Cerebral Cooling Deactivation: Guided Behaviors Revealed by Restricted Cerebral Cooling Deactivation

The purpose of the present study was to test the hypothesis that superficial and deep layers within a single cerebral region influence cerebral functions and behaviors in different ways. For this test, we selected posterior middle suprasylvian (pMS) sulcal cortex of the cat, a suspected homolog of the area V5 complex of primates, because the region has been implicated in several visually guided behaviors. Cats were trained on three tasks: (1) discrimination of direction of motion; (2) discrimination of static patterns partially obscured by static or moving masks; and (3) visual detection and orienting. Cooling of cryoloops in contact with pMS sulcal cortex to 8 ± 1°C selectively and completely impaired performance on the two motion discrimination tasks (1 and 2), while leaving the detection and orienting task (task 3) unimpaired. Further cooling to 3°C resulted in an additional complete impairment of task 3. The 8°C temperature resulted in silencing of neuronal activity in the supragranular layers (I–III) and the 3°C temperature silenced activity throughout the thickness of pMS sulcal cortex. The variation in behavioral performance with covariation of cryoloop temperature and vertical, but not lateral, spread of deactivation shows that deactivation of superficial cerebral layers alone was sufficient to completely impair performance on the two motion discrimination tasks, whereas additional deactivation of the deep layers was essential to block performance on the detection and orienting task. Thus, these results show a functional bipartite division of labor between upper and lower cortical layers that is supported by efferent connectional anatomy. Similar bipartite division into upper and lower layers may be a general feature of cerebral cortical architecture, signal processing and guidance of behavior.

Introduction

For the past century a massive effort has been invested in examining the functional contributions different regions across the cerebral cortical sheet make to brain function, with the hope of linking the functional contributions to underlying differences in cortical architecture (Diamond, 1979; Finger, 1994; Gross, 1998). The principal method of investigation was to ablate regions of cortex and test for specific deficits in behavior, or symptoms, caused by the ablation. The resulting conclusions from these studies always related the symptoms to the position of the ablation in the cortical sheet. The third dimension, cortical depth, was not and could not be studied with the traditional ablation method. In other words, the cortical sheet was studied in the two-dimensional x,y-coordinate system only, without consideration for the third dimension, the z-coordinate, although the possibility that the upper layers may be sensorial and the deep layers may be motor was discussed by William James in 1890 in his Principles of Psychology.

We decided to investigate the third dimension of cortex and test the relative contributions more superficial cortical layers make to visually guided behaviors versus all cortical layers, using the reversible cooling method of Lomber et al. (Lomber et al., 1999) to reversibly deactivate, in a restricted region of cortex, either the superficial layers alone, or in combination with the deep layers. This was achievable because we can apply lesser or greater levels of cooling to a limited region of the cerebral surface and deactivate, in a graded yet consistent way, neurons at progressively deeper locations in cortex. Thus, we can de-activate the more superficial layers alone or in combination with the deep layers.

For the behavioral analyses, we examined a region of extra-striate cortex bounding the posterior two-thirds of cat middle suprasylvian (pMS) sulcus (Fig. 1), which is located at the junction of temporal, occipital and parietal cortices. We consider pMS sulcal cortex to be equivalent to the combined lateral suprasylvian and posterior ectosylvian visual areas as defined by Grant and Shipp (Grant and Shipp, 1991). This region has been well characterized on the basis of anatomical connections (Rosenquist, 1985; Sherk, 1986), retinotopic representation (Palmer et al., 1978; Grant and Shipp, 1991; Sherk and Mulligan, 1993) and receptive field properties (Spear, 1991; Dreher et al., 1996). Moreover, it has several biological features in common with the area V5 complex of primates (Payne, 1993), which broadens the applicability of our results and conclusions. But more importantly, full cooling deactivation of pMS sulcal cortex induces deficits in a number of visually guided behaviors. The behaviors sensitive to pMS sulcal cortex cooling deactivation include abilities to: (1) discriminate between two different directions of motion [30° difference (Lomber et al., 1996b)]; (2) discriminate between patterns when they are partially obscured by moving masks (Lomber et al., 1994b, 1996b); and (3) detect and orient toward stimuli introduced into the visual field when attention is initially fixed (Lomber et al., 1996b; Lomber and Payne, 1996; Payne et al., 1996). We applied these tasks to four trained cats and used cryoloops cooled to different temperatures to investigate the contributions the superficial, and the superficial and deep, layers make to the successful execution of these three behaviors. Our results show that deactivation of the superficial layers alone greatly impaired performance on the direction of motion and the pattern/moving-mask tasks, while at the same time leaving the ability to detect and orient toward stimuli completely or partially unimpaired. Full impairment of the latter task was only achieved when the cooling deactivation penetrated to include the deep layers in addition to the super-ficial layers.

