SUPERIOR COLLICULUS LESIONS PREFERENTIALLY DISRUPT MULTISENSORY ORIENTATION

L. R. BURNETT,* B. E. STEIN,* D. CHAPONISb AND M. T. WALLACE**

*Department of Neurobiology and Anatomy, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA
bDepartment of Psychology, Wake Forest University, Winston-Salem, NC, USA

Abstract—The general involvement of the superior colliculus (SC) in orientation behavior and the striking parallels between the multisensory responses of SC neurons and overt orientation behaviors have led to assumptions that these neural and behavioral changes are directly linked. However, deactivation of two areas of cortex which also contain multisensory neurons, the anterior ectosylvian sulcus and rostral lateral suprasylvian sulcus have been shown to eliminate multisensory orientation behaviors, suggesting that this behavior may not involve the SC. To determine whether the SC contributes to this behavior, cats were tested in a multisensory (i.e. visual-auditory) orientation task before and after excitotoxic lesions of the SC. For unilateral SC lesions, modality-specific (i.e. visual or auditory) orientation behaviors had returned to pre-lesion levels after several weeks of recovery. In contrast, the enhancements and depressions in behavior normally seen with multisensory stimuli were severely compromised in the contralesional hemisphere. No recovery of these behaviors was observed within the 6 month testing period. Immunohistochemical labeling of the SC revealed a preferential loss of parvalbumin-immunoreactive pyramidal neurons in the intermediate layers, a presumptive multisensory population that targets premotor areas of the brainstem and spinal cord. These results highlight the importance of the SC for multisensory behaviors, and suggest that the multisensory orientation deficits produced by cortical lesions are a result of the loss of cortical influences on multisensory SC neurons. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: corticotectal, cross-modal, visual, auditory, excitotoxins, ibotenic acid, lesions.

The superior colliculus (SC) plays an important role in integrating information from multiple sensory systems (i.e. vision, audition and somatosensation), as well as in generating signals that result in movements of the eyes, ears, head and body (see Stein and Meredith, 1993). Subserving the multisensory role of the SC is a substantial population of neurons that respond to stimuli from more than one sensory modality. These multisensory neurons exhibit significant changes in their responses when presented with stimuli from multiple sensory modalities (Meredith and Stein, 1983, 1986b; King and Palmer, 1985; Wallace et al., 1996). Their responses can be substantially enhanced or depressed depending on the spatial, temporal and physical characteristics of the stimuli that are combined (Meredith and Stein, 1986a,b; Meredith et al., 1987; Kadunce et al., 1997). The same factors that govern multisensory integration in single SC neurons also govern multisensory orientation behaviors (Stein et al., 1988). Thus, multisensory stimulus combinations that enhance neural activity in SC neurons also enhance behavioral performance, whereas combinations that depress activity in SC neurons result in degraded performance (Stein et al., 1988, 1989; Wilkinson et al., 1996; Jiang et al., 2002).

The most straightforward interpretation of the parallels between neuronal activity and behavior is that changes in the responses of multisensory SC neurons are directly responsible for changes in behavior (Stein et al., 1988, 1989). However, recent work has shown that two areas of cortex, the anterior ectosylvian sulcus (AES) and the rostral lateral suprasylvian cortex (rLS) play a critical role in these processes (Wilkinson et al., 1996; Jiang et al., 2002). Both of these cortical areas project heavily to the multisensory layers of the SC (Tortelly et al., 1980; Stein et al., 1983; Clemo and Stein, 1984; Norita et al., 1986; McHaffie et al., 1988; Harting et al., 1992, 1997), where they appear to gate the integrative properties of their SC target neurons (Wallace and Stein, 1994; Jiang et al., 2001; Jiang and Stein 2003). Thus, deactivation of AES or rLS disrupts multisensory integration in SC neurons, while having little effect on their responses to modality-specific cues. Similarly, deactivation of these areas compromises multisensory orientation behavior, but has little effect on modality-specific behavior (Wilkinson et al., 1996; Jiang et al., 2002). A recent neural network model of multisensory integration has been constructed in which response enhancements in SC neurons can be gated by corticotectal inputs (Anastasio and Patton, 2003).

