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Multisensory Input to the Lateral Rostral Suprasylvian Sulcus (LRSS) in Ferret

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MULTISENSORY INPUT TO THE LATERAL ROSTRAL SUPRASYLVIAN SULCUS (LRSS) IN FERRET

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

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ABSTRACT

MULTISENSORY INPUT TO THE LATERAL ROSTRAL SUPRASYLVIAN SULCUS (LRSS) IN FERRET

By Elizabeth White Hagood, B.A.

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For the brain to construct a comprehensive percept of the sensory world, information from the different senses must converge onto individual neurons within the central nervous system. As a consequence, how these neurons convert convergent sensory input into multisensory information is an important question facing neuroscience today. Recent physiological studies have demonstrated the presence of a robust population of multisensory neurons in the lateral bank of the rostral suprasylvian sulcus (LRSS) in adult ferret (Keniston et al, 2008). The LRSS is a region situated between somatosensory and auditory cortices, where bimodal (somatosensory-auditory) neurons occupy the greatest percentage of the sensory-responsive cell population. The present study was designed to

evaluate the anatomical connections that underlie these multisensory features. Injections of neuroanatomical tracer were first made into the LRSS. After transport and histological processing, microscopy revealed retrogradely-labeled cell bodies in identified regions of cortex and thalamus. The resultant analysis showed that the greatest number of projections to LRSS originated in auditory and somatosensory cortex. Of these, auditory cortex contributed a greater proportion of inputs. These anatomical data support the idea that LRSS is a multisensory cortex that receives primarily bimodal input from auditory and somatosensory sources.

INTRODUCTION

Our notions of the senses and the manner in which distinct environmental stimuli are processed in the brain have been largely shaped by the idea that the sensory pathways exist as independent transit systems for dedicated signals, and that very little overlap occurs between them. This mode of thinking is thoroughly engrained in our understanding of neuroscience, and has been reinforced by textbooks and even by the laboratory experiments that seek to better understand the underlying phenomena of sensory processing from the level of transduction to perception.

In the external environment, events occur constantly. These events effect changes in light, airborne and ground vibrations, or the dispersal of particulate matter in air or water. Furthermore, they can be categorized according to the specific type of energy that they represent. Photons in a beam of sunlight and a sound wave from a barking dog are distinct forms of energy that exist separately, meaning that the presence of one event has no bearing on the presence of another. Additionally, each of the sensory systems is 'tuned' by its particular receptor to sample only a specific segment of the variety of environmental 'energies.' For example, the retina is sensitive only to photic energy at specific wavelengths along the electromagnetic spectrum, and the cochlea is sensitive only to airborne vibrations of specific frequency. However, this energy-specific segregation of stimuli does not continue once these inputs are relayed into the central nervous system. In numerous areas of the brain, inputs from different sensory modalities converge on

individual neurons to produce a unique pattern of activity that is unlike the signal received from those modalities in isolation. Thus, convergence of inputs from these different sensory modalities gives rise to multisensory processing, and it will be shown here that this convergence produces interactions between modalities that do not occur in the natural environment. Given that this integration of responses to different sensory stimuli only occurs upon arrival in the CNS, multisensory processing is an emergent property of the brain.

In the context of these issues, a definition of multisensory processing is necessary. Multisensory processing is the neural condition through which inputs or responses to one sensory modality are influenced by the presence of another sensory modality. In macroscopic terms, this phenomenon is readily apparent in the events of daily life. For example, the ventriloquist effect (Howard and Templeton, 1966) occurs every time we watch a movie or television, whereby we perceive that the conversation that we are hearing is actually emanating from the image of the speaker's lips (and not from the loudspeaker under the screen). The ventriloquism effect subscribes to the idea of intersensory bias, in which the perception of a stimulus from one modality is accompanied and altered by the presence of a stimulus from another. The result of these simultaneous processing events is often a bias towards the perception of one sensory stimulus. The extent to which an intersensory bias will exist depends on the intensities of the respective stimuli and their spatial and temporal presentation with respect to one another (Welch and Warren, 1986). The most classical example of this phenomenon is the simultaneous presentation of visual and auditory stimuli that arise from slightly different spatial origins (as in the case of a

ventriloquist, in which the skilled entertainer will throw his voice such that it appears visually to originate at the mouth of the dummy). The audience of such an experiment perceives that both stimuli arise from the visual stimulus. This intersensory manipulation is the basis of the so-called ventriloquism effect, which is but one example among a host of multisensory effects.

Another practical example of multisensory integration is the cocktail party effect. This is the scenario in which a conversation takes place in a noisy environment, making it difficult to discern what a friend is saying. In such a situation, interpretation of speech is aided by visualization of the speaker's face. Neuroimaging studies have confirmed this to be true in demonstrating that activity in auditory cortex is enhanced by the sight of lip movements (Sams et al, 1991). A corollary of this finding was shown in the work of McGurk and MacDonald (1976). Their study of non-matching visual and auditory stimuli is further proof that multisensory integration of these distinct cues can generate a consequently unique sensory percept. For example, when one sees a video image of a person saying the phrase, "Ga-ga," superimposed with an auditory track that concurrently plays "Ba-ba," the percept is a fusion of the two, giving "Da-da" as the perceived phrase.

Perhaps the most clinically relevant example of multisensory processing is found in the phenomenon of sensory compensation. Sensory compensation can be defined as the substitution of inputs for a deficient sensory system with inputs from another operative modality. This takes place via the adaptive processes of neural plasticity, by which both physiological and anatomical changes in neuronal structure and activity occur. For instance, in individuals with congenital deafness, sign language (a visual cue) can trigger

the activation of supposedly dedicated regions of auditory cortex, as seen with positron emission tomography (PET) (Nishimura et al, 1999; Petitto et al, 2000). Similarly, Braille reading can generate activity in the visual cortices of the blind but not seeing subjects (Sadato et al, 1996; Sathian et al, 1997).

Yet another multisensory phenomenon is that of cross-modal matching. This is the task in which a subject must match two objects presented to him using cues from two different sensory channels. The first stimulus might be a visual image of the object to be identified, which is followed by the blindfolded task of identifying that same object on the basis of its tactile qualities only (Stein and Meredith, 1993). Animal studies demonstrate that an intact amygdala is important for this task, since monkeys trained to execute cross-modal masking lose that ability if the amygdala is damaged (Murray and Mishkin, 1985).

To study the mechanism by which cues from different sensory channels are integrated to form the unique sensory percepts described above, the midbrain structure of the superior colliculus has served as a productive model. The superior colliculus is structurally and functionally unique in that it contains neurons within its deeper laminae that respond to visual, auditory, and somatosensory stimuli. The overlapping receptive fields for each of these sensory channels make the superior colliculus particularly well-suited to the study of multisensory processing. The physiological activity of multisensory neurons in the superior colliculus is altered drastically by the concurrent arrival of combined visual, auditory, or somatosensory stimuli, and this, in turn, can generate altered patterns of neural activity known as response enhancement and response depression. These multisensory interactions will be discussed subsequently in terms of their

physiological significance, but it should be noted at present that the multisensory activities of neurons in the superior colliculus generate behavioral adaptations that are due to the multisensory phenomena occurring there. The superior colliculus in general is responsible for mediating behaviors relating to orientation and attention. When presented with a stimulus, be it auditory, visual, or somatosensory, the superior colliculus directs the animal's attention and bodily orientation towards that point in the surrounding space. As can be deduced from this information, the function of the superior colliculus is crucial to the development of survival strategies once a threat is perceived and its location identified. Early work of the 1960's demonstrated that lesions to the superior colliculus induced contralateral visual neglect of extrapersonal space surrounding test animals (Sprague and Meikle, 1965; Schneider, 1967, 1969).

Other regions in the mammalian brain have also been identified as sites of multisensory integration, such as the superior temporal sulcus (STS). The STS is known as a site of auditory and visual sensory convergence, and it has been likewise implicated in mediating cross-modal interactions between these two modalities. Silent reading, or lip reading, is an activity in particular that has been found to elicit cross-modal facilitation in STS (Calvert et al, 2000) by virtue of the fact that it involves the concurrent processing of auditory and visual cues.

Multisensory processing is an important factor in our daily responses to demanding situations and helps to promote more rapid physiological reactions under stress. Multisensory effects are known to cause a decrease in reaction time after exposure to two coincident stimuli. For instance, when visual and auditory cues are presented in close

proximity to one another, the combined stimuli will produce a faster response than either of the same stimuli presented independently (Bell, Munoz, Corneil, and Meredith, 2003). This effect is in accord with physiological evidence for multisensory response enhancement, which proffers that two stimuli can generate a multiplicative response when presented together, provided that the stimuli are delivered to the target neuron in an appropriate spatial and temporal pattern (Meredith and Stein, 1983).

From an evolutionary perspective, multisensory processing confers an adaptive advantage upon those organisms that exhibit it. Patterns of sensory convergence have been identified in numerous phyla, including organisms such as the paramecium, crayfish, moth, rattlesnakes, frogs, rodents, carnivores, and humans (Stein and Meredith, 1993). In the face of a threatening stimulus, it is to the organism's benefit that multiple sensory pathways exist. In this manner, the animal can rely on one sensory system in the event that another is compromised, or the animal may perceive a heightened signal of the sensory event at hand. The convergence of multiple stimuli upon a single neuron ultimately translates to a behavioral response that enhances survival strategies for predator and prey alike.

Given these many and different examples, it is important to recognize that multisensory processing underlies many of the most frequent and indispensable activities of daily life. In a more sophisticated sense, multisensory processes are the mechanism by which stimuli from disparate sensory sources are synthesized into a single perceptual fabric that forms our impressions of the world.

It is currently known that multisensory convergence occurs in locations throughout the central nervous system in higher organisms, from the spinal cord to the neocortex. Multisensory processing has been shown to occur between all sensory modalities and has been identified thus far in all organisms that possess nervous systems (Stein and Meredith, 1993). In addition to its evolutionary and adaptive significance, the study of multisensory processing has important potential applications in both clinical settings and in technology. For example, in disease states that induce sensory losses, multisensory areas of the brain can function to compensate for these deficits through the phenomenon of cross-modal plasticity, discussed previously. This is most widely cited in the case of individuals who develop blindness. Without visual input, areas of visual cortex are observed to transition to tactile responsivity, as in the reading of Braille type (Sadato et al, 1996). This example of cross-modal plasticity between visual and somatosensory cortices is but one instance of this phenomenon, which has a high degree of clinical applicability in the context of sensory losses. Recent work has shown that auditory cortex can make a similar transition to tactile responsiveness in deafened adult ferrets (Allman et al, 2009). In addition, multisensory circuitry may prove important in the development of robotic technologies that are aimed at simulating brain functions to study neurodegenerative disorders such as Alzheimer's disease (Marks, 2008).

At the neuronal level, multisensory processing has distinctive functional features. Physiological studies have done much to elucidate the patterns of activity that are elicited from multisensory neurons when they receive stimulation from multiple sensory modalities. It is well known that multimodal stimulation of a neuron produces a distinct

pattern of activity that cannot be predicted according to the stimulation of that neuron by a single modality (Meredith and Stein, 1983). Early work in the superior colliculus was instrumental in discerning the response properties of multisensory neurons when those cells encountered multimodal stimulation. Multisensory neurons can be distinguished from unimodal neurons (those that are responsive to stimulation from only one modality) in that they contain multiple receptive fields that are each responsive to stimulation from a single sensory modality. Consider, for example, a bimodal multisensory neuron that is responsive to input from two sensory channels (for instance, auditory and visual). If both stimuli fall within the neuron's excitatory receptive fields for these modalities, and are properly coincident in terms of their spatial and temporal arrival, then the combination will likely generate a multisensory interaction known as response enhancement. Response enhancement occurs when two distinct stimuli fall within their receptive fields upon convergence in a single neuron, producing a substantial increase in the number of discharges generated. This produces a multiplicative, or super-additive, response that is typically much greater than the sum of the two responses alone. In this manner, the multisensory response that is generated is very much non-linear (Meredith and Stein, 1986). In addition to this characteristic, the response enhancement is proportionally greatest when the stimulation of either sensory channel alone is just above threshold, or minimal (Meredith and Stein, 1983). Response depression is also an observed effect of multisensory convergence. The outcome of response depression can be inferred from its name; the result is a decrease in the number of elicited discharges. Experimental evidence suggests that this phenomenon occurs when one stimulus falls outside of its receptive field

and strikes an inhibitory surround. This has most often been seen in neurons that are highly responsive to stimulation from one particular modality (usually the visual modality), but are nearly unresponsive to stimulation from others (often the auditory or somatosensory modalities). The result upon combining the stimuli is a great reduction in the overall rate of discharge, which can be attributed to inhibition of the effective stimulus by the ineffective one(s) (Meredith and Stein, 1983). Whether or not the multimodal stimuli arrive in ideal spatial and temporal register will determine if a multisensory interaction is to occur at all, and these factors merit further consideration here. A multisensory interaction is more likely to occur when the multimodal stimuli arrive in close temporal proximity to one another than when they are separated by larger breaks in time. The likelihood that a multisensory interaction will occur is also favored when the different stimuli originate at closer spatial locations (Meredith, 2002). These spatial, temporal and physical constraints on multisensory integration are regarded as the underlying physiological principles of multisensory processing.

Multisensory processing is defined by a fundamental and prerequisite step, which is the convergence of information from different sensory pathways onto individual neurons. The body of research on multisensory integration contains surprisingly little information on the actual neural and membrane properties that underlie multisensory convergence. Electron microscopy has yielded only one documented example of convergence at the neuronal level, in the cochlear nucleus. There, trigeminal synapses are found in contact with the membrane of neurons in the dorsal cochlear nucleus (Shore et al, 2000). Most other studies identify inputs from different sensory modalities that converge into a

common brain region, a pattern of integration called areal convergence. Notable convergent regions include the superior colliculus in the midbrain (Wallace et al, 1993) and the superior temporal sulcus (Barraclough et al, 2005), which were noted previously in the context of the behavioral consequences of multisensory processing.

The goal of the present experiment was to identify a region of cortex in adult ferret that receives convergent multisensory information and then document the sources of those inputs. This effort was guided by physiological experiments that have identified multisensory responses in ferret cortex. Preliminary studies in ferret suprasylvian cortex (Keniston et al, 2008) showed the lateral portion of the rostral suprasylvian sulcus (LRSS) to exhibit a large proportion of multisensory neurons. This area of cortex was targeted as a potential multisensory zone due to its location between adjacent representations of audition and somatosensation (see Figure 1). In the LRSS, bimodal multisensory neurons were frequently identified. These neurons responded to the presentation of either a somatosensory stimulus or an auditory stimulus presented alone (see Figure 2) and frequently showed a significant response change (integration) when the same stimuli were combined. In addition, subthreshold multisensory neurons were also identified, which were vigorously activated by only a somatosensory stimulus, but had that response modulated when combined with an otherwise ineffective auditory stimulus.

