sherwood and Hof, unpublished observations). When observed, VENs unambiguously showed all of the features of the typical morphology described in hominids, some cetaceans, and elephants (von Economo 1926; Nimchinsky et al. 1995,1999; Hakeem et al. 2009; Butti et al. 2009). Moreover, with the exception of the manatee, they were observed in clusters of 3–5 neurons mainly concentrated at the crown of the gyri as previously reported in cetaceans (Hof and Van der Gucht 2007; Butti et al. 2009), and few isolated VENs were also visible. It is worth mentioning that an unusual numbers and distribution of VENs were observed in the insula of the manatee and pigmy hippopotamus. In the manatee, unlike in the other species, VENS were extremely rare and scattered, and did not group in clusters. In contrast, in the pigmy hippopotamus, although still restricted to layer V and grouped in clusters, VENs were observed not only in the insula, but throughout the entire neocortex, similarly to the situation in the common zebra (Butti, Sherwood and Hof, unpublished observations). A quantitative assessment of the VENs and a detailed description of their cortical distribution in each species in which we observed them are beyond the scope of the present study and will be necessary to elucidate further the evolutionary significance of VENs in these species.

Discussion

Multiple selective pressures influenced, during evolution, the structure and connectivity of the mammalian brain, resulting in a large variety of brain sizes and complexity, cortical organization, and connectivity. The degree to which this variable organization is related to different functions or represents evolutionary alternatives supporting the similar functions is difficult to assess. The major factor limiting our understanding the many changes that the mammalian brain (and specific brain regions) underwent during evolution is the restricted number of species commonly available in research. Commonly used laboratory species, although considered representative of their taxa, are often used for general (and reductionist) models of the situation in mammals. Another issue in studying large and unconventional species is the impossibility to perform experiments that could provide evidence of brain connectivity. Thus, the functional significance of mammalian brain organization can be elucidated only through a comprehensive comparison among as many species as possible and inferences based on connectivity in laboratory animals.

In the present study we described the cytoarchitecture of the insular cortex of unconventional and rare mammals in light of what is available from laboratory animals (such as mouse, cat, and macaque) and humans. The species we focused on represent interesting points in the context of brain evolution and in the understanding of the selective pressures that shaped different organizational schemes. Cetaceans are fully adapted to aquatic life, they possess the largest brains in absolute size and show an extreme degree of gyral complexity (Morgane et al. 1980; Marino 1998, Marino 2008; Marino et al. 2004, 2007; Hof and Van der Gucht 2007; Oelschläger and Oelschläger 2008); the pigmy hippopotamus is a semiaquatic mammal and the closest relative of cetaceans (Gatesy 1997; Boisserie et al. 2005; Agnarsson and May-Collado 2008); the Western bongo is a terrestrial artiodactyl, which represents a good comparison for the pigmy hippopotamus and for the previously described insula cytoarchitecture in the bull (Russo et al. 2008) and sheep (Rose 1942); the Atlantic walrus is a large, mostly aquatic, carnivore mammal that has a relatively large brain (Marino 2008); and the Florida manatee is a large aquatic herbivore, closely related to elephants, that presents a unique condition of lissencephaly in a large brain (Reep and O'Shea 1990).