Materials and Methods

Subjects

Four domestic cats were examined. Earlier reports of motion and orienting-related deficits from these four animals have been published previously (Lomber et al., 1994b, 1996b). All treatments on the cats met the guidelines published in the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication 86-23). In all cats, crossing visual fibers in the optic chiasm and corpus callosum were cut.
along the midline to create a ‘split-brain’ preparation. The cooling loops were placed unilaterally into the posterior two-thirds of the middle suprasylvian sulcus of the left hemisphere, and in contact with ventral posterior suprasylvian (vPS) cortex of the right hemisphere (control deactivation site). In split-brain cats, visual stimuli were restricted to one hemisphere by the use of an opaque contact lens placed over the locally anesthetized (2–3 drops of sterile 0.5% ophthalmic proparacaine) cornea of the opposite eye, making it possible to test for visual deficits with a single cooling loop.

Experimental Apparatus and Behavioral Training
All initial training of the experimental subjects was done binocularly, with additional binocular and monocular training following the optic chiasm and corpus callosum sections as needed. For the final training on both discrimination tasks, monocular viewing conditions were applied. For the orienting task, binocular viewing was used to maximize the extent of the visual field that could be used. In both testing situations the cat received a minimum reward level for ~50% of the trials, even when a region of cortex was maximally cooled. During both training and testing, cats were rewarded with soft or moist commercial cat food and their diet was supplemented with dry cat chow, to which they had free access at the end of each day until they were satiated.

Discriminations
A two-choice Pennsylvania General Testing Apparatus (PGTA) was used and discriminanda were displayed on 17 cm diagonal black-and-white video monitors. For more details, an explanation of the training procedures and an illustration, see Figure 2 of Lomber et al. (1996b).

For task 1 (direction of motion, Fig. 2A), the cats were trained to discriminate between horizontal rightward motion of a high-contrast display, the designated positive (rewarded) direction, and movement 90° (upward) or 45°, or 30° (upward and rightward), the designated negative (unrewarded) directions. The discriminda consisted of black irregular shapes at various orientations distributed at random on a white background that moved at a constant velocity of 6.4 cm/s, were viewed through a 10.5 cm diameter circular aperture and were identical in all respects except direction of motion. At this speed, the stimulus moved at 13.6°/s when viewed from the decision line. A circular aperture is used because the straight edges of a rectangular aperture can be used by the cat to determine the absolute directions of movement, and comparison between discriminda is not a requirement for solution of the discrimination.

For task 2 (Motion and pattern, Fig. 2B), the stimuli were high contrast and consisted of stationary patterns comprised of an outline I or O partially occluded by a grid-like mask. The mask grid was either held stationary or oscillated (up and down) at a variable rate (1–4 cycles/s).

Visual Orienting
A white semicircular arena was used to test the cat’s ability to detect and orient toward visual stimuli introduced upwards through one of 13 openings spaced along the circumference of the arena at 15° intervals with respect to the arena’s center (task 3, Fig. 2C). For more details and an illustration, see Figure 2 of Lomber and Payne (1996). Cats were pretrained in the orienting arena to stand at its center, attend to a piece of dry cat chow, which was held on a metal rod and presented as the cynosure at the 0° position, and then approach the cynosure when the rod was tapped on the side of the opening. After pretrained, the same procedure was followed except that a second, higher-incentive, moist morsel of food was raised through the same or one of the 12 peripheral openings. Rapid and accurate turning of the head, or head and body, and subsequent approach toward the locus of the second stimulus constituted a correct response. To ensure high proficiency, cats were trained for >1000 trials after mastering the detection and orienting task.
et al. using procedures similar to those described elsewhere (Payne sectioned in the midline after taking the transoral, transpharyngeal approach). Discriminant and discriminative function analysis was performed as described previously (Lomber et al., 1994b). Cooling loops (Lomber et al., 1996) were shaped to lie in contact with both the right ventral-posterior suprasylvian gyrus, on the lateral side of the hemisphere, and its continuation into the hippocampal fusiform gyrus, on the tentorial surface. The vPS loop was used to deactivate a control region of cortex which is not involved in any substantive way in the region of cortex which is not involved in any substantive way in processing motion or the detection and orientation toward stimuli (Lomber et al., 1996b). Cooling of vPS cortex to any temperature should be without impact on any of the selected tasks.