The loss of multisensory orientation behavior following AES and/or rLS deactivation has been interpreted as a functional loss of these critical corticofugal influences. However, there are also populations of multisensory neurons in AES (Wallace et al., 1992; Jiang et al., 1994a,b) whose projections do not target the SC (Wallace et al., 1993). These cortical neurons integrate their multisensory inputs in much the same way as SC neurons (Wallace et
al., 1992). Hence, it is possible that the loss of multisensory orientation following AES/rLS deactivation reflects the loss of these cortical multisensory populations. Although little is known about the projections of these cortical multisensory neurons, AES and rLS target a number of cortical and subcortical loci in addition to the SC (Stein et al., 1983; Micelli et al., 1985; Norita et al., 1986; Olson and Graybiel, 1987; Gimenez-Amaya, 1988; McHaffie et al., 1988; Tamai and Miyashita, 1989; Scannell et al., 1995), and may comprise a multisensory circuit independent of the SC.

The current study sought to distinguish between these possibilities by producing excitotoxic lesions of the SC and examining the consequent effects on multisensory-mediated behaviors. A portion of this work has been published in abstract form (Burnett et al., 2000).

EXPERIMENTAL PROCEDURES

General description

All experimental procedures were conducted using aseptic techniques and were in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 86-23) and an approved Wake Forest University School of Medicine Animal Care and Use Committee protocol and all efforts were made to minimize the number of animals used. Using standard shaping methods, cats (n=7) were trained with food rewards to orient to either visual or auditory stimuli presented within a perimetry device (Stein et al., 1989; Wilkinson et al., 1996; Jiang et al., 2002). Animals were selected for the ease with which they could be handled and were prescreened to ensure that they had no visual or auditory deficits. Six animals were trained on an orientation task in which the target was a visual stimulus. In this paradigm the animals also learned that responses to auditory stimuli would not be rewarded, and consequently learned to “ignore” the auditory stimuli. Following training to criterion (see below), the intensities of the visual targets were lowered and the effects of spatially coincident and spatially disparate auditory stimuli on orientation responses were examined. An additional animal was trained with the stimulus conditions reversed. In this case the auditory stimulus was the target, and the visual stimulus was unrewarded. Following the collection of a baseline behavioral data set, lesions of the multisensory SC layers (i.e. below stratum opticum) were performed via injections of excitotoxic agents (see below). Unilateral lesions to the left SC were performed in six animals and one animal received a bilateral lesion. Following a recovery period, all animals were tested in the same apparatus using the same paradigms. At the end of the data collection, animals were killed, perfused and their brains were processed histologically in order to assess the extent and location of the damage.

Apparatus

The perimetry device used in behavioral testing is identical to that previously described (Stein et al., 1989; Wilkinson et al., 1996; Jiang et al., 2002). Briefly, the apparatus consisted of light-emitting diodes (LEDs) and small speakers that were positioned on a semicircular array at a fixed distance from the animals start position (50 cm). Target LEDs and speakers were arranged at 15° intervals from center and were mounted in the same horizontal plane as the cat’s eyes. The entire apparatus was situated inside a darkened, sound-attenuated chamber (auditory background 47 dB sound-pressure level [SPL]).

Training/testing procedures

The six cats trained on the visual task learned to orient to a briefly (40 ms) illuminated target LED presented at one of five locations (0°, ±30° and ±45° where (+) represents locations to the right of center and (−) represents locations to the left of center). At the beginning of each trial, the cat was held at the start position so that its head was facing the central (i.e. 0°) LED. One of the possible targets was then illuminated and the animal was required to make an orientation movement to it in order to receive a food reward. A correct response was scored when a movement was made within 5° of the target within 3 s of its presentation. The incidence and location of correct and incorrect responses were recorded manually by the experimenter or by an assistant in single blinded trials. Training continued until the animal reached a criterion performance level (>95% correct at each target location). Once criterion was reached, the intensity of the visual stimulus at each location was reduced until the animal was working near behavioral threshold (i.e. 50% correct responses). Threshold intensities were typically in the range of 0.07–0.09×10−3 foot candles. Once visual thresholds were established, the unrewarded auditory stimulus was introduced into the training process. Auditory stimuli could originate from any of seven locations (−45°, −30°, −15°, 0°, 15°, 30°, 45°). The stimulus consisted of a 40 ms broad-band noise burst at a constant intensity of 52 dB SPL.

