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The Morphology of Supragranular Pyramidal Neurons in the Human Insular Cortex: A Quantitative Golgi Study

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Although the primate insular cortex has been studied extensively, a comprehensive investigation of its neuronal morphology has yet to be completed. To that end, neurons from 20 human subjects (10 males and 10 females; $N = 600$) were selected from the secondary gyrus brevis, precentral gyrus, and postcentral gyrus of the left insula. The secondary gyrus brevis was generally more complex in terms of dendritic/spine extent than either the precentral or postcentral insular gyri, which is consistent with the posterior–anterior gradient of dendritic complexity observed in other cortical regions. The male insula had longer, spinier dendrites than the female insula, potentially reflecting sex differences in interoception. In comparing the current insular data with regional dendritic data quantified from other Brodmann's areas (BAs), insular total dendritic length (TDL) was less than the TDL of high integration cortices (BA6β, 10, 11, 39), but greater than the TDL of low integration cortices (BA3-1-2, 4, 22, 44). Insular dendritic spine number was significantly greater than both low and high integration regions. Overall, the insula had spinier, but shorter neurons than did high integration cortices, and thus may represent a specialized type of heteromodal cortex, one that integrates crude multisensory information crucial to interoceptive processes.

Keywords: basilar dendrite, dendritic spine, interoception, microanatomy, morphometry

Introduction

Although the architectonics and connections of the nonhuman primate insular cortex have been extensively documented (Mesulam and Mufson 1982a, 1982b; Mufson and Mesulam 1982), much less is known about the dendritic morphometry of the region's pyramidal neurons. A quantitative morphological understanding of the cortex is the basis for accurate delineation of structure–function relationships (Ramón y Cajal 1899). Historically, a variety of functions have been attributed to the human insula (for review, see Augustine 1985, 1996; Shelley and Trimble 2004), including audition (Wong et al. 2004), gustation (Yaxley et al. 1990), language (Nestor et al. 2003; Habib et al. 1995), motor speech (Dronkers 1996), somatosensation (Robinson and Burton 1980), and visceral control (King et al. 1999). The current, more integrative opinion urges that the insula may be crucial to multimodal interoceptive processing (Craig 2002; Critchley, Weins, et al. 2004; Paulus and Stein 2006), emotionally driven visceral reactions (Critchley, Rotshtein, et al. 2004), and empathy (Gallese et al. 2004). The neural substrate supporting these functions constitutes the focus of the present study. Along these lines, recent morphological accounts (Scheibel et al. 1990; Jacobs et al. 1997, 2001; Elston and Rosa 1998b) have uncovered differences in dendritic/spine extent across the cortex, revealing that functionally more integrative regions contain pyramidal cells with more extensive basilar dendritic arbors than less integrative regions. These quantitative investigations supplement classic architectonic, comparative, and qualitative morphological undertakings (Clark 1896; Brodmann 1909; Ramón y Cajal 1909, 1911; Rose 1928) and provide a broader morphological basis for understanding cortical functioning. In the present study, we explored 3 distinct human insular gyri in an attempt to uncover quantitative variations in the morphology of the basilar dendrites of supragranular pyramidal neurons.

The human insula, or Island of Reil (Reil 1809), is buried beneath the frontoparietal and temporal opercula (Türe et al. 1999; Naidich et al. 2004) and typically contains between 4 and 6 gyri that fan outwards from the insular apex (Clark 1896). The central insular sulcus divides the insula into a larger anterior region and a smaller posterior region. The present study, focusing on the left human insula (Fig. 1), investigated 2 anterior insular gyri (the secondary gyrus brevis and the precentral gyrus) and 1 posterior insular gyrus (the postcentral gyrus). In terms of cytoarchitectonics, the insular cortex changes from an anteroventral agranular zone (roughly, Brodmann's area, BA, 14–16) to a posterodorsal granular region (roughly, BA13), with the middlemost region representing transitional dysgranular cortex (Rose 1928; Mesulam and Mufson 1982a; Fig. 1*B*). This transition from primitive allocortex to full-fledged isocortex is not limited to the insula. Cortices surrounding the insula (e.g., the lateral and medial orbitofrontal cortex and the temporal pole) display a similar agranular–granular architectonic flow, which led Mesulam and Mufson (1982b, 1985) to propose the existence of a functionally intertwined, "paralimbic" insulo-orbito-temporopolar region, or at least an "orbital prefrontal network" that includes the insula (Amaral and Price 1984; Öngür and Price 2000).

Tract tracing studies of nonhuman primates have revealed intricate reciprocal insular connections with sensory cortices, limbic structures, and autonomic brainstem nuclei (Kapp et al. 1985; for review, see Mesulam and Mufson 1985). Most insular connections to other cortical regions are architectonically organized (i.e., insular efferents and afferents will begin and terminate in the same type of architectonic area; Mesulam and Mufson 1982b). The agranular and anterior dysgranular insular region is extensively connected with almost all subnuclei of the amygdala (Mufson et al. 1981; Höistad and Barbas 2007), agranular/dysgransular orbitofrontal cortices (Öngür and Price 2000), the anterior cingulate gyrus (Mesulam and Mufson 1982b; Vogt and Pandya 1987), the caudal ventral striatum (Fudge et al. 2005), the nucleus of the solitary tract and the vagal dorsal motor nucleus (Kapp et al. 1985), the piriform

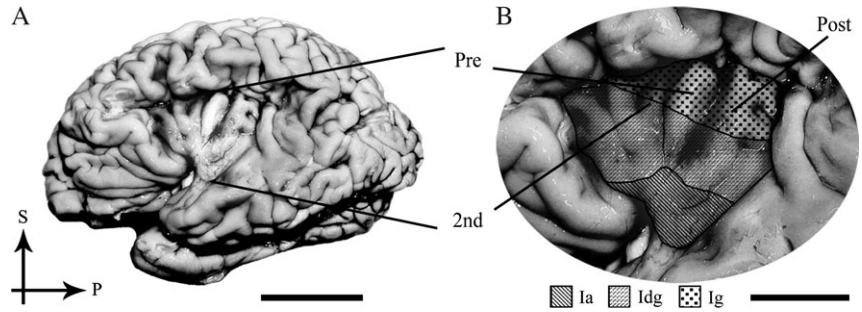

Figure 1. (*A*) The left hemisphere of a human brain with the opercula of the lateral fissure retracted to reveal the insula. Scale bar = 5 cm. (*B*) The insula is enlarged to display anatomical and cytoarchitectonic regions as labeled: 2nd = secondary gyrus brevis; Pre = precentral insular gyrus; Post = postcentral insular gyrus; Ia = Agranular; Idg = Dysgranular; Ig = Granular. Cytoarchitectural boundaries are approximated from Mesulam and Mufson (1982a, 1982b). Scale bar = 2.5 cm.

cortex (PC), temporopolar regions, and the prorhinal–entorhinal cortex (Mufson and Mesulam 1982; Insausti et al. 1987). The granular and posterior dysgranular insular region is connected with the granular and dysgranular orbitofrontal cortices, premotor cortex, first and second somatosensory cortices, auditory cortices, the superior temporal sulcus, mid- and posterior cingulate cortices, and the lateral and central amygdaloid nuclei (Mufson et al. 1981; Mesulam and Mufson 1982b; Mufson and Mesulam 1982). In general, olfactory and gustatory connections are concentrated within the anterior insula, whereas somesthetic and auditory connections remain in the posterior insula (Mesulam and Mufson 1982b). These diverse connections imply that the insula is a heteromodal association area (Mesulam and Mufson 1985) that exhibits extreme architectural and connectional heterogeneity (Clascá et al. 1997, 2000), with recent studies suggesting that it may be crucial for modulating behavior and learning based on internal states (Nerad et al. 1996; Reynolds and Zahm 2005).