Our results shows that the general organization of the insular cortex into agranular, dysgranular, and granular fields observed in most laboratory animals (Rose 1928; Mesulam and Mufson 1982a; Augustine 1985; Chikama et al. 1997; Clasca et al. 1997; Aleksandrov and Fedorova 2003) cannot be generalized to all mammals. The insular cortex of the two artiodactyls we examined, pigmy hippopotamus and Western bongo, is completely agranular, similarly to what was reported in the bull by Russo et al. (2008). Agranularity in the complete rostrocaudal extent of the insular cortex was also observed in the sirenian Florida manatee, and in a mysticete, the minke whale, in agreement with previous descriptions in other cetaceans such as the bottlenose dolphin and the humpback whale (Jacobs et al. 1984; Manger et al. 1998; Hof and Van der Gucht 2007). Although we were able to study only the most rostral part of the insular cortex of the beluga whale and have no direct evidence of the cytoarchitectonic organization of its posterior sector in this species, previous studies in odontocete cetaceans reported the whole rostrocaudal extent of the insular cortex in these species as agranular (Jacobs et al. 1984; Manger et al. 1998; Hof et al. 2005). Moreover, given the largely documented lack of layer IV throughout the cetacean neocortex, it can be assumed that the posterior part of the insular cortex of the beluga whale is agranular as well. Interestingly, the cytoarchitecture of the insular cortex of the pigmy hippopotamus resembles in many ways that of cetaceans. Large clusters of pyramidal cells of layer V and a high degree of modularity in the upper cortical layers due to clustering of layer II are observed in the anteroventral part of the insular cortex. This is particularly true for the minke whale as the pattern of clustering in the smaller beluga whale is less evident. Moreover, layer I of the insular cortex of the pigmy hippopotamus is very thick, as in cetaceans.

The Florida manatee exhibits a unique cortical architecture characterized, in addition to clustering of layer II comparable to cetaceans and the hippopotamus, by an extreme modularity throughout the entire cortical plate, and by the consistent presence of well-defined, large neuronal clusters in layer VI, originally described (Dexler 1913), and considered typical of the organization of the sirenian neocortex. Finally, it is intriguing that the anterior insular cortex of the pigmy hippopotamus, Atlantic walrus, Florida manatee and the two cetacean species here studied contained VENs, similarly to humans, great apes, elephants, and several species of cetaceans previously described (Nimchinsky et al. 1995, 1999; Hof and Van der Gucht 2007; Fajardo et al. 2008; Hakeem et al. 2009; Butti et al. 2009; Allman et al. 2010). Moreover, it is interesting that VENs were observed also in the insular cortex of a perissodactyl, the common zebra (Butti, Sherwood and Hof, unpublished observations). A simplified phylogeny of placental mammals for which information on the presence of VENs and granularity of the insular cortex exist is shown in Fig. 11.

Although the functional significance of the differences in cytoarchitecture and neuronal specialization observed among these species cannot be firmly established, some speculations can be made. Considering the major role of the insula in the autonomic control of bodily functions and body homeostasis, reviewed in this issue of *Brain Structure and Function*, its cytoarchitectural variability among species suggests the existence of a comparable degree of adaptive differences in the physiology of these functions. Thus, variations in the structure of the insular cortex among mammals could have evolved in relation to homeostatic needs and ecological niches. In an effort to understand the significance of such variability in cytoarchitecture and neuronal specialization, we summarize in Table 1 the available data on diet, behaviors related to sociality, vocalization, and ecological niche of the species we studied, and we pair this information with details on the presence and pattern of distribution of VENs, and with the granularity/agranularity of the insular cortex. Although a clear pattern does not emerge, it is possible to draw some inferences from these data and previous literature. From a cytoarchitectural point of view it is worth noting that Russo et al. (2008), considering the evidence that links the agranular insular cortex to the modulation of gastroenteric motility in laboratory animals (Vjacheslav et al. 1996; Hoffman and Rasmussen 1953; Penfield and Faulk 1955; Showers and Lauer 1961), and assuming a preserved involvement of the insular cortex in the visceral gastroenteric control among mammals, proposed that the extensive (or complete) agranularity of the insular cortex reported in the sheep (Rose 1942) and bull (Russo et al. 2008), could be related to the need of controlling very complex

