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Dendritic Spine Density Varies Between Unisensory and Multisensory Cortical Regions

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

at Virginia Commonwealth University

by

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ABSTRACT

DENDRITIC SPINE DENSITY VARIES BETWEEN UNISENSORY AND MULTISENSORY CORTICAL REGIONS

By Moazzum Nabeel Bajwa, B.S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
at Virginia Commonwealth University

Virginia Commonwealth University, 2010

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In the brain, the dendritic spine is a point of information exchange that extends the neuronal surface on which synapses occur, as well as facilitates and stabilizes those contacts. Furthermore, dendritic spines dynamically change in shape and number in response to a variety of factors. Dendritic spine numbers are reduced in mental retardation, enhanced during development, sensory enrichment or physical exercise, or fluctuate during the reproductive cycle. Thus, for a given neuron type, it might be expected that dendritic spine number might achieve a dynamic optimum. Indeed, many studies of spine density of pyramidal neurons in sensory cortex indicate that an average of ~1.4 spines/micron occurs is present (Briner et al., 2010). Most such studies examined dendritic spines from primary sensory areas which are dominated by inputs

from a single sensory modality. However, there are a large number of neural regions that receive inputs from more than one sensory modality and it is hypothesized that spine density should increase to accommodate these additional inputs. To test this hypothesis, the present experiments used Golgi-Cox stained layer 2-3 pyramidal neurons from ferret primary somatosensory (S1) and auditory (A1) cortical regions, as well as from the higher-level rostral posterior parietal (PPr) and lateral rostral suprasylvian (LRSS) multisensory areas. Spine densities in S1 (avg 1.309 ± 0.247 spines/micron) and A1 (avg 1.343 ± 0.273 spines/micron) were measured to be significantly greater ($p < 0.05$, t-test) than those observed in multisensory regions PPr (avg 1.242 ± 0.205 spines/micron) or LRSS (avg 1.099 ± 0.217 spines/micron). These results also indicate that spine densities are greater in primary (S1, A1) than in higher-level (PPr, LRSS) sensory areas. The functional consequences of such unexpected findings are discussed in light of potential biophysical differences between unisensory and multisensory neurons.

INTRODUCTION

Dendritic spines are primary sites of excitatory synaptic transmission in the central nervous system (Harris 1999). As such, they are essential to the transmission of neural signals and, thereby, the process of sharing information from one neuron to another. In addition, much like their parent dendrites, dendritic spines function to amplify the surface area upon which synaptic contacts can occur. The classical dendritic spine consists of a head connected to a dendrite by a narrow neck. The head contains the post-synaptic density that anchors the receptor channels that receive the pre-synaptic contacts and transmissions. In pyramidal cells, the three main types of dendritic spines are described: simple, pedunculated, and filopodic. Simple spines are protrusions from the dendrite that are usually no more than 2 microns in length.

Pedunculated spines are more numerous and have a more bulbous, mushroom-shaped enlargement at the tip (Stuart et al 2008). Filopodia are long, thin and highly motile forms that, given their lack synaptic contacts, represent an immature stage in spine development (Arellano et al., 2007). There are many morphological variations among these basic spine shapes, leading some investigators to catalog and define up to 23 different types! However, it has been shown recently that dendritic spines change dynamically between the three basic forms defined above (Parnass et al., 2000).

The maturational sequence of dendritic spines is still debated, with at least three possible theories to account for this process. In the first, highly motile filopodia (Dailey et al. 1996) seek synaptic partners in the developing neuropil. When a partner is found, the filopodium shortens

and draws the axonal element closer to the parent dendrite. Eventually, a fully mature synapse is formed on the spine head. In the second theory, filopodia also seek out synaptic partners, which form synapses on them not necessarily at the tip (Fiala et al 1998). The filopodium retracts completely, which leads to the formation of an asymmetric shaft synapse. Then a spine emerges at that site with a mature synapse at its head (Fiala 1996). The third theory shows continued spine turnover throughout the lifetime of the animal, a capacity that is greatest during synaptogenesis (Lendvai et al 2000). In this scheme, spines constantly form by seeking out presynaptic partners and stabilizing into functional spines of any morphology. The size, not the morphology, is the more relevant characteristic and is related to the strength of the synapse at any point in time. Therefore, although the mechanism remains to be determined, dendritic spines clearly exhibit characteristic shapes related to their maturity and function.

It is well established that dendritic spines vary in shape. In addition, dendritic spine numbers (or density, measured in spines per unit length of dendrite) also can vary in response to a large number of conditions. Spines are regulated by numerous extracellular factors such as neurotransmitters, growth factors, and hormones. Development, maturation and aging also affect dendritic spine number (Jacobs et al 1997). In mammals, dendritic spines begin to increase in density during the first week after birth and reach the peak in the third week, which corresponds roughly to the peak of synaptogenesis (Harris 1999; Nimchinsky et al 2002). Spine density is also influenced by environmental signals including stress (Segal 2005), sensory loss or deprivation (Briner et al., 2010), and by various types of mental disorders (Fiala et al 1998). Because of the central role occupied by dendritic spines in synaptic transmission, it is not surprising that a number of human disease states are associated with changes in dendritic spine density, such as the link between abnormal dendritic spine shape/number and mental retardation

(Purpura 1974). Studies have shown decreases in spine density in neocortical pyramidal neurons in patients with schizophrenia. One study of schizophrenics found 59 and 66% decreases in spine density in temporal and frontal cortical regions, respectively (Garey et al 1998). Another study (Glantz et al 2000) showed a 21% decrease in spine density in schizophrenic patients compared with age-matched controls. It is well known that the cognitive functions are impaired during normal aging. Some studies have suggested that the cell loss associated with normal aging may be insufficient to account for the observed cognitive changes (Morrison et al 1997). The list of neurologic disorders associated with changes in dendritic spines continues to expand. The highly plastic nature of dendritic spines makes these changes difficult to interpret. As a consequence, it is very difficult to determine whether a given abnormality is due to a primary defect of the spine rather than a response of the spine to some other perturbation.