Surgical Procedures
For all procedures, cats were fully anesthetized with sodium pentobarbital (25 mg/kg, i.v.) and antiseptic precautions were exercised throughout. Detailed surgical procedures have been described previously (Lomber et al., 1996b). The optic chiasm was sectioned in the midline after taking the transoral, transpharyngeal approach through the body of the sphenoid bone (Myers, 1955). The visual fibers of the corpus callosum (Lomber et al., 1994a) were sectioned using procedures similar to those described elsewhere (Payne et al., 1984). Cooling loops (Lomber et al., 1999), 9–10 mm in length, fabricated from 23G stainless steel hypodermic tubing, were implanted in the left pMS sulcus (Fig. 1) between coronal levels AP-0 and A+10 in Cat P, AP-0 and A9 in Cat D, P1 and A9 for Cat Sd, and P2 and A7 for Cat V. In addition, a second loop was shaped to lie in contact with both the right ventral-posterior suprasylvian gyrus, on the lateral side of the hemisphere, and its continuation into the hippocampal fusiform gyrus, on the tentorial surface. The VPS loop was used to deactivate a control region of cortex which is not involved in any substantive way in processing motion or the detection and orientation toward stimuli (Lomber et al., 1996b). Cooling of vPS cortex to any temperature should be without impact on any of the selected tasks.

Behavioral Testing
After each surgical procedure, and prior to any cortical deactivations, baseline performance levels (monocularly for tasks 1 and 2, binocularly for task 3) were re-established. The re-establishment of prior baseline levels of performance indicated that neither the surgical procedures nor the presence of the cryoloop interfered with performance. During monocular (tasks 1 and 2) or binocular (task 3) testing, a three-step cooling cycle paradigm was applied to all sessions: (1) baseline period with all cortical areas active; (2) testing period with the cryoloop cold; and (3) baseline period following release from cooling and resultant rewarming of cortex. Cooling of cortex was effected by circulating chilled methanol through the lumen of the loop. Each period (1, 2 or 3) in the cooling cycle consisted of one or more blocks of trials. For tasks 1 and 2, a testing block consisted of 25 trials and for task 3, a block consisted of 28 trials (two presentations at each of the 12 peripheral locations and four presentations adjacent to the cynosure). Generally, cortex was cooled for 60–90 min in an individual testing session and encompassed 4–6 blocks of trials each of 10–15 minutes in duration. Many blocks of trials were collected at one cryoloop temperature or one or more blocks were collected at sequentially lower temperatures. Approximately 5 min were permitted to elapse between testing blocks to ensure equilibration of neural tissue at a new temperature. Temperature measurements show that this period of time is more than adequate for cortex to stabilize at a new temperature.

Thermodynamic Measurements
At the conclusion of behavioral testing the cats were anesthetized (sodium pentobarbital, 25 mg/kg, i.v.) and we measured temperatures beneath the cooling loops to estimate the region deactivated during cooling of the cryoloops to 8 ± 1°C and 3 ± 1°C for each cat. Cortical temperatures during cooling were measured simultaneously at four different coronal levels in the brain using multiple microthermocouples (150 µm in diameter) manufactured for us by Omega Engineering (Stanford, CT). These microthermocouples were first positioned, then the loop was cooled to several temperatures. Between 50 and 60 sites were sampled at each of the coronal levels. This procedure ensured that temperature measurements for a given cryoloop temperature setting were taken at exactly the same sites in cortex. For each measurement, cortex was cooled for ~5 min prior to a recording being made, as occurred in the behavioral component of the study. This protocol was then repeated at multiple, sequentially sampled sets of sites.

Tissue Fixation and Processing
At the conclusion of all experimental procedures, the cats were injected intravenously with sodium pentobarbital anesthetic (50 mg/kg), heparin anticoagulant (5000 units) and 1% sodium nitrite vasodilator (1 ml). The cat’s vascular system was perfused with saline followed by 10% formalin. Tissue fixation and processing are detailed. Following histological processing, the temperatures at the sampled loci were plotted on camera lucida reconstructions of pMS cortex. The position of the 20°C thermocline was interpolated between relevant points by eye.

