Auditory Space Map in the Guinea Pig Superior Colliculus

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Ingham, Neil J., Sally K. Thornton, Damian McCrossan, and Deborah J. Withington. Neurotransmitter involvement in development and maintenance of the auditory space map in the guinea pig superior colliculus. J. Neurophysiol. 80: 2941–2953, 1998. The mammalian superior colliculus (SC) is a complex area of the midbrain in terms of anatomy, physiology, and neurochemistry. The SC bears representations of the major sensory modalities integrated with a motor output system. It is implicated with saccade generation, in behavioral responses to novel sensory stimuli and receives innervation from diverse regions of the brain using many neurotransmitter classes. Ethylene-vinyl acetate copolymer (Elvax-40W polymer) was used here to deliver chronically neurotransmitter receptor antagonists to the SC of the guinea pig to investigate the potential role played by the major neurotransmitter systems in the collicular representation of auditory space. Slices of polymer containing different drugs were implanted onto the SC of guinea pigs before the development of the SC azimuthal auditory space map, at ~20 days after birth (DAB). A further group of animals was exposed to aminophosphonopentanoic acid (AP5) at ~250 DAB. Azimuthal spatial tuning properties of deep layer multiunits of anesthetized guinea pigs were examined ~20 days after implantation of the Elvax polymer. Broadband noise bursts were presented to the animals under anechoic, free-field conditions. Neuronal responses were used to construct polar plots representative of the auditory spatial multiunit receptive fields (MURFs). Animals exposed to control polymer could develop a map of auditory space in the SC comparable with that seen in unimplanted normal animals. Exposure of the SC of young animals to AP5, 6-cyano-7-nitroquinoxaline-2,3-dione, or atropine, resulted in a reduction in the proportion of spatially tuned responses with an increase in the proportion of broadly tuned responses and a degradation in topographic order. Thus N-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors and muscarinic acetylcholine receptors appear to play vital roles in the development of the SC auditory space map. A group of animals exposed to AP5 beginning at ~250 DAB produced results very similar to those obtained in the young group exposed to AP5. Thus NMDA glutamate receptors also seem to be involved in the maintenance of the SC representation of auditory space in the adult guinea pig. Exposure of the SC of young guinea pigs to γ-aminobutyric acid (GABA) receptor blocking agents produced some but not total disruption of the spatial tuning of auditory MURFs. Receptive fields were large compared with controls, but a significant degree of topographical organization was maintained. GABA receptors may play a role in the development of fine tuning and sharpening of auditory spatial responses in the SC but not necessarily in the generation of topographical order of the these responses.

INTRODUCTION

The mammalian midbrain nucleus, the superior colliculus (SC) is well established as a center for the integration of multisensory input (for extensive review, see Stein and Meredith 1993). The SC bears mutually aligned neural representations of the world in the form of visual and auditory space maps and a somatosensory map of the body surface (Dräger and Hubel 1975, 1976; King and Palmer 1985). The auditory space map, present in the deeper layers of the SC, has been described clearly in several species, especially the guinea pig (for example, Withington-Wray et al. 1990c), ferret (for example, King and Hutchings 1987), cat (Middlebrooks and Knudsen 1984) and in the avian homologue of the SC, the optic tectum (for example, Knudsen 1982). The guinea pig SC map of auditory space is highly dependent on visual and auditory experience for its correct developmental emergence. At 32 days after birth (DAB) (Withington-Wray et al. 1990a,b). This experience-dependent development also is seen in the other species mentioned above (King et al. 1988; Knudsen 1985). The ferret SC auditory space map also emerges during postnatal development, becoming well defined ~1 mo after the opening of the eyes and ears (King 1993). In the guinea pig, a short crucial period, from 26 to 30 DAB, has been demonstrated when the spatial auditory responses recorded from the deep SC undergo dramatic change, indicative of plastic modification (Withington-Wray et al. 1990d). Before this crucial period, auditory responses are broadly tuned for spatial location and do not show any topographical organization. During the 4-day crucial period, events occur that result in the responses becoming sharply tuned for a spatial position and finally becoming organized topographically to form a map of auditory space, clearly evident at 32 DAB. During the first month of life, the fast head growth of the guinea pig results in rapidly changing intensity and timing of interaural cues for sound position. This has been proposed as one reason that the SC map of auditory space is not present at birth but develops over a prolonged period, as described earlier, finally emerging when head growth (and therefore the rate of change of the interaural cues) has slowed sufficiently to allow mechanisms of neuronal plasticity to track these changes and still generate the space map. Similar periods also have been described for the development of sound localization behavior in the barn owl and for recovery of this behavior after sensory deprivation (for example, Knudsen and Knudsen 1990; Knudsen et al. 1984a,b). Several studies have contributed to the idea that the spatially tuned auditory responses in the deep layers of the SC develop under instructive influences derived mainly from the overlying visual space map (for example, King et al. 1988; Knudsen 1994). The maintenance of the SC map of auditory space in the adult guinea pig has been investigated in some detail. The map remains susceptible to deprivation in two modalities, although over different time courses: to auditory deprivation, before 100 DAB (Binns et
pared containing 5-aminophosphonopentanoic acid (AP5, Sigma) or atropine sulfate (Sigma), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, in a water soluble form, complexed with hydroxypropyl-β-cyclodextrin, Tocris Cookson) or both bicuculline methiodide (Sigma) and 2-hydroxyxyscophalen (Tocris Cookson). Aliquots of 10 µl of each stock solution were added per 10 ml of the polymer solution. Control polymer was prepared using the same volume of distilled H₂O instead of drug solution. The mixture was frozen in a bath of dry ice in acetone and the frozen polymer transferred to a −20°C freezer for 48 h. The polymer then was placed under a mild vacuum for a further 48 h. This process resulted in 1 ml of Elvax polymer per 10 ml of polymer solution. Therefore the final concentration of the drug in the polymer was 0.1 mM, a concentration of AP5 shown previously to affect axonal arborization in the superficial SC (Simon et al. 1992). At the time of our experiments, there was no information on the release characteristics of drugs from the polymer and no indication of IC₅₀ values for this preparation. The different drug containing polymers therefore were formulated to the same drug concentration as the AP5-Elvax (0.1 mM). The flexible Elvax polymer was cut into small squares of side 4 mm and sectioned into 90-µm sections on a freezing microtome. The polymer sections were rehydrated in physiological saline for 30 min before implantation into the brain.