Dendritic spines are also differentially distributed along the dendritic tree of a neuron. For neocortical pyramidal neurons, there is a relative absence in the initial portion of the apical dendrite. There is an exponential increase in apical dendritic spine density with distance from the soma, a peak reached in the middle cortical layers, and a subsequent drop-off in distal dendrites (Valverde 1967). Spine density varies not only within individual dendritic trees but also across cortical areas. A recent study (Elston 2000) demonstrated that spine density on basal dendrites of layer III neurons in several cortical areas of the frontal pole and orbitofrontal cortex in the macaque monkey was generally threefold greater than neurons of the primary visual cortex and twofold greater than neurons in a parietal visual cortical region. In the human brain, among eight cortical areas sampled, layer III basal dendritic spine density was greatest in areas in the prefrontal and orbitofrontal cortex and up to 40% greater in the frontal cortex than in the primary somatosensory cortex (Jacobs et al 1997). It is suspected that these higher order, associational,

multi-sensory areas may require more synapses and thus more dendritic spines (Nimchinsky et al 2002).

In multisensory cortical areas, inputs from different sensory pathways converge onto individual neurons to generate multisensory processing. Multisensory processing is defined as an effect in which stimuli from one modality influence the activity induced by stimuli from another sensory modality. Multisensory processing occurs between all sensory modalities (Stein and Meredith, 1993) and produces a distinct pattern of activity that cannot be predicted with stimulation by a single modality (Meredith and Stein 1983). Perhaps the best known multisensory area is that of the superior temporal sulcal cortex in primates (Benevento et al., 1977) or the anterior ectosylvian sulcal area of cats (Meredith, 2004). In the ferret brain, the lateral rostral area of the suprasylvian sulcal cortex, (LRSS; Keniston et al., 2008), and the rostral posterior parietal cortex (PPr; Manger et al., 2002) are cortices that exhibit multisensory properties and process multisensory information. All of these multisensory cortical areas are considered to be higher-order cortices, not only for the increased number of synaptic relays used to reach them, but also for their inputs from non-primary thalamic regions. Therefore, it is logical to expect that multisensory neurons would exhibit greater dendritic spine densities than unimodal neurons in their primary sensory counterparts simply because multisensory neurons appear to receive more inputs. This hypothesis will be tested in the present study, which will examine the dendritic spine density found in ferret multisensory cortical areas LRSS and PPr and compare those measures with that obtained in primary somatosensory (S1) and auditory (A1) cortex.

METHODS

All procedures were conducted in accord with the National Research Council's *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (2003) and with the approval of the Animal Care and Use Committee of the Virginia Commonwealth University.

Animals and Histology

Young, adult (~132 days old; 1.4 kg weight) male ferrets (n=3) were deeply anesthetized (sodium pentobarbital, 60 mg/kg i.p.) and, when areflexive, were perfused transcardially with 0.9% saline followed by 0.4% paraformaldehyde. Brains were stereotaxically blocked in situ, removed from the cranium, and post-fixed for 24 hours in 0.4% paraformaldehyde.

Blocks (7-10mm) of cortex from both hemispheres were prepared for a Golgi-Cox stain using the FD Rapid GolgiStain Kit (FD NeuroTechnologies, Ellicott City, MD, USA). Specifically, tissue was rinsed briefly in double-distilled water (used throughout) to remove any remaining blood products from the surfaces. The tissue was then immersed in the Golgi-Cox impregnation solution (5% potassium dichromate, 5% mercuric chloride, 5% potassium chromate in ddH₂O). The solution was refreshed after 24 hours and stored in the dark for 14 days. The tissue was then transferred to Solution "C" (FD Rapid GolgiStain Kit), refreshed after 24 hours, and incubated for a total of 7 days in the dark. The block was then sectioned serially (100µm thickness) using a vibratome and the sections mounted on gelatin-coated glass

microscope slides. The sections were moistened with Solution “C” but excess was removed using filter paper. Mounted sections were dried slowly overnight in a humidior at room temperature. Rinsed sections (2x, 2 minutes in ddH₂O) were reacted in equal parts of Solutions “D” and “E” (Rapid GolgiStain Kit; contains ammonium hydroxide, 30%) for 10 minutes in the dark. Sections were then rinsed (2x, 4 minutes in ddH₂O), dehydrated in an ascending alcohol series, cleared in xylene and coverslipped with permount. Finished slides were archived for later analysis. The mechanism by which this method stains a limited and random number of neurons is still largely unknown.

Data Collection

Golgi-Cox stained tissue sections containing the desired cortical regions (A1, S1, PPr, LRSS) were surveyed using low magnification light microscopy to identify candidate neurons for measurement and evaluation. In each region, pyramidal layer 2-3 neurons were sought that demonstrated intact apical and basilar dendrites containing dendritic spines. Once a neuron was selected, the entire section was traced under light microscopic guidance using Neurolucida (MBF MicroBrightfield, Willston VT, USA) software. The section outline and the gray-white border was plotted under low magnification (40x) while the neuronal soma and its dendritic branches were subsequently plotted using 200x magnification. Candidate portions of apical and basilar dendrites were selected and marked on the neuronal tracing, and measured using Neurolucida. Using high magnification (1000x, oil), identified dendritic segments were scanned for the presence of dendritic spines. The Neurolucida was used to mark the location of each visible dendritic spine; no attempt was made to resolve spines hidden by the thickness of the dendrite

itself. Dendritic spines were classified and marked according to the criteria of Stuart et al., (2008), as follows. Simple (or sessile) spines occurred as protrusions from the parent dendrite that lacked a neck constriction and could be short, bent or straight. Pedunculated spines occurred as protrusions that exhibited a neck constriction, usually with a bulbous enlargement at the tip. Occasionally, filopodic spines were observed as long, thin, sometimes wavy protrusions but, because they represent immature spines, were not marked. In addition, because spine densities diminish at locations close to the neuronal soma, no spines were evaluated $<100\mu\text{m}$ from the soma. Spines were only marked if a dendritic segment could be followed for $> 50 \mu\text{m}$. This process was repeated until at least one apical and 2-4 basilar dendrites were examined in 6-7 neurons for each of the selected cortical regions for each animal.

