Multisensory Orientation Behavior Is Disrupted by Neonatal Cortical Ablation

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Jiang, Wan, Huai Jiang, Benjamin A. Rowland, and Barry E. Stein. Multisensory orientation behavior is disrupted by neonatal cortical ablation. J Neurophysiol 97: 557–562, 2007. First published September 13, 2006; doi:10.1152/jn.00591.2006. The integration of visual and auditory information can significantly amplify the sensory responses of superior colliculus (SC) neurons and the behaviors that depend on them. This response amplification depends on the development of SC inputs that are derived from two regions of cortex: the anterior ectosylvian sulcus (AES) and the rostral lateral suprasylvian sulcus (rLS). Neonatal ablation of these cortico-collicular areas has been shown to disrupt the development of the multisensory enhancement capabilities of SC neurons and the present results demonstrate that it also precludes the development of the normal multisensory enhancements in orientation behavior. Animals with neonatal ablation of AES and rLS were tested at maturity and found unable to benefit from the combination of visual and auditory cues in their efforts to localize targets in contralesional space. In contrast, their ipsilesional multisensory orientation capabilities were indistinguishable from those of normal animals. However, when only one of these cortical areas was removed during early life, later behavioral consequences were negligible. Whether similar compensatory processes would occur in adult animals remains to be determined. These observations, coupled with those from previous studies, also suggest that a surprisingly high proportion of SC neurons capable of multisensory integration must be present for orientation behavior benefits to be realized. Compensatory mechanisms can achieve this if early lesions spare either AES or rLS, but even the impressive plasticity of the neonatal brain cannot compensate for the early loss of both of them.

INTRODUCTION

Having multiple sensory systems allows the brain not only to monitor a variety of environmental cues in parallel, and to substitute one system for another when necessary (e.g., hearing and touch substitute for vision in darkness), but also to benefit from the pooling and integration of the information they contain. The integration of multisensory information is a characteristic property of neurons in the superior colliculus (SC) and can be seen as a mechanism for modulating the physiological salience of external events (see Stein and Meredith 1993). Thus the same stimulus conditions that enhance or depress the responses of SC neurons (e.g., Bell et al. 2001; Binns and Salt 1996; Kadunce et al. 1997; King and Palmer 1985; Meredith and Stein 1983, 1985; Peck 1996; Perrault et al. 2003; Stanford et al. 2005; Wallace et al. 1996; Zangenehpour and Chaudhuri 2001) also enhance or depress SC-mediated orientation behaviors (Frens and van Opstal 1998; Jiang et al. 2002; Stein et al. 1988, 1989).

The ability of SC neurons to engage in normal multisensory integration is dependent on influences from two adjacent cortical regions in the cat: the anterior ectosylvian (AES) and the rostral lateral suprasylvian (rLS) sulci (for a review, see Stein 2005). These cortico-collicular influences are particularly evident in the enhanced responses of multisensory SC neurons: when they are eliminated by reversible cortical deactivation, multisensory stimuli evoke responses that are no greater than those produced by the most effective unisensory component stimulus (Jiang and Stein 2003; Jiang et al. 2001; Wallace and Stein 1994). The physiological consequences resulting from disrupting these cortico-collicular influences in adults are paralleled by disruptions in their SC-mediated orientation behavior (Jiang et al. 2002; Wilkinson et al. 1996).

Although critical for normal SC function, cortical influences develop only gradually during early life (Stein et al. 1973; Wallace and Stein 1997; 2000) when the brain is maximally plastic (Buonomano and Merzenich 1998; Rauschecker 1999; Wickelgren and Sterling 1969) and the cortex is first gathering experience with cross-modal cues (Perrault et al. 2004; Wallace and Stein 1997). The plasticity of cortico-collicular circuits in multisensory processing became evident when neonatal ablation of either AES or rLS was shown to produce no obvious alteration in the development of the multisensory enhancement capabilities of SC neurons (Jiang et al. 2006), a finding that was unexpected in light of the serious consequences of deactivating either of these areas in normal adults (Jiang et al. 2001, 2002). These data suggested that the developing brain compensates for the loss of either AES or rLS. In contrast, when neonatal lesions included both AES and rLS, little developmental compensation was noted: a permanent physiological disruption of SC multisensory enhancement capabilities was produced. Thus few SC neurons developed this capability and those that did appeared to do so because of islands of spared tissue at the lesion site (see Jiang et al. 2006). The present study was initiated to determine the effect of these early lesions on the animals’ ability to use cross-modal cues to control overt orientation behavior.