**Implantation surgery**

Pigmented guinea pigs (Cavia porcellus) were used in this study. Various groups of animals were used to investigate the developmental effects of chronic exposure to the receptor antagonists. These animals were aged 19–21 DAB at the time of implantation of the polymer, either control-Elvax or drug-Elvax (i.e., before the crucial period of 26–30 DAB for the development of the SC auditory space map in this species) (Withington-Wray et al. 1990d). This allowed a period of time for the drug concentration to build up in the SC (by diffusion) before the crucial period began. Another group of guinea pigs was implanted with AP5-Elvax at 250–263 DAB (by which age the SC map of auditory space is thought to have become stabilized against the effects of deprivation of auditory and visual experience) (Withington et al. 1994) to investigate NMDA-receptor involvement in the maintenance of the SC auditory space map in the adult.

Guinea pigs were anesthetized using a combination of fentanyl citrate/hypnase (Hypnorm, Janssen) and midazolam hydrochloride (Hypnovel, Roche). The induction dose was administered as an intramuscular injection of Hypnorm (1 ml/kg) with intraperitoneal Hypnovel (2 ml/kg). Droxapram (Dopram, Willow Francis Veterinary; a short-term respiratory stimulant) 10 mg/kg iv was administered routinely to help to prevent respiratory insufficiency. The animal was placed in a minimal head holder. After administration of xylocaine (2%, Astra) under the scalp, an incision was made along the midline and the skin drawn back to expose the skull. Connective tissue and muscle were cleared from the surface of the skull, and a craniotomy was performed to expose the cortex overlying the right SC. After removal of the dura, the cortex was aspirated carefully, under a dissecting microscope, to expose the dorsal surface of the right SC. A 90-µm section of Elvax polymer (prepared as described in the previous section) then was positioned over the exposed surface of the SC. The wound was packed with Sterispon (Allen and Hanbury, London), the skull repaired using dental cement and the scalp sutured. The opioid antagonist, naloxone (Sigma) 0.1 mg/kg iv, was given to promote recovery from the analgesic.

**SC space mapping experiments**

The terminal electrophysiology was performed ≥20 days after the implantation of the Elvax polymer, i.e., after the normal emergence of the map, or after an equivalent period for the adult AP5-Elvax implants (details of animal ages are given in the results). Anesthesia was induced as described above, using intramuscular and intraperitoneal doses of Hypnorm and Hypnovel, respectively. In the majority of cases, the left external jugular vein was cannulated to enable anesthesia to be maintained using intravenous doses of Hypnorm and Hypnovel in distilled H₂O (1.6 ml/kg; 50% vol/vol Hypnorm/Hypnovel in water). If cannulation was not attempted, the anesthesia was maintained using intramuscular doses of Hypnorm (0.7 ml/kg) and intraperitoneal doses of Hypnovel (1.4 ml/kg) hourly. As before, with the animal in the minimal