Data Analysis

Plots of dendritic segments marked with simple and pedunculated spines were analyzed with NeuroExplorer (MBF MicroBrightfield, Willston VT, USA) software. This program determined the length of dendrite measured, and counted the number of spines that occurred along that segment. The program also indicated the dendritic location (apical, basilar) as well as spine type (simple, pedunculated). For each neuron, these values were exported to a spreadsheet in which data from neurons from the same cortical region and animal were tabulated. Statistical methods were used to determine the average, and standard deviation, of the spine counts per dendritic length obtained in relation to the variables of spine type, dendrite location (apical vs. basilar), specific modality of a particular cortical region, unisensory or multisensory nature of the

cortical region, and the hierarchical position of the region. Data within these groups were statistically compared using a t-test ($p < 0.05$ = significant).

Following data analysis, representative neurons and dendritic segments were photographed (Nikon Eclipse 60) or reconstructed using camera lucida techniques using a drawing tube attached to a light microscope. Images were imported to Photoshop (Adobe Systems) for graphic manipulation and display. Data was tabulated or graphed using Excel (MicroSoft).

RESULTS

The dendritic spine density (spines/micron of dendrite length) was measured from Golgi-stained sections through cortex of three, age-matched adult male ferrets. Selected, matched populations of neurons from the primary sensory areas of S1 and A1 and the higher-level multisensory areas, PPR and LRSS, were examined, as detailed in Table 2. Each measured neuron was required to have a pyramidal-shape soma that was located in cortical layers II-III with attached, intact apical and basilar dendrites, like those illustrated from camera-lucida renderings in Figure 2. Furthermore, each selected neuron was mapped using NeuroLucida to quantify dendritic branching features and measure dendritic dimensions, as illustrated in Figure 3. The statistics for the dendrites that were sampled are displayed in Table 3, indicating that nearly the same dendritic length (overall average= 78.9 microns, ± 2.7 STD) was measured in each of the neuronal populations. In addition, similar numbers of apical (avg=1) and basilar (avg=3.3) dendrites were examined from each region.

Neurons that met the selection criterion were then examined for the presence of spines along their apical and basilar branches using 1000x-oil light microscopy, an example of which is presented in Figure 3. Using NeuroLucida, the locations of pedunculated and simple spines were differentially marked along a selected dendrite. Filopodia-type spines were not included, nor were spines measured within 100 μ m of the soma due to their known reduction in density in that region. For the entire population of neurons sampled, a total of 37513 dendritic spines were examined and marked. Consequently, the average number of spines over the average measured dendritic length represented an average dendritic spine density of 97.69 spines/length. The

following procedures were used to assess whether this value varied by spine type, dendritic location, sensory modality, unisensory versus multisensory region, or by hierarchical level.

Dendritic Spine Type. Although both pedunculated and simple spines were observed in abundance, pedunculated spines consistently outnumbered their simple counterparts in each region. This relationship is obvious when pedunculated and simple spine densities are compared for each region, as listed in Table 4. For each area, the difference in pedunculated versus simple spine density was statistically significant ($p < 0.05$, t-test). This relationship was maintained when dendritic position was considered: the dominance of pedunculated over simple spines on apical or basilar dendrites (listed in Table 5) was statistically significant ($p < 0.05$, t-test).

Dendritic Location. When the total number of dendritic spines (both pedunculated and simple) were evaluated by dendritic location (apical versus basilar), there was not a statistically significant difference ($p > 0.05$, t-test) in their density by location. These values for all cortical regions are listed in Table 6. In addition, when the results were separated according to cortical region (S1, A1, PPr, or LRSS), there was no statistical difference between spine densities on apical versus basilar dendrites for any of these regions.

Sensory Modality. When the total number of dendritic spines (both pedunculated and simple, on apical and basilar dendrites) was compared by sensory modality, the density of spines in the primary somatosensory area (avg S1= 1.309 spines/micron; ± 0.247 STD) was not statistically

different ($p=0.355$, t-test) from that measured for the primary auditory area (A1, avg 1.344 spines/micron; ± 0.273 STD). This comparison is depicted in Figure 4.

Unimodal versus Multisensory. When the total number of dendritic spines (both pedunculated and simple, on apical and basilar dendrites) was compared between the unisensory (S1, A1) and multisensory regions (PPr, LRSS), there was a statistically significant difference between the different unisensory and multisensory areas, as summarized in 5. Unisensory regions S1 (avg 1.309 ± 0.247 spines/micron) and A1 and (avg 1.343 ± 0.273 spines/micron) individually and collectively exhibited significantly higher spine densities than did their multisensory counterparts (PPr avg 1.242 ± 0.205 spines/micron; LRSS avg 1.099 ± 0.217 spines/micron)(t-test, $p<0.05$). Furthermore, the spine density values for the multisensory regions PPr and LRSS were statistically different ($p<0.05$, t-test) from each other.

Hierarchical Level. When the total number of dendritic spines (both pedunculated and simple, on apical and basilar dendrites) was compared between the lower level primary sensory cortices (S1, A1) and the higher-level multisensory regions (PPr, LRSS), there was a statistically significant difference (t-test, $p<0.05$). The lower level regions (avg= 1.327 spines/micron; ± 0.246 STD) exhibited higher spine densities than did their higher level counterparts (avg= 1.169 spines/micron; ± 0.222 STD).