During behavioral testing, performance (i.e. percent correct responses) for three stimulus conditions was examined for each visual target location. Stimulus conditions were: 1) visual alone, 2) spatially coincident visual–auditory (both stimuli presented at the same spatial location) and 3) spatially disparate visual–auditory (stimuli separated by 45°). This resulted in a total of 14 stimulus combinations that were interleaved in a pseudo-random order and repeated twice daily. The total data set consisted of 44 trials for each of the combinations, and took 22 days to acquire. In addition, auditory catch trials were presented as a block following each day’s testing session to ensure that the animal was performing at chance to the auditory stimulus. Following the collection of this baseline data set, SC lesions were made as described below and the animal was allowed to recover. Once visual orientation responses recovered (generally within a month), each animal was then re-tested in an identical set of post-lesion trials. The same experimental protocol was employed for the animal trained in the auditory task except for the reversal of the valence of the auditory and visual stimuli.

Excitotoxic injection surgical procedure

Animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and maintained with supplemental doses (1–2 mg/kg, i.v.) as needed. Following the establishment of a stable plane of anesthesia, animals were placed in a stereotaxic head holder using atraumatic ear bars. To maintain adequate levels of hydration, lactated Ringer’s was administered i.v. throughout the procedure. Core body temperature was monitored and maintained between 38 and 39 °C by means of a thermostatically regulated heating pad. A craniotomy was made to allow access to the SC, and the placement of the excitotoxic injections was guided by electrophysiological recordings. A coordinate grid was constructed based on these recordings and was used to guide the placement of the injection microsyringe (5 μl total volume, 30 gauge needle). A total of nine penetrations were made in a 3 mm×3.5 mm grid with 1 mm between injection sites. These injections were made in each penetration at depths of 1.5, 2.5 and 3.5 mm below the dorsal surface of the SC. Lesions were made using injections of 0.05 μl of a 1% ibotenic acid (IBO)–5% N-methyl-D-aspartate (NMDA) solution (see Berson and Graybiel, 1991). The final concentrations of IBO and NMDA were 10 μg/μl and 50 μg/μl, respectively. IBO was
chosen because of its effectiveness in destroying cell bodies but sparing fibers of passage (Schwarcz et al., 1979).

**Behavioral data analysis**

For the five animals with unilateral SC lesions and trained in the visual task, across subject comparisons were evaluated using a $2 \times 2 \times 9$ repeated measures analysis of variance. Post hoc differences were then analyzed using paired-sample t-tests for two measures: 1) pre- vs. post-lesion performance for each stimulus condition at each location, and 2) modality-specific vs. modality-specific performance at each location. For the animal trained to the auditory target, the animal with a bilateral lesion and the animal with a small lesion (see Results), within-subject data comparisons were analyzed using McNemar’s exact change tests for correlated proportions (Rosner, 1994). The same two measures described above were analyzed using these tests.