Insular functions are multifaceted (Shelley and Trimble 2004). The anterior insula appears particularly involved in emotion related processes, including anxiety (Reiman 1997; Stein et al. 2007), social cognition (Gallese et al. 2004), and facial expression recognition (Krolak-Salmon et al. 2003; Wicker et al. 2003). As such, the anterior insula serves as a cortical locus for homeostasis (Craig 2002, 2003; Hua et al. 2005), whereby one's sense of self arises from a combination of general sensory signals, including the highly emotional sense of smell (Mufson and Mesulam 1982), and interoceptive signals (Critchley, Weins, et al. 2004; Brooks et al. 2005; Craig 2005). This view is consistent with the region's connections to the autonomic regulatory nuclei of the dorsal medulla (Kapp et al. 1985) and hypothalamic autonomic nuclei (Barbas et al. 2003). Insular regions near the postcentral insular gyrus have been implicated in language (Bennet and Netsell 1999; but see also Hillis et al. 2004; Shuster and Lemieux 2005), specifically with regards to motor aspects of speech articulation (Dronkers 1996). A parsimonious account here is that the insula creates a sense of self by relating the internal milieu with the external world (Mesulam and Mufson 1985; Karnath et al. 2005; Esslen et al. 2008). The insula is especially tuned to sensory feedback, including language, that relates to self rather than nonself (McGuire et al. 1996; McCabe et al. 2008). Thus, the insular activation that occurs just prior to speech (Kuriki et al. 1999) may be related to the motivational and emotional aspects of language. Finally, the posterior insula appears to be a site of convergence of auditory and somatosensory information, and may also be involved in motor control and some aspects of vision (Mesulam and Mufson 1982b; Ostrowsky et al. 2002; Rodgers et al. 2008).

The present study examined dendritic systems from both the anterior (specifically, the secondary gyrus brevis and the precentral insular gyrus) and the posterior insula (specifically, the postcentral insular gyrus; Fig. 1*B*). Supragranular pyramidal cells were chosen because they are the numerically dominant cortical neuron (Peters and Kara 1985), are crucial integrators of corticocortical activity (Meyer 1987) and, in terms of methodology, are more clearly stained in Golgi preparations of adult tissue than are infragranular cells. Basilar dendrites, which develop well into adulthood (Nakamura et al. 1985), were examined because they appear to be a primary target of intracortically derived connections (Globus and Scheibel 1967; Douglas et al. 1995; Feldmeyer et al. 2002), and thus may reflect the amount of information convergence on a neuron (Elston and Rosa 1997; Jacobs et al. 1997). Given the variation in architectonics and connectivity between anterior and posterior insular regions, we hypothesized intrainsular dendritic differences. First, we predicted greater dendritic/spine complexity in the anterior insula because 1) the majority of intrainsular connections begin in the anterior insula and terminate in the more posterior insular regions (Mesulam and Mufson 1982b) and 2) more rostral cortical regions have typically exhibited more intricate dendritic/spine systems (Elston and Rosa 1997, 1998a, 1998b; Elston et al. 2005). Because previous morphological studies of the human cerebral cortex have not indicated any sexual dimorphism (Jacobs et al. 1993), there was no a priori reason to anticipate sex differences in the insula. Finally, we examined insular dendritic/spine complexity vis-à-vis other cortical areas (BA6β, 10, 11, 39, 3-1-2, 4, 22, and 44; Jacobs et al. 2001). Briefly, Jacobs et al. (2001) found that primary and unimodal (low integration) cortices were significantly less complex than heteromodal and supramodal (high integration) cortices. Given the insula's heteromodal interconnectivity, we expected its dendritic/spine complexity to fall between that of low and high integration cortices.

Materials and Methods

Subjects

Tissue was obtained from 20 neurologically normal subjects (M_{age} = 39 ± 10 years; 10 males: M_{age} = 44 ± 6 years; 10 females: M_{age} = 35 ± 10 years; Table 1). Autolysis time (AT) averaged 19 ± 8 h. Tissue was provided by Dr R. Bux of the El Paso County coroner's office. Relevant historical

Table 1
Subject summary

Subject[a]	Body weight (in kg)	AT (in h)	Cause of death	Occupation
M34	92	7.5	Smoke inhalation	Military
M39.1	93	17	Cardiac arrest	Labor finder
M39.2	79	19	Cardiac arrest	Construction worker
M39.3	75	26	Alcohol intoxication	—
M43.1	61	17	Hypertension; enlarged heart	Military
M43.2	57	21	Cardiac arrest	Homeless
M46[b]	87	19	Hypertension; cardiac arrest	Cement truck driver
M51[b]	68	21	Drowning (suicide)	State employee
M52	75	9	Choking	—
M53	—	22	Atherosclerotic cardiovascular disease	Car salesman
F20[b]	59	24	Hanging (suicide)	—
F23[c]	80	30	Renal failure	—
F25[b]	54	8.5	Motor vehicle accident	—
F28[b]	75	31	Accident trauma	—
F34	84	30	Motor vehicle accident	—
F37	102	6	Myocarditis	—
F41	109	10.5	Motor vehicle accident	—
F43	81	18	Gunshot wound to chest	Dental assistant
F47	97	17	Postoperative arrhythmia	—
F48	52	19	Drug overdose (lorazepam; tizanidine)	—

[a]Subjects are designated by sex (M = male; F = female) and by age. For example, M34 refers to a 34-year-old male. Subjects of the same age are differentiated by arbitrary 0.1 or 0.2 designations.
[b, c]Subjects are Caucasian unless denoted as [b]Hispanic or [c]African-American.

information (e.g., age, sex, cause of death) for each subject was obtained from autopsy reports and medical records. Tissue was excluded if there were any signs of trauma, cerebral edema, or chronic illness with central nervous system involvement. The research protocol was approved by The Colorado College Human Subjects Review Board (#H94-004).

Tissue Selection and Processing

All brains were immersion fixed in 10% neutral buffered formalin ($M_{fixation}$ = 34 ± 28 days) prior to staining. Tissue blocks (1–2 cm long) were taken from anterior and posterior insular regions (Fig. 1). The 3 sampled gyri were consistently identified according to their position around the middle cerebral artery, which lies lengthwise on top of the central insular sulcus (Türe et al. 1999). Two anterior short insular gyri and 1 posterior long insular gyrus were represented, as described below:

Secondary Gyrus Brevis

The secondary gyrus brevis (*Gyrus brevis secundus*; Rose 1928), also known as the middle short insular gyrus (Türe et al. 1999), was the most anterior component of the insula examined in the present study. This gyrus appears, grossly, to be the least developed of the 3 anterior short insular gyri (Türe et al. 1999).

Precentral Insular Gyrus

The precentral insular gyrus, or posterior short insular gyrus (Türe et al. 1999), lies within the anterior insula, directly anterior to the central insular sulcus and posterior to the secondary gyrus brevis. Unlike the secondary gyrus brevis, the precentral insular gyrus generally appears well structured and convex (Türe et al. 1999).

Postcentral Insular Gyrus

The postcentral insular gyrus, or anterior long insular gyrus (Türe et al. 1999), is immediately posterior to the precentral insular gyrus, separated by the central insular sulcus. The postcentral insular gyrus generally appears larger, more convex, and better developed than the posterior long insular gyrus (Türe et al. 1999).

There appears to be no isomorphic mapping between the insular gyri and their cytoarchitecture (Mesulam and Mufson 1982a), as indicated in Figure 1. Thus, the present study made no attempt to align the 3 selected gyri with cytoarchitectonic boundaries.

Tissue blocks were coded to prevent experimenter bias, trimmed to 3–5 mm in anteroposterior thickness, and processed by a modified rapid Golgi technique (Scheibel and Scheibel 1978). To be consistent with previous research (Jacobs et al. 1997, 2001), processed tissue was serially sectioned at 120 μm with a vibratome such that the preparation was vertical to the pial surface and perpendicular to the long axis of the gyrus.

Cell Selection Criteria and Dendritic/Spine Quantification

Ten relatively isolated supragranular pyramidal cells per tissue block (i.e., 30 cells per brain) were randomly chosen for analysis following previously established criteria (Jacobs et al. 1997, 2001; see also Petanjek et al. 2008). Briefly, selected neurons needed to be relatively isolated and unobscured, appear fully impregnated, morphologically complete, and have the soma located centrally within the 120 μm section depth, with the apical dendrite perpendicular to the pial surface. Apical branches were not examined because they were often incomplete due to sectioning. To assure a relatively homogeneous cell population, all cells expressed a minimum of 3 basilar dendrites with at least 2 naturally terminating higher-order branches.

Cells were quantified along *x*-, *y*-, and *z*-coordinates on a Neurolucida system (Microbrightfield, Williston, VT) interfaced with an Olympus BH-2 microscope under a planachromat ×40 (0.70) dry objective. Tracings began at the soma, which was traced at its widest point in the 2-dimensional plane to provide an estimate of its cross-sectional area. After drawing the apical shaft, basilar dendrites were traced in their entirety along with all visible spines, regardless of spine type. Dendritic processes were not followed into adjacent sections nor was dendritic diameter examined. Broken tips and unclear terminations were identified as incomplete endings.