DISCUSSION

The results of the present study did not support the hypothesis that multisensory cortical regions exhibit higher dendritic spine densities than their unimodal counterparts. The data showed that multisensory cortical neurons exhibited significantly lower spine densities than did those from unimodal cortical areas. The other major findings were that dendritic spine densities were significantly greater in low-level versus higher-level (hierarchical) cortices, but that no differences were detected among neurons that process different sensory modalities. These observations were obtained from layer 2-3 pyramidal neurons from normal, adult, age-matched male ferrets.

The observation that multisensory cortices had lower spine densities than their unimodal counterparts is counterintuitive to the assumption that, because of increased modality number (e.g., multisensory), there are an increased numbers of inputs. The present data question this assumption. First, it is not known if a multisensory area actually has an increase in the absolute number of inputs it receives when compared with a unisensory area. In fact, it would be very difficult to make such an assessment. One possibility might be to count the density of axons entering a multisensory versus a unimodal region by examining the white matter directly beneath them. However, these values would be influenced by the numbers of axons that exit the examined areas. Alternatively, if the absolute number of inputs were relatively the same across different areas, the relative number of modality-specific inputs would be inversely related to the number of sensory modalities involved. In this case, something must change in the neurons themselves to allow both unisensory and multisensory neurons to be activated by the same

sensory stimulus. It is completely unknown at this time how, or if, this happens. However, preliminary evidence from recordings in multisensory cortices (Meredith lab, unpublished data) show that multisensory neurons exhibit higher spontaneous discharge rates, and higher response rates, than do their unimodal counterparts. This observation would suggest that the threshold for action potential generation in multisensory neurons might be different from unimodal neurons, a condition that can be achieved by changing the membrane resistance of the neuron. It is well known from basic electrophysiology that neurons with higher resistances discharge to lower levels of synaptic or electrical inputs (Johnston et al., 1995). In this way, a neuron with reduced numbers of inputs from one modality would still, like its unimodal counterpart, be able to evoke a response from a given stimulus. This possibility needs to be developed further both in concept and experimentation.

Similarly, dendritic spine density varied by hierarchical level, with higher spine densities observed in lower-level (S1, A1) than in higher-level (PPr, LRSS) sensory areas. This observation actually complicates the interpretation of the unimodal-multisensory issue described above. Because both variables of modality number and hierarchy covary, it is not possible to distinguish them at this time. However, it is curious that although other studies have observed similar values for spine density in primary areas (Briner et al., 2010) and (Larkman 1991), higher measurements were obtained for higher-level frontal and orbital cortices (Nimchinsky et al 2002). The differences between the reported values for frontal/orbital areas and the present higher-level measurements may be due to the LRSS and PPr being from a different part (parietal) of cortex. It has been hypothesized that higher order processing and convergence results from the integration of more inputs per neuron (Elston 2000) and it is thought that one way to achieve this increase in inputs is to increase the density of spines along the dendrite (Ballesteros-Yanez,

et al 2002). However, the results of the present study show that the areas of higher order processing have lower spine densities. Further experiments are necessary to resolve this issue.

There was not a significant difference in the dendritic spine density measured in somatosensory, S1, and auditory, A1, cortices. These results indicate that spine density is not influenced by the sensory modality of the input received. This is consistent with essentially all of the previous work, where none of the reviewed studies indicated there would be changes in density between primary sensory modalities (Ballesteros-Yanez, et al 2002). This observation was further emphasized by the similar lack of difference in density of the different types of dendritic spine. Regardless of the modality of the cortical area examined, complex pedunculated spines always outnumbered the simple spines.

There were no differences observed when dendritic spine density was examined in relation to dendritic spine location: either apical or basilar segments of the dendrite. It was expected that dendritic location could affect dendritic spine density, because spines immediately around the soma is diminished while their density increased further along the apical dendrite (Valverde 1967). Nonetheless, for the cortical pyramidal neurons analyzed, the area of the dendrite was not a significant factor in the variance of dendritic spine density. However, this may be an indication of the care that was taken to examine the same dendritic segments and segment lengths in the different populations of neurons (see Table 3).

For each neuron, the spine counting effort was concentrated on a relatively small sample of spiny segments in parallel with the plane of focus. This was the preferred method because it was difficult to reliably count the density of spines running perpendicular to the plane of focus. While this may indicate that the sample size was not representative of the entire neuron, it has

been shown previously that the branching patterns and morphology of particular types of dendrites maintain a high degree of stereotypy (Larkman 1991). Furthermore, it has been demonstrated that peak dendritic spine density in different cortical areas is found between 60 and 100 microns from the soma (Ballesteros-Yanez, et al 2002). This coincides with the average measurements in the present study. Therefore, the present spine density results as categorized by location and type are considered a strong representation for cortical pyramidal neurons.

CONCLUSIONS

In cortical neurons of normal, adult, age-matched, male ferrets, the dendritic spine density observed was similar to those observed in cortical areas of other species. However, in the present study, the density of dendritic spines was compared for representations of different sensory modalities (which did not vary), for hierarchical level (which did vary), and for unimodal versus multisensory representation (which did vary). Furthermore, the dendritic spine density was significantly higher in unimodal than multimodal areas. Therefore, although dendritic spine density has been demonstrated to be sensitive to a variety of conditions, the particular sensory modality of a given region does not appear to be an influential factor.