Approximately 60 percent of the sensory-responsive neurons in LRSS demonstrated some form of multisensory response. Of these, approximately 80 percent were bimodal neurons, while 20 percent were unimodal somatosensory neurons with subthreshold auditory effects (Keniston et al, 2008). Collectively, these multisensory

proportions render the LRSS one of the densest multisensory areas known in the brain. Other cortical regions contain approximately 20 to 30% multisensory neurons, such as the auditory field of the anterior ectosylvian sulcus (FAES, Meredith and Allman, 2009; Carriere et al., 2007), posterolateral lateral suprasylvian visual area (PLLS, Allman and Meredith, 2007), and the rostral suprasylvian auditory area (RSp, Clemo et al., 2007). In addition, the archetypal multisensory area of the midbrain, the superior colliculus, contains approximately 55% multisensory neurons (Meredith and Stein, 1986). Therefore, the high proportion of multisensory neurons in the ferret LRSS makes it a likewise attractive site for examination of the properties of multisensory convergence.

Ferret sensory cortex has been analyzed in some depth in recent years, which has promoted the analysis of the sensory regions and their borders (see Figures 1, 3, and 4). Most anteriorly, the prefrontal cortex has been demarcated as the area immediately anterior and posterior to the presylvian sulcus, including portions of the orbital, anterior sigmoid, posterior sigmoid, and coronal gyri (Duque et al, 2009). Primary motor cortex in ferret has not been extensively studied, but it thought to be concentrated in the anterior sigmoid, posterior sigmoid, and coronal gyri toward the anterior pole of the brain. Somatosensory cortex has been defined on the posterior sigmoid, coronal/suprasylvian, and anterior ectosylvian gyri. These gyri contain the functional zones of the primary somatosensory cortex (SI), secondary somatosensory cortex (SII), third somatosensory area (SIII), and the posterior parietal rostral area (PPr) (Leclerc et al, 1993). Auditory cortex, meanwhile, is concentrated on the ectosylvian gyrus, including the anterior, middle, and posterior ectosylvian gyri. These gyri house the functional regions for audition, including the

anterior ventral field (AVF), the anterior dorsal field (ADF), anterior auditory field (AAF), primary auditory cortex (AI), posterior pseudosylvian field (PPF), and the posterior suprasylvian field (PSF) (Bizley et al, 2005). Finally, visual cortex has been identified on the lateral and suprasylvian gyri and includes the posterior parietal rostral area (PPr), the posterior parietal caudal area (PPc), the anteromedial and anterolateral lateral suprasylvian sulci (AMLS and ALLS, respectively), the posteromedial and posterolateral lateral suprasylvian sulci (PMLS and PLLS, respectively), and visual areas 17, 18, 19, 20, and 21 (Manger et al, 2002). Finally, thalamic nuclei in ferret have been assessed and identified as well (see Figure 5). These include the pulvinar (Pul), posterior nucleus (Po), ventral anterior nucleus (VA), ventrobasal complex (Vb), reticular nucleus (Ret), lateral posterior nucleus (LP), medial geniculate nucleus (MG), and the A, A1, and C lamina of the lateral geniculate nucleus (LG) (Manger et al, 2002).

Given the established sensory organization of the ferret cortex and the preliminary electrophysiological data, the multisensory area of the ferret LRSS was examined using neuroanatomical tracing methods to determine the cortical and thalamic sources of its incoming multisensory information.

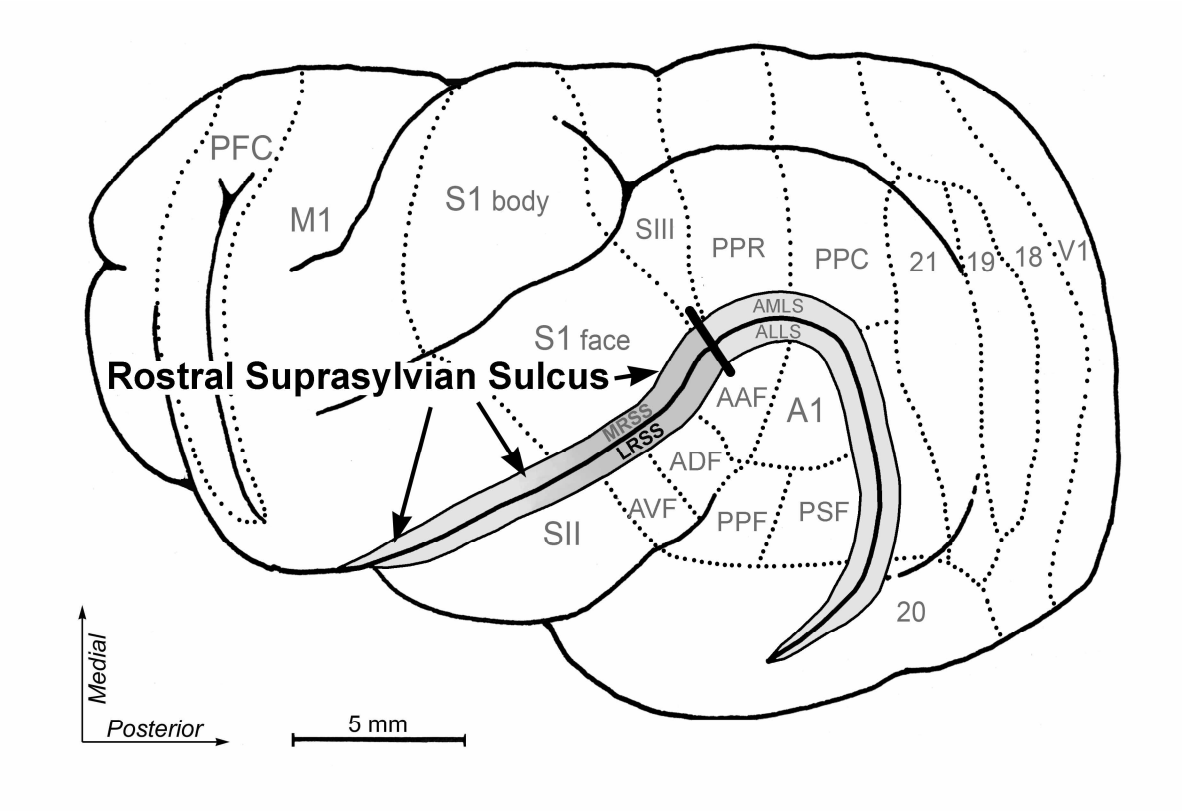

Figure 1. Lateral View of Ferret Cortex

Lateral aspect of the left hemisphere of the ferret brain with emphasis on the suprasylvian sulcus (shaded in gray). The rostral portion of the suprasylvian sulcus (RSS) is demarcated and subdivided into medial and lateral banks (MRSS and LRSS, respectively). Both MRSS and LRSS lie situated between somatosensory cortex (SI, face representation) and auditory cortex.

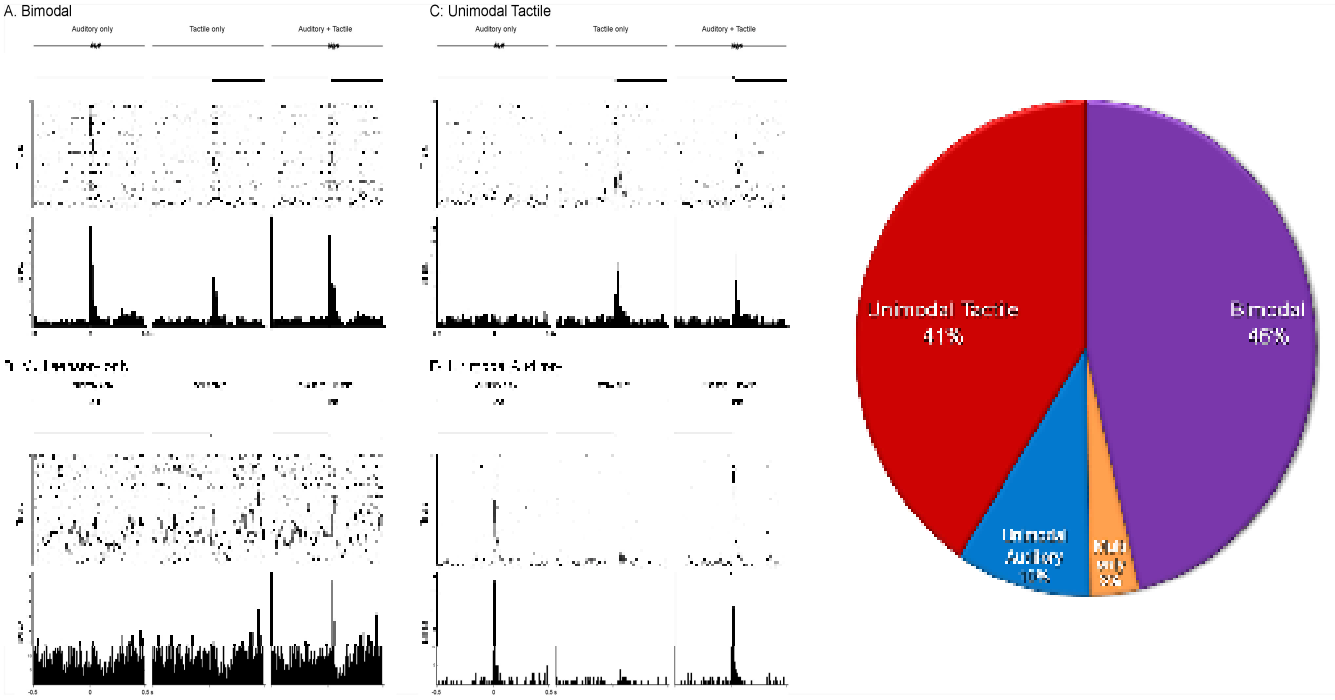

Figure 2. Breakdown of Sensory-Responsive Neurons in LRSS

The distribution of sensory-responsive cells in LRSS is shown, with the response activity of the individual neuronal types shown in the panels to the left. Bimodal neurons are shown in panel A, neurons classified as purely multisensory are shown in panel B, unimodal somatosensory (tactile) neurons in panel C, and unimodal auditory neurons in panel D. The proportional breakdown of cells by sensory response is also given in the chart at the right. (From Keniston et al, 2008)

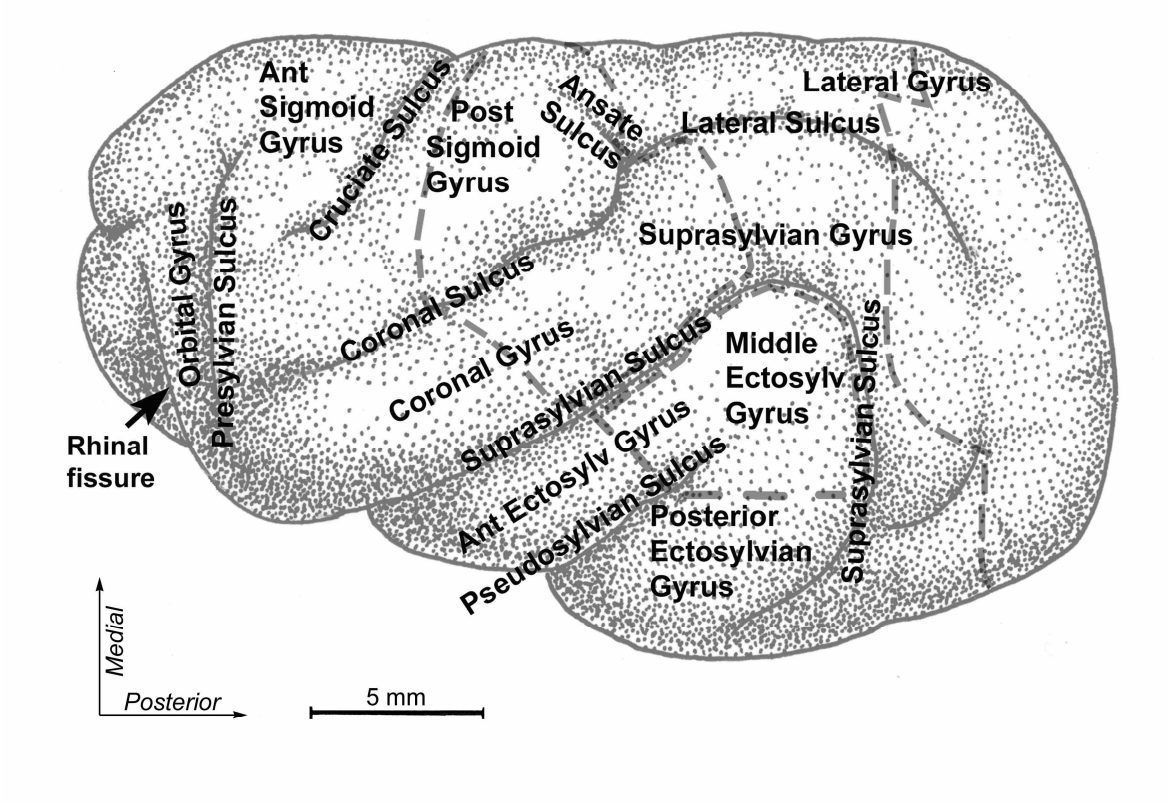

Figure 3. Lateral View of Ferret Cortex with Labeled Gyri and Sulci

Lateral aspect of the left hemisphere of the ferret brain with emphasis on the major gyri and sulci.