Cells were traced by 7 individuals. To determine intrarater reliability, each individual traced the same dendritic system 10 times. There was little variation in tracings. The average coefficient of variation across all raters for soma size, total dendritic length (TDL), and dendritic spine number (DSN), was 4%, 2%, and 5%, respectively. To further test intrarater reliability, a split plot design ($\alpha = 0.05$) compared the first 5 tracings with the second 5 tracings; no significant difference was found among raters for any of these measures. For interrater reliability, all raters were normed before quantification by comparing their tracings to those of the primary investigator (B.J.). In tracings of 10 different dendritic systems, Pearson product correlations across soma size, TDL, and DSN averaged 0.96, 0.99, and 0.97, respectively. The tested agreement among raters was further evaluated by using an analysis of variance (ANOVA; $\alpha = 0.05$), which indicated no significant difference among raters on these measures. Lastly, all final tracings of neurons were reexamined by the primary investigator to assure quality control.

Dependent Dendritic/Spine Measures

Dendritic systems were quantified according to a centrifugal nomenclature (Bok 1959; Uylings et al. 1986): dendritic branches arising from the soma are first-order segments until they bifurcate into second-order segments, which branch into third-order segments, and so on. The raw data were analyzed along 5 previously established measures, some of which are interrelated (Jacobs et al. 2001). TDL is the sum of all traced dendrites. Mean segment length (MSL) is a mean of each dendritic segment. Dendritic segment count (DSC) is a measure of the number of segments per cell. DSN represents to the total number of spines per cell; similarly, dendritic spine density (DSD) is the average number of spines per micron of dendrite.

Independent Variables and Statistical Analyses

The first analysis treated each insular gyrus as a within-groups measure. Specifically, the raw data were aggregated by neuron (CELL) to investigate the effects of insular gyri on the dependent measures. The data were analyzed using a nested ANOVA design (Proc Nested, SAS, 9.0, Cary, NC), where CELL was nested within AREA (each individual gyrus), and AREA was nested within BRAIN (Jacobs et al. 2001). This nested, repeated-measures design afforded each dependent measure its own nested analysis, thereby increasing the ability to identify how

much each independent variable contributes to the values found for the dependent measures. A Bonferroni–Dunn correction ($\alpha = 0.01$) was used to ensure an experiment-wise α of 0.05. Next, the aggregated data set for each insular gyrus was examined using a 2 × 3 MANOVA for a main effect of sex (SPSS 15.0 for Windows, Chicago, IL).

Finally, in order to provide a more comprehensive understanding of the insula in relation to other cortical areas, the current data were collapsed across gyri and compared with the regional data from other BAs (specifically, BA6β, 10, 11, 39, 3-1-2, 4, 22, 44; Jacobs et al. 2001). To control for a significant difference in mean AT and age between the insular data set (M_{age} = 39 ± 10 years, M_{at} = 19 ± 8 h) and the regional data set (Jacobs et al. 2001: M_{age} = 30 ± 17, M_{at} = 12 ± 6 h), the analysis was run with age and AT as covariates. Both the present study and Jacobs et al. (2001) are nested designs with multiple cells measured within different areas of the brain. When analyzing the insular data alongside the regional data (Jacobs et al. 2001), the areas are confounded with BRAIN because the insular and regional data were not collected from the same brains. That is, insular cells were not traced from the "regional brains" and regional cells were not traced from the "insular brains." For the purpose of a combined, exploratory analysis, the cell measurements were averaged for each area and compared using a between-means ANOVA analysis. This analysis is not ideal given the dependence of measurements within the multiple insular and regional areas as well as the independence of measurements when comparing means between insular and regional cell areas. The choice of a between-levels analysis, although violating independence assumptions, is a more conservative approach to the analysis than if all means were compared in a repeated-measures (or within) ANOVA analysis. Given the unavoidable confounding of BRAIN with AREA and the violation of independence assumptions in the ANOVA, the interpretation of these statistical comparisons is considered a tentative, albeit relatively conservative choice of analysis.

Results

Summary of Neuronal Sample

Golgi-stained tissue (Fig. 2) did not exhibit the autolytic changes (e.g., irregular varicose enlargements, constriction of dendrites) described by Williams et al. (1978). Of the 5 dependent measures, only MSL exhibited a significant (negative) correlation with AT [$r(600) = -0.15$, $P < 0.0001$]. There were significant correlations between age and 3 of the dependent measures [TDL: $r(600) = 0.16$, $P < 0.0001$; DSN: $r(600) = -0.24$, $P < 0.0001$; DSD: $r(600) = -0.35$, $P < 0.0001$], suggesting that TDL increased slightly with age whereas spine number and especially density decreased with age. To document homogeneity among the sample, 2 additional measures were analyzed in a 2-tailed Pearson product correlation: 1) soma depth from pial surface and 2) soma size. A Bonferroni–Dunn correction ($\alpha = 0.0001$) was used to maintain an experiment-wise α of 0.05.

Laminar Thickness and Soma Depth

To provide a more accurate account of neuronal sampling, insular gyri from 3 brains were stained with a Nissl technique. Laminar and cortical thickness remained relatively constant across the 3 sampled gyri (Table 2). Moreover, soma depth of sampled neurons varied minimally among the 3 insular gyri, with the somata located primarily in upper layer III. There was a significant correlation between soma depth and soma size, such that soma depth increased slightly with soma size [$r(600) = 0.17$, $P < 0.0001$]. TDL also increased with soma depth [$r(600) = 0.26$, $P < 0.0001$]. These correlations underscore the importance of controlling for soma depth across the 3 insular gyri.

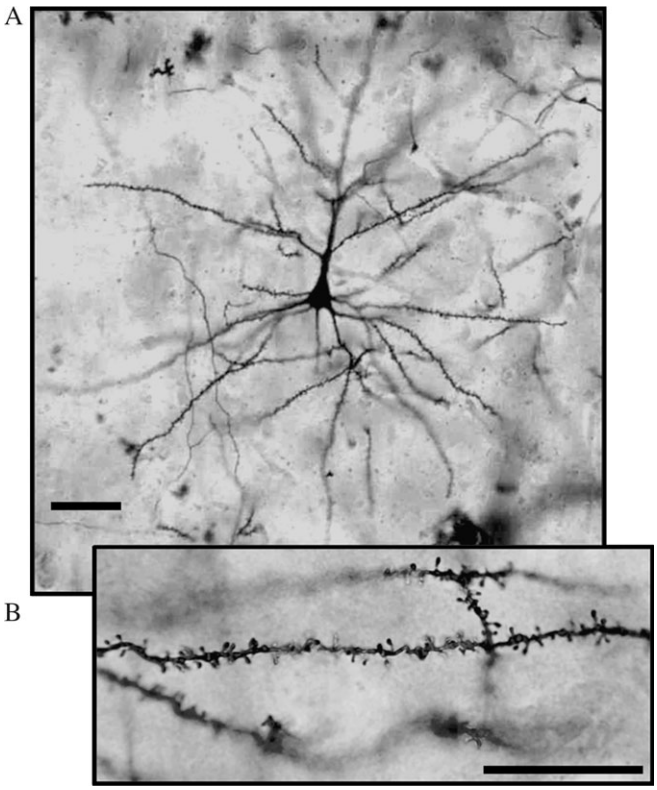

Figure 2. Photomicrographs indicating the overall quality of a successful Golgi impregnation. (*A*) A supragranular pyramidal cell from the precentral insular gyrus of subject F28. Scale bar = 50 μm. (*B*) A dendritic arbor from a supragranular pyramidal cell in the precentral insular gyrus of subject F34 illustrating dendritic spines. Scale bar = 25 μm.

Table 2
Laminar and sampled soma depths (μm) and soma size (μm^2)[a]

	Secondary gyrus brevis	Precentral	Postcentral
Layer I depth	310 ± 94	278 ± 95	284 ± 33
Layer II depth	200 ± 46	229 ± 66	206 ± 51
Layer II/III junction	510 ± 135	508 ± 153	490 ± 20
Sampled soma depth	836 ± 56	836 ± 59	834 ± 56
(Sampled soma size)	(348 ± 53)	(339 ± 66)	(334 ± 77)
Layer III depth	1127 ± 325	690 ± 164	976 ± 365
Layer III/IV junction	1637 ± 317	1197 ± 233	1466 ± 351
Gray/white matter junction	3043 ± 306	2872 ± 250	3155 ± 60

[a]Values represent mean ± SD.