FIGURES

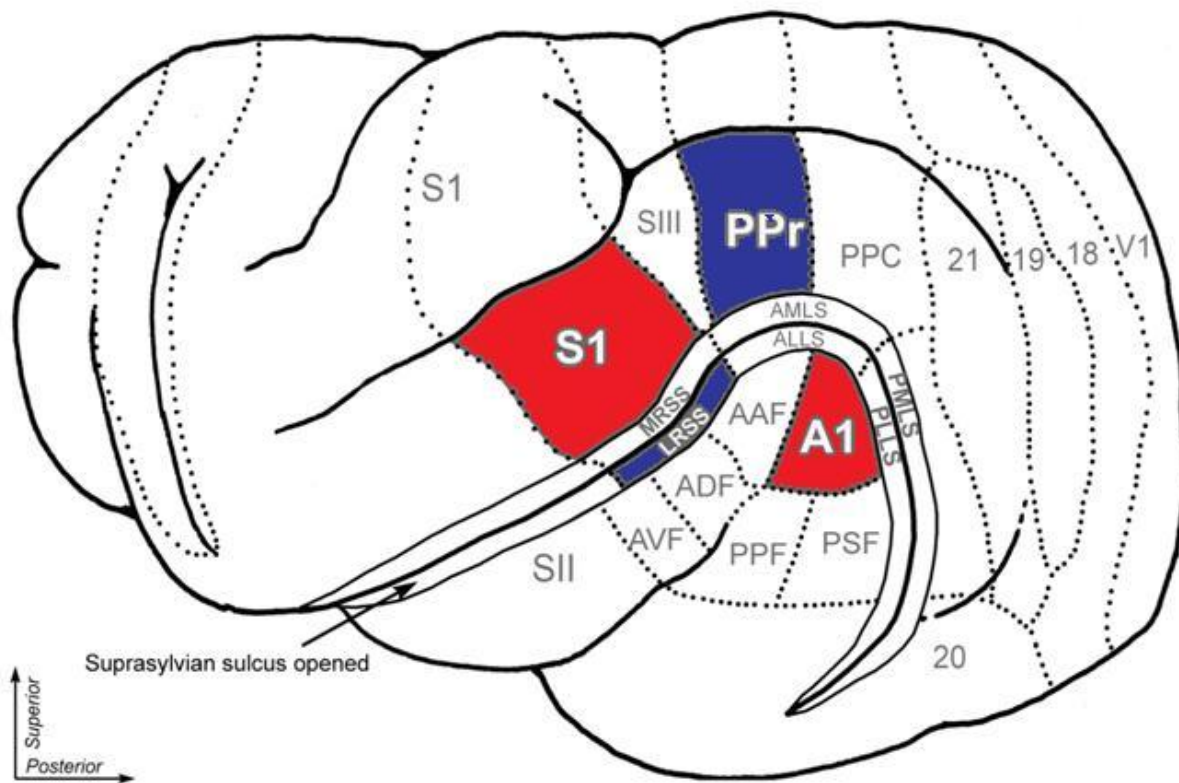


Figure 1. Lateral view of ferret brain with the functional subdivisions marked by dotted lines. The locations of the primary cortical sensory areas S1, A1 (colored red) and the multisensory cortical regions PPr, LRSS (colored blue) emphasize the regions examined in this study.