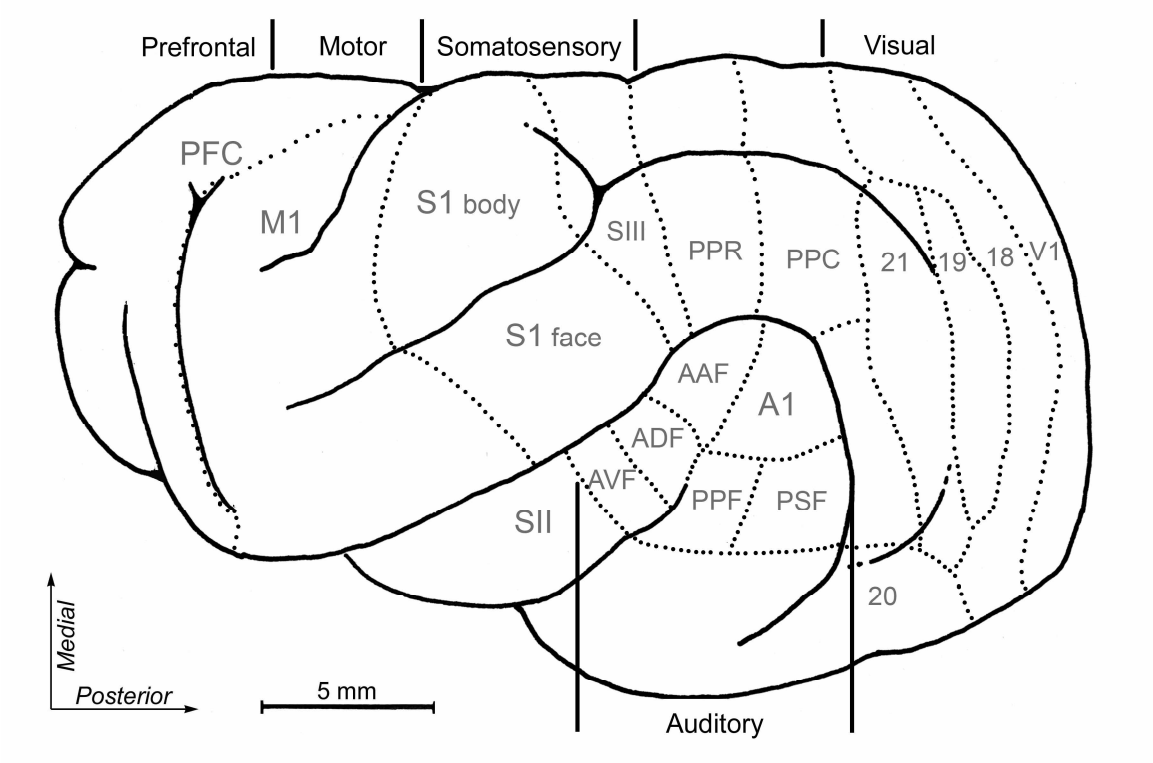

Figure 4. Lateral View of Ferret Cortex with Demarcation of Sensory Borders

Lateral aspect of the left hemisphere of the ferret brain shown with sensory areas indicated by vertical lines. Prefrontal cortex is shown at the anterior pole of the brain, followed by motor, somatosensory, auditory, and visual cortices. Functional areas within each of these sensory regions are indicated by dotted lines.

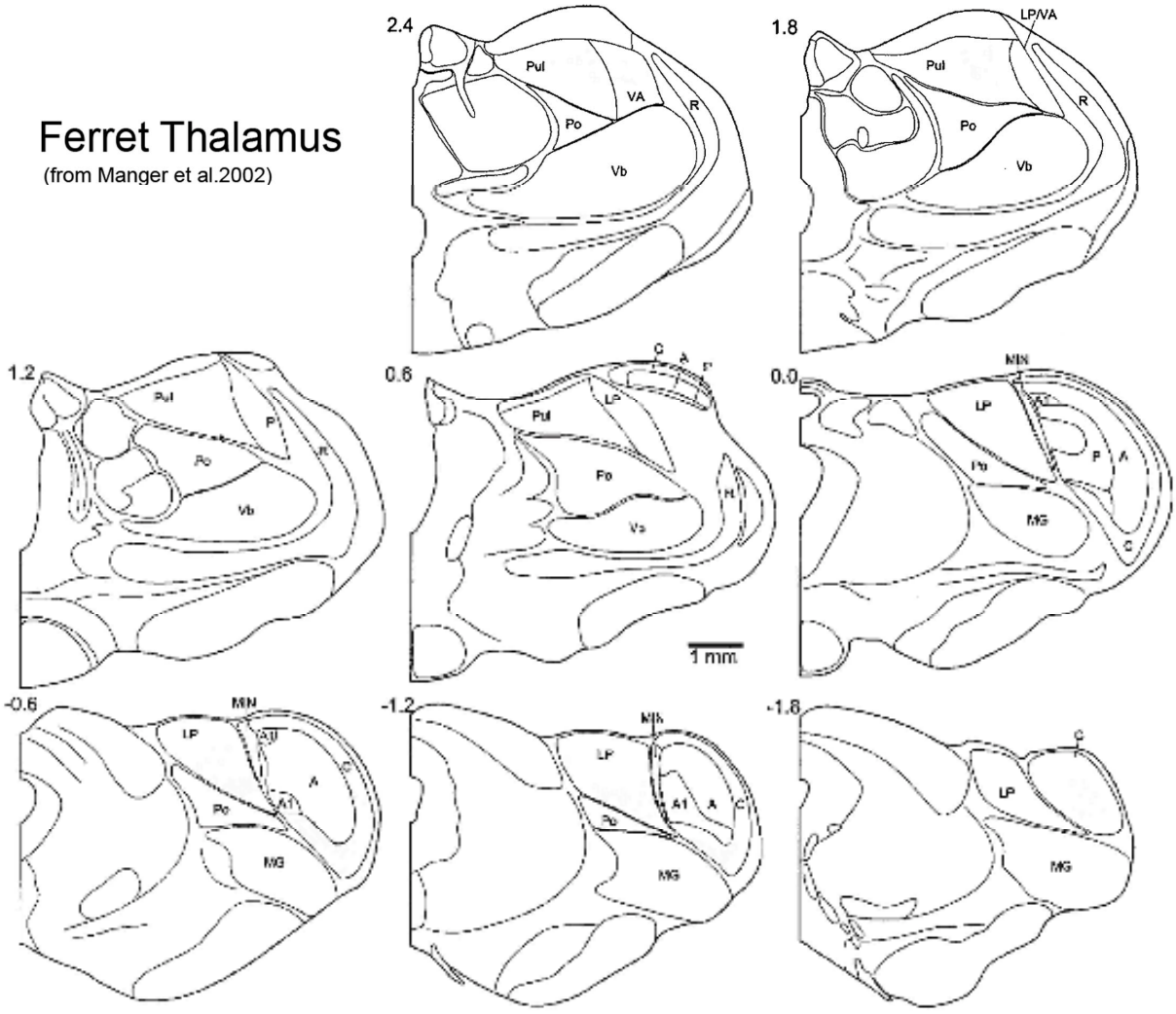

Figure 5. Representation of Thalamic Nuclei in Ferret

Serially arranged (anterior at top middle; posterior at bottom right) coronal sections of the ferret thalamus (From Manger et al, 2002). Shown are the pulvinar (Pul), posterior nucleus (Po), ventral anterior nucleus (VA), ventrobasal complex (Vb), reticular nucleus (Ret), lateral posterior nucleus (LP), medial geniculate nucleus (MG), and the A, A1, and C lamina of the lateral geniculate nucleus (LG).

MATERIALS AND METHODS

All procedures were performed in compliance with the Guide for Care and Use of Laboratory Animals (National Institutes of Health, publication 86-23), the National Research Council's Guidelines for Care and Use of Mammals in Neuroscience and Behavioral Research (2003), and the Institutional Animal Care and Use Committee at Virginia Commonwealth University. Data for the present study were derived from archived tissue obtained as follows below.

Surgical Procedures

Approximately seven days prior to surgery, pigmented ferrets (n=5) were anaesthetized with sodium pentobarbital (40 mg/kg) and their heads positioned in a stereotaxic frame. Under aseptic conditions, a craniotomy and durectomy were performed to expose the region of sulcal cortex between primary somatosensory cortex (SI) and the anterior auditory field (AAF), which corresponds to the target area, the lateral rostral suprasylvian sulcus (LRSS). A modified electrode carrier was used to support a Hamilton syringe (5 μl) and its needle (31-gauge), which was inserted at the targeted site to a depth between 1.0 mm and 2.0 mm. In order to examine the retrograde projections of LRSS, the neuroanatomical tracer biotinylated dextran amine (BDA; 3,000 mol. wt.; lysine fixable; 10% in 0.1 M phosphate buffered saline (PBS)) was pressure injected at a rate of 15-20

nl/min. BDA was selected for its appropriateness as a long-term retrograde and anterograde tracer and also for its low toxicity, solubility in water, and unique α-1,6-polyglucose linkages. After injecting the desired volume of tracer, the needle was retracted, the exposed area of cortex covered with gel foam, and the scalp sutured closed. Standard post-operative procedures were then followed.

Histological Processing

Following a 7-10 day survival period, the animals received a barbiturate overdose (120 mg/kg sodium pentobarbital) and were then intracardially perfused with heparinized saline followed by fixative (4.0% paraformaldehyde). The brain was then removed and cryoprotected in 25% sucrose in PBS (0.1 M) at 4˚C. Coronal sections were cut with a freezing microtome to a thickness of 50 μm and collected serially at 250-μm intervals. Visualization of BDA reaction product was achieved using the avidin-biotin peroxidase method (after the protocol of Veenman et al., 1992). Cut sections of tissue were rinsed in PBS and incubated overnight at 4˚C in an ABC kit (Elite; Vector Labs) with 0.3% Triton-X under gentle agitation. The following day, the sections were again rinsed in PBS followed by visualization of the peroxidase with a DAB reaction intensified with nickel-cobalt. The sections were rinsed and mounted on chrome-alum treated glass slides and then dehydrated with serial alcohol treatments in ascending concentration. The slides were then coverslipped without counterstain. An additional set of sections, at 250-μm intervals, was processed using standard histological techniques and then counterstained with cresyl violet to visualize cytoarchitecture and laminae.

Data Analysis

BDA-labeled neurons were visualized under a standard light microscope (Nikon Eclipse E-600) with a PC-driven digitizing stage controlled by Neurolucida software (MicroBrightfield, Inc., Williston, VT, USA). Selected sections were digitally traced, from anterior to posterior, at approximately 750-μm intervals. Tracings were completed using the Neurolucida software to plot the tissue outline, grey-white border, ventricle outline, injection site location, and labeled neurons. BDA-labeled neurons were clearly identifiable with distinctly black cell bodies and often darkly-stained dendrites. In some cases, BDA labeling was somewhat less pronounced but still quite visible. Tissue and ventricle outlines, the grey-white border, the injection site, and labeled neurons were all traced using a magnification of 10X. Tracking of labeled neurons by both number and distribution was accomplished by the Neurolucida software. These tracings were completed for each of five cases and included analysis of all cortical areas plus serial sections of thalamus for each case. Following completion of tracing procedures, the tracings were exported to a graphics program (Adobe Photoshop, Adobe Systems, Inc., San Jose, CA, USA) for arrangement and display. In correlating observed patterns of neuronal distribution to areas of functional significance, gyral and sulcal landmarks were used.

RESULTS

Tracer injections centered on the LRSS regions produced retrograde neuronal labeling, as depicted in Figure 6. These labeled neurons showed filled somata as well as dendrites. Dendrites were often filled far along their distal extents. Furthermore, there was a stark contrast between labeled neurons and the unlabeled neuropil, indicating that labeling was specific to neurons connected with the injection site.

As a consequence of the high signal-noise ratio provided by this technique, there was little ambiguity when plotting the data points (labeled neurons) on the tissue section reconstructions. A representative example of the tissue reconstructions from one case (FRSS9) is illustrated in Figure 7. These coronal sections, arranged serially from anterior (left) to posterior (right) for the hemisphere ipsilateral to the injection site (top row) as well as contralateral (bottom row) plot the locations of labeled neurons (1 black dot = 1 neuron) from an LRSS injection (black area). This figure shows that retrograde neuronal labeling occurred primarily in those areas closest to the injection site, and then progressively decreased at further anterior and posterior levels. Portions of the brain at its anterior and posterior poles that did not contain labeled neurons are not pictured. These data are described in detail below for case FRSS9 relative to the functional organization of the cortex.

Somatosensory Cortex

Retrogradely labeled neurons in somatosensory cortex were found throughout the posterior sigmoid, coronal/suprasylvian, and anterior ectosylvian gyri, as illustrated in Figure 8. Within these gyri, the functional regions of the primary somatosensory cortex (SI; body and face representations), secondary somatosensory cortex (SII), third somatosensory area (SIII), and the posterior parietal rostral area (PPr) are represented. BDA-labeled neurons were identified in each of these regions, but labeling appeared to be most concentrated in the sulcal areas between SI and SII, identified as the MRSS (Keniston et al., 2009a). In contrast, few labeled neurons were identified in somatosensory areas SIII or PPR.

Auditory Cortex

Of the cortical areas examined, projections to LRSS from auditory cortex were perhaps the most robust. Figure 9 shows the pattern of retrograde labeling observed in auditory cortex, which occurred in dense aggregates of neurons in all functional subdivisions. BDA-labeled neurons were found in the anterior, middle, and posterior ectosylvian gyri. The functional regions of the anterior ventral field (AVF), anterior dorsal field (ADF), anterior auditory field (AAF), primary auditory cortex (AI), posterior pseudosylvian field (PPF), and the posterior suprasylvian field (PSF) all demonstrated retrograde neuronal labeling. Projections to LRSS chiefly arose from the ADF and AAF (nearest to the injection site), while small densities of neurons also occurred within the areas of AI and PPF. Regions such as the AVF and the PSF were much less densely

populated with labeled cells. In addition, it should be noted that labeling did occur in as yet unmapped higher auditory centers located in the ventral-most portions of the posterior ectosylvian gyrus.

Visual Cortex

Labeling in visual cortex was less pronounced than in other cortical areas, but still apparent in higher-level, extrastriate areas, as depicted in Figure 10. Projections to LRSS were observed to originate from the posterior parietal rostral area (PPr), posterior parietal caudal area (PPc), anterolateral lateral suprasylvian sulcus (ALLS), posterolateral lateral suprasylvian sulcus (PLLS), and visual area 21. These visual regions occur chiefly within the lateral and suprasylvian gyri and the adjoining suprasylvian sulcus. Among these cortical areas, the transitional zone between ALLS and PLLS, as well as area 21, contributed the greatest number of projections to LRSS. While very small aggregates of labeled cells did arise from the PPr and PPc, these regions were otherwise unlabeled.

It should be noted that the LRSS shares a border with the anterior-most aspects of the visual ALLS. As a consequence, injections into LRSS that spread into ALLS were found to produce considerably more label in these same visual cortical areas (as illustrated in the Appendix, particularly in Appendices D and F). Under no conditions were retrogradely-labeled neurons found in primary visual cortex (V1).

Motor Cortex

A small number of retrogradely labeled neurons projecting to LRSS were found throughout the posterior sigmoid and coronal gyri, corresponding to motor cortex. As shown in Figure 11, both subregions displayed consistent labeling, with the posterior sigmoid gyrus in particular bearing a large number of projections. The portion of motor cortex on the anterior sigmoid gyrus, meanwhile, lacked labeled cells entirely. Labeling patterns in motor cortex appeared to occur in more dispersed patterns rather than in distinct, densely-labeled foci.

Prefrontal Cortex

Analysis of the presence and distribution of multisensory neurons projecting to LRSS from prefrontal cortex (PFC) was not carried out in all cases, including case FRSS9. PFC projections are visible in cases FRSS1, FRSS2, FRSS3, and FRSS4, which are shown in Appendices A, B, D, and F, respectively. Neuronal labeling in PFC was sparse and was chiefly observed within portions of the PFC on the coronal gyrus. Labeling in this gyrus was found in both medial and lateral zones.