**METHODS**

**Preparation of implant polymer**

The method used to synthesize the polymer was essentially that of Rhine et al. (1980). Beads of Elvax-40W (a gift from DuPont UK) were dissolved in methylene chloride (Sigma) to give a 10% solution. Stock drug solutions (10 mM for each drug) were prepared, dissolved in distilled H₂O. Batches of polymer were prepared containing 5-aminophosphonopentanoic acid (AP5, Sigma; dissolved in 1 equivalent of NaOH and the pH adjusted accordingly), atropine sulfate (Sigma), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, in a water soluble form, complexed with hydroxypropyl-β-cyclodextrin, Tocris Cookson) or both bicuculline methiodide (Sigma) and 2-hydroxyxyscophalen (Tocris Cookson). Aliquots of 10 µl of each stock solution were added per 10 ml of the polymer solution. Control polymer was prepared using the same volume of distilled H₂O instead of drug solution. The mixture was frozen in a bath of dry ice in acetone and the frozen polymer transferred to a −20°C freezer for 48 h. The polymer then was placed under a
head holder, xylocaine was infused under the skin, and the scalp was reopened to reveal the skull. The dental cement and sterspon were removed to re-expose the SC. In all cases the polymer implant was found to be still lying in position over the SC. No movement of the polymer had taken place during the period after implantation. The polymer sheet was lifted carefully out, ensuring no damage to the superficial layers of the SC. Body temperature was maintained at 38 ± 1°C with a thermostatically controlled heating blanket and a rectal thermometer.

After surgery, the animal was transferred to the center of a large anechoic chamber, 3.1 × 3.1 × 2.2 m high, for the free-field presentation of auditory stimuli. Broad band noise stimuli (1.2–25 kHz), presented in 100-ms bursts (with 5 ms rise and fall times) repeated at 1 Hz, were played through an array of loudspeakers (Kef T27A) covering the entire 360° of the animals’ azimuthal plane at a distance of 1.2 m. The loudspeakers previously had been calibrated to produce an equal intensity stimulus in the center of the array; that is, in the center of the animal’s head. A series of penetrations were made, using glass-coated tungsten microelectrodes (∼1.0 MΩ), under direct visual control into the deeper layers of the SC. The entire rostrocaudal extent of the SC was visible from its border with the roof of the thalamus to the posterior border with the inferior colliculus. The rostrocaudal axis was normalized to a length of 4 mm for each of the animals used, and the recording position noted as a proportion of this normalized distance from the border with the inferior colliculus. Multiunit activity from the auditory responsive regions of the SC was amplified and then subjected to a signal processing technique, allowing neuronal activity to be distinguished from background noise (Chung et al. 1987).

Under software control, 100-ms segments of response were digitized using aCED 1401 interface, and a discrete fast Fourier transformation applied to the filtered (50 Hz to 3 kHz) neuronal activity to allow the power spectrum to be calculated. The neuronal signal was filtered to ensure that the stopband was below the Nyquist frequency (5 kHz) for the sampling rate of 10 kHz employed here to remove potential aliasing frequencies >5 kHz. It also is known that the frequency spectrum of individual action potentials lies in the range of 100 Hz to 3 kHz (Chung et al. 1987). After a number of iterations (usually 3), the responses were transformed and the resulting spectra averaged. The power spectral density was calculated using Simpsons’ rule. Background (spontaneous) neural activity was recorded as the spectral density of the signal recorded with no noise presentation. Activity also was recorded as the signal spectral density recorded in response to presentation of 100-ms noise bursts from each loudspeaker location at an isointensity level of 20 dB above auditory threshold (triggered at the same time as the onset of the noise burst). Stimulus-evoked activity then was calculated as the difference between the stimulus-induced and background activity spectral density measurements. The sound-intensity level needed to elicit the threshold response of each multiunit was recorded using a Bruel and Kjaer (type 4133) 0.5-in condenser microphone, placed over the animals’ head 4 cm above the midline, linked to a Bruel and Kjaer Type 2610 measuring amplifier, in decibels (dBA, re reference pressure of 20 μPa). Responses to visual stimulation (light flashes) also were obtained from the superficial layers of the SC in all animals. However, these responses were not specifically charted; only their presence or absence was noted.