Soma Size

Soma size varied minimally between the samples (Table 2). Soma size increased with 3 of the 5 dependent measures [TDL: $r(600) = 0.18$, $P < 0.0001$; DSC: $r(600) = 0.41$, $P < 0.0001$; and DSN: $r(600) = 0.14$, $P < 0.0001$], suggesting that more complex dendritic arbors accompanied increases in soma size.

An overview of the main effects, discussed in detail below, is provided in Table 3.

Intrainsular Analyses

In general, insular gyri were not uniform in dendritic/spine complexity. The secondary gyrus brevis generally had the most complex basilar dendritic/spine systems of the 3 sampled gyri.

Dendritic Length

There was a significant difference for TDL ($F_{40,540}$ = 2.06, P < 0.0001) and MSL ($F_{40,540}$ = 1.81, P < 0.003) across the 3 insular gyri. The secondary gyrus brevis was more complex in terms of TDL than the precentral (by 9.6%) and postcentral (by 8.8%) insular gyri (Fig. 3*A*). MSL appeared to be slightly greater in the secondary gyrus brevis than in either the precentral or postcentral insular gyri (Fig. 4*A*).

Table 3
Overview of main effects

	Dependent measures[a]				
	TDL	MSL	DSC	DSN	DSD
Within insula	***	***	**	***	—
Sex (males vs. females, pooled insula)	***	***	—	—	—
Interaction (sex × insular gyrus)[b]	**	—	—	*	—
Insula versus high/low integration regions[c]	***	—	***	***	***

[a]Significance levels: *P < 0.05; **P < 0.01; ***P < 0.005.
[b]See text (also Fig. 8) for details of the sex by insular gyrus interaction.
[c]See text (also Figs 3–7, 9) for comparisons of individual insular gyri to low and high integration regions (Jacobs et al. 2001).

Dendritic Number

Across the 3 gyri, there was a significant difference for DSC ($F_{40,540}$ = 1.70, P < 0.006). The secondary gyrus brevis exhibited a greater DSC than the precentral (by 7.6%) and postcentral (by 5.2%) insular gyri (Fig. 5*A*).

Dendritic Spines

There was an overall significant difference for DSN in the insula ($F_{40,540}$ = 2.33, P < 0.0001). The secondary gyrus brevis exhibited a higher DSN than the precentral (by 8.9%) and postcentral (by 10.9%) insular gyri (Figs. 6*A*). Results for DSD were not significant (Fig. 7*A*).

Sex Differences within the Insula

Males and females displayed a different pattern of dendrite/spine complexity across insular gyri. These tests were evaluated with an α = 0.05. There was a significant interaction between sex and insular gyri for TDL ($F_{2,36}$ = 6.96, P < 0.005,

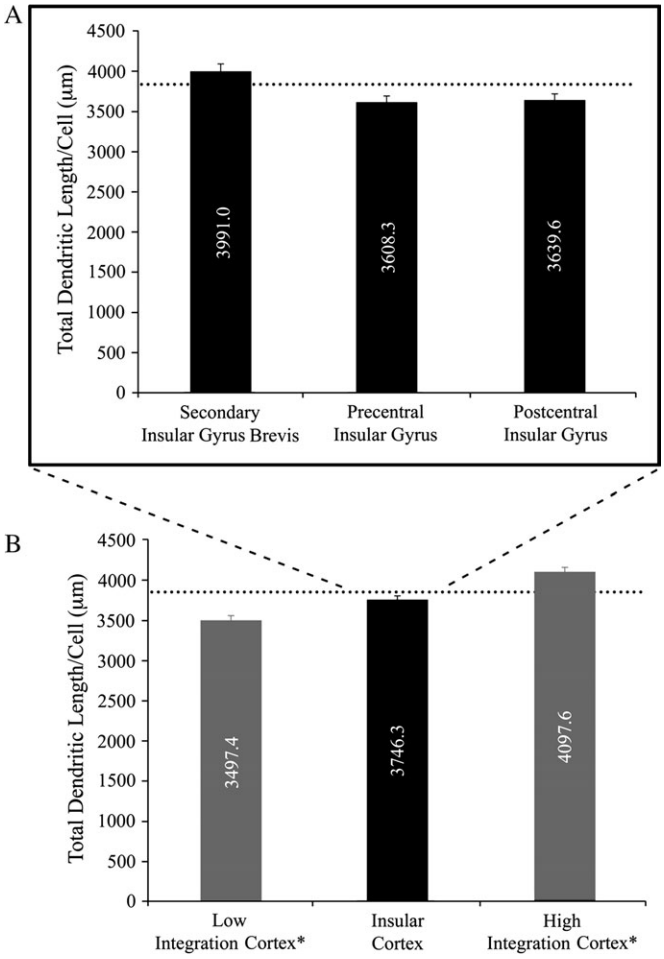

Figure 3. (*A*) Bar graph of relative TDL (µm) within the insular cortex, specifically the secondary gyrus brevis, precentral insular gyrus, and postcentral insular gyrus. The secondary gyrus brevis had significantly longer basilar dendrites than either the precentral or postcentral insular gyri. (*B*) Bar graph of relative TDL for the insular cortex and previously quantified cortices, that is, Low Integration Cortex and High Integration Cortex. Insular TDL values fell between those of high integration cortices and those of low integration cortices. *Denotes data from Jacobs et al. (2001). All Error bars represent SEM.

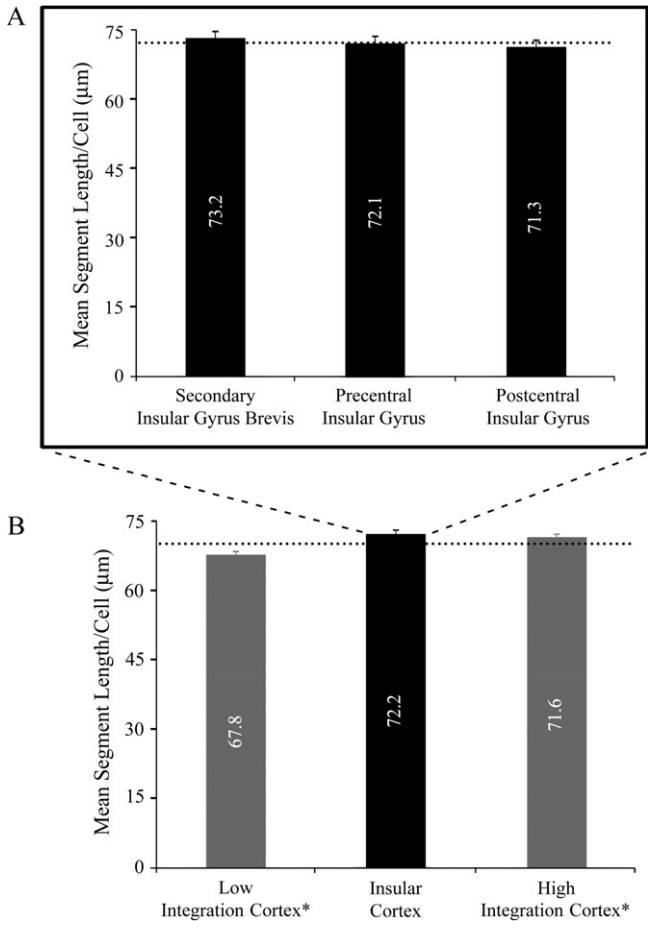

Figure 4. (*A*) Bar graph of relative MSL (µm) within the insular cortex, specifically the secondary gyrus brevis, precentral insular gyrus, and postcentral insular gyrus. There was a nonsignificant increase in complexity in the secondary insular gyrus brevis that was similar to the pattern observed in TDL. (*B*) Bar graph of relative MSL for the insular cortex and previously quantified cortices, that is, Low Integration Cortex and High Integration Cortex. Although not significant, insular MSL was greater than that of the low and high integration cortices. *Denotes data from Jacobs et al. (2001). All Error bars represent SEM.