Callosal Connections with Opposite Hemisphere

LRSS connections with the opposite hemisphere through the corpus callosum were primarily observed in the homotypical region of cortex, the LRSS, as shown in Figure 12 (bottom row). Heterotypical connections occurred mostly with the MRSS and portions of

auditory cortex corresponding to AI. Callosal projections to LRSS were not observed to any significant degree from any other part of contralateral cortex.

Thalamus

Figure 13 depicts a serial arrangement of coronal sections through the ferret thalamus illustrating the major relay nuclei as well as the location of neurons retrogradely labeled from the LRSS. Sections of thalamus were processed for analysis of retrograde labeling of thalamic neurons in their respective nuclei. Dense aggregates of labeled neurons were identified within the following thalamic nuclei: the posterior nucleus (Po), the ventrobasal complex (Vb), the lateral posterior nucleus (LP), and the medial geniculate nucleus (MG). When the overall number of neurons projecting to the LRSS was assessed, the majority originated from the auditory MG. Labeled neurons were never observed in the contralateral thalamus.

Areal Counts of Labeled Neurons

For each of the functional divisions of cortex listed above, the number of labeled neurons contained within was counted for each case. These results are provided in Table 1, which shows the mean, standard deviation, and percentage values for the labeled neurons by area for each case. These data are represented graphically in Figure 14. These analyses confirm what was visibly apparent from Figure 7, that projections to the LRSS primarily arise from auditory and somatosensory cortices.

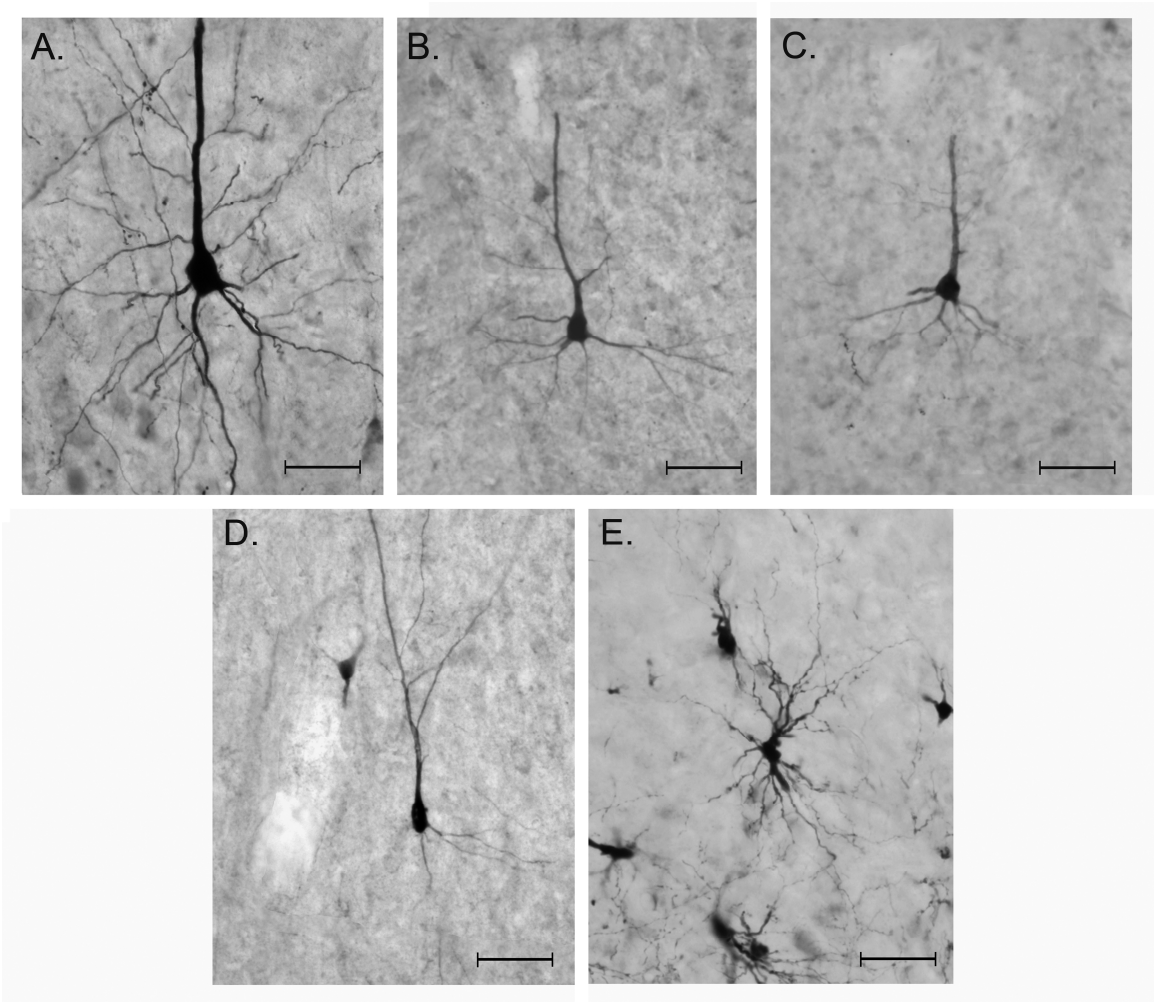

Figure 6. Photomicrographs of Retrogradely-Labeled Neurons from Injections Centered on LRSS

Representative pyramidal neurons from selected areas of cortex, plus thalamic neurons. Starting at top left and moving clockwise are neurons sampled from somatosensory cortex (A), auditory cortex (B), motor cortex (C), prefrontal cortex (D), and thalamic neurons from the medial geniculate nucleus (E). BDA-labeled neurons pictured here show filled somata as well as filled apical and basilar dendrites. Note stark contrast between labeled neurons and the surrounding neuropil. Scale bar = 100 um.

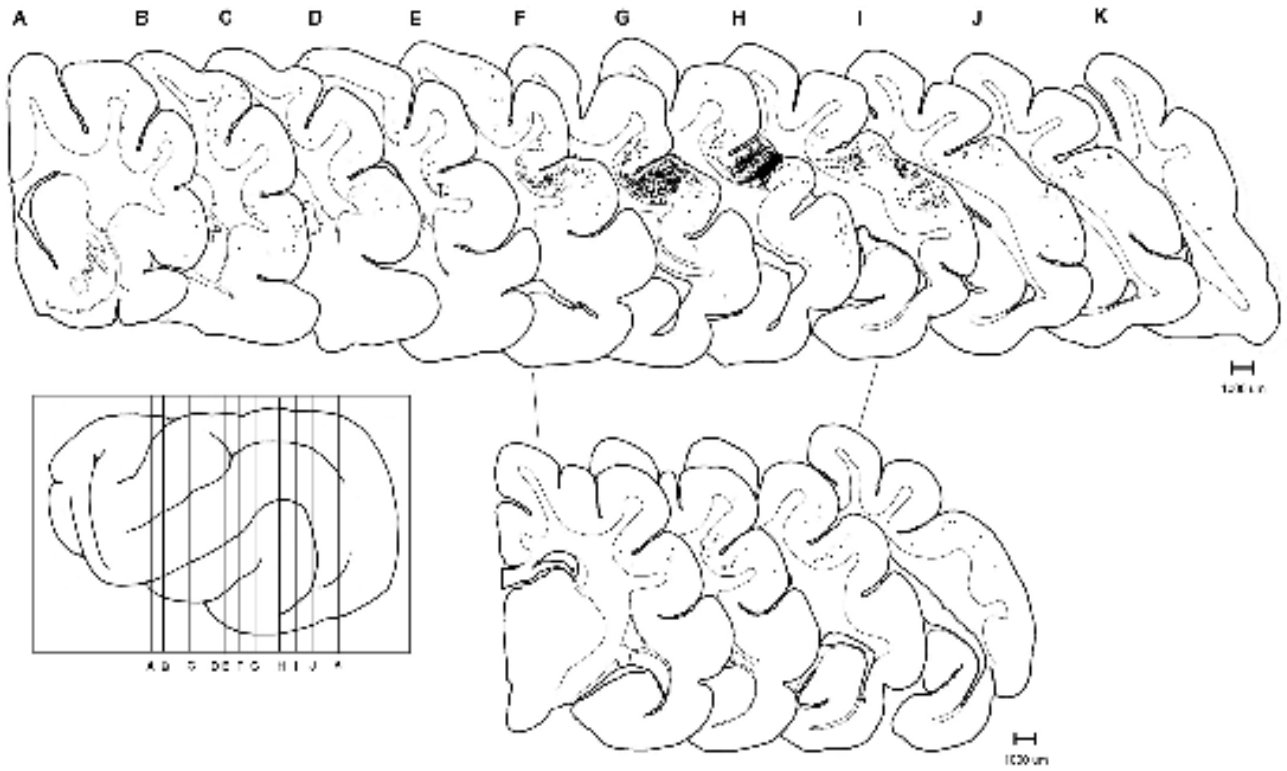

Figure 7. Case FRSS9 as an Example of Retrograde Labeling Throughout Cortex After Injection to LRSS

Inset of the lateral aspect of the ferret brain at the lower left demonstrates the levels of section from which the coronal sections shown in the upper row are derived. The bottom row displays coronal sections from contralateral cortex that correlate to the same level of section as their ipsilateral partners above. Injection site at LRSS is shown outlined in black in section G. One black dot = one retrogradely-labeled neuron from LRSS injection. Scale bar = 1000 μm.

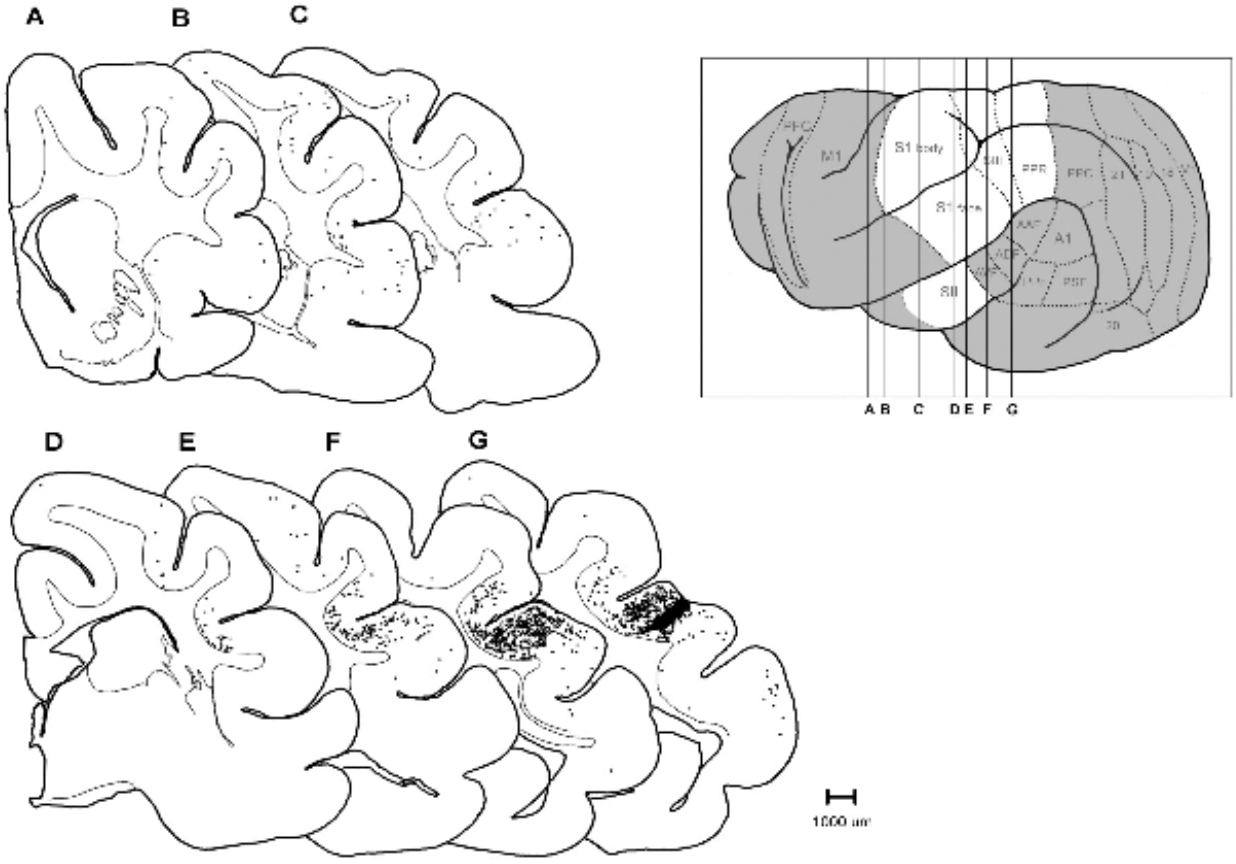

Figure 8. Retrograde Labeling in Somatosensory Cortex as Observed in Case FRSS9

Inset of the lateral aspect of the ferret brain is shown at the top right. Areas of somatosensory cortex are highlighted in white, while all other regions of cortex are shown in gray. The levels of section for the coronal slices shown to the left are indicated by letter labels. Section A is the most anterior, while section G is the most posterior. Retrogradely-labeled neurons from LRSS injections are shown throughout somatosensory cortex. Note the high degree of labeled cells in regions proximal to the injection site, with considerable labeling also occurring in the region of sulcal cortex between SI (face representation) and SII, known as the medial bank of the rostral suprasylvian sulcus, or MRSS.