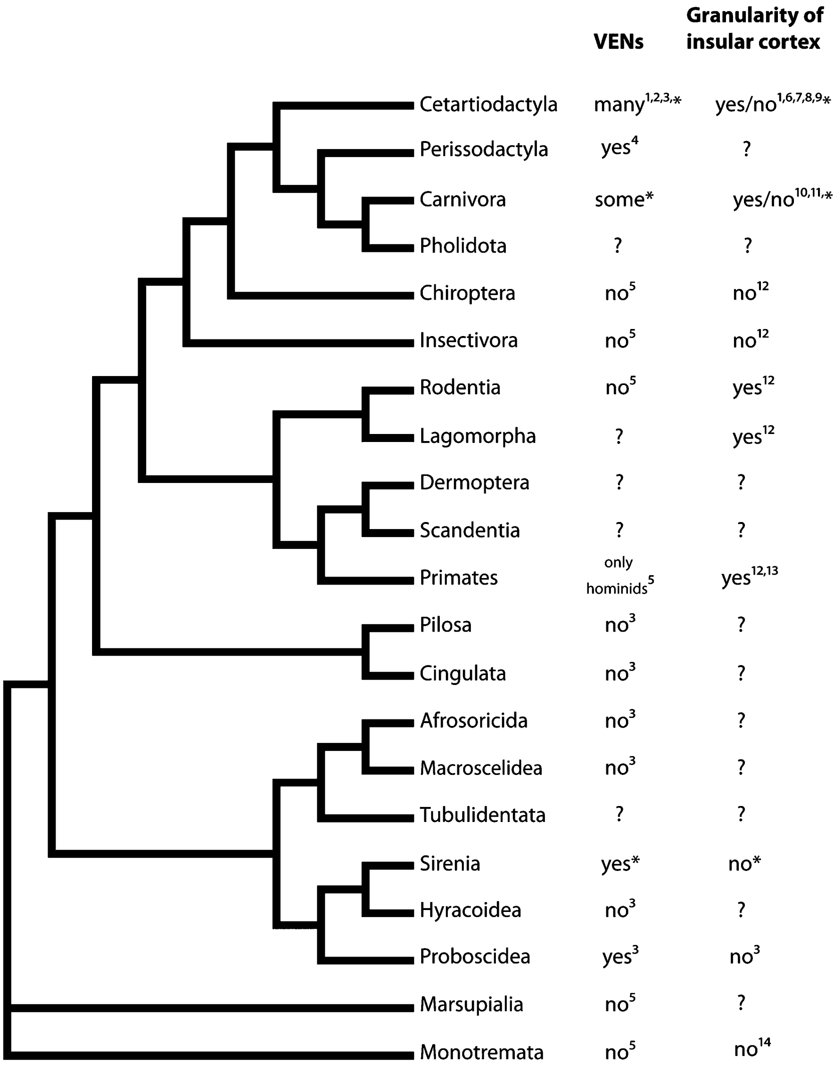

Fig. 11 Phylogeny of living placental mammals adapted from Murphy et al. (2001) showing phylogenetic distribution of the VENs and the granularity of the insular cortex. *1* Hof and Van der Gucht (2007), *2* Butti et al. (2009), *3* Hakeem et al. (2009), *4* Butti, Sherwood, and Hof (unpublished observations), *5* Nimchinsky et al. (1999), *6* Jacobs et al. (1984), *7* Manger et al. (1998), *8* Russo et al. (2008), *9* Rose (1942), *10* Salazar et al. (1988), *11* Clasca et al. (1997), *12* Rose (1928), *13* Mesulam and Mufson (1982a, 1985), *14* Hassiotis et al. (2004), *asterisk* refers to specimens analyzed in the present study. Note that "yes/no" in the Cetartiodactyla group refers to the fact that within this clade, cetaceans have an agranular insular cortex whereas some artiodactyls present both granular and agranular insular cortices