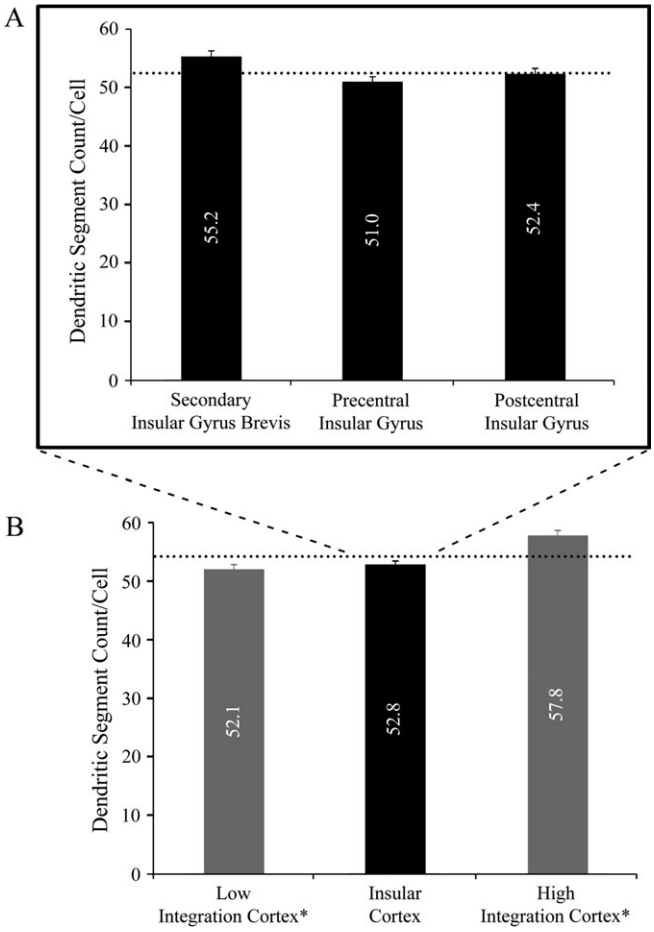

Figure 5. (*A*) Bar graph of relative DSC within the insular cortex, specifically the secondary gyrus brevis, precentral insular gyrus, and postcentral insular gyrus. Data for DSC mirrored the pattern observed in TDL and MSL; the secondary gyrus brevis expressed greater DSC than either the precentral or postcentral insular gyri. (*B*) Bar graph of relative DSC for the insular cortex and previously quantified cortices, that is, Low Integration Cortex and High Integration Cortex. The insula exhibited significantly lower DSC than the high integration regions, whereas the DSC of INSULA and low integration cortices was similar. *Denotes data from Jacobs et al. (2001). All Error bars represent SEM.

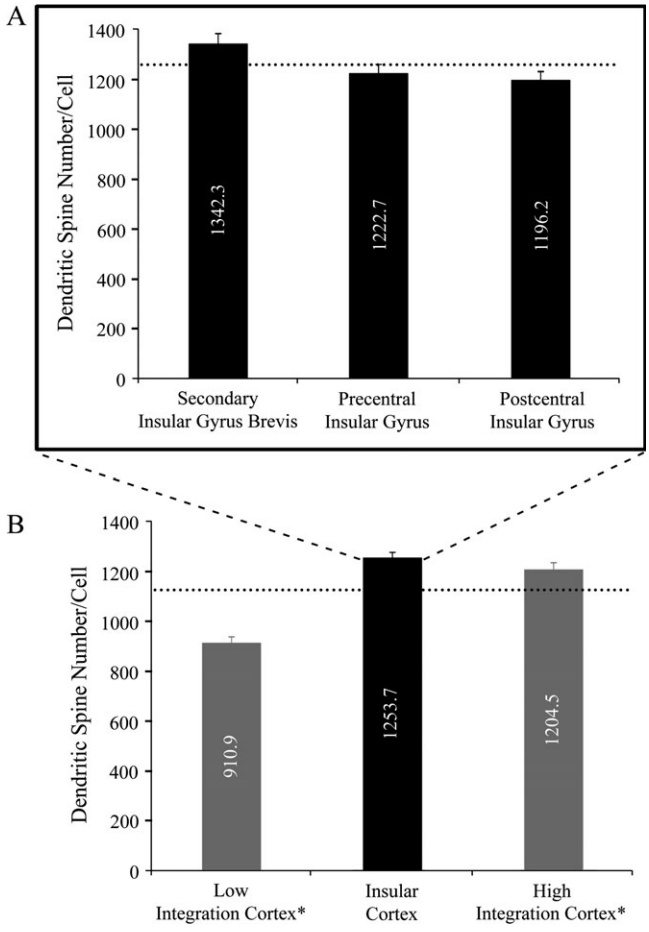

Figure 6. (*A*) Bar graph of relative DSN within the insular cortex, specifically the secondary gyrus brevis, precentral insular gyrus, and postcentral insular gyrus. The secondary gyrus brevis exhibited more spines than either the precentral or postcentral insular gyri. (*B*) Bar graph of relative DSN for the insular cortex and previously quantified cortices, that is, Low Integration Cortex and High Integration Cortex. The insula exhibited a significantly greater DSN than both the low and high integration regions. Whereas the secondary gyrus brevis exhibited a greater DSN than either the low or high integration cortices, the precentral and postcentral insular gyri exhibited a greater DSN than only the low integration cortex. *Denotes data from Jacobs et al. (2001). All Error bars represent SEM.

partial eta^2 = 0.28) and DSN ($F_{2,36}$ = 3.59, P < 0.05, partial eta^2 = 0.17), with MSL approaching significance ($F_{2,36}$ = 2.84, P < 0.07, partial eta^2 = 0.14). A test of between-subjects effects, whereby the 3 insular gyri were pooled into a single measurement (INSULA), revealed significant differences between males and females for TDL ($F_{1,18}$ = 14.52, P < 0.001, partial eta^2 = 0.45) and MSL ($F_{1,18}$ = 11.96, P < 0.005, partial eta^2 = 0.40) but not for DSN. Neither of these analyses revealed a significant difference for DSD.

Post hoc analyses of the sex by insular area interaction for TDL found that, among males, the secondary gyrus brevis exhibited a significantly greater TDL than the precentral and postcentral insular gyri (P < 0.05; Fig. 8). In females, the TDL difference among gyri was not significant. The female secondary gyrus brevis and postcentral insular gyri were nearly indistinguishable for measures of TDL, and although not significant, both gyri appeared to be more complex than the precentral insular gyrus (Fig. 8). In an independent samples *t*-test of insular gyri by sex, males displayed significantly greater TDL (P < 0.01) than females in the secondary insular gyrus brevis (by 26.7%) and precentral insular gyrus (by 22.6%).

Post hoc analyses of the sex by insular area interaction for DSN found that, for males, DSN in secondary gyrus brevis was marginally larger than DSN in the postcentral insular gyrus (P < 0.10). For females, DSN in secondary gyrus brevis was marginally larger than DSN in the precentral insular gyrus (P < 0.10).

Due to marginal differences between samples from males and females in age and AT, covariate ANOVA analyses were conducted to equate males and females on these variables. This covariate analysis was not found to alter the significance of the findings. In fact, in some cases TDL and DSN differences between males and females actually increased.

Comparison of the Insula with Other Cortical Regions

The insular data were subsequently compared with the regional data of Jacobs et al. (2001), which also examined the dendrites/spines of supragranular pyramidal neurons.

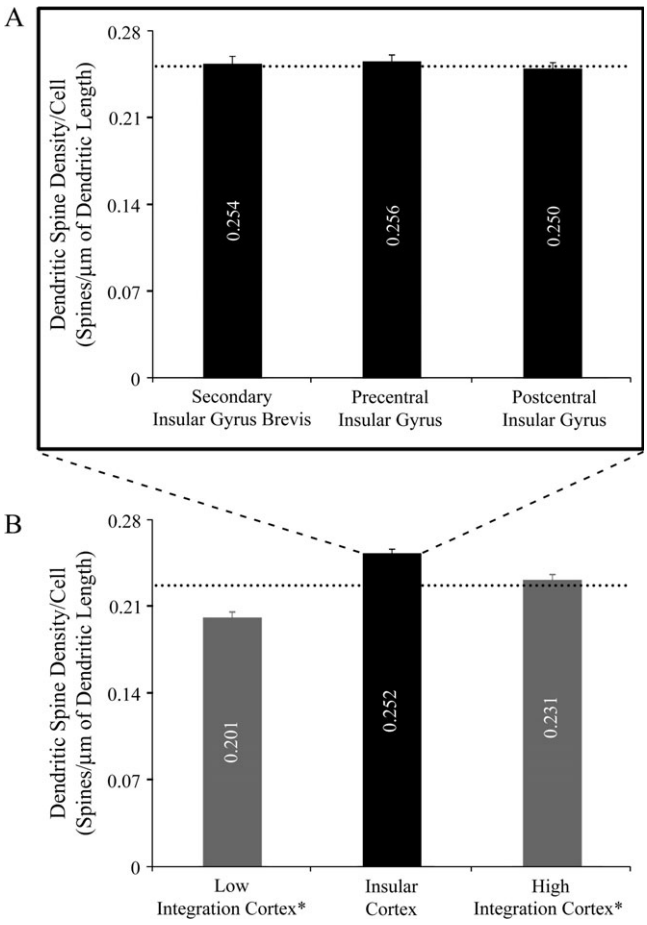

Figure 7. (*A*) Bar graph of relative DSD (spines/mm of dendritic length) within the insular cortex, specifically the secondary gyrus brevis, precentral insular gyrus, and postcentral insular gyrus. There appeared to be no differences in DSD among the 3 insular gyri. (*B*) Bar graph of relative DSD for the insular cortex and previously quantified cortices, that is, Low Integration Cortex and High Integration Cortex. DSD patterns were nearly identical to those of DSN. *Denotes data from Jacobs et al. (2001). All Error bars represent SEM.