METHODS

All survival surgery was conducted using aseptic techniques and in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 86-23) and an approved IACUC protocol at Wake Forest University School of Medicine, an AAALAC-accredited institution. These experiments were conducted with the same animals used previously to study the responses of SC neurons (see Jiang et al. 2006). Thus the ablation procedures are only briefly summarized here.

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Neonatal cortical ablation

Cortical ablations were performed at 3 wk postnatal. This is before visual stimuli can activate neurons in the multisensory (i.e., deep) layers of the structure and 1 wk before cortex first begins to mediate multisensory integration in SC neurons (Kao et al. 1994; Stein et al. 1973; Wallace and Stein 2000; Wallace et al. 1997). Each animal was anesthetized with ketamine hydrochloride (15–30 mg/kg, administered intramuscularly) and isoflurane (0.2–3%, inhalation). A unilateral craniectomy exposed the cortex over AES, rLS, or both areas. The area of interest was removed by aspiration. An attempt was made to remove all cortical gray matter and the ablations often included some of the underlying white matter. The surgical area was then covered with Gelfoam, the bone was replaced, and the skin was sutured closed. Animals were given postsurgical analgesics (butorphanol tartrate, 0.1–0.4 mg·kg⁻¹·h⁻¹) as needed and received antibiotic treatments for 7–10 days (ceftriaxone 20 mg·kg⁻¹·b.i.d. or enrofloxacin 5 mg·kg⁻¹·b.i.d.).

Behavioral training and tests

Animals were allowed to mature to 1–2 yr of age, at which time they were trained on an SC-mediated spatial localization task using previously established methods (e.g., see Jiang et al. 2002; Stein et al. 1988, 1989; Wilkinson et al. 1996). Training and testing took place within a 90-cm semicircular array of light-emitting diodes (LEDs) and speakers placed at 15° intervals to the left (–), right (+), and centered on a fixation point at 0° (see Fig. 1). Animals were trained to fixate on the fixation point and, within 3 s of the appearance of a briefly (40-ms) illuminated LED, to approach that LED. The animals were also trained to maintain fixation during trials in which no stimulus appeared (“catch” trials) and also in response to brief (40-ms) bursts of broadband noise (60 dB). In all cases correct performance was rewarded with a small food reward (175-mg pellet). During training, LEDs appeared at maximum intensity (3.0 × 10⁻³ fc), first at 0° then randomly at other eccentricities (±15, ±30, and ±45°). Training was complete when an animal’s responses were accurate on 95% of the trials. Visual intensities were then lowered to elicit between 30 and 50% response accuracy to the visual target stimulus at each location. The intensity of the auditory stimulus was unchanged. During testing, visual and auditory stimuli could appear alone (as in training), simultaneously at the same location, or not at all (catch trials).

When all experiments were terminated, the animals were deeply anesthetized (sodium pentobarbital, 100 mg/kg, administered intravenously) and perfused transcardially with formalin (10%). The brain was removed, cut into 50-μm frozen sections, and stained with cresyl violet or neutral red. The cortical tissue was examined with low-power and high-power microscopy and reconstructed serially by projecting and tracing scanned sections through the lesion site. Reconstructions of the lesions were previously described (see Jiang et al. 2006).

Data acquisition and analysis

Unisensory and multisensory orientation behavior in contralesional and ipsilesional space was evaluated so that each animal served as its own control. These data were also compared with controls drawn from previous studies with normal animals performing the same tasks in this apparatus (Jiang et al. 2002; Stein et al. 1988, 1989; Wilkinson et al. 1996). Error patterns (erroneous localizations vs. No-Go responses) were also recorded to determine the possible influence of response bias. Approximately four to eight trials per stimulus type were conducted per day, allowing estimation of daily accuracy between 13 and 25%. The data were analyzed separately for each
animal. For each stimulus location within the perimetry device for each animal, the following was calculated.

1) Response accuracy: This represents the percentage of correct responses, defined as accurate localization of the visual target when only the visual stimulus was present (unisensory) and when the visual stimulus was accompanied by a spatially and temporally coincident auditory stimulus (multisensory). The response accuracies under the different stimulus conditions were compared using t-test after arcsine transformation to stabilize variance.