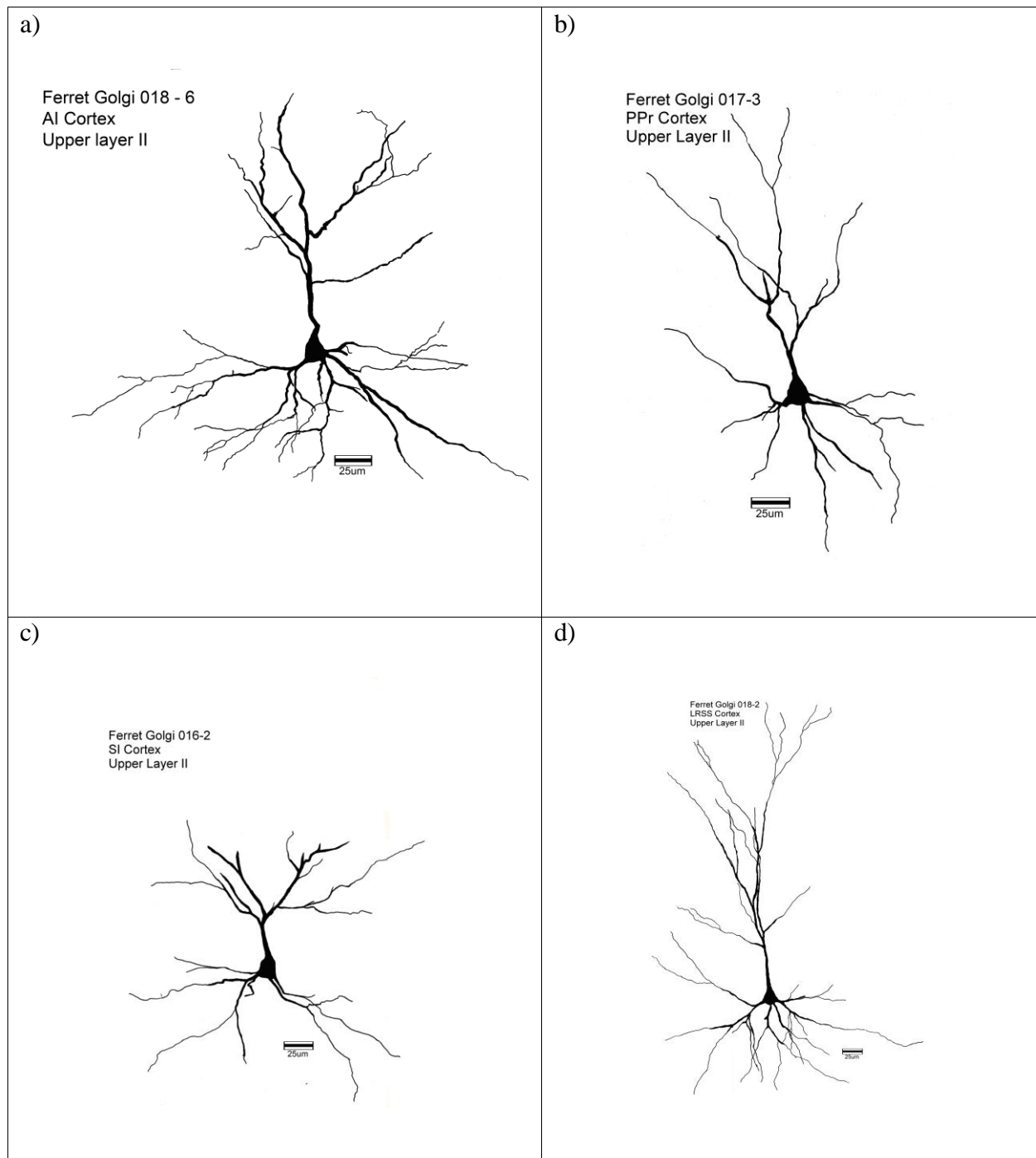


Figure 2. Camera-lucida renderings of representative Layer 2-3 pyramidal neurons from cortical areas a) A1, b) PPr, c) S1, d) LRSS. Scale bar = 25 microns.

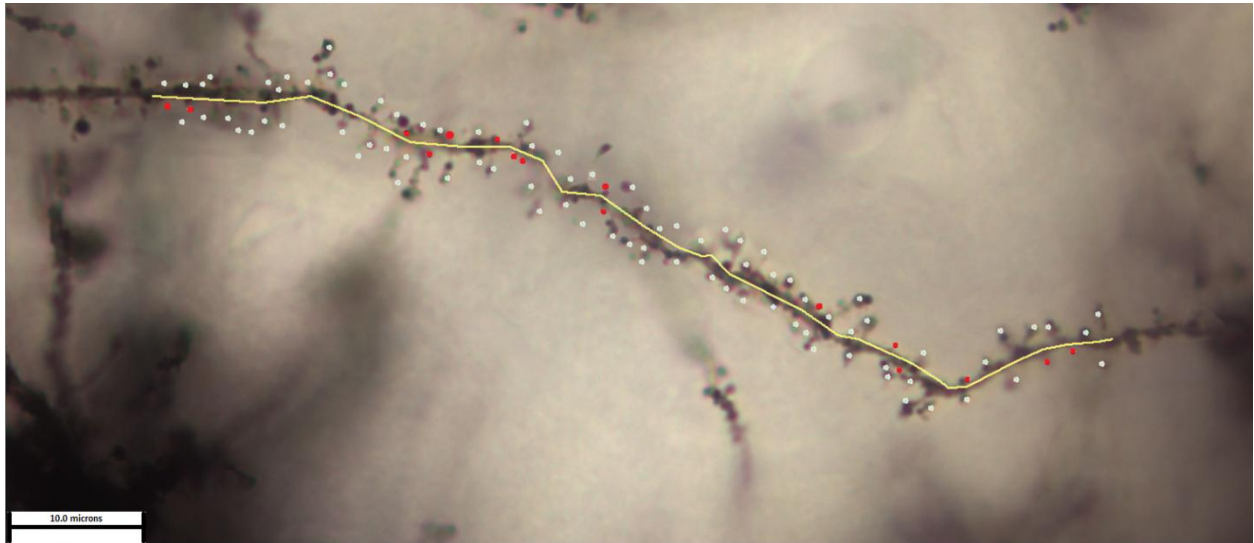


Figure 3. A photomicrograph (100x, oil) of a basilar dendrite (indicated by yellow line, from boxed area in Figure 2) exhibiting dendritic spines. White markers = Pedunculated Spines, Red markers = Simple spines. Scale = 10 microns.