gastroenteric activities in polygastric species. This is interesting in light of the fact that, despite the differences in their diet (see Table 1 for details) cetaceans and hippopotamuses are polygastric (Clemens and Malioy 1982; Reynolds and Rommel 2008) and, incidentally, possess completely agranular insular cortices. However, based on this theory, we would expect monogastric species, regardless of their phylogenetic position, to present at least some extent of granular cortex within the insula. This is in contrast with the complete agranularity that we observed in the insular cortex of the Florida manatee, an herbivorous monogastric species (Reynolds and Rommel 1996, 2008). It would be interesting, in this context, to compare the insula of the manatee to that of large terrestrial monogastric herbivores (hindgut fermenters) such as elephants, horses, and rhinoceroses (Hungate et al. 1959; Getty 1975; Stevens and Hume 1995). Moreover, it has to be considered that some taxa, such as cetaceans and hippopotamuses, simply evolved completely agranular cortices, as alternative strategies to generate similar outcomes to those generated by granular cortices in other species. As a consequence, the variability in cytoarchitecture and granularity of cortical regions (including the insular cortex) are more likely related to different strategies of cortical typology and wiring adopted to process information, as it has been suggested to be the case in cetaceans (Voronov et al. 1985; Revishchin and Garey 1990). It is suspected in fact, that the unusual organization of the cortex in cetaceans, with the lack of layer IV and the presence of a tick layer I, reflects an unusual wiring pattern with a shift of the terminations of thalamic afferent from layer IV to layers I and II (Voronov et al. 1985; Revishchin and Garey 1990). However, what are the main driving forces of these evolutionary adaptations is still unknown.

The presence in the insula of the pigmy hippopotamus and Florida manatee, similarly to cetaceans (Manger et al. 1998; Hof and Van der Gucht, 2007), of a high modularity of layer II, which is not observed to a comparable extent in

Table 1 Details on the diet, socially relevant behaviors, ecological niche, presence of VENs and their clustering, and granularity of the insular cortex in the species studied

Species	Diet	Behaviors related to sociality	Vocalization	Ecological niche	VENs in the insula	VENs layering distribution	VENs clusters	Granularity of the insular cortex
Beluga whale	Fish, cephalopods, crustaceans[a]	Large pods (up to 1,000); stable associations; cooperative behaviors	Extremely diverse vocal repertoires[a]	Coastal and offshore Arctic waters[a]	Yes	Layer V	Yes	No (only AI available)
Minke whale	Krill (euphausiids) and fish[b]	Solitary or small groups up to three individuals[b]	Complex and stereotyped sound sequences possibly related to mating[b,c]	Coastal and offshore waters throughout the world[b]	Yes	Layer V	Yes	No
Pigmy hippopotamus	Herbivorous[d,e]	Solitary[d,e]	Elaborate vocal repertoire used in social context[m,f,g]	Swamps and heavily forested regions near water[e]	Yes (but also in other cortical regions)	Layer V	Yes	No
Western bongo	Herbivorous[h]	Large groups[h]	Limited number of vocalisations[h]	Females in herds/matu-re males are solitary[h]	No	–	–	No
Atlantic walrus	Benthic invertebrates, mostly bivalve mollusks, but also shrimps, crabs and occasionally hunting/scavenging seals, cetaceans and sea birds[i,j,k]	Highly gregarious; one or two males dominate herds of 20–50 females[i]	Acoustic signals used in social contexts (e.g., reproductive period)[i]	Shallow arctic waters[i]	Yes	Layer V	No	No (only AI available)
Florida manatee	Herbivorous but occasionally reported to consume fish[l]	Form temporary groups depending upon water temperature[l]	Acoustic signals, tactile, visual and chemical cues are used in social context[l]	Subtropical and tropical coastal and riverine waters[l]	Yes	Layer V	No	No

AI anterior insular cortex

[a] O'Corry-Crowe (2008)

[b] Perrin and Brownell (2008)

[c] Gedamke et al. (2001)

[d] Eltringham (1999)

[e] Eltringham (1993)

[f] Barklow (2004)

[g] Barklow (1997)

[h] Kingdon (1982)

[i] Kastelein (2008)

[j] Sheffield et al. (2001)

[k] Gray (1927)

[l] Reynolds et al. (2008)

[m] Literature reporting data on the larger river hippopotamus, *H. amphibius*

other species in the present study, is worth noting. Hof and Van der Gucht (2007) suggested that the development of small and organized modules in the neocortex of cetaceans could be related to a cost-effective strategy to obtain economic and efficient wiring in large brains. The presence of similar modules in the anterior insular cortex of pigmy hippopotamus and Florida manatee, is interesting given the different phylogenetic relationships that these two species have with cetaceans (Fig. 11) and their common aquatic lifestyle. The presence of such modules in the pigmy hippopotamus, their absence in the Western bongo and their poor development in the Atlantic walrus, supports the speculation that such strategy could be related to the adaptation to the aquatic environment in cetartiodactyls, but not in mostly aquatic carnivores. A description of the insula of seals, otters, and polar bears would be required to explore this possibility further. Similarly, a comparative study of the insula in manatees and their closest relatives, the terrestrial elephants (de Jong et al. 1981), would have a particular significance in this context.