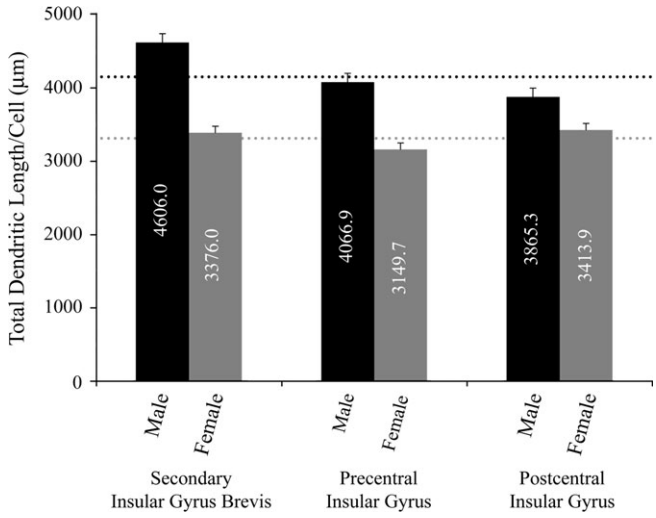

Figure 8. Bar graph of relative TDL (µm) within the insular cortex between sexes, specifically the secondary gyrus brevis, precentral insular gyrus, and postcentral insular gyrus. The black bars and dotted average line represent male TDL values, whereas the gray bars and dotted average line represent female TDL values. Males had significantly greater TDL in the secondary gyrus brevis and precentral insular gyrus than did females. Male TDL followed a postero-anterior increase in complexity, whereas the TDL of the female secondary gyrus brevis and postcentral insular gyri were nearly identical. The female precentral insular gyrus exhibited the lowest TDL of all male and female gyri. Error bars represent SEM.

Specifically, previously quantified Brodmann's areas were grouped into 2 classifications: areas representing primary (BA3-1-2 and 4) and unimodal cortices (BA22 and 44) were designated as low integration regions (n = 400 neurons); areas representing heteromodal (BA6β and 39) and supramodal cortices (BA10 and 11) were designated as high integration regions (n = 400 neurons). This comparison provided a broader cortical context for understanding insular dendritic extent. Sample tracings depict an increase in overall dendritic complexity from primary and unimodal cortices, to the insula, and finally to heteromodal and supramodal cortices (Fig. 9). There were significant differences between INSULA and the 2 cortical integration regions for TDL, DSC, DSN, and DSD, but not for MSL. Details of these comparisons are provided below.

Dendritic Length

There was a significant difference for TDL ($F_{2,135}$ = 8.73, P < 0.001) such that the high integration regions displayed the greatest TDL, followed by INSULA, and finally the low integration regions (Fig. 3*B*). Pairwise comparisons did not reveal any significant differences between INSULA (or the 3 individual insular gyri) and low or high integration regions. The secondary gyrus brevis appeared to be quantitatively similar to the more dendritically complex high integration regions, whereas the precentral and postcentral insular gyri aligned with the less complex low integration regions.

Dendritic Number

Results for DSC were significant ($F_{2,135}$ = 10.35, P < 0.001) with INSULA DSC appearing similar to the DSC of low integration regions. Post hoc analyses indicated that DSC for INSULA was significantly lower than that of the high integration regions (P < 0.001). DSC in both the pre- and postcentral insular gyri was significantly lower than in the high integration regions (P < 0.05). However, DSC of the secondary gyrus brevis appeared to be comparable to that of high integration regions (Fig. 5*B*).

Dendritic Spines

Results for DSN were significant ($F_{2,135}$ = 25.30, P < 0.001), and unlike measures of length, the DSN for INSULA was significantly higher than the DSN for both low integration and high integration regions (P < 0.05). The secondary gyrus brevis was significantly spinier than both low (by 32.1%) and high (by 10.3%) integration regions (P < 0.05, Fig. 6*B*). Although the precentral and postcentral insular gyri expressed significantly greater DSN values than the low integration regions (25.5% and 23.9%, respectively; P < 0.05), these insular gyri did not have significantly more spines than the high integration regions.

INSULA exhibited a significant difference for DSD ($F_{2,135}$ = 27.23, P < 0.001). INSULA was significantly more spine dense than high (by 8.3%) and low (by 20.2%) integration regions (P < 0.05). All 3 insular gyri expressed a greater DSD than either of the 2 cortical integration regions (P < 0.05; Fig. 7*B*).

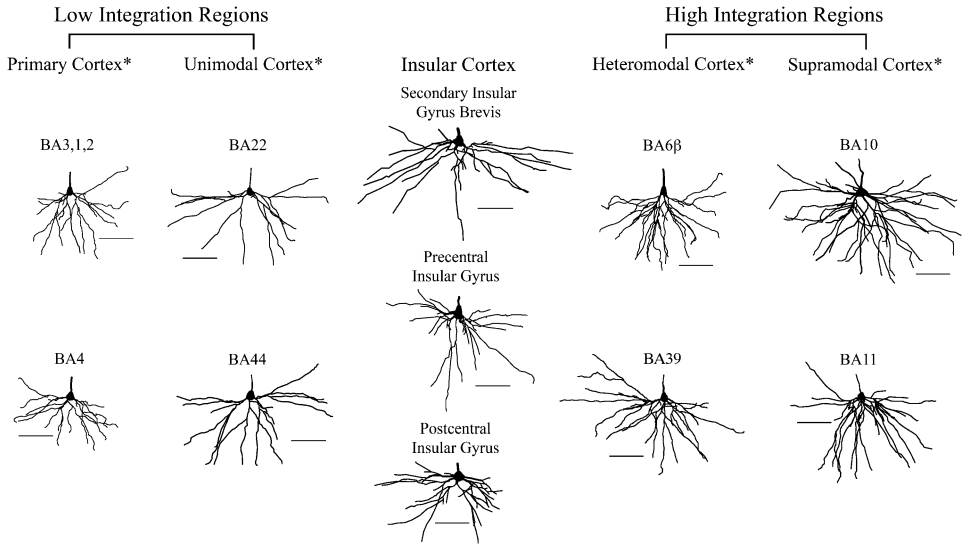

Figure 9. Sample tracings from the insular cortex of subject F25 compared with tracings of high and low integration cortices from a previous subject (F47) quantified by Jacobs et al. (2001). Primary (BA3-1-2, 4) and unimodal cortices (BA22, 44) represent low integration regions, whereas heteromodal (BA6β, 39) and supramodal cortices (BA10, 11) represent high integration regions. The cells have been arranged from left to right to highlight the increase in dendritic complexity from primary and unimodal cortices, to the insula, and finally to heteromodal and supramodal cortices. *Denotes tracing from Jacobs et al. (2001). Scale bars = 100 μm.

Discussion

Intrainsular analyses revealed significant differences among the 3 insular gyri, with basilar dendrites in the secondary gyrus brevis generally being more complex than those in the precentral and postcentral insular gyri. In addition, analyses revealed significant sex differences, indicating that cells in the male insula were more complex than those in the female insula, with a different pattern of dendritic complexity across the 3 insular gyri. Finally, comparisons of insular gyri with other cortical regions (Jacobs et al. 2001) revealed that the insular cortex generally fell between low and high cortical integration regions in measures of dendritic extent; however, spine measures indicated that insular cortex was generally more complex than both low and high integration regions. Before addressing the implications of these findings, potential limitations need to be addressed.