Statistical Analyses
Performance on complete blocks of trials, or groups of blocks, was compared both within and between testing sessions. For each animal, within-subject tests were initially carried out between the pre- and post-cooling baseline conditions with the cortex active or released from cooling respectively. Since there were no statistically significant differences between data collected during the pre- and post-cooling baseline periods for any cat, data were pooled to create a more comprehensive measure of baseline performance. Individual means and their standard errors were calculated from equal numbers of cooling cycles (a minimum of three) for the active, deactivated and released conditions.
periods. For task 1 (directions of motion), we conducted a separate 2 × 3 analysis of variance (ANOVA) for each of the two conditions (warm and cold) for the three differences in direction of motion examined (90°, 45° and 30°). For task 2 (patterns obscured by moving masks), we carried out a 2 × 2 analysis of variance. When a difference was detected with the ANOVAs, we conducted follow-up within-subject $t$-tests. In the Results, unless otherwise stated, if a difference is described as being significant or reliable it was at the $P \leq 0.01$ level. If a cooling-induced deficit was significant, additional $t$-tests were performed to determine if performance was different from chance.

Results
The Results section is divided into two parts. In the first part, we show that cooling of the cryoloops to different temperatures differentially impairs performance on the three tasks, and data from all four cats is presented in a summary of their behavioral performance. In the second part, we estimate the extent of cortex deactivated during cooling of the cryoloops to 8° and 3°C.

Comparison of Behavioral Performance during Cooling of pMS Cryoloops to Different Temperatures

Task 1: Direction of Motion
With pMS cryoloop temperatures >20°C, all four cats were highly proficient (80-86% correct responses) at discriminating differences in movement direction of 30° for two identical non-systematic patterns moving at 6.4°/s (Fig. 3, task 1, filled circles and solid lines). Cooling to progressively lower temperatures <20°C first initiated and then maximized an impairment
in motion processing, which was reduced to a complete impairment, and chance levels of performance, at a cryoloop temperature of 7–9°C (Fig. 3, task 1, filled circles and solid lines). Similar results were obtained with angular differences in motion direction of <30°. In contrast, performance on an easier 45° version of the task was completely unimpaired even when the cryoloop was cooled to 3 ± 1°C (Fig. 3, task 1, open circles and interrupted line). Presumably other regions of cortex are sufficient to maintain high performance when angular difference in direction is ≥45° (Lomber et al., 1996b). Also, cooling of an alternate site in visuotemporal cortex (vPS cortex), which is not involved in motion processing, had no effect on performance at any temperature or any angular difference in motion direction, thus attesting to the specificity of the effect to the blockade of pMS cortex and angular differences in motion of ≤30°.

**Task 2: Motion and Pattern**

With pMS cryoloop temperatures >15°C, all four cats were also highly proficient (80–90% correct responses) at discriminating...
The length of each bold line corresponds to the percentage of responses at each midline. The two concentric semicircles represent the 50% and 100% response levels. Curved arrows indicate movement of the right-most boundary of the attended visual field towards the midline. The two concentric semicircles represent the 50% and 100% response levels. Curved arrows indicate movement of the right-most boundary of the attended visual field towards the midline.

**Figure 4.** Visual orienting responses during cooling of the pMS cryoloop to different temperatures. Left cryoloop at (i) ≥9°C, (ii) 7°C, (iii) 5°C and (iv) 3°C. Curved arrows indicate movement of the right-most boundary of the attended visual field towards the midline. The two concentric semicircles represent the 50% and 100% response levels. The length of each bold line corresponds to the percentage of responses at each peripheral location tested. Data from Cat P. Note progressive reduction of visual field as the cryoloop temperature was lowered, until orienting to stimuli presented in the contracooled hemifield was abolished.

By recording neural activity immediately adjacent to a microthermocouple in these same cats (Lomber et al., 1999), we verified that the critical neuropil temperature for the silencing of neurons is 20°C (Jasper et al., 1970; Benita and Condé, 1972).

We identified that: (i) neuropil temperatures of 24°C or higher had no obvious effect on neuron discharges; (ii) neuropil temperatures >20°C, but <24°C, resulted in a graded weakening of evoked activity; and (iii) neuropil temperatures of 20°C or below induced neurons to become unresponsive to visual stimuli (Lomber et al., 1999).