**Euthanasia, immunohistochemistry and cell counts**

Animals were killed by means of an i.v. overdose of sodium pentobarbital (100 mg/kg) and were perfused intracardially with buffered saline followed by a fixative solution containing 4% paraformaldehyde and 0.25% glutaraldehyde. The SC was blocked and cut coronally on a vibratome (section thickness 60 μm). The tissue was then counterstained using Neutral Red, and was reacted immunohistochemically for the calcium binding proteins calbindin-D28k (CB) and parvalbumin (PV; Mize et al., 1991; Henkel et al., 1997; McHaffie et al., 2001). Because PV neuron labeling showed the clearest differentiation between areas of presumptive damage and those outside of the lesion, it was chosen as the marker to delimit the extent of the lesions that were drawn using Neurolucida. In an attempt to better identify the extent of the functional damage to the SC, the incidence of PV- and CB-labeled cells was quantified using a technique similar to that described previously (Benson et al., 1997; Henkel et al., 2003). To count the cells within each section, color images were acquired using a Nikon EFD-3 microscope equipped with a Diagnostic Instruments Inc. Spot digital camera (resolution $1024 \times 768$). Images were taken at $4 \times$. A total of eight images were required to view both the left and right SC and care was taken to align each section so that there was no overlap. Using Scion Image, the deep SC layers were drawn and a macro was used to normalize the selected area within each image. A threshold mean and standard deviation were established using a control image. The threshold for labeled cells was 1 standard deviation above the normalized image mean. Cells touching the edge of each image were not counted. Counted elements were eliminated from the data set if their maximum width was less than 25% of their maximum length. Cell count differences between lesioned and unlesioned sides were analyzed using the chi-square statistic.

**RESULTS**

**Pre-lesion behavior**

In the six animals trained to orient to visual stimuli (five with unilateral lesions and one with a bilateral lesion), pre-lesion data were consistent with previous reports examining multisensory orientation behaviors (Stein et al., 1988, 1989; Wilkinson et al., 1996; Jiang et al., 2002). Thus, when an unrewarded (i.e. neutral) auditory stimulus was combined with a visual stimulus at the same location (spatially coincident trials), there was a significant increase in the percentage of correct responses (Fig. 1A), indicating that the target had become more salient. In contrast, when the neutral auditory stimulus was presented at a location 45° disparate from the visual stimulus (spatially disparate trials), there was a significant decrease in the percentage of correct responses, indicating that the target had become less salient (Fig. 1B). This auditory modulation of the effectiveness of a visual stimulus in eliciting behavior was highly reliable across animals and testing sessions, and was seen at each of the tested locations.

**Transient behavioral deficits following unilateral SC lesions**

Following unilateral SC lesions, each of the animals showed a characteristic pattern of immediate but transient deficits that included ipsiversive circling in four of the six cases. Following the resolution of such circling (range 0–2 days), the deficits were characterized by a lack of orientation toward sensory stimuli in the contralesional hemisphere. This sensory neglect generally continued for a number of weeks. Although animals showed some variability in the duration of neglect, responses to generalized sensory stimuli (e.g. hand waves, finger snaps or body touches) presented in the affected hemisphere typically returned within 1 month (mean 29 days, range 12–35 days). Once an animal’s responses to these stimuli returned in the affected hemisphere, and there were no apparent left-right asymmetries in response to these highly salient stimuli, they began retraining in the perimetry device.

In the initial days of this retraining, it was necessary for stimuli in the contralesional hemisphere to be much more intense when compared with pre-lesion values. Thus, sensory thresholds were initially much higher in the affected hemisphere (thresholds were unchanged in the ipsilesional hemisphere). However, after an average of 1 week of training (range 2–12 days) this asymmetry disappeared and animals responded to stimuli in the contralesional hemisphere at thresholds comparable (±10%) to those used during pre-lesion tests. Once criterion performance was reached during the retraining phase (i.e. >95% correct responses), each animal was then tested using an identical set of trials to those used prior to the lesion to assess multisensory performance.