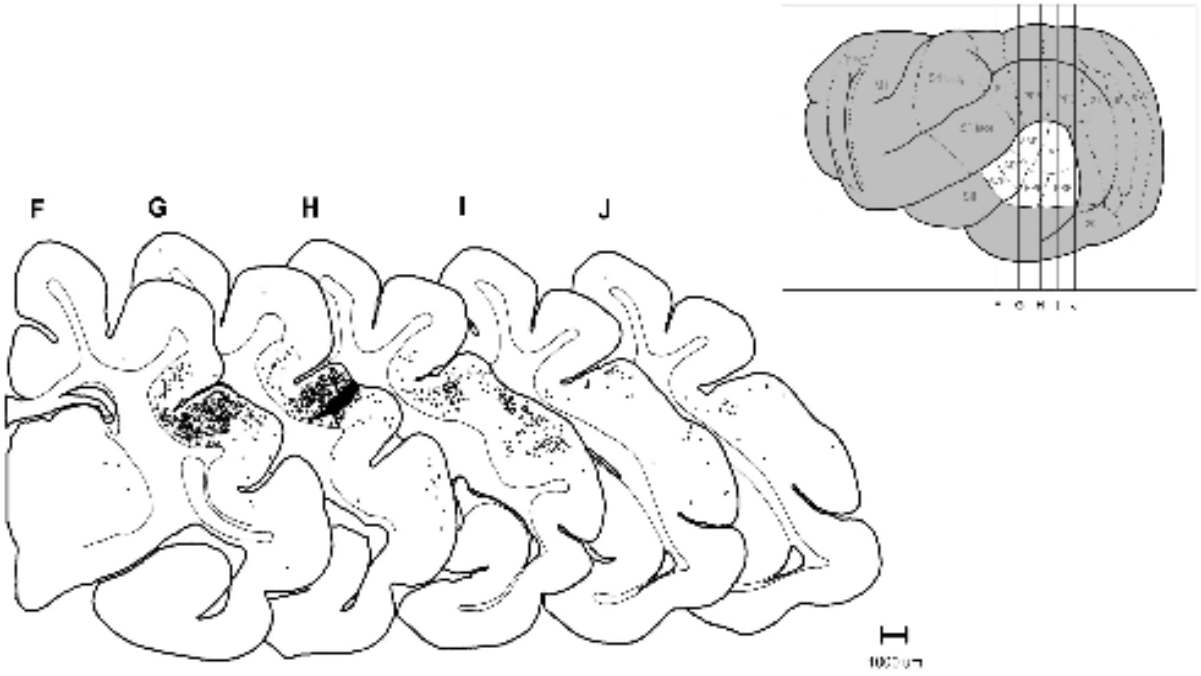

Figure 9. Retrograde Labeling in Auditory Cortex as Observed in Case FRSS9

Inset of the lateral aspect of the ferret brain is shown at the top right. Areas of auditory cortex are highlighted in white, while all other regions of cortex are shown in gray. The levels of section for the coronal slices shown to the left are indicated by letter labels. Section F is the most anterior of these, while section J is the most posterior. Retrogradely-labeled neurons from LRSS injections appear throughout auditory cortex and extend into its most posterior regions. Note the high degree of labeled cells in regions proximal to the injection site, with significant labeling occurs in regions that correlate to the ADF and AAF. Lesser but still considerable labeling is present in AI and PPF. Minor labeling was observed in the AVF and PSF.

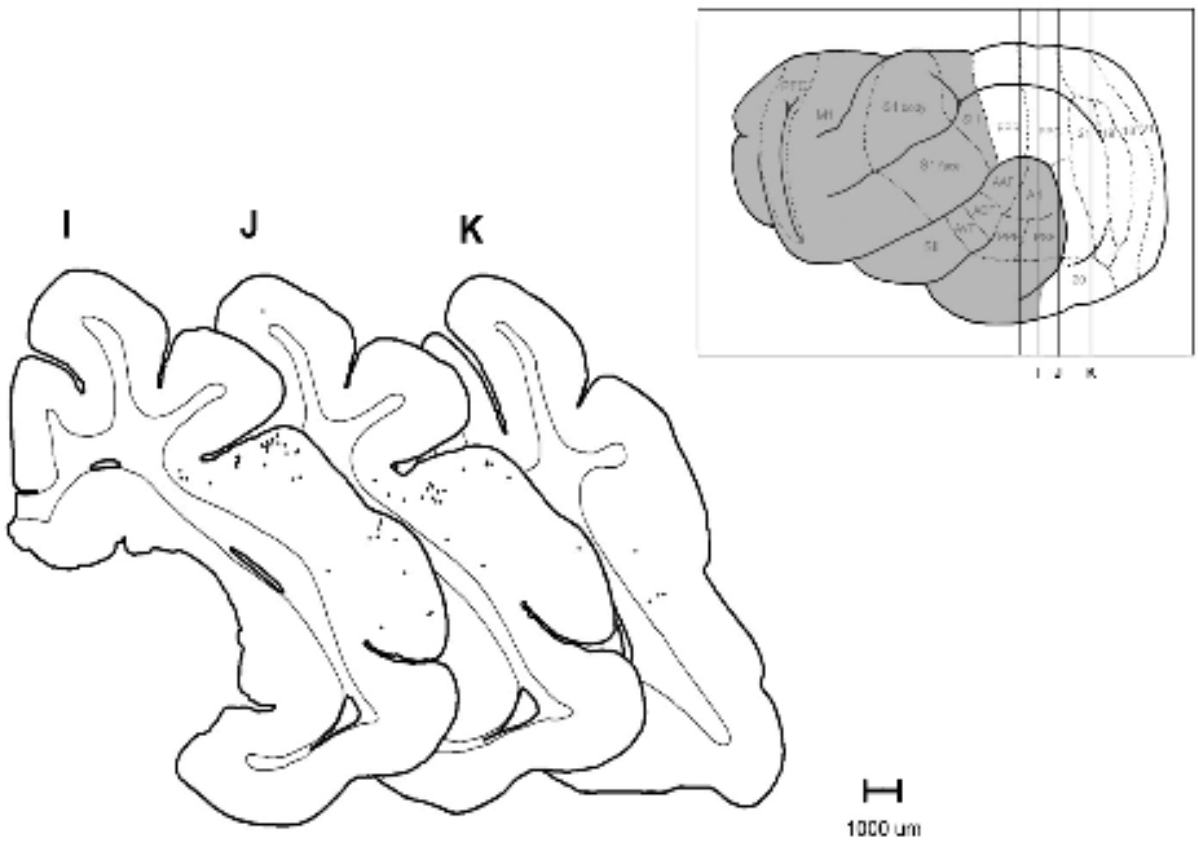

Figure 10. Retrograde Labeling in Visual Cortex as Observed in Case FRSS9

Inset of the lateral aspect of the ferret brain is shown at the top right. Areas of visual cortex are highlighted in white, while all other regions of cortex are shown in gray. The levels of section for the coronal slices shown to the left are indicated by letter labels. Section I is the most anterior of these, while section K is the most posterior. Retrogradely-labeled neurons from LRSS injections appear sparsely in visual cortex in this particular case. Labeling was observed chiefly in higher-level, extrastriate areas. Projections from the ALLS and PLLS, as well as visual area 21, are most dense. Note the absence of labeled cells in primary visual cortex (VI).

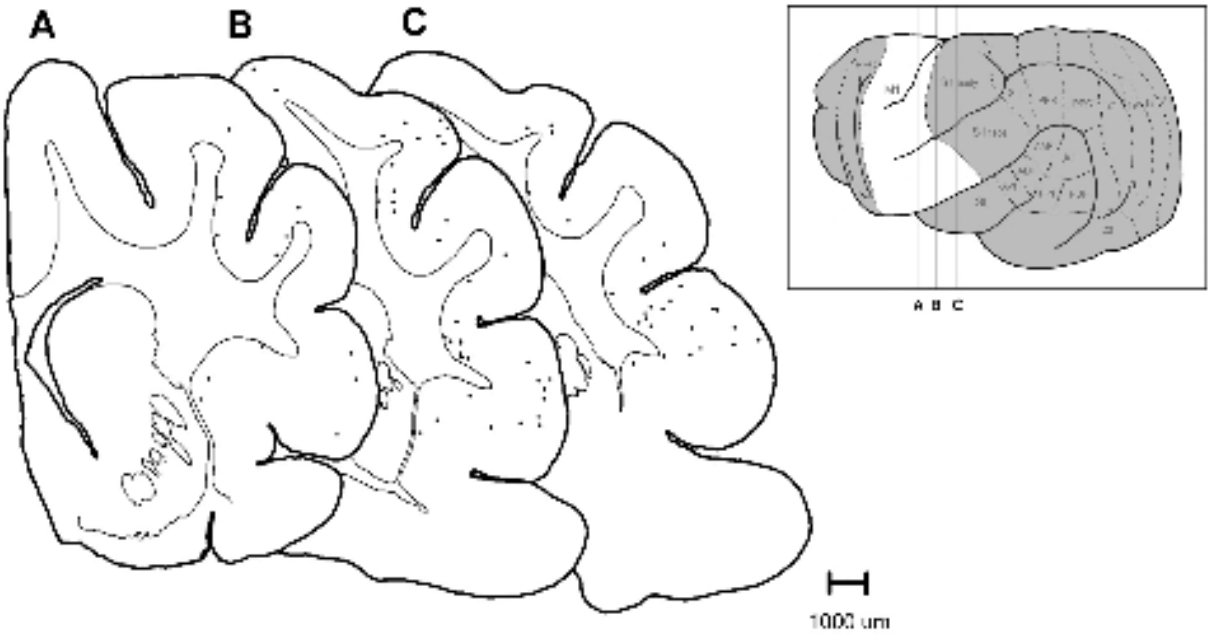

Figure 11. Retrograde Labeling in Motor Cortex as Observed in Case FRSS9

Inset of the lateral aspect of the ferret brain is shown at the top right. Areas of motor cortex are highlighted in white, while all other regions of cortex are shown in gray. The levels of section for the coronal slices shown to the left are indicated by letter labels. Section A is the most anterior of these, while section C is the most posterior. Retrogradely-labeled neurons from LRSS injections appear in small numbers in primary motor cortex. Labeling was observed in the posterior sigmoid and coronal gyri, while the anterior sigmoid gyrus lacked labeled cells entirely.

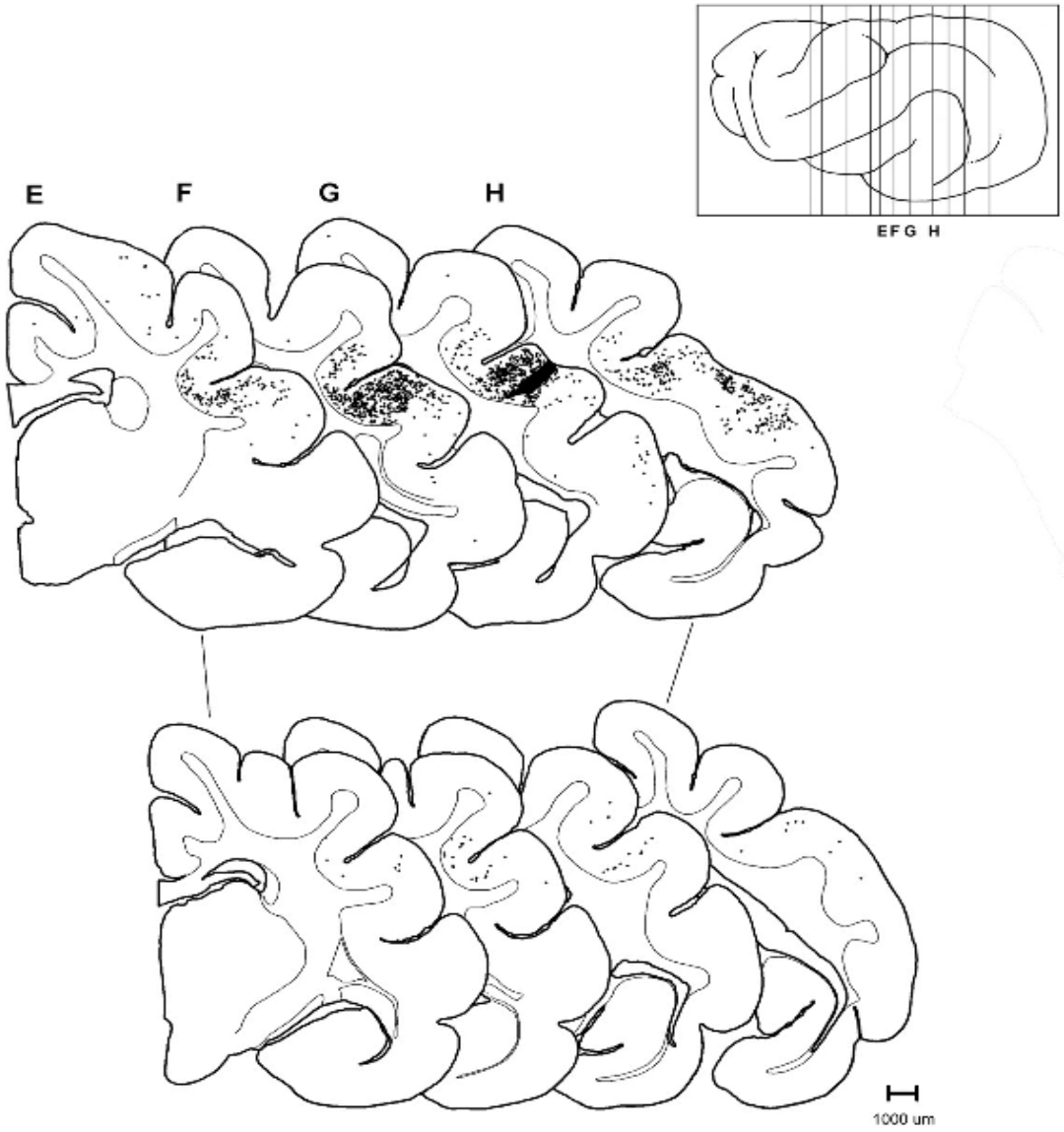

Figure 12. Retrograde Labeling in Contralateral Cortex as Observed in Case FRSS9

Inset of the lateral aspect of the ferret brain at the upper right demonstrates the levels of section from which the coronal sections shown in the upper row are derived. The bottom row displays coronal sections from contralateral cortex that correlate to the same level of section as their ipsilateral partners above. Labeling in contralateral cortex was chiefly confined to homotypical regions of cortex (LRSS), while heterotypical labeling occurred in MRSS and in portions of auditory cortex corresponding to AI.

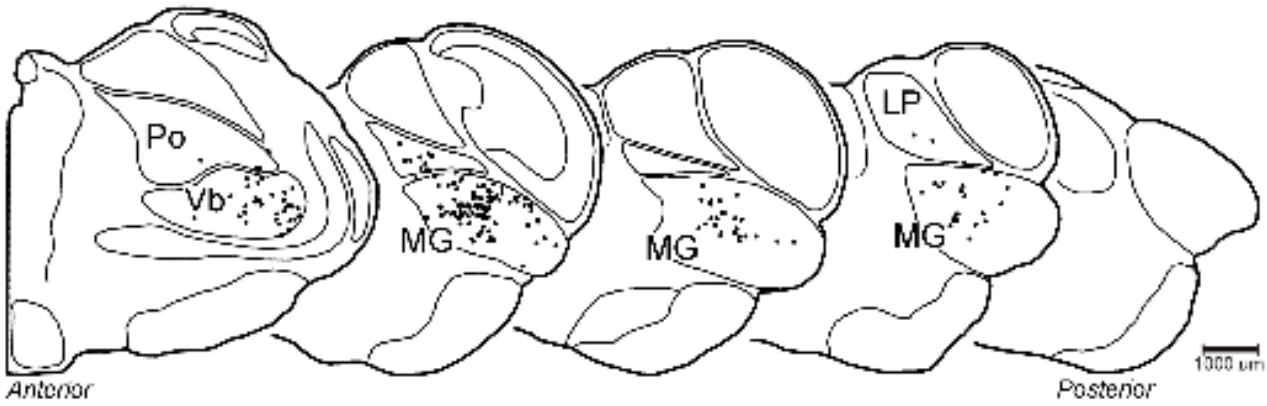

Figure 13. Retrograde Labeling in Thalamus as Observed in Case FRSS9

Sections of thalamus shown here demonstrate retrograde labeling in the following thalamic relay nuclei: the posterior nucleus (Po), the ventrobasal complex (Vb), the medial geniculate nucleus (MG), and the lateral posterior nucleus (LP). Labeling in thalamic nuclei was primarily confined to the Vb and MG, which represent the relay nuclei for somatosensory and auditory stimuli, respectively.