**Behavioral Summary**

Therefore, all four cats were completely impaired (at chance levels of performance) on the discrimination tasks when the pMS cooling loop was cooled to 7–9°C. This was the case for the 30° difference-in-motion task (task 1) and the patterns obscured by a moving grid task (task 2), but not for the visual detection and orienting task (task 3). For the latter task, it was necessary to cool the cryoloops to 2–3°C to prevent visual orienting responses into the contralateral hemifield. In contrast, cooling of the loops to 1°C was without impact on the performance of the cats on any of the control tasks: (1) the 45° difference in direction-of-motion; (2) the pattern obscured by the static grid-mask; or (3) detecting and orienting toward stimuli introduced into the ipsilateral hemifield.

**Determination of Extent of Cortex Deactivated**

**Cortical Activity and Cortical Temperature**

By confirming that 20°C is the critical temperature for deactivating neurons, we set out to determine the position of the 20°C thermocline and estimate the extent of the cortex deactivated by cooling the cryoloop to the two temperatures (8°C and 3°C) that differentially blocked the three visual tasks (1 and 2 versus 3). We made several simultaneous tracks with microthermocouples through cortex adjacent to the cooling loops at several coronal levels, and measured temperatures at multiple depths along each track with the cryoloops chilled to 8°C and then to 3°C. An example of one of these microthermocouple tracks, with attendant temperatures, is shown in Figure 5. In this example, the microthermocouple penetrated through the superficial-most layers of pMS sulcal cortex and the
cryoloop was maintained at 8°C throughout. From the figure, it is immediately evident that the cooling of the loop to 8°C did not cool even the immediately adjacent cortex to 8°C, but rather to 12°C. Moreover, temperatures in excess of 20°C were measured at distances of between 1 and 1.5 mm away from the cryoloop. These observations tell us that cooling effects are extremely restricted in extent, presumably because the cooling must counter the constant delivery of 38°C blood to the region.

Previous studies have provided valuable data on the spread of cooling across the cortical sheet when a cooling device is placed in contact with its exposed surface (Alexander and Goldman, 1978; Lomber et al., 1996b), but none to our knowledge have mapped either the extent of the cooling when a cryoloop is placed in a sulcus and is cooled to different temperatures. To obtain these figures and locate the critical 20°C thermocline, we made 50–60 thermal measurements at four coronal levels with the cryoloop temperature held constant at either 8°C or 3°C, the temperatures that differentially abolish proficient performance on the three behavioral tasks. Examples of the temperatures recorded at cryoloop temperatures of 8°C and 3°C at one coronal level in one example cat are shown in Figure 6A,B respectively. Cooling of the cryoloop to 8°C placed the 20°C thermocline in the mid-depths of cortex (interrupted line in Fig. 6A), whereas cooling to 3°C pushed the 20°C thermocline to the vicinity of the gray/white matter interface (interrupted line in Fig. 6B). In the former instance, upper layer temperatures were <20°C, whereas deep layer temperatures were in the range of 20–30°C. In the latter instance, cortex throughout the thickness of both cortical banks was at a temperature <20°C. Companion histology from the same cat and a second cat (Fig. 7A,B) show that these deactivations silenced superficial layers I–III alone (8°C) and superficial and deep layers together (3°C) respectively. In addition, there was a slight lateral expansion in the extent of layer I–III cortex that was deactivated as cryoloop temperature was reduced from 8°C to 3°C. This lateral expansion amounted to 9.4% of the surface line deactivated, and contrasts with the expansion of 129.6% across the depth of the cortex during the same increase in cooling. When summed across the full extent of cortex deactivated by the 8°C and 3°C temperatures, the change in surface area deactivated by the increased cooling amounts to a net increase of ∼3% of the total initial large volume of cortex deactivated. In contrast, the same increase in cooling more than doubles (∼2.65) the already large volume of deactivated cortex as the 20°C thermocline spreads from the middle layers through to the gray/white interface. The difference in the scale of these values convinces us that there is an extremely strong causal linkage between the expansion of the cooling across the deep layers and the abolition of contralateral orienting. It is unlikely that the decrease in orienting performance can be linked in any substantive way to the slight lateral expansion of deactivated layers I–III, although there is no doubt

Figure 5. Coronal section through the MS sulcus, stained for myelin to show a microthermocouple penetration to record temperatures in the superficial layers of pMS cortex during cooling of the cooling loop to 8°C. Track is identified by densely staining glial cells along the initial portion and lighter staining along distal portion of track. Circles show the position of the cooling loop in the sulcus. Left is medial and scale bar = 2 mm.