2) The Multisensory Index (MSI): This reflects the difference between the multisensory and unisensory response accuracies, divided by the unisensory (visual) response accuracy. This measure parallels that used for assessing multisensory integration at the level of the single neuron (see Meredith and Stein 1983) and provides a measure of the percentage change in the localization of the visual stimulus caused by the addition of the auditory stimulus

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\text{MSI(\%)} = \left( \frac{\text{Accuracy}_{\text{MSI}} - \text{Accuracy}_{\text{uni}}}{\text{Accuracy}_{\text{uni}}} \right) \times 100
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3) Percentage of No-Go responses: No-Go “responses” were trials in which the animal did not move from the start position within 3 s of the appearance of the target stimulus.

4) Percentage of incorrect orientations: This represents the percentage of orientation responses that were incorrect (removing all No-Go responses) when only the visual stimulus was present (unisensory) versus when it was accompanied by a spatially and temporally coincident auditory stimulus (multisensory trial). A value of 50\% indicates that half the orientation responses were to the visual target location and half were to some other location. This metric examines possible auditory-induced shifts in the willingness of the animal to make an orientation response (i.e., its “response bias”), irrespective of whether it knows the target location. For example, if multisensory response accuracy was better than unisensory accuracy but there was no corresponding decrease in the percentage of incorrect orientations, this was taken as evidence of an increase in response bias. In such a case the addition of the auditory stimulus would simply have increased the overall number of localization responses by decreasing the number of No-Go trials. The higher level of “response accuracy” would have spurious; that is, it would not be indicative of enhanced performance as a consequence of multisensory integration. These data were also compared with t-test after arcsine transformation.

R E S U L T S

Animals and lesions

Four of six animals receiving neonatal lesions were later found suitable for behavioral training as adults: two had dual cortical ablations (i.e., both AES and rLS were removed) and two had single cortical ablations (i.e., either AES or rLS was removed). All data are organized so that contralosional space is always to the left (−) and ipsilesional space is always to the right (+). All cortical ablations were performed at 3 wk postnatal and all animals were allowed to reach maturity (i.e., 1–2 yrs of age) before beginning training and subsequent behavioral testing. The histological reconstructions of these lesions and their consequences for multisensory integration in single SC neurons of these animals were published previously (see Jiang et al. 2006). Schematics of lesion locations are reproduced as insets in Fig. 1. Briefly, the extensive nature of the lesions and the early maturational stage at which they were made produced developmental changes in the geometry of nearby sulci and gyri and distinguished the ipsilesional and contralosional cortices. These conformational changes in cortical morphology were likely enhanced by the inclusion of the underlying white matter in some of the lesions and would have been expected to produce functional changes that were greater than those associated with the dimensions of the lesions on the cortical surface. Thus it was surprising to find only minimal changes in the properties of multisensory SC neurons (for discussion, see Jiang et al. 2006) and the behaviors that depend on them (see following text) after early ablation of either AES or rLS.

Behavioral consequences of neonatal lesions

Although the behavioral consequences of combined neonatal AES and rLS ablation matched the severity of the effects observed with adult animals when these cortices were temporarily deactivated (Jiang et al. 2002), the effects of treating these regions individually were strikingly different in adults and neonates. In adults, the deactivation-induced loss of AES or rLS influences severely disrupted multisensory orientation behavior, whereas neonatal lesions of either area alone had no apparent effects on these behaviors. The performance data for animals with each of the neonatal ablation conditions (AES and rLS, AES alone, and rLS alone) are plotted in Fig. 1.

The figure illustrates that all animals exhibited the typical enhancement in the localization of an ipsilesional visual target when it was accompanied by a simultaneous and spatially coincident auditory stimulus (15° increments in eccentricity from 0 to 45° were tested). Multisensory response enhancement as measured by the Multisensory Index (MSI) ranged from 68 to 160% and all differences between multisensory and unisensory performance were statistically significant (P < 0.05). This response enhancement occurred despite the fact that the auditory stimulus was “neutral” and was not, itself, a target for a response. Furthermore, the MSI was comparable to that obtained previously with normal adult cats (see Jiang et al. 2002; Stein et al. 1988, 1989; Wilkinson et al. 1996). Therefore the response scores at each location for each individual animal were used as a control to predict that animal’s overall performance accuracy as well as to predict the MSI in the matching contralosional locations.