Methodological Considerations

General methodological limitations relevant to the present study (i.e., small sample sizes, issues with Golgi stains, effects of post-mortem delay) have been extensively discussed elsewhere, and need not be readdressed (de Ruiter 1983; Jacobs and Scheibel 1993; Jacobs et al. 1993, 2001). It should be noted, however, that the present dendritic and spine values are attenuated. Indeed, actual intrainsular differences are likely greater than observed because 1) sectioning at 120-μm disproportionately cuts dendrites with greater extent (Jacobs et al. 1997) and 2) one cannot visualize spines extending directly above and below the dendrite with light microscopy (Horner and Arbuthnott 1991). These general limitations accepted, we focus here on 3 broader issues.

The present study examined the basilar dendrites of Golgi-stained supragranular pyramidal neurons. Although the Golgi stain permits a relatively random sampling of neurons (Scheibel and Scheibel 1978), it provides no information about the projections or interconnectivity of these cells. As such, the present sample could constitute different subpopulations of neurons rather than a homogenous sampling. Moreover, by focusing on basilar dendrites—one of the most investigated of all cortical components (Benavides-Piccione et al. 2006)—the present study excluded other neural structures (e.g., apical dendrites, axonal plexus) and cell types (e.g., interneurons, infragranular pyramidal neurons), as well as potential differences in cytoarchitectonics, neuronal density, and neurochemistry, all of which clearly bear on the functional characteristics of a particular cortical area. Consequently, the current results open but a narrow window into microstructure–functional relationships within the insula.

A second limiting factor of the present study is that it examined only the left insula. Several recent investigations have found gross morphological and/or functional differences between left and right insular cortices (Critchley, Weins, et al. 2004; Wicker et al. 2003). For example, right insular activation may be involved in sympathetic nervous system control whereas left insular activation is associated with parasympathetic activity (Ostrowsky et al. 2002; Craig 2003, 2005). Clearly, further morphological research on both left and right insulae would be illuminating, particularly given that cytoarchitectonic differences may obtain despite a lack of gross morphological asymmetries (Gannon et al. 2008).

Although there is substantial interindividual variability among gyral and sulcal patterns (Bartley et al. 1997; Lohmann et al. 1999), as well as dendritic samples (Jacobs et al. 1993) taken from homologous areas, each individual in the present study served as his/her own control. Because each brain provided samples from all 3 insular gyri, differences in dendritic extent among gyri were not likely the result of individual history. However, because of considerable interindividual variation among insular gyri (Türe et al. 1999), the current study selected gyri that were consistently identifiable based on their position around the central insular sulcus, which restricted the range of sampled tissue. Obviously, a broader perspective of pyramidal cell morphology in the insula would

have been obtained if tissue had also been examined from other insular gyri.

Intrainsular Analysis

Dendritic systems within the 3 insular gyri appear to be roughly organized in a postero-anterior pattern of increasing complexity in terms of TDL, MSL, and DSC, which is reminiscent of dendritic patterns observed in the supragranular pyramidal neurons of nonhuman primates. A postero-anterior progression in basilar dendritic complexity has been observed in the visual processing stream (Elston and Rosa 1997, 1998a, 1998b), cingulate cortex (Elston et al. 2005), and motor system (i.e., from BA4 to BA6; Elston and Rockland 2002). In contrast, dendritic progression in the somatosensory system proceeds in an anteroposterior direction (i.e., from BA3b, BA5, to BA7b; Elston and Rockland 2002). Similarly in humans, basilar dendrites are more complex in BA6β over BA4, and in BA39 over BA3-1-2 (Jacobs et al. 2001). Thus, the emergent pattern is one of increasing basilar dendritic extent in cortical regions of successively higher processing, with the most complex dendrites generally, but not always, found in granular prefrontal cortex (Jacobs et al. 2001; Elston 2007).

The current results indicate that measures of DSN, which were strongly correlated with TDL, produced a similar, significant morphological pattern to those reported above. Note, however, that there were no significant differences in DSD among insular gyri. Given that larger dendritic trees appear to be a morphological manifestation of enhanced integrative capacity (Jacobs et al. 1997, 2001; Elston 2007), the heightened complexity of dendritic/spine systems in the anterior insula may be indicative of more complex processing than occurs in the posterior insula. Both intrinsic and extrinsic connectivity may underlie these morphological and functional differences.

Although relatively little is known about intrinsic connections within the insula (Bennet and Netsell 1999), the literature suggests there may be distinct patterns of connectivity in this region (Kapp et al. 1985). In fact, specific regions of the cat insula appear to be more closely related to noninsular modality-specific cortices than to each other (Clascá et al. 2000). In terms of intrinsic connections, however, there appear to be strong recurrent synaptic connections between pyramidal neurons in layers II/III of the rodent insula that are responsible for layer-dependent synchronization of gustatory barrel cortex (Sato et al. 2008). In addition, axon collaterals in the guinea pig anterior PC project caudally and nonreciprocally to the posterior PC (Chen et al. 2003). The macaque anterior agranular insula is reciprocally connected and is adjacent to the PC (Mesulam and Mufson 1985) and exhibits a pattern of caudally directed efferent innervation similar to that found in the PC of guinea pigs (Mesulam and Mufson 1982b). Thus, the increased dendritic complexity observed in the secondary gyrus brevis may be due, in part, to the innervation of anterior insular regions by recurrent collaterals. In fact, Mesulam and Mufson (1985) proposed that components of the hippocampocentric paralimbic areas, which include the PC and insula, may be functionally and architectonically related. Within these paralimbic areas, caudally directed intrainsular efferents might represent a pathway by which anterior signals are sent to the more posterior regions.

What remains unclear are the factors that determine dendritic extent in a given cortical region. On the one hand, an increase in layer III pyramidal neuron dendritic complexity has been shown to accompany long-range corticocortical projections, as opposed to local corticocortical connections (Duan et al. 2002), a finding that seems consistent with the possibility that the long-range corticocortical connections of the anterior insula may exceed those of the posterior insula. On the other hand, other research indicates that basilar dendritic complexity is not significantly affected by the long distance projections of the pyramidal neurons themselves (Elston and Rosa 2006). Instead, it appears that the local cortical matrix and intrinsic genetic determinants are crucial factors in shaping dendritic morphology (Vercelli and Innocenti 1993). Although this issue cannot be resolved here, it is certainly possible that basilar dendritic morphology in the present study may also be representative of local, intrinsic signals operating within each of the insular gyri examined.

In terms of extrinsic connections, processing demands on the anterior insula may exceed those of more posterior regions. Given the anterior insula's extensive connections with the prefrontal cortex (Öngür and Price 2000), it may be in a position not only to direct the multimodal convergence of information from all sensory modalities (Mesulam and Mufson 1985), but also to contribute to high-level cognition, decision-making, and hedonic experience (Kringelbach 2005). Insofar as basilar dendritic extent appears to be more complex in supramodal prefrontal cortices than in primary and unimodal cortices (Jacobs et al. 2001), it follows that the anterior insula may also exhibit greater dendritic complexity and contribute to supramodal-type processes. In fact, activation in the anterior insula appears to be necessary for successful decision-making under risky circumstances (Clark et al. 2008; Preuschoff et al. 2008), a process that requires integrative capacities beyond those that occur in multimodal cortices. Rolls (2008), in fact, has hypothesized that binary decision-making is an additional level of representation over simple affective assignment, a distinction that underscores the ability of the anterior insula to function beyond multimodal integration. Similarly, the insula appears to combine information regarding anticipatory states with simultaneous sensory and autonomic signals (Yasui et al. 1991; Chua et al. 1999), and is activated during emotionally charged thought caused by the expectation of visceral pain (Berman et al. 2006). The anticipatory state may also arise from an additional level of representation beyond actually experiencing pain. Activation in the anterior insula has been shown to signal pain's anticipation (Ploghaus et al. 1999), whereas pain itself activates the middle insula (specifically the secondary gyrus brevis; Afif et al. 2008). Importantly, both intrinsic and extrinsic factors connote that the anterior insula is in a position to direct the convergence of visceral (Cechetto and Saper 1987; Dupont et al. 2003), somatosensory, auditory (Mesulam and Mufson 1982b), thermosensitive (Craig et al. 2000), and painful (Ostrowsky et al. 2002; Brooks et al. 2005) signals, all of which have been localized to the posterior insula. If the anterior insula oversees a caudally directed efferent pathway, this model would be consistent with heightened dendritic complexity in this region.