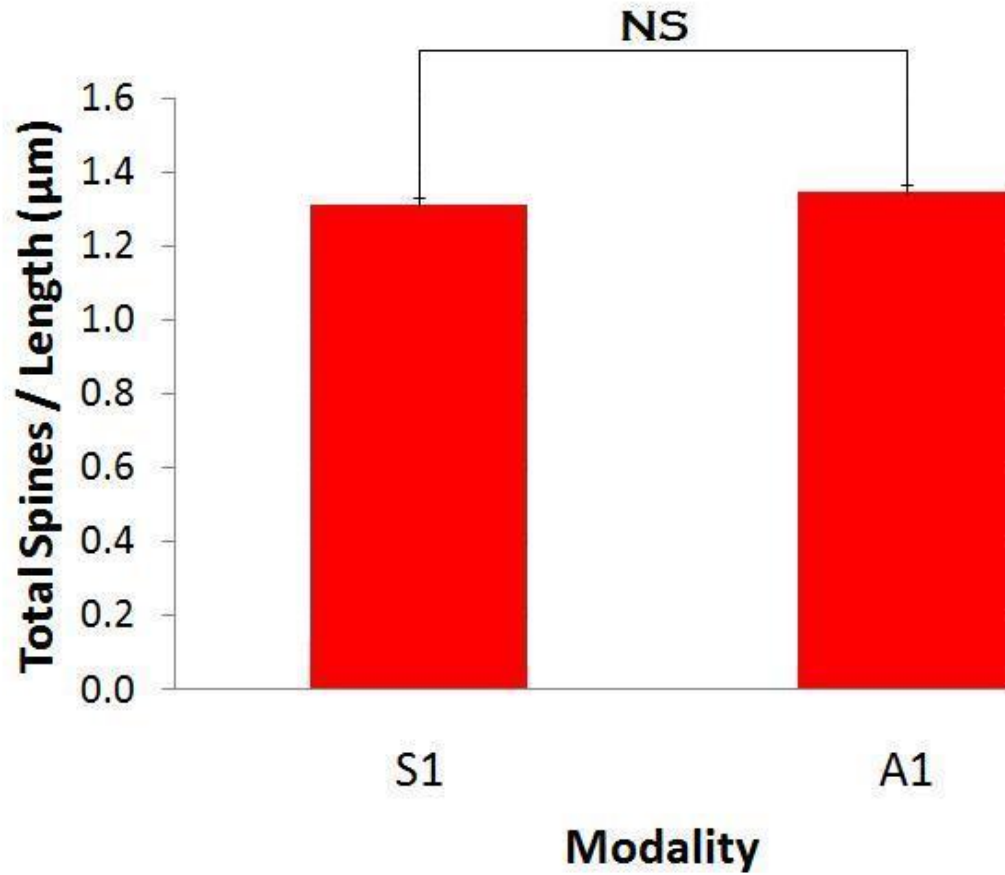


Figure 4. Dendritic spine density (spines/micron) does not vary between sensory modality representations. S1=primary somatosensory cortex; A1=primary auditory cortex. NS= not significant; t-test $p > 0.05$. Error bars = standard error of the mean.

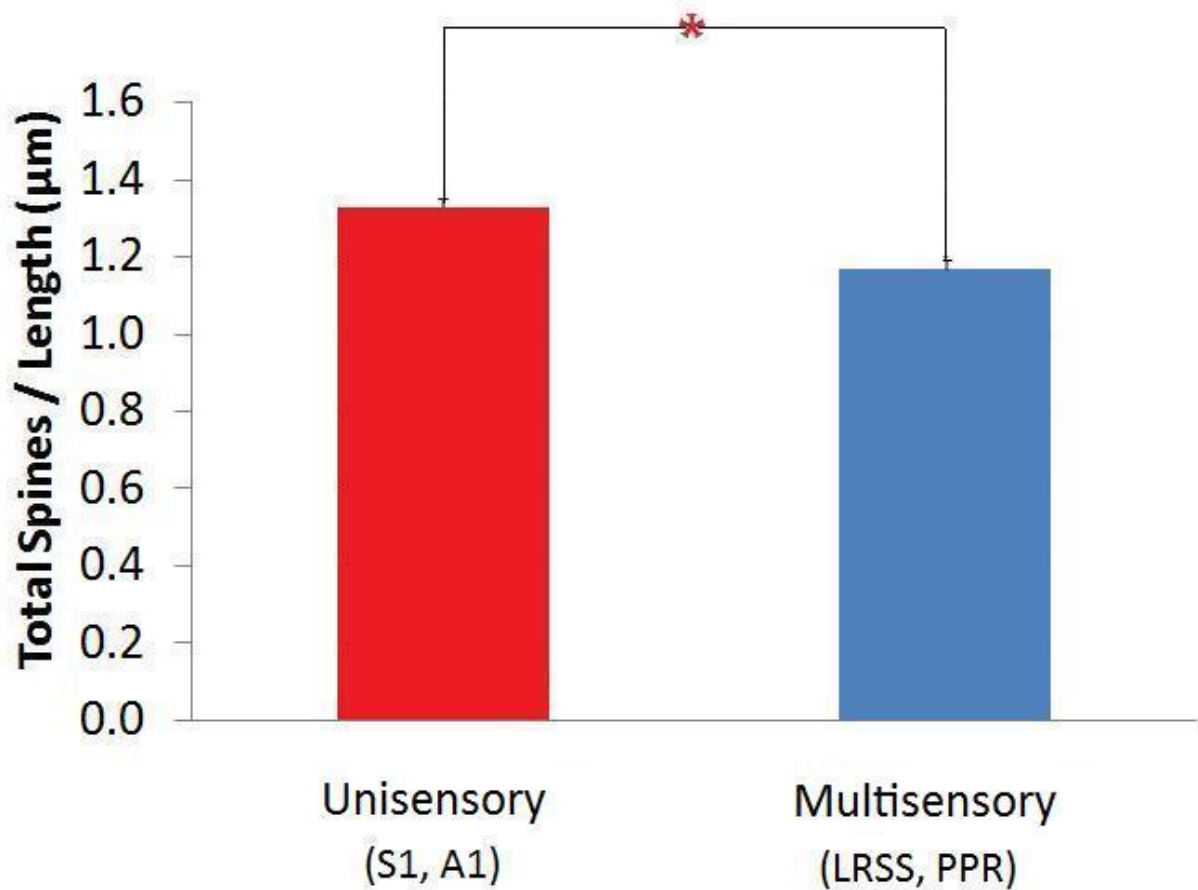


Figure 5. Dendritic spine density varies significantly (*, $p < 0.05$, t-test) between unimodal (S1, A1) and multisensory (PPr, LRSS) cortical regions. In addition, spine density also varies significantly between the two multisensory regions (PPr vs LRSS). Error bars = standard error of the mean.