Figure 6. Temperature measurements recorded from identical sites in the cortex lining the middle suprasylvian sulcus while the cooling loop (circles) was cooled to 8° (A) and 3°C (B) in Cat P. Dashed line indicates the position of the 20°C thermocline estimated from these measurements. Scale bar = 1 mm.
Figure 7. (A, B) Estimated position of the 20°C thermoclines for cats P (A) and D (B) during cooling of the loop to 8° and 3°C in cat P (i) and cat D (ii). With cooling to 8°C the 20°C thermocline lay in the mid-depths of the cortex, whereas cooling to 3°C pushed the position of the 20°C to the vicinity of the gray–white matter junction. Dark stipple demarcates region of cortex with temperatures <20°C when the cryoloop was cooled to 8°C. Combined, the dark and light stipple zones demarcate the region of cortex <20°C when the cryoloop was cooled to 3°C. (C, D) Cortical histology following post-mortem removal of the cryoloop. Coronal sections, near those used for the thermocline interpolations shown in (A) and (B), stained for Nissl substance to show the integrity of the region after cooling loop implantation and multiple cooling cycles. Circles show the position of the cooling loop in the sulcus. Outside diameter of cryoloop tubing is 0.63 mm. During tissue processing the sulcus opened slightly to displace the lateral bank away from the estimated cryoloop position in contact with the medial bank. Impressions in the cortical surface indicate accurately the position of the cooling loops. Left is medial and scale bar = 2 mm.
that the impairments on the two ‘motion’ tasks are included primarily by deactivation of these layers alone.

Discussion
Our results show that cooling of cryoloops in contact with the medial and lateral banks of the pMS sulcus to 8°C or 3°C differentially impairs the discrimination (tasks 1 and 2) and detection and orienting (task 3) tasks in a temperature-dependent way (Fig. 3). Moreover, cooling loops to these temperatures places the 20°C thermocline, the critical temperature for blocking stimulus evoked activity in the anesthetized cat, either in the mid-depths of cortex (8°C cryoloop) or near the base of layer VI (3°C cryoloop; Fig. 7). We interpret these results to show that blockade of visually evoked activity in the superficial layers fully impairs the difference-in-motion (task 1) and the pattern-and-motion (task 2) discriminations, while leaving performance on the detection and orienting task (task 3) unimpaired. Full impairment on this latter task was only achieved when the deactivating temperatures included the deep cortical layers.

Functional Differentiation of the Cortical Layers
It is worth acknowledging that the determination of 20°C as the critical temperature for complete neuronal blockade in the anesthetized animal may not completely reflect the critical temperature for identical effects in awake cats. This possibility exists because anesthesia, like cooling, tends to depress neuronal activity and the two effects may be additive. In that event, we may have mildly overestimated the volume of cortex deactivated during cooling of the cryoloop to both 8°C and 3°C. Notwithstanding that possibility, we still conclude that a given thermocline lies much deeper in the cortex beneath a 3°C cryoloop than beneath an 8°C cryoloop, and that our reliance on the terms ‘superficial’ and ‘superficial and deep’ are adequate descriptions of the deactivation.

The inclusion of the deep layers in the deactivation, even in awake cats, is accurate because cooling of pMS cryoloops to 1–3°C virtually abolishes uptake of 2-deoxyglucose (2DG) in cortex in contact with, and in the immediate vicinity of, the cooling loop and the diminution in uptake approximates the regions delineated by the thermal measurements. 2DG uptake in all other brain regions is in the normal range (Payne and Lomber, 1999). 2DG is a relevant marker because it accurately represents metabolic activity (Sokoloff, 1981a,b) and it is an excellent secondary marker of neural activity with very active neurons taking up very little (Schoppmann and Stryker, 1981).

Residual Activity in Deep Layers during Silencing of Superficial Layers
During cooling of the cryoloop to 8°C and the silencing of the superficial layers, what is the evidence that neuronal activity is preserved in deep cortical layers? We can partially answer this question directly because of the previously described link between temperature and neuronal activity, and because we know that temperatures in the deep layers were 20–30°C when the superficial layer temperatures were <20°C (Fig. 8a). However, we do not have direct evidence from these cats on this point, and we must consider the data of others. For example, Schwark and co-workers provide electrophysiological evidence from area 17 that neuronal activity is present in the deep layers in the absence of activity in the superficial layers (Schwark et al., 1986). As in the present study, Schwark et al. cooled the superficial layers and most neurons in layers V and VI remained highly active and responded well to visual stimuli. Equivalently, many deep layer neurons remain active when the superficial layers have been destroyed by a cryolesion (Schwark et al., 1986). We presume that deep layers in middle suprasylvian cortex behave in a similar way during limited superficial deactivation.