**Long-term multisensory behavior deficits following unilateral SC lesions**

Although modality-specific (i.e. visual) performance had returned to pre-lesion levels following retraining in all animals, there was a substantial change in both behavioral indices of multisensory integration (multisensory enhancement and multisensory depression) in four of the five animals. In these animals, enhanced performance in the presence of spatially coincident multisensory stimuli was lost at all tested contralesional locations, but remained at pre-lesion levels in response to stimuli presented in the ipsilesional hemisphere (Fig. 2A). A similar pattern of post-lesion performance changes was evident in the responses to spatially disparate stimuli. In these cases a contralesional auditory stimulus no longer had a significant depressive impact on responses...
Fig. 1. Control (i.e. pre-lesion) orientation responses to visual and spatially-coincident (A) and spatially disparate (B) multisensory stimuli. In the center of each figure is a representation of the perimetry device used for behavioral testing and a drawing of a normal cat brain. Bar graphs summarize pooled behavioral data from five animals, and illustrate the percentage of correct behavioral responses for visual (V) and multisensory (VA) conditions, as well as the proportionate change in response upon multisensory stimulation (interactive index, calculated using the formula $[((VA-V)/V)\times100]$. (A) Responses are plotted for visual and spatially coincident visual–auditory combinations at five locations (arrows from perimetry device show stimulus location). Note the significant enhancement in correct responses to the multisensory stimulus at each location; t-test, * P<0.01, ** P<0.001. (B) Responses are plotted for visual and 45° spatially disparate visual–auditory combinations at four locations. Note here that the locations on the perimetry device represent the location of the unrewarded auditory stimulus. For each combination, this auditory stimulus significantly depresses responses to the visual stimulus.
Fig. 2. Orientation responses to visual and spatially coincident (A) and spatially disparate (B) multisensory stimuli following unilateral SC lesions. Data are from the same five animals shown in Fig. 1. All conventions are the same as in Fig. 1, except that the shading on the cat brain shows the side of the SC lesion and the darker shading on the perimetry device denotes the affected (i.e. contralesional) hemifield. Bars outlined by dashes and shaded in light gray depict pre-lesion behavior (see Fig. 1). Post-lesion multisensory behavior is represented with the darker shading and the black bars. (A) Note that following the lesion, multisensory enhancement is lost for locations in the affected hemifield. ‡ Denotes significant differences between pre- and post-lesion multisensory performance; t-test, ‡ P<0.01, ‡‡ P<0.001. In contrast, multisensory enhancement remains robust in the unaffected hemifield. As in Fig. 1, asterisks denote significant differences between visual and multisensory performance. (B) Following the lesion, the unrewarded auditory stimulus, when positioned in the affected hemifield, lost its ability to depress responses to the spatially disparate visual stimulus. In contrast, when the auditory stimulus was positioned in the unaffected hemifield, significant response depression was still seen.
The effects of bilateral SC lesions were assessed in an additional animal. Prior to the lesion, the animal was trained in the visual task (see above), and exhibited the characteristic enhanced orientation behavior to spatially coincident multisensory stimuli (Fig. 6), and depressed orientation behavior to spatially disparate multisensory stimuli (data not shown). Immediately following the lesion, the animal exhibited a profound inability to orient to novel stimuli anywhere in its home environment. However, much like the unilateral lesion cases described above, the animal began responding to visual stimuli within 27 days and was retrained to criterion performance on the visual orientation task on day 32. When examined in the multisensory paradigm, there was a complete absence of multisensory enhancements to spatially coincident stimuli at all tested locations (Fig. 6). Although there was a similar trend toward a loss of multisensory depression in response to spatially disparate stimuli, the effects failed to reach statistical significance for this single case.

**Extent and specificity of the lesions**

Despite the extensive multisensory behavioral deficits that were produced by the lesions, histological examination revealed that in each case only portions of the SC were damaged (Fig. 7). Parvalbumin (PV) neurolabeling provided the best contrast for assessing areas of presumptive damage. In the five unilateral cases showing profound multisensory behavioral deficits, quantitative analyses of the area of clearly diminished PV immunoreactivity revealed that the average lesion encompassed only 40% (range 15–86%) of the volume of the multisensory (i.e. intermediate and deep) layers of the SC. In the fifth unilateral case, which showed minor behavioral change (see above), the lesion encompassed only 8% of these layers. In the bilateral case...
(Fig. 7B), the damage on the left and right sides was 42% and 21%, respectively.

Although PV neuropil immunoreactivity was the most obvious histological marker for delimiting the region of excitotoxic-induced damage, cell counts revealed that the area of functional damage likely extended well beyond this boundary. Most notable was the loss of PV-immunoreactive neurons, which included large pyramidal elements located in the intermediate layers (Fig. 8A). In contrast, although reduced in number, the loss of CB-immunoreactive neurons was much less severe (Fig. 8B). A quantitative analysis of these two neuronal populations in three animals revealed that whereas the loss of PV-immunoreactive neurons extended for more than 1 mm beyond the neuropil-delimited lesion border, CB-immunoreactive neurons were reduced in number only within the region of neuropil damage (Fig. 9).