Animals that had neonatal ablation of both AES and rLS exhibited profound deficits in contralosional multisensory performance. The multisensory response accuracy was on average 38\% less than that predicted based on ipsilesional performance, a difference that was statistically significant at each perimetric location examined for each animal (P < 0.05). Similarly, the MSI was 76\% less than that expected. In contrast, animals that lost only one of these areas (either AES or rLS) were unimpaired. Their average multisensory response accuracy was equivalent to that in ipsilesional space (the average <9\% difference observed was not statistically significant) and the same was true of their average MSI (<14\% decrement was not statistically significant). In short, no significant deficits in contralosional multisensory orientation performance were noted in the single cortical lesion animals.

Despite the magnitude of the multisensory dysfunction in animals with combined AES and rLS ablation, close inspection of the data revealed that averaged multisensory response accuracy was slightly, albeit statistically significantly, enhanced at two contralosional locations (~15° and ~45°) in one animal. The underlying cause of these small putative multisensory response “enhancements” was evaluated by considering the possible influence of response bias. In Fig. 2, the percentage of...
increasing the overall number of orientations, without actually increasing the number of accurate responses by in-creasing the percentage of orientation errors. Apparently, the auditory enhancement (i.e., the MSI) against unisensory response accuracy for each location in contralesional and ipsilesional space was plotted in that animal (Cat 9907).

At every ipsilesional location the multisensory stimulus produced a robust increase in response accuracy and a decrease in the percentage of incorrect orientations. This is consistent with data from normal animals and indicates that any change in response bias induced by the addition of the auditory stimulus was relatively small (e.g., see Jiang et al. 2002; Wilkinson et al. 1996). In contrast, response bias proved to be a major factor in the marginally enhanced performance in response to contralesional stimuli. Whenever this animal showed what appeared to be a statistically significant contralesional multisensory enhancement, there was no corresponding significant decrease in the percentage of orientation errors. Apparently, the auditory stimulus increased the number of correct responses by increasing the overall number of orientations, without actually improving the overall degree of response accuracy.

The results of the various lesions on multisensory performance are summarized in Fig. 3. Here the index of enhanced multisensory performance, MSI, is plotted against unisensory response accuracy for each location in contralesional and ipsilesional space for each animal. The data in each hemifield were collapsed across perimetric locations and then fit by linear regression. Animals with ablation of AES and rLS showed significant ipsilesional–contralesional differences (F-test, $P < 0.05$) that were not seen in animals that lost only one of these cortical regions. The “principle of inverse effectiveness” (see Stein and Meredith 1993) was also evident in the performance of all animals on both sides of space; that is, the MSI was greatest when the unisensory response accuracy was lowest. The figure also shows a great deal of consistency in ipsilesional performance across animals, which is consistent with previous observations (see Jiang et al. 2002; Stein et al. 1988, 1989; Wilkinson et al. 1996).

**DISCUSSION**

Animals in which AES and rLS were removed unilaterally during neonatal life proved to be severely impaired as adults in using multiple sensory cues to guide orientation behavior. Their deficits were quite specific. Whereas their orientation responses were normal to unisensory visual targets anywhere in space, and were also normal in response to ipsilesional visual–auditory stimuli, they failed to develop the characteristic ability to benefit from this cross-modal stimulus combination in contralesional space (for normative data, see Stein et al. 1988, 1989). As a consequence, the coupling of the neutral auditory cue with the visual target in contralesional space failed to enhance orientation performance, a result that closely parallels that obtained during reversible deactivation of AES and rLS (see Jiang et al. 2002; Wilkinson et al. 1996). This developmental failure did not appear to be attributable to a lesion-induced hearing loss. The animals showed no obvious impairments in responses to sound, an auditory stimulus could induce shifts in their response bias, and the incidence of auditory-responsive neurons in the ipsilesional SC was similar to that of normal animals (see Jiang et al. 2006). In addition, previous studies showed that deactivation of AES and rLS compromises neither SC auditory responses nor orientation to an auditory target in these perimetric tests (Jiang et al. 2001, 2002; but also see Malhotra et al. 2004). Rather, the contralesional anomaly was likely a result of the failure to develop normal ipsilesional multisensory processes as indicated by the
misregister in the visual and auditory receptive fields of multisensory neurons here and the inability of these neurons to integrate cross-modal stimuli (see Jiang et al. 2006). Yet, the unisensory responses of these neurons were like those of normal animals, thereby paralleling their overt orientation responses to modality-specific stimuli.