However, deep cortical layers do not go completely unaffected by deactivation of the superficial layers. For some neurons, levels of activity were reduced, but for many others activity levels remain very high and the neurons have normal receptive field properties. Such reductions that are identified are understandable because many deep layer neurons have apical dendrites that ascend into the superficial layers where they receive many signals. Moreover, many superficial layer neurons project excitatory axons into the deep layers which also activate neurons. Even so, the feature most relevant to the current study is not that activity is reduced in the deep layers, but that many neurons retain strong visual responsiveness. These observations imply that there are pathways to deep layer neurons which bypass the superficial layers, and that these pathways are capable of activating deep layer neurons alone. Moreover, sufficient circuitry remains unaffected by the cooling to permit the emergence of sophisticated receptive field properties, such as orientation and direction selectivity. Virtually identical results have been obtained by Ferster et al. in area 17 (Ferster et al., 1996).

Direct projections from extrinsic sources are likely to be the strongest source of visual signals activating deep layer pMS neurons in the absence of superficial layer activity. Unfortunately information on projections from extrinsic sources directly into deep pMS cortex is not available. However, it is known that the visual thalamus and areas 17, 18 and 19 project into layer IV (Sugiya, 1979; Symonds et al., 1981; Sherk, 1989; Lowenstein and Somogyi, 1991; Payne et al., 1991; Shipp and Grant, 1991). Many of these same axons may possess collaterals that terminate in the deep layers, just as their equivalents entering area 17 do (Lund et al., 1979; Gilbert and Wiesel, 1983; Martin and Whitteridge, 1984).

Even so, it is likely that layer IV is an essential link for visual signals to reach the deep layer neurons in the absence of superficial layer activity. Supporting evidence for this proposal is again provided by Schwark and co-workers (Schwark et al., 1986). They showed that increasingly greater levels of deactivation of layer IV progressively decreased the number of deep layer neurons that remained active in the absence of superficial layer activity. The source of this deep layer driving activity was the LGN (Malpeli, 1983; Malpeli et al., 1986), which also projects axons to pMS cortex (Rosenquist, 1985; Lomber et al., 1995; Kawano, 1998). These signals are of the Y and W types (MacNeil et al., 1997), and the Y-signals may contribute significantly to both rapid activation and much of the visual responsiveness in both upper and lower layers of pMS cortex.

Electrophysiological and Anatomical Evidence for Laminar Dissociations of Function
The bipartite functional division of cortex into upper and lower layers has been recognized for a long time (James, 1890), but has gone untested. Electrophysiological studies have hinted at the division by demonstrating differences in the functional properties of neurons in upper, middle and deep layers (Hubel and Wiesel, 1962; Gilbert, 1977; Berman et al., 1982; Payne and Berman, 1983). Moreover, anatomical data reveal that neurons
located in different layers have distinct projection targets. Upper layer neurons target, via lateral and ascending pathways, other regions in cerebral cortex (Rosenquist, 1985); and deep layer neurons target, via feedback projections, lower order cortical areas and, via descending projections, thalamus and midbrain (Gilbert and Kelly, 1975). Ascending projections have a large functional impact and probably drive higher order neurons (Bullier et al., 1994; Vanduffel et al., 1997; Payne and Lomber, 1999). The lateral pathways appear to contribute significantly to the formation of associations between neuron populations that permit the detection of coherence, and segmentation. In contrast, feedback and descending projections appear to regulate transmission of ascending signals by turning the ‘neural attentional spotlight’ onto specific groups of neurons to enhance neural activity, and thereby salience. Increases in salience probably contribute to ‘pop-out’ effects, breaks of camouflage and increased visibility under conditions of poor contrast (McClurkin et al., 1994; Sillito et al., 1994; Hupe et al., 1998; Merabet et al., 1998). With reversible deactivation techniques we have had the opportunity, hitherto unavailable, to test and confirm with behaviors that upper and lower cortical layers make different contributions to brain function (Lomber, 1999).