	Motor	Somatosensory	Auditory	Visual	Prefrontal	Commissural
FRSS1	4.25	63.18	148.89	95.71	10.67	*Not quantified
FRSS2	9.33	124.14	215.67	107.75	4.00	38.63
FRSS9	5.33	116.28	120.60	34.00	0.00	19.50
Mean	6.31	101.20	161.72	79.15	4.89	29.07
St. Dev.	2.68	33.16	48.81	39.57	5.39	13.53
Percentage	1.65	26.47	42.30	20.70	1.28	7.60

Table 1. Summary Data of Retrograde Projections from LRSS to Cortical Regions

Data shows the summed mean and standard deviation values for projections to LRSS observed to originate in each of the following cortical areas: motor, somatosensory, auditory, visual, prefrontal, and commissural cortex. Data were obtained via areal counting of these cortical regions for cases FRSS1, FRSS2, and FRSS9. Cases FRSS3 and FRSS4 were excluded from this analysis because the injections in these cases did not accurately target LRSS. Percentage values were calculated here and are represented graphically in a subsequent figure. Note that commissural projections were not evaluated in case FRSS1; this was taken into account in the analysis conducted above.

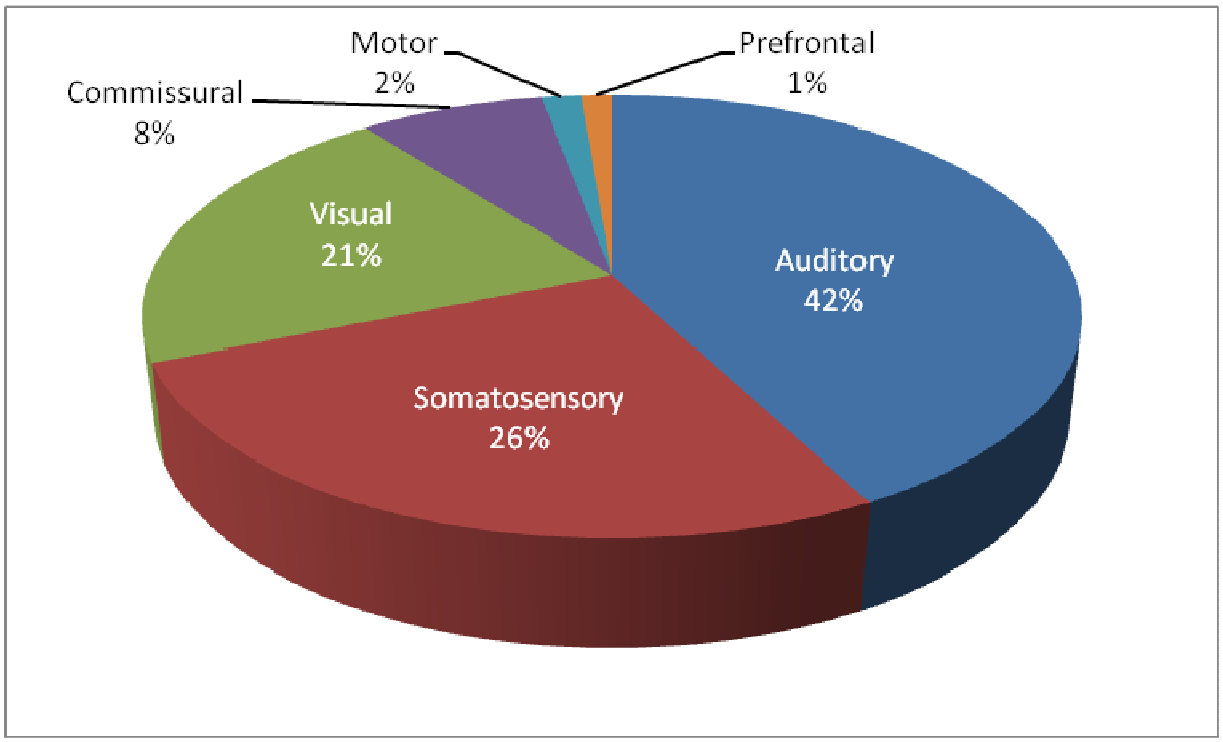

Figure 14. Summary of Projections to LRSS by Cortical Area

Distribution of inputs to LRSS by cortical area (including auditory, somatosensory, visual, commissural, motor, and prefrontal cortex). As demonstrated here, auditory and somatosensory inputs occupy the greatest percentage of the whole, representing 42 and 26 percent, respectively.

DISCUSSION

The results of the present anatomical study indicate that the major sources of input to the physiologically-defined multisensory area located in the lateral bank of the rostral suprasylvian sulcus (LRSS) largely mirror its auditory-somatosensory functional properties (Keniston et al., 2008). These data suggest that the multisensory inputs of LRSS are derived largely from auditory and somatosensory cortices. Of these cortical regions, the greatest proportion of afferents was typically found in auditory cortex. Specifically, retrograde labeling from LRSS was most densely concentrated in the anterior dorsal field (ADF) and anterior auditory field (AAF), while substantial neuronal labeling was also seen in primary auditory cortex (AI) and the posterior pseudosylvian field (PPF). In somatosensory cortex, projections to LRSS were primarily found in the region of sulcal cortex between primary somatosensory cortex (SI, face representation) and secondary somatosensory cortex (SII), identified as the medial bank of the rostral suprasylvian sulcus (MRSS) (Keniston et al, 2009a).

Preliminary physiological investigation of sensory responses in LRSS in adult ferret (Keniston et al, 2008) first demonstrated the presence of multisensory neurons. Multisensory neurons are those whose activity in one modality can be influenced by the presence of stimuli from another sensory modality (Meredith and Stein, 1986). Studies in ferret and cat cortex have established that approximately 25 percent of neurons within

specific regions are multisensory in nature (AES = 27%: Wallace et al, 1992; 25%: Meredith, 2004; RSp = 24%, Clemo et al, 2007). However, studies have confirmed that the number of neurons showing multisensory responses in LRSS is much higher (Keniston et al, 2008, 2009b). Of the cells displaying sensory responses in LRSS, approximately 60 percent were shown to respond to multimodal stimulation. Over three-fourths of the population of multisensory neurons were bimodal, responding both to auditory and to somatosensory stimuli presented independently, and displaying integration when these same stimuli were presented simultaneously. The remaining portion was constituted by unimodal somatosensory neurons that showed some degree of subthreshold auditory influence. In the context of these findings, the anatomical work conducted in the present study supports the idea that the majority of multisensory responses in LRSS are elicited from somatosensory and auditory sources. How these inputs from different sensory modalities interact within the LRSS and how synaptic architecture might underlie these effects are questions currently under investigation.

Physiological studies of the LRSS have revealed that the majority (90%) of responses in this region are somatosensory (either unisensory or multisensory; Keniston et al., 2008) However, in the present anatomical study, nearly all cases with reliable injections to LRSS showed a greater number of projections to originate from auditory cortex than somatosensory cortex. While the reasons for this apparent contradiction are unknown, there are several reasonable possibilities. It is possible that projections from somatosensory cortical sources are more highly branched (divergent) than their auditory counterparts. This condition would allow fewer somatosensory neurons to contact the

same or greater number of LRSS targets. However, the branching pattern of sensory inputs to this, or to any other multisensory cortical area, are currently unknown. Similarly, the synaptic activation conveyed by the somatosensory afferents may be more potent than those from the auditory modality, making it easier for fewer somatosensory neurons to activate the same (or a larger) volume of target tissue. Alternatively, the explanation may be more experimentally based: the path to inject the LRSS must traverse through the auditory cortices ADF and AAF. In this way, tracer not only directly labeled LRSS but also portions of adjoining auditory cortex. This condition is evident in case FRSS3, where the injection included the lateral lip of the suprasylvian sulcus and the adjoining auditory cortices. Additional control experiments that access the LRSS without traversing the auditory cortices are needed to resolve this issue.

Other cortical afferents to the LRSS include motor and prefrontal areas, but these represented only a minor source of inputs. Motor cortex is known for its somatosensory responsivity, and prefrontal cortex is characterized by both auditory and somatosensory properties. In some cases (but not all), projections to LRSS from regions of visual cortex were often considerable, but these did not match the scale of inputs that arose in auditory and somatosensory cortex and are likely to result from the spread of tracer from the LRSS injection site into adjoining visual areas. Commissural projections to the LRSS were surprisingly sparse, but, as expected, arose from homotypical areas of the contralateral cortical hemisphere. In sum, these results demonstrate that LRSS receives a significant degree of convergent input, and the primary origin of these projections in auditory and

somatosensory cortices suggests that LRSS is very likely a site of the integration of multimodal stimuli from these key sources.

The nature and degree of projections from LRSS to ipsilateral and contralateral thalamus were investigated in the present study, and it was determined that projections to LRSS did arise from select relay nuclei in the ipsilateral thalamus. The majority of thalamic projections arose from the medial geniculate nucleus (MG) and the ventrobasal complex (Vb). Given that these are relay nuclei for auditory and somatosensory stimuli, respectively, this information supports the finding mentioned previously, that LRSS receives the most significant degree of convergent input from auditory and somatosensory sources. The total absence of projections from contralateral thalamus confirms that LRSS receives only ipsilateral thalamic input.

Multisensory Cortical Organization

Efforts to understand the organization of multisensory cortical areas has not led to consensus across studies. It appears that the organization and properties of multisensory areas may differ. For instance, between the auditory area of the field anterior ectosylvian sulcus (FAES) and the anterior ectosylvian visual area (AEV), the population of multisensory neurons is constituted by both bimodal cells and unimodal subthreshold cells interspersed together (Carriere et al, 2007; Meredith and Allman, 2009). This also appears to be the arrangement that exists in MRSS (Keniston et al, 2009a). However, in the transitional area between the dorsal auditory zone (DZ) and the visual posterolateral lateral suprasylvian sulcus (PLLS), it appears that bimodal neurons exist in an isolated band that does not include those multisensory neurons with subthreshold effects. Similarly,

somatosensory area SIV in the cat rarely exhibits bimodal neurons except at its posterior border with auditory FAES or visual AEV, but over 66% of neurons contained within SIV show subthreshold multisensory properties (Dehner et al., 2004). Thus, the relative distribution of bimodal and subthreshold multisensory neurons appears to vary according to area. Preliminary evidence indicates that these multisensory neuron types are intermingled within the LRSS (Keniston et al., 2008). This observation suggests that a tracer injection that fills the LRSS would label inputs to both bimodal and subthreshold forms of multisensory neurons.

Related Findings

A 2009 study by Keniston and colleagues investigated the multisensory nature of the medial bank of the ferret rostral suprasylvian sulcus (MRSS), which is the medial counterpart of LRSS. The MRSS is situated between SI medially and LRSS laterally, with auditory cortex bordering LRSS further posterolaterally. Given its location, it is logical that MRSS would be a site of multisensory convergence and integration, like the LRSS. However, the MRSS was found to be a higher-order somatosensory area with only slight multisensory effects. Somatosensory, auditory, and multisensory neurons were all identified in MRSS, but only a small proportion of neurons here were multisensory and the level of integrated activity elicited by combined somatosensory and auditory cues there was modest at best (Keniston et al, 2009a). It should be pointed out that despite the presence of auditory and multisensory neurons in the MRSS, no cortical source of auditory inputs could be determined (except perhaps for the LRSS) using anatomical techniques identical to the present study.

Anatomical and physiological studies of the cat rostral suprasylvian sulcal areas (RSp; Clemo et al., 2007) suggest that it may be homologous to the ferret MRSS/LRSS regions (Keniston et al., 2009a). The cat RSp contains unisensory (primarily auditory or somatosensory), bimodal, and subthreshold multisensory neurons, and multisensory interactions there are characteristically weak (Clemo et al., 2007). Anatomically, injections made into the somatosensory regions of the fourth and fifth somatosensory areas (SIV and SV, respectively); the auditory areas of primary and secondary auditory cortex (AI and AII, respectively), anterior auditory field (AAF), field anterior ectosylvian sulcus (FAES), and the posterior auditory field (PAF); as well as the visual regions of the anterior ectosylvian visual area (AEV), posteromedial lateral suprasylvian sulcus (PMLS), and posterolateral lateral suprasylvian sulcus (PLLS) all projected to the RSp (Clemo et al, 2007). Although the present study describes the projections of ferret LRSS via retrograde tracing experiments, the data nonetheless provide comparable results to these orthograde projections to cat RSp. Retrograde tracing from LRSS in ferret gave terminal labeling concentrated in somatosensory, auditory, and visual cortices. In the study conducted by Clemo and colleagues, orthograde injections to auditory cortex gave the most widespread labeling in RSp. Similarly, injections to LRSS in ferret gave the most pronounced degree of retrograde label in auditory cortex, as evidenced by the preponderance of labeled neurons there. The most striking similarity between the two studies, therefore, is their descriptions of significant reciprocal labeling between the respective sulcal areas (LRSS in ferret and RSp in cat) and auditory cortex.

In addition, studies in cat by Monteiro and colleagues (2003) documented the connectivity between the rostral suprasylvian sulcal cortex (RSS) and the anterior ectosylvian sulcal cortex (AESc). Retrograde injections made into RSS in this body of work showed there to be significant projections to AESc from the former structure. Of the injections made into the anteromedial bank, fundus, and posteromedial bank of the RSS, all except those made in the anteromedial bank produced terminal labeling in the auditory field of the anterior ectosylvian sulcus (FAES). Similar results were observed after injections were made into LRSS in the present study. While no direct analogue of the FAES exists in the ferret, the site of intersection between the ADF and PPF is its ostensible location, and distinct clusters of labeled cells resulting from LRSS injections are present there, particularly in cases FRSS2 (shown in Appendix B) and FRSS9 (see Figures 7 and 9). Therefore, as the RSS projects to FAES in the cat, LRSS appears to project substantially to the ADF/PPF intersection site in the ferret.