It is interesting to note that physiological evaluation of these animals showed that some (15%) SC neurons did develop the capability to integrate cross-modal cues, a result attributed to the sparing of isolated areas of AES and/or rLS (see Jiang et al. 2006). The present observations indicate, however, that the proportion of these neurons was far too low to support the typical enhancement in multisensory orientation behavior that is seen in intact animals. Indeed, more than a majority of the normal complement of such neurons appears to be required to support this capability. Previously it was shown that although the deactivation of rLS in normal adult cats affected fewer ipsilateral multisensory SC neurons than did deactivation of AES, it still eliminated this capability in roughly 44% of these neurons. Behavioral tests demonstrated that the remaining 56% of the multisensory integrating population was insufficient to support enhanced multisensory orientation behaviors. The animals showed no better orientation responses to the cross-modal stimulus combination than to its unisensory component stimuli (Jiang et al. 2002). These observations indicate that cortico-collicular influences from both AES and rLS are normally necessary to support multisensory enhancement in orientation behavior and that even when they are both lost during early life, the brain does not craft alternate circuits to substitute for them in this role.

However, these observations do not indicate that AES and rLS are insensitive to early lesion-induced compensatory changes. In contrast to the dysfunctions in the animals with combined AES and rLS removal discussed above, those in which only one (either AES or rLS) area was removed appeared to be quite normal; the different receptive fields of multisensory SC neurons overlapped one another and these neurons showed the typical benefit from the combination of cross-modal cues in both hemifields. Apparently, the loss of potential cortical influences essential for SC multisensory integration (see Wallace and Stein 2000) and SC-mediated multisensory behavior during neonatal life is less disruptive than when their actual influences are removed in adulthood.

The contrast between the effects of dual and single cortical lesions during early life suggests that the compensatory processes initiated by single cortical lesions involved changes in the cortico-collicular projections from the remaining area. It is not yet known whether these cortico-collicular changes involved the retention of normally exuberant projections and/or the sprouting and growth of new projections from the intact cortical area and/or the involvement of other developmental changes. Furthermore, it is not yet known whether the capacity to initiate such compensatory changes is restricted to early development. Although this seems likely given the high degree of plasticity in the neonatal as compared with the adult brain, the possibility that similar lesion-induced compensatory mechanisms, even if less robust, could be initiated in adult animals cannot yet be excluded. This is because the comparison observations currently available in adult animals involve acute deactivation of AES and/or rLS—a method that not only retains the anatomical integrity of the pathways involved in SC multisensory integration, but one that also does not allow assessing possible long-term functional changes in that circuitry.

Irrespective of whether these compensatory changes are restricted to early life, however, their presence is surprising: comparable changes in the unisensory properties of ipsilesional SC neurons are not seen after early ablation of the cortico-collicular regions on which they normally depend (see Stein and Gallagher 1981; Stein and Magalhães-Castro 1975). This distinction may reflect differences in the inherent plasticity of these cortical areas, but it seems highly likely that the critical factors include the unique and shared relationship between AES and rLS and the SC neurons involved in multisensory integration. SC multisensory integration depends on nearly equivalent influences from AES and rLS (Jiang et al. 2001), cortices that are not only highly interconnected (see Meredith 2004; Scannell et al. 1995), but also have overlapping terminal territories in the SC (Segal and Beckstead 1984; Stein et al. 1983). Indeed, they sometimes target the same multisensory SC neurons; a “redundant” input (see Jiang et al. 2001) that in this context would be expected to support the normal development of these “shared” SC target neurons as well as those with which they are uniquely coupled. Given the similarities in their tectopetal roles and territories, they are also well suited to substitute for one another by expanding or retaining their exuberant projections to SC neurons that normally are coupled uniquely with their cortical counterpart. In doing so they provide an alternate source of the higher-order influences that these SC neurons need to synthesize their cross-modal inputs, a role that other cortical regions appear unable to duplicate.

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