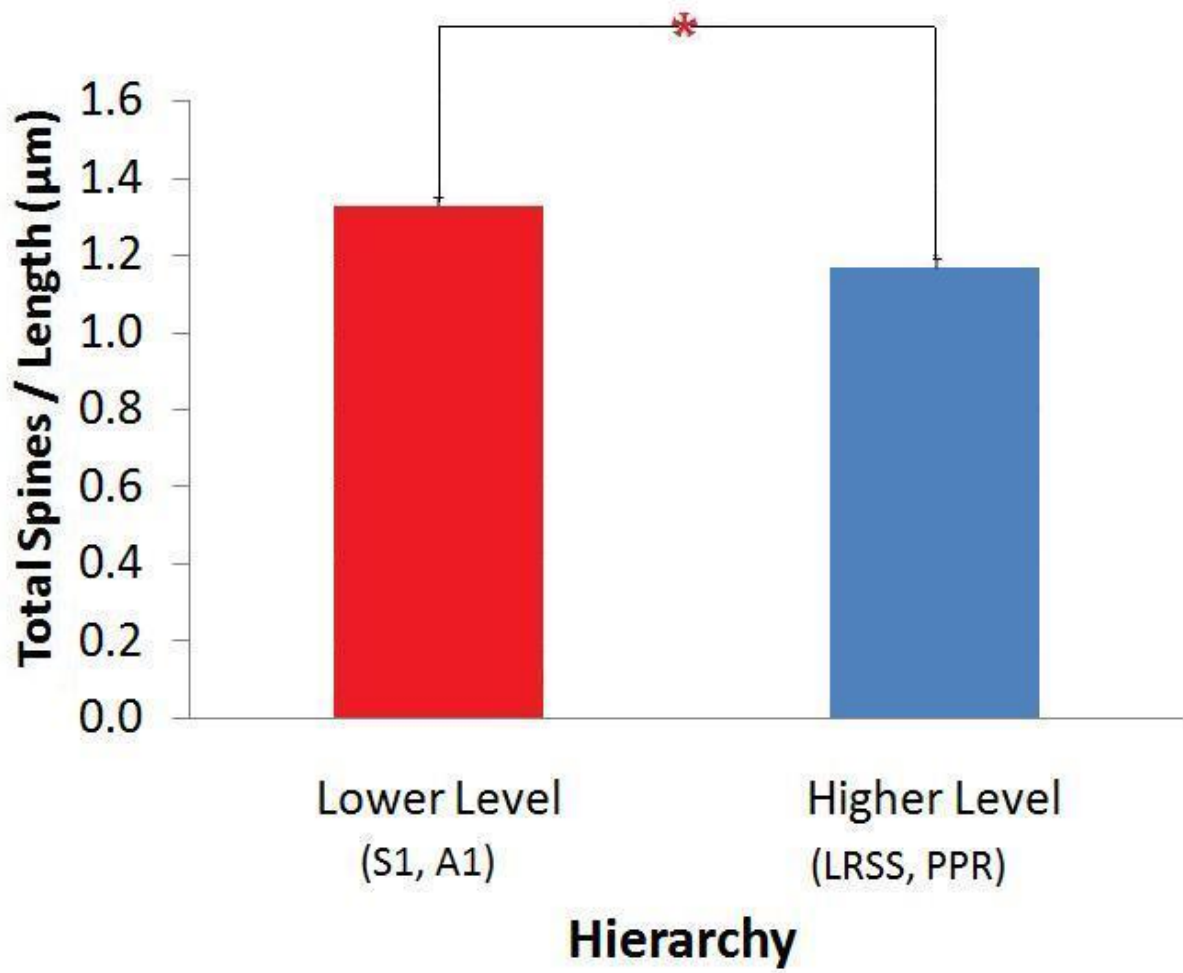


Figure 6. Graph of spine density comparison based on hierarchal order of coritcal areas. Error bars = standard error of the mean.

TABLES

TABLE 1

Ferret ID	Age (days)	Weight (kg)	Sex
F09016	135	1.3	Male
F09017	126	1.3	Male
F09018	134	1.6	Male

Table 1. Animal characteristics

TABLE 2

Cortical Region	Cases	Neurons	Avg Soma Area	Dendrites
S1	3	17	181.55 (\pm 34.92)	93
A1	3	18	175.75 (\pm 25.69)	100
PPr	3	18	205.59 (\pm 27.77)	94
LRSS	3	17	208.94 (\pm 58.86)	97

Table 2. Statistics for animals, neurons and dendrites included in sample.

TABLE 3

Cortical Region	Average Measured Apical Branch (μm)	Avg # Apical	Average Measured Basilar Branch (μm)	Avg # Basilar
S1	83.26 (\pm 14.81)	1	78.33 (\pm 12.73)	3.71
A1	76.30 (\pm 13.86)	1	75.03 (\pm 13.88)	2.72
LRSS	77.26 (\pm 13.87)	1	79.82 (\pm 13.36)	3.41
PPr	80.63 (\pm 12.88)	1	80.91 (\pm 13.31)	3.39

Table 3. Statistics for dendrites included in sample.

TABLE 4

Area	Average Total Spine/Length (μm)	Average Pedunculated Spine/Length (μm)	Average Simple Spine/ Length (μm)
S1	1.309 (\pm 0.247)	1.077 (\pm 0.215)	0.232 (\pm 0.076)
A1	1.343 (\pm 0.273)	1.115 (\pm 0.258)	0.229 (\pm 0.066)
LRSS	1.099 (\pm 0.217)	0.893 (\pm 0.205)	0.205 (\pm 0.083)
PPr	1.242 (\pm 0.205)	1.043 (\pm 0.199)	0.199 (\pm 0.075)

Table 4. Average (and standard deviation) spine density measures by region according to spine type.

TABLE 5

Dendritic Location	Average Total Spine/Length (μm)	Average Pedunculated Spine/Length (μm)	Average Simple Spine/ Length (μm)
Apical	1.261 (\pm 0.114)	1.041 (\pm 0.109)	0.220 (\pm 0.012)
Basilar	1.237 (\pm 0.103)	1.025 (\pm 0.087)	0.212 (\pm 0.021)

Table 5. Average (and standard deviation) spine density measures by dendritic location according to spine type

TABLE 6

Cortical Region	Average Total Spine per Length (μm)		
	Total	Apical	Basilar
S1	1.309 (± 0.247)	1.328 (± 0.248)	1.288 (± 0.246)
A1	1.344 (± 0.273)	1.345 (± 0.315)	1.343 (± 0.216)
LRSS	1.099 (± 0.217)	1.094 (± 0.219)	1.104 (± 0.217)
PPr	1.242 (± 0.204)	1.276 (± 0.198)	1.214 (± 0.208)
ALL	1.249 (± 0.108)	1.261 (± 0.114)	1.237 (± 0.103)

Table 6. Average (and standard deviation) spine density measures (both pedunculated and simple) by dendrite location.

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VITA

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