The concept of functionally differentiable laminar subdivisions within pMS sulcal cortex is supported by anatomical evidence which shows that the efferent connections of the superficial and deep layers differ substantially. The dominant targets of superficial layer neurons are area 19 and the splenial visual area, whereas targets of deep layer neurons include areas 17 and 18, the thalamus, superior colliculus and pontine nuclei (Rosenquist, 1985; Kawamura et al., 1978; Bjaalie, 1986). In other words, signals transmitted from the superficial layers of pMS cortex to area 19 or forward to splenial visual cortex are retained within the cerebral network and are likely to be of a different character from signals emanating from deep pMS layers transmitted backwards to either thalamus and areas 17 and 18, or downwards to the superior colliculus and pontine nuclei. In functional terms, the forward and lateral projections may be considered associative and convey information on segmentation and coherence, between elements in the displays, whereas the ‘backwards’ signals may shift attention and the ‘descending’ signals may result in the direct activation of motor programs. The latter suggestion is supported by two deactivation studies (Ogasawara et al., 1984; Hardy and Stein, 1988), both of which show that cooling deactivation of middle suprasylvian cortex results in the depression of neuronal activity in intermediate and deep collicular layers, the origin of premotor and motor outputs from the superior colliculus (Stein and Meredith, 1993).

An alternative view, which we must address, postulates that the progressive cooling gradually degrades a unitary network within pMS cortex that is required for all three behaviors, and that difficult tasks are more fragile and are more readily blocked before the less demanding tasks. This view is supported by the differences in performance on the control and experimental tasks, and in this view it is reasonable to think that some discriminations are more demanding than some detection tasks, and that our results reveal nothing more than that some tasks are more difficult for the cat than others. However, this view is extremely tentative and it ignores the numerous covariations in behavior, cryoloop temperatures, extent and completeness of deactivation, and the overall anatomy of these aspects. Moreover, it ignores the link between our measures and the demonstrably different anatomical circuits. Together, observations reveal so much more about the system than the tentative view expressed above.

We postulate that it is the parallel covariation in behavioral performance on three tasks — covariation with cryoloop temperature, covariation with vertical but not lateral spread of cortical deactivation and covariation with connectional anatomy of efferent projections from pMS cortex — that strongly reinforce the strength of our conclusion. For some though, the differences in behavior that we have identified by the two deactivation temperatures reflect the impact of the cooling on the medial and lateral banks of the pMS sulcus. At present, there is no reason to adopt this view because the receptive field properties of neurons in the medial and lateral banks are largely indistinguishable (von Grünau et al., 1987). Moreover, both medial and lateral banks are critical for guiding detection and orienting (Hardy and Stein, 1988; Lomber et al., 1996b) and both banks appear to be critical for motion processing (Pasternak et al., 1989; Lomber et al., 1996b). In the event that the reasoned explanation is correct, it does not diminish our overall argument for superficial and deep layer contributions to visual processing, it merely separates them into upper layers of one pMS sulcal bank and lower layers of its opposite bank.

Collectively, our reflections on this study remind us of the words of William James (James, 1890) and the aphorism of Irving Diamond:

To summarize, layer V projects to brainstem centers in the tectum, pons, and medulla which have direct or indirect connections to motor neurons. Thus, every area of the cortex can be viewed as a motor area, or layer V itself could be termed ‘motor cortex’. Every area of the cortex is also an association area inasmuch as each receives corticocortical fibers from other subdivisions of the same field. Finally . . . every area of the neocortex is sensory in as much as each is the target of sensory impulses that arise in the thalamus. (Diamond, 1979)

Notes
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Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Cg</td>
<td>cingulate gyrus</td>
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<tr>
<td>pMS</td>
<td>posterior-middle suprasylvian</td>
</tr>
<tr>
<td>MEG</td>
<td>middle ectosylvian gyrus</td>
</tr>
<tr>
<td>Mg</td>
<td>marginal gyrus</td>
</tr>
<tr>
<td>MSt</td>
<td>middle suprasylvian gyrus</td>
</tr>
<tr>
<td>MSs</td>
<td>middle suprasylvian sulcus</td>
</tr>
<tr>
<td>MT</td>
<td>middle temporal visual area</td>
</tr>
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<td>V5</td>
<td>fifth visual area</td>
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<tr>
<td>wm</td>
<td>white matter</td>
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</table>

References


Diamond IT (1979) The subdivisions of the neocortex: a proposal to revise the traditional view of sensory, motor, and associative areas. Prog Psychobiol Physiol Psychol 8:1–45.