Sex Differences in the Insula

Assuming the present results are not an artifact of the small sample size, the current data indicate an interaction of sex by insular gyrus. The secondary gyrus brevis of males had

consistently longer basilar dendrites than that of females. Moreover, males exhibited a postero-anterior trend of increasing dendritic extent, whereas the complexity of basilar dendrites in the 3 gyri remained relatively stable in females. Although significant sex-related dendritic differences in humans have not emerged in previous quantitative studies (Jacobs et al. 1993, 2001), several differences between the brains of males and females have been well documented (for review, see Cahill 2006). For example, the female cortex is often thicker than the male cortex (Luders et al. 2005) with different gray–white matter ratios (Allen et al. 2003). Structural and chemical sexual dimorphisms have also been noted in the hippocampus (Juraska et al. 1985; Madeira and Lieberman 1995), amygdala, medial paralimbic cortex (Goldstein et al. 2001), and prefrontal cortex (Bixo et al. 1995), all structures closely linked to emotions and, potentially, along with the insula, to interoception. Indeed, studies with emotional or interoceptive components have found sex differences in the activation of the insula and related limbic structures (Naliboff et al. 2003; Li et al. 2005).

From a functional perspective, one could infer that the greater dendritic extent of the male anterior insula may be 1 correlate of enhanced interoceptive abilities in males vis-à-vis females, who may instead rely more on environmental and contextual cues to create their sense of self (Roberts and Pennebaker 1995; Labus et al. 2008). This speculative observation is based on tests of context-free interoception, such as heartbeat detection tasks (Katkin 1985). The insula has long been implicated in cardiovascular control (Critchley, Rotshtein, et al. 2004), and recent studies suggest that one's heartbeat detection abilities correlate positively with the gray matter volume of the right anterior insula (Critchley, Weins, et al. 2004). Males are better at accurately perceiving and learning to detect their resting heartbeat than are females (Katkin 1985; Blascovich et al. 1992). In fact, in laboratory-based tests that provide no available external cues, males surpass females in other signal-detection paradigms (e.g., stomach contraction and respiratory resistance), several of which have been functionally linked to the insula. As suggestive as these findings may be, further quantitative morphological research, particularly in the right insula, is necessary to confirm whether there is indeed a relationship between insular dendritic extent and sex-related interoceptive abilities.

Insula versus Low and High Integration Regions

Accepting that structural specializations in cortical pyramidal cells are associated with phenotypic expression of processing demands (Elston 2003, 2007; Elston et al. 2005), the present findings suggest that certain morphological variations (e.g., a greater number of spines) may characterize insular pyramidal neurons, particularly when juxtaposed next to the results of previous quantifications (Jacobs et al. 2001). Jacobs et al. (2001) found greater dendritic/spine complexity in heteromodal (BA6β and 39) and supramodal (BA10 and 11) cortices as compared with primary (BA3-1-2 and 4) and unimodal cortices (BA22 and 44), reflecting potential differences in the integrative demands on these regions. In the present study, insular basilar dendrites generally fell between regions of low (primary and unimodal cortices) and high (heteromodal and supramodal cortices) integration for measures of length. In terms of spines, however, the insula was consistently more complex than both low and high integration regions. For individual insular gyri, the secondary gyrus brevis was similar to high integration regions in dendritic length, whereas the precentral and postcentral insular gyri were more similar to low integration regions. This analysis of the 3 individual insular gyri alongside these 2 regions reinforces the theory that the greater dendritic extent found in the anterior insula is functionally related to the high-level processes that have been attributed to that region (Wicker et al. 2003; Kringelbach 2005). For reasons that remain unclear, the only measure where the secondary insular gyrus brevis did not approach the levels of high integration regions was DSC. Given that an increase in spine number allows for enhanced integration without greatly increasing cellular volume (Jaslove 1992), it is possible that the large number of dendritic spines in the insula mitigates the need for more branches. All 3 insular gyri, however, exhibited essentially the same DSD, which suggests that the middle and posterior insular regions have proportionally fewer afferent inputs than the anterior insula and that increased afferent sampling may be a hallmark of insular dendritic function.

It was no surprise that a region as diversely connected as the insula was as morphologically complex as other multimodally responsive cortices. Not only autonomic/visceral afferent systems (Oppenheimer et al. 1992; Naliboff et al. 2003), but also somesthetic, auditory, olfactory, and gustatory signals are processed in the insula (Mesulam and Mufson 1985). The morphology of insular basilar dendrites suggests that these neurons are not merely a spinier version of those from other heteromodal cortices, but rather a potentially distinct variation of the pyramidal cell type that may be fine-tuned for enhanced afferent sampling and integration. Along these lines, Mesulam and Mufson (1985) noted that the insula received strong innervation directly from primary sensory cortices. They further suggested that, unlike other heteromodal cortices, which receive previously processed sensory information, the insula may be one of the few cortical regions to multimodally integrate crude information directly from primary sensory cortices. The present results indicate that the main difference between basilar dendrites in the insula and those in the dorsolateral cortex (Jacobs et al. 2001) is that insular dendrites tend to be more spinous, an adaptation that may be due to extensive afferent innervation (Ramón y Cajal 1896; García-López et al. 2007). On the contrary, it is possible that dendritic architecture is not merely a function of afferent input. For example, the insula may have a fundamentally different synaptic integration strategy than other heteromodal cortices: one that is specialized for combining raw sensory and interoceptive signals. The cortical representation of interoceptive signals relaying pain and temperature information appear to be localized to the middle and posterior insula (Brooks et al. 2005; Hua et al. 2005), as are auditory and somesthetic afferents (Mesulam and Mufson 1985). Dendritic spines in the middle/posterior insula may be the sites of informational convergence regarding internal and external states. From there, the information may be fed forward to receive conscious awareness (Critchley, Weins, et al. 2004) and emotional coloring (Krolak-Salmon et al. 2003) in the anterior insula, which could ensure the emergence of an accurately formed self-image (Craig 2005).

Whether these insular neurons constitute a particular subclass of pyramidal cells remains to be seen. Certainly,

subpopulations of pyramidal neurons have been observed in neocortex. For example, Hof et al. (2001) have documented calretinin-positive pyramidal cells unique to the hominid anterior cingulate cortex. Given the strong anatomical and functional connections between anterior cingulate and anterior insular cortices (Mesulam and Mufson 1982b), it is worth noting that there may be neurochemical differences among insular subregions similar to those of the cingulate cortex.

Finally, it is unclear if insular basilar dendrites are in some way functionally distinct from other pyramidal neurons. In the visual processing stream, for example, the extensive basilar dendrites in inferior temporal lobe (i.e., area TEO), in conjunction with receptive field map compression, have a 100-fold greater sampling region than do the relatively simple basilar dendrites in primary visual cortex (Elston and Rosa 1998a). In accordance with the theory of dendritic compartmentalization (Häusser and Mel 2003), a neuron's computational abilities increase as branch/spine numbers increase. A theoretical example of a functional compartment is the clustered synapses on dendritic sub-branches, which appear to represent a basic unit of cortical networks (Larkum and Nevian 2008). Insular dendritic spines, which are even denser than those quantified in prefrontal cortices (Jacobs et al. 2001), may provide for a large degree of such clustering. Given the insula's interconnections with primary sensory areas, the increase in spine density in the insula vis-à-vis other cortical areas may be associated with the computational resources provided by spine clusters. Just as spine number and density influence a neuron's integrative capacity, so too does the distance of a synapse from the soma, with the emergence of a gradient whereby long-term potentiation is more common in proximal dendrites and long-term depression in distal dendrites (Sjöström and Häusser 2006). Thus, a large dendritic arbor could have associative learning properties distinct from those of a smaller arbor. This observation potentially supports the parallels we suggested between basilar dendritic extent in the secondary gyrus brevis and the functional attributes of the anterior insula.

In conclusion, the present morphological findings support 4 observations. First, the anterior insula in the left hemisphere, as represented by the secondary gyrus brevis, is morphologically more complex than more posterior insular regions. Second, the anterior insula appears as morphologically complex as other hetero- and supramodal cortical regions. Third, there are differences between the male and female left insulae that may be related to sex differences in interoceptive ability. Finally, the insula may represent a distinct type of heteromodal cortex that is characterized by a large number of basilar dendritic spines, suggesting that insular pyramidal neurons may not be solely scaled versions of the neurons in other cortices. These markedly spiny insular dendrites may represent part of the microanatomical circuitry involved in the merging of primary sensory signals into the sense of self.

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Notes

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