Methodological Considerations

The results generated in the present study depend heavily upon the precise injection of the tracer dye into the desired target (LRSS). Any errors in the accuracy of this injection represent a potential confound to the experimental results. Of the five cases in which retrograde projections from LRSS were examined, three (FRSS1, FRSS2, and FRSS9) made visibly accurate injections into the target site. On the other hand, cases FRSS3 and FRSS4 showed injections that included the LRSS, but they were centered on the posterior aspects of that region. Until recently, the posterior border of the LRSS had been unmapped (Manger et al., 2008), and this matter was not a significant concern during

the initial injection experiments. Analysis of the injection sites and the resulting data now indicate that these injections (FRSS3 and FRSS4) were made in the transitional region between LRSS (anteriorly) and the visual region of sulcal cortex that corresponds to the anterolateral lateral suprasylvian area (ALLS), posteriorly. As a result, the pattern of retrograde labeling produced from these two injections was different from that observed in cases FRSS1, FRSS2, and FRSS9. In particular, more dense patterns of labeling were seen in the more posterior regions of cortex (particularly in visual and auditory cortex). Additionally, these two cases produced more significant commissural labeling than was observed in the remaining three cases. However, these cases showed nearly identical labeling of auditory and somatosensory areas to those derived from more anteriorly-placed injections (cases FRSS1, FRSS2, FRSS9). Therefore, given these considerations, it seems appropriate to include all of these studies in their assessment of auditory and somatosensory corticocortical connections, but to reserve judgment regarding connections to the visual cortices (for which there are no physiological bases in the LRSS) until more sophisticated experimental procedures are approved and conducted. These future experiments would require functional mapping of the LRSS and ALLS border to guide injections in a manner that would avoid that area.

CONCLUSION

The present results show that the LRSS receives its inputs largely from auditory and somatosensory cortical and thalamic regions. Combined with data from prior physiological studies, these observations indicate that the LRSS is a highly multisensory cortical area. As such, the region appears to represent a viable model with which to evaluate features of neuronal processing that may ultimately underlie multisensory perception.

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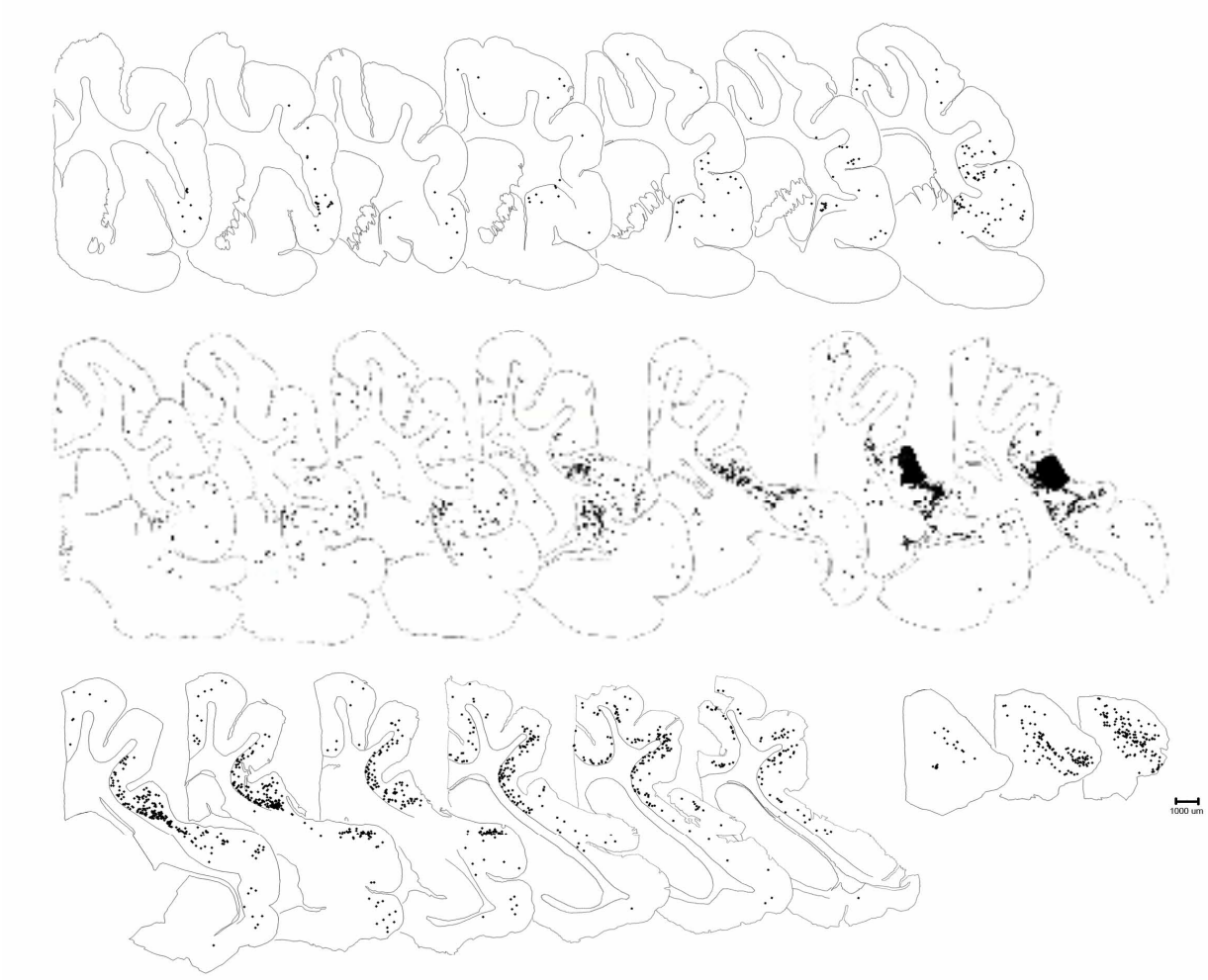
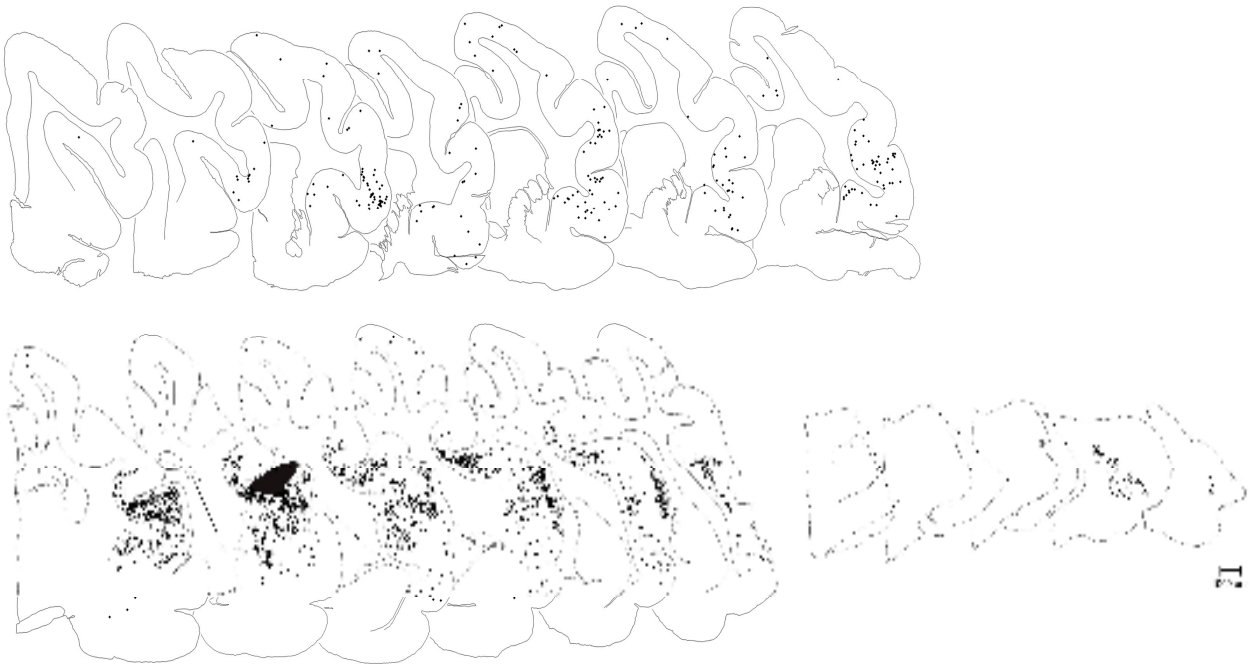
APPENDIX A



Retrograde Labeling from LRSS as Observed in Ipsilateral Cortex, Case FRSS1

Coronal sections of cortex and thalamus are shown, with the most anterior sections shown at the top left and the most posterior sections shown towards the bottom right (excluding the thalamic sections, shown at extreme bottom right). 1 black dot = 1 retrogradely-labeled neuron; injection site outlined in black.

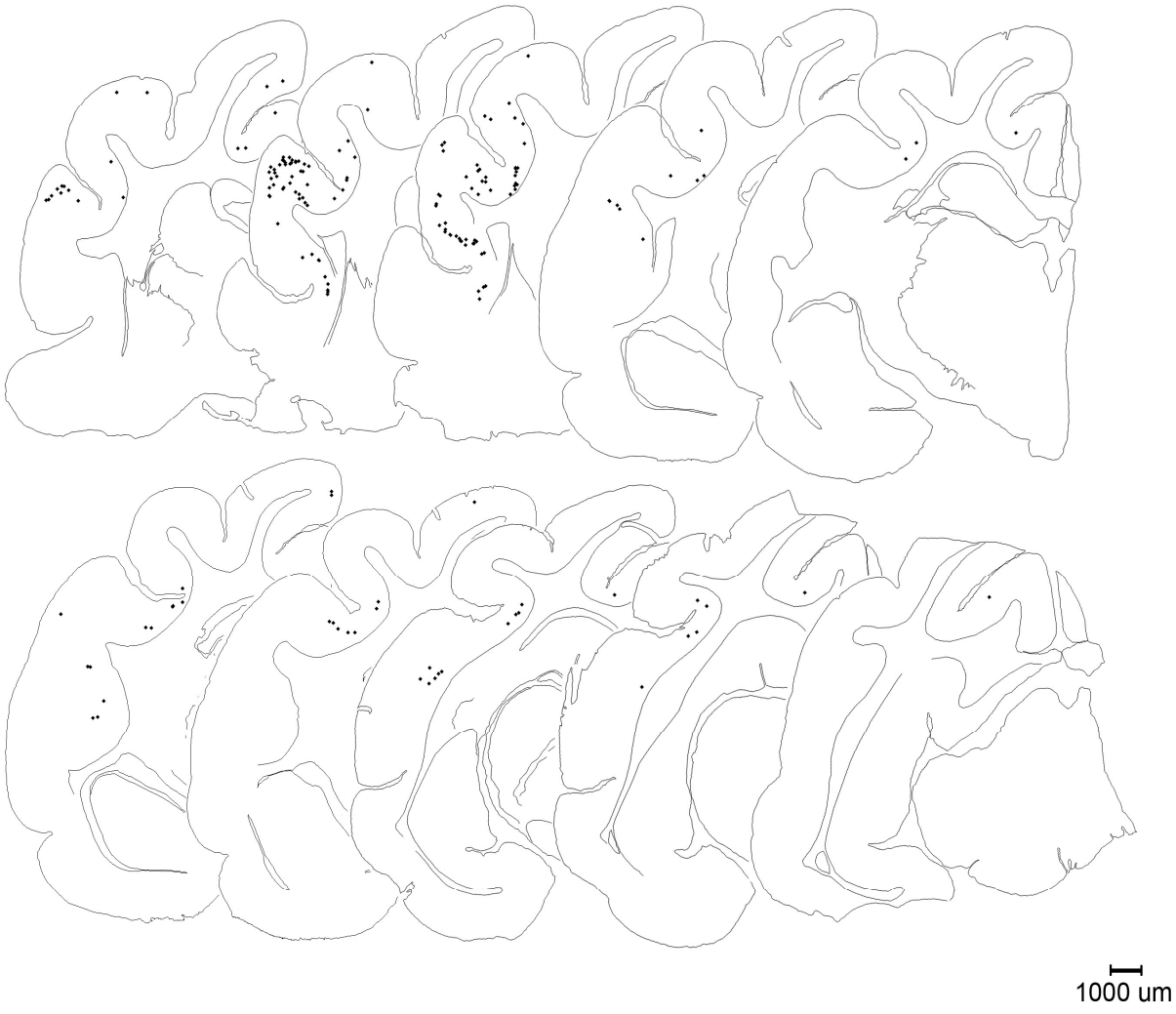
APPENDIX B



Retrograde Labeling from LRSS as Observed in Ipsilateral Cortex, Case FRSS2

Coronal sections of cortex and thalamus are shown, with the most anterior sections shown at the top left and the most posterior sections shown towards the bottom right (excluding thalamic sections, shown at extreme bottom right). 1 black dot = 1 retrogradely-labeled neuron; injection site shown outlined in black.