**DISCUSSION**

The results of the current study demonstrate that the integrity of the SC is essential to maintain an animal’s ability to integrate visual and auditory cues in the control of orientation behavior. Excitotoxic lesions of the multisensory layers of the SC eliminated this ability, thereby rendering a stimulus from one modality (e.g. auditory) incapable of modulating the salience of a target stimulus from another modality (e.g. visual). This result is consistent with earlier suggestions regarding the multisensory role of the SC (Stein et al., 1988), the general role of this structure in sensory-evoked orientation behavior (see Stein et al., 2002), and with the ability of individual SC neurons to integrate information from different sensory modalities (Stein and Meredith, 1993).

Immediately after the unilateral lesions, ipsiversive circling followed by a profound neglect for contralesional stimuli was typically seen. Consistent with prior reports, these initial deficits were transient, lasting between 2 and 5 weeks (Sprague and Meikle, 1965; Tunkl, 1980; Flandrin and Jeannerod, 1981; Vievard et al., 1986; Rosenquist et al., 1996). Subtler but more permanent deficits, such as alterations in saccade dynamics (Schiller et al., 1980) and response biases when two competing stimuli are presented (i.e. perceptual rivalry, see Sprague and Meikle, 1965; Sprague, 1972; Rosenquist et al., 1996) were not examined in the current study.

Once the initial deficits had resolved, the animals were readily retrained to orient to a visual or auditory target, and returned to performance levels equivalent to those seen prior to the lesion. This result suggests that the integrity of the SC is not necessary for the performance of modality-specific orientation tasks, a conclu-
sion in keeping with previous reports (Sprague and Meikle, 1965; Tunkl, 1980; Flandrin and Jeannerod, 1981; Vievard et al., 1986; Midgley et al., 1988; Rosenquist et al., 1996). Likely candidates for subserving these modality-specific behaviors following SC lesions include the geniculostriate system, the frontal eye fields, and auditory cortical projections that bypass the SC, possibly targeting the inferior colliculus. Alternatively, the lesions may have spared neuronal elements in the SC that subserve modality-specific function (for an elaboration on this see the discussion below on the possible specificity of the lesions). However, in contrast to this lack of effect on modality-specific behaviors was the finding that the lesions resulted in what appears to be a permanent loss in the animal’s ability to integrate visual and auditory inputs in order to enhance (for spatially coincident stimuli) or degrade (for spatially disparate stimuli) orientation performance.

One possible explanation for the loss of multisensory orientation was that the lesions induced a consistent response bias. For example, the lesions could have induced a sensory- and/or motor-bias such that responses were systematically shifted by 10°, thus falling outside of the 5° window required to be scored as a correct. However, no orientation difficulty was noted in modality-specific trials and no systematic response biases were noted following the lesions. A second possibility was that the lesions selectively compromised the access of auditory information to the SC. It has been shown previously that transient deactivation of the SC induces a loss of auditory orientation (Lomber et al., 2001). However, no post-recovery orientation impairment was noted when an animal was trained to use an auditory stimulus as a target. Thus, recovery from the SC lesion-induced hemineglect (see also Lomber and Payne, 1996; Payne et al., 1996) appears to have selectively involved modality-specific mechanisms.

Somewhat perplexing was the “all-or-none” nature of the changes seen to stimuli presented in the contralateral hemifield. Thus, despite the fact that in no animal did the damage encompass all of the multisensory SC layers (on average the loss was 40%), in all but one case the loss of multisensory enhancement was complete for the affected hemifield(s). Furthermore, there appeared to be no regional specificity to these effects, since both rostrally biased and caudally biased lesions resulted in a complete behavioral effect. Though these results seem counterintuitive in light of the topographic organization of the SC, they are consistent with previous studies of visual neglect (Sprague and Meikle, 1965; Sprague, 1972; Kirvel et al., 1974; Dean and Redgrave, 1984; Midgley et al., 1988; Payne et al., 1996; Rosenquist et al., 1996; Lomber et al., 2001). These results are also consistent with models of SC organization in which both sensory and motor function are coded by activity.
within large neuronal ensembles (Hikosaka and Wurtz, 1986; Lee et al., 1988; Badler and Keller, 2002).