When considering the presence or absence of VENs in the species we studied (see Table 1 for details) it is notable that although their function it is not yet fully understood, VENs do not seem to be a common feature among artiodactyls as they are present in the semiaquatic hippopotamus, a close relative of cetaceans, but not in the Western bongo. Moreover, the presence of VENs throughout the neocortex of the pigmy hippopotamus, suggests an evolutionary difference with the regionally specific distribution observed in cetaceans (and in fact in primates as well), which may have important implications in regard of the possible connectivity and function of these neurons in different species. The significance of these neurons has been tentatively related to certain behavioral and social specialization in the few species that have them, by comparison to the function of the cortical regions in which they occur inferred from studies of human subjects (Nimchinsky et al. 1999; Hof and Van der Gucht 2007; Seeley et al. 2007; Seeley 2010; Craig 2010; Allman et al. 2010). Interestingly, Hof and Van der Gucht (2007) suggested that VENs could be involved in the control of non-conscious vocal behaviors, given their presence in cortical regions from which vocalization can be elicited in primates (Jürgens 1982), and remarkably, all the species in which VENs were documented in this study present complex and elaborate vocalization repertoires (see Fig. 11; Table 1 for details). However, it has to be mentioned that the only behavioral data available for the hippopotamuses, and reported in Table 1, are based on studies of the large river hippopotamus (*Hippopotamus amphibius*), much more gregarious and aquatic than the pigmy hippopotamus (Eltringham 1993). Therefore, although the river hippopotamus possesses an elaborate vocal repertoire producing sounds that transmit both in air and water simultaneously (Barklow 1997, 2004) and that include clicks and pulses that have been compared to those produced by cetaceans (Barklow 1997, 2004), it is not known whether comparable vocalizations are present in the more solitary pigmy hippopotamus. These issues will require a comprehensive assessment of VENs occurrence in mammalian lineages and a much more extensive knowledge of the eco/ethology of these species.

In summary, based on the cytoarchitectural features observed in the species available to this study, the concept, originating from analyses of a few murid rodents, one felid carnivore, and anthropoid primates, that three main subdivisions (agranular, dysgranular and granular) of the insular cortex represent a conserved evolutionary feature among mammals (Clasca et al. 1997), is questioned by evidence of clear departures from this general concept. Whereas the features of a specific cortical region may have been retained from the ancestral type in some species, they likely have been subject to adaptive pressures in others, leading to considerable structural modifications from the primitive plan. It may also be that the pattern seen in rodents, terrestrial carnivores, and many primates represents a derived state compared to other species such as cetaceans, semiaquatic mammals, and large terrestrial herbivores. These data, together with previous observations on cortical cytoarchitecture in cetaceans (Manger et al. 1998; Hof et al. 2005; Hof and Van der Gucht 2007; Butti et al. 2009), certainly point to the fact that diverging patterns of cellular distribution and possibly of cortical wiring organization exist even within a given order, which reflects in part the extreme diversity of anatomical and behavioral adaptation among mammals. The present study was limited, given the rarity of the species investigated, by the number of brain specimens available for each species. Although we were not able to assess possible intraspecific variability in the pattern of organization of the insular cortex, our results can be considered representative of the species described. Our observations reveal however that generalizations about brain architecture based on a few common taxa are not tenable. While we presented evidence from a limited number of species, additional studies exploring broadly the diversity of brain organization, and including quantitative studies of cortical subdivision, are crucially needed to understand fully the evolutionary complexity of the cerebral cortex.

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