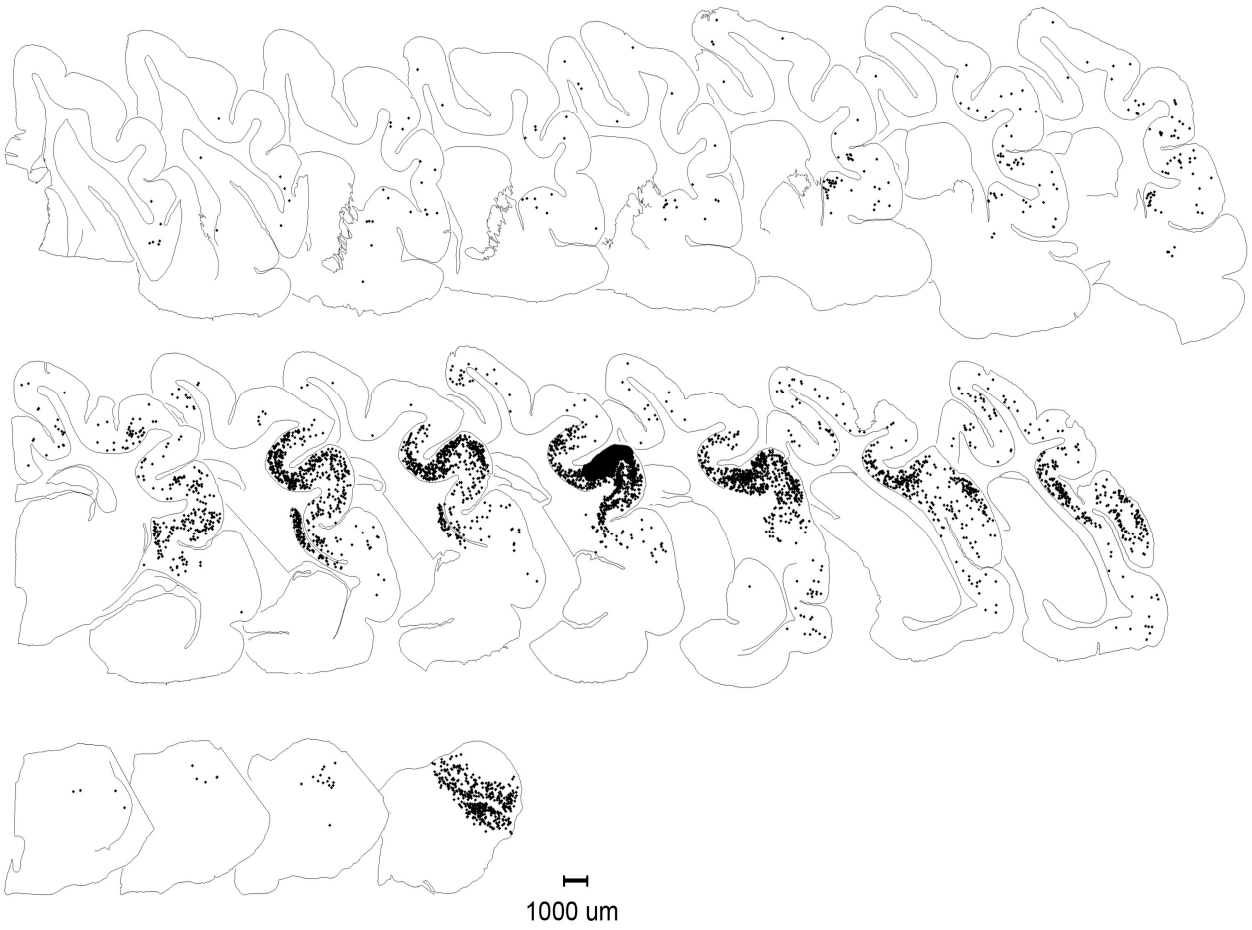
APPENDIX C



Retrograde Labeling from LRSS as Observed in Contralateral Cortex, Case FRSS2

Coronal sections of contralateral cortex are shown, with the most anterior sections shown at the top right and the most posterior sections shown towards the bottom right. 1 black dot = 1 retrogradely-labeled neuron.

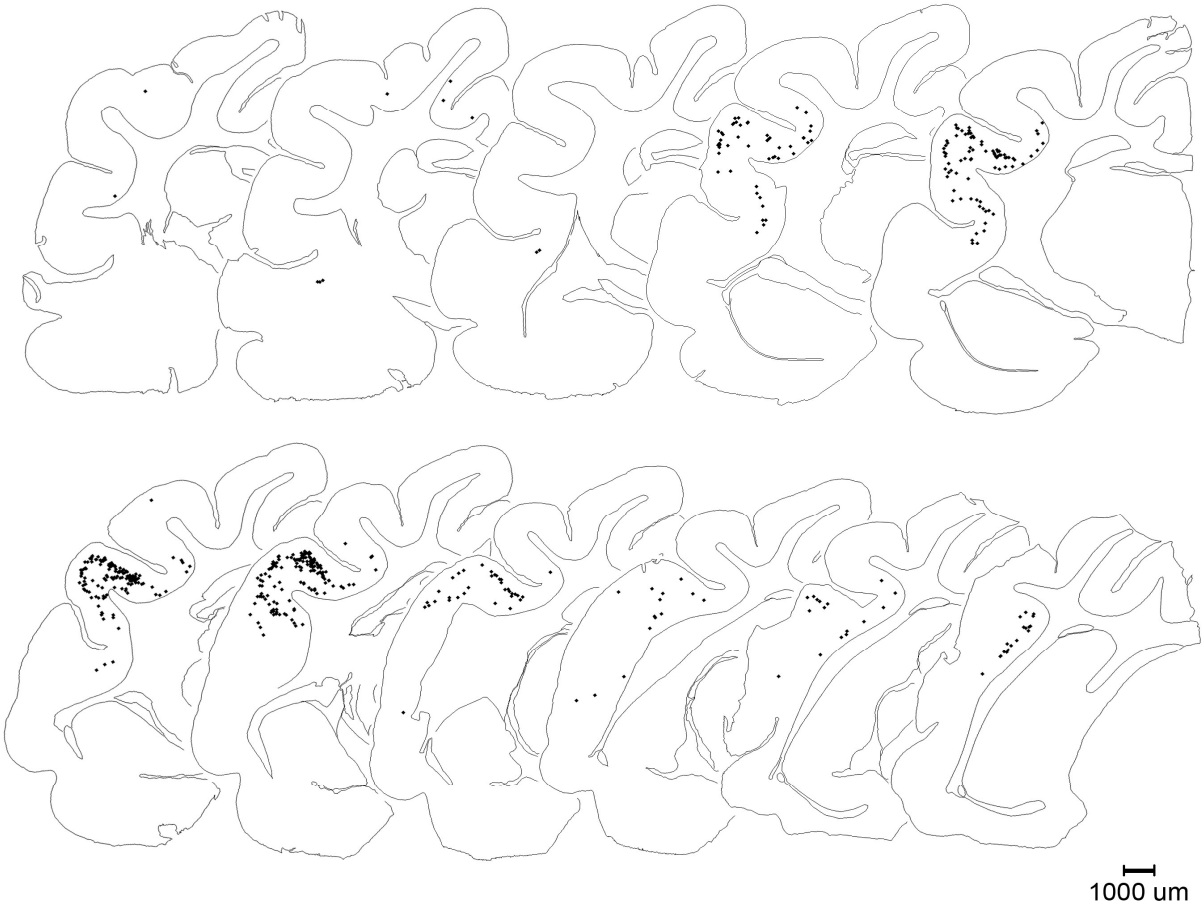
APPENDIX D



Retrograde Labeling from LRSS as Observed in Ipsilateral Cortex, Case FRSS3

Coronal sections of cortex and thalamus are shown, with the most anterior sections shown at the top left and the more posterior sections shown on the second row at the right (excluding thalamic sections, shown on third row). 1 black dot = 1 retrogradely-labeled neuron; injection site outlined in black.

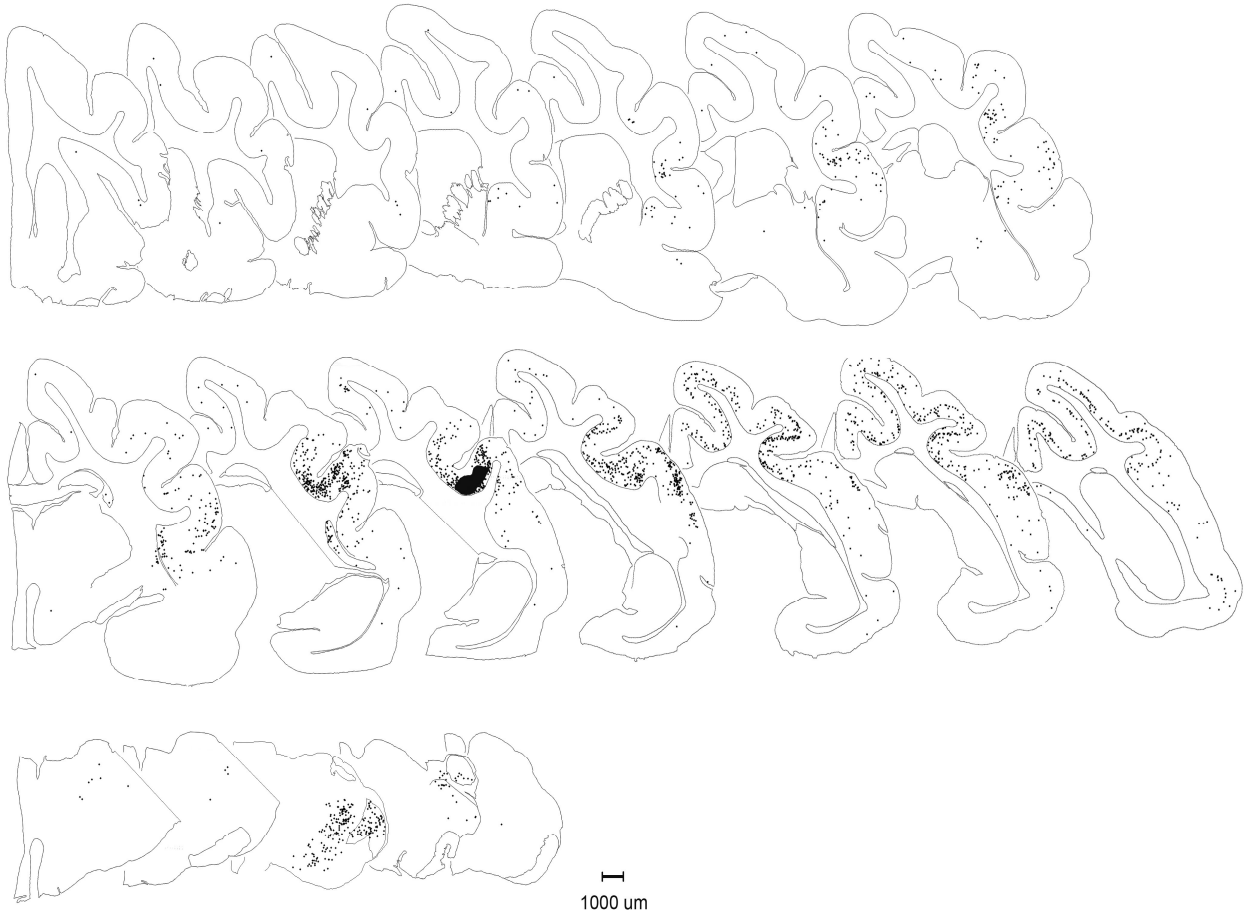
APPENDIX E



Retrograde Labeling from LRSS as Observed in Contralateral Cortex, Case FRSS3

Coronal sections of contralateral cortex are shown, with the more anterior sections shown at the top left and the most posterior sections shown at the bottom right. 1 black dot = 1 retrogradely-labeled neuron.

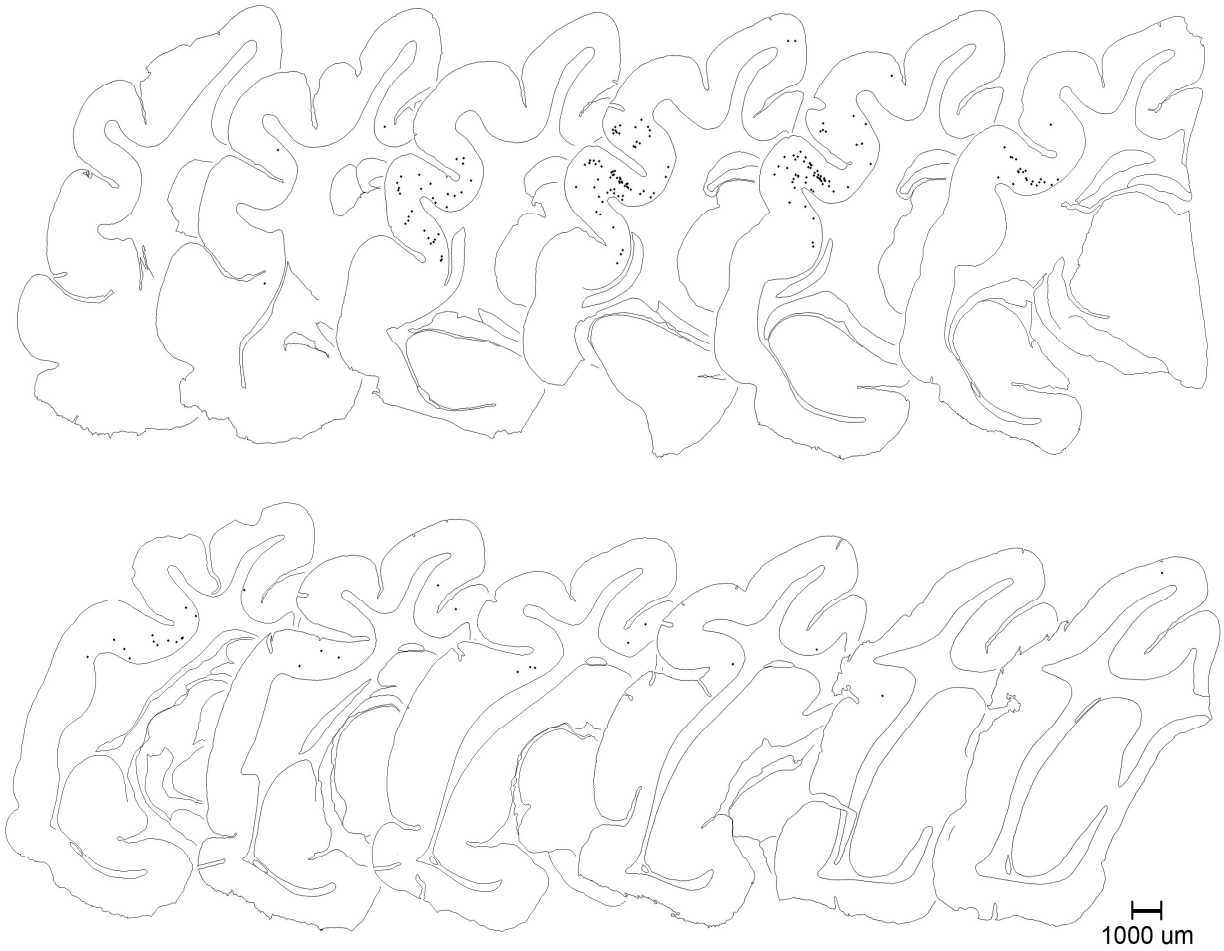
APPENDIX F



Retrograde Labeling from LRSS as Observed in Ipsilateral Cortex, Case FRSS4

Coronal sections of cortex and thalamus are shown, with the most anterior sections shown at the top left and the more posterior sections shown on the second row at the right (excluding thalamic sections, shown on third row). 1 black dot = 1 retrogradely-labeled neuron; injection site outlined in black.

APPENDIX G



Retrograde Labeling from LRSS as Observed in Contralateral Cortex, Case FRSS4

Coronal sections of contralateral cortex are shown, with the more anterior sections shown at the top left and the more posterior sections shown at the bottom right. 1 black dot = 1 retrogradely-labeled neuron.

VITA

Elizabeth White Hagood was born in Salem, Virginia, on August 12, 1983, and is an American citizen. She grew up in Shawsville, Virginia and graduated from Eastern Montgomery High School in 2002. She received her baccalaureate degree in English from Wake Forest University in May of 2006, graduating with a minor in biology. She subsequently worked in the Virginia State Senate and in breast cancer research throughout 2006 and 2007 before entering graduate school at the Medical College of Virginia at Virginia Commonwealth University. She completed her graduate certificate in the Department of Anatomy and Neurobiology in May of 2008 and has spent the last year in pursuit of her Master's degree in the same department. Elizabeth will pursue a career in geriatric health care in the coming years.