The excitotoxic cocktail used here to produce the lesions preferentially targeted a specific neuronal subpopulation, those whose cell bodies were strongly immunoreactive for the calcium binding protein PV. Although the precise mechanism for this selectivity is not known, there is evidence that excitotoxic analogs of glutamic acid are more effective in destroying glutamatergic neurons that receive glutamatergic afferents (Nadel and Cuthbertson, 1980), a profile that is consistent with the large PV-immunoreactive pyramidal neurons of the deep SC (Mize, 1996). Most interesting in the context of the current study is the finding that a subpopulation of PV-immunoreactive SC neurons project to brainstem regions involved in the control of eye, ear and head movements (Mize et al., 1992, 1996), and have the same profile and laminar distribution as multisensory neurons (Huerta and Harting, 1984; Moschovakis and Karabelas, 1985; Wallace et al., 1993; McHaffie et al., 2001). Taken together, these results suggest that the lesions may have had a preferential impact on multisensory neurons, and that the consequence of depriving the SC of neurons capable of integrating visual–auditory signals was a loss of multisensory-mediated behavior. In contrast, the selectivity of the lesions may have left

---

**Fig. 6.** Orientation responses to spatially coincident multisensory stimuli from an animal with a bilateral SC lesion. Conventions are the same as Fig. 2. Note the abolition of multisensory enhancement at all tested locations following the lesions.

**Fig. 7.** Neurolucida drawings of selected coronal sections through the SC depicting the area of clear histological damage following the unilateral (A) and bilateral (B) SC lesions. The extent of the lesions (black shading) was drawn from sections stained for PV (see text for detail). Unilateral cases are aligned in descending order of damage, and the percentage values represent the extent of the damage in the multisensory SC layers.
many modality-specific visual and auditory neurons intact. Whether such neurons actually participated in the recovery of modality-specific orientation is not known. In light of the present findings, the cortical lesion-induced losses of multisensory behavior previously reported (Wilkinson et al., 1996; Jiang et al., 2002; Jiang and Stein, 2003) most likely reflected the functional loss of cortical inputs to the SC. Influences from the AES and rLS are known to be essential for multisensory SC neurons to integrate cross-modal stimuli (see Wallace and Stein, 1994; Jiang et al., 2001, 2002). Depriving these multisensory SC neurons of the ability to integrate visual–auditory signals would be reflected in the loss of this ability in the control of SC-mediated orientation responses, a behavioral result that would parallel that induced by the present lesion-induced loss of these critical SC neurons.

Fig. 8. Immunohistochemical staining for the calcium-binding proteins PV (A) and CB (B) on the lesioned (left) and unlesioned (right) sides following a unilateral SC lesion. (A) In the larger photomicrograph of a coronal section, note the clear area of lighter PV neuropil staining on the lesioned side (scale bar = 1 mm). Boxes show the location of the higher power insets (scale bar = 100 μm). In these, note the absence of large heavily PV-immunoreactive pyramidal neurons (arrows) in the intermediate layers of the lesioned SC. (B) An adjacent section immunoreacted for CB. Note that although apparent, the loss in neuropil immunoreactivity is not as obvious on the lesioned side as for the PV. Also note in the insets that although there is a clear difference in the number of small CB-immunoreactive neurons on the lesioned side, a number of these neurons are still present.
Acknowledgements—We would like to acknowledge Dr. John McHaffie for his assistance with the histology, and Dr. Craig Henkel for his assistance with the cell count analysis. This work was supported by NIH MH63861, NS22543 and NS36916.

REFERENCES


(Accepted 11 December 2003)