**Data analysis**

The results obtained for each azimuthal sound source location were combined to produce a normalized polar plot. The radius of the polar plot represents the maximum neuronal activity recorded at that site. Submaximal values (normalized) were plotted along the radius corresponding to the appropriate loudspeaker position. Connecting each point plotted gives the auditory spatial multuni receptive field (MURF). From the polar plot, several indices of the spatial tuning of the MURF can be computed. These tuning parameters include the response area (RA), the area bound by the perimeter of the MURF, measured in arbitrary units; the Q50 angle, the angular spread of the response at a level of 50% of maximum; and the Q75 angle, the angular spread at 75% of the maximum response level. The magnitude of the RA and Q50 and Q75 angles are inversely related to the sharpness of spatial tuning of the MURF. These values were analyzed statistically using the following tests. The Kolmogorov-Smirnov test (α = 0.05) was used to test for normal distribution of the data. If a group of values passed this test, they were tested using analysis of variance (ANOVA, Tukey’s method for multiple comparisons). Data that did not follow a normal distribution were analyzed using Kruskal-Wallis nonparametric ANOVA (Dunn’s method for multiple comparisons).

The tuning parameters provide a measure of the degree of directional tuning exhibited by the neurons in the deeper layers of the SC. The topographical organization of the spatial responses was investigated by plotting the recording position within the SC against the peak angle of the response (taken from the polar plot). The data from normal control animals were found to have a linear progression of peak angle with regard to recording position. Thus it is valid to represent the normal control topography data using linear regression analysis. To compare other groups with the normal control data, the same linear regression analysis was used. Regression analysis of the peak angle/collital position data indicated how closely correlated these variables were; i.e., the degree of precision of the topographical organization. ANOVA of the regression results indicated whether the slope of the line was significantly different from zero.

Each MURF was allocated a grade, I-V, according to the magnitude of the each tuning parameter. Grade allocation (Binns et al. 1992) was based on values obtained from 295 responses from 53 normal guinea pigs. Values less than the mean were allocated grade I; grade II included those having a Q50 angle of grade III or higher. And grade IV was given to values lying between the mean ± 2 SD or greater. Individual MURF response profiles also were analyzed. MURFs were classified into several groups. 1) “Tuned” profiles had a single major peak, with a Q50 angle of grade II or lower. 2) “Bilobed” response profiles were classed as having an additional secondary peak reaching >75% of the maximum response amplitude. Bilobed profiles were of interest as they give information on the population of spatially ambiguous auditory receptive fields in a group of data. 3) “Broad” MURFs included those having a Q50 angle of grade III or higher. And 4) “omni directional” responses were defined as having no spatial positions resulting in <50% of the maximum response amplitude. These response profiles provide more information about the shapes of the individual receptive fields. This contrasts the grade allocations, which indicates the size of the angular spread of the receptive field.

**Methodological considerations**

Elvax is an excellent tool for delivery of substances over a prolonged period and is itself inert, causing no inflammatory responses or toxicity. In the studies described here, no attempt was made to assess the release of the drugs from the polymer, either in situ or in vitro. A recent study by Smith et al. (1995) demonstrated the release characteristics of glutamate receptor antagonists from Elvax. Aqueous drugs in the polymer showed relatively constant release levels for periods >60 days. Schmapp et al. (1995) demonstrated the release of [3H]-MK801 from 400-μm-thick Elvax containing 10 mM of the drug. In vitro studies showed that the polymer was still releasing 250 pmol MK801 per square
millimeter of polymer per day after 50–60 days incubation. Diffusion of the drug into the SC also was assessed, and significant levels were found in the first 800 μm of tissue from the pial surface of the SC. In the guinea pig SC, this diffusion range would include the superficial layers and the dorsal extreme of intermediate layers. Thus the drug essentially is restricted to the superficial layers of the SC. However, there are certain differences in the polymers used here. Only 0.1 mM of drug was included in the polymer, which was sectioned into 90-μm slices for implantation. Considering the just-mentioned findings and the fact that very clear differences were found between the SC auditory spatial properties of guinea pigs exposed to Elvax containing drugs and those exposed to Elvax loaded with water, it appears that the drugs within the polymers used here are being released into the SC and at a level sufficient to produce pharmacological effects. It is highly unlikely that the drugs penetrate into the intermediate layers of the SC, at least in effective concentrations. Therefore it is assumed that the implanted drugs are affecting the superficial layers only. We cannot say with certainty which synapses are likely to be affected.

There is a possibility that the larger MURFs seen in drug-exposed animals may result from small tuned single-unit receptive fields (SURFs) away from the best position of the MURF, producing a larger aggregate receptive field, recorded in the MURF. This possibility has been investigated in some detail in a previous paper (Withington-Wray et al. 1990c). The data presented in Withington-Wray et al. (1990c) illustrate clearly that in the guinea pig SC (unlike in other species, such as the frog, *Xenopus laevis*) broad MURFs, such as those recorded from young animals before map emergence, are composed of similarly broadly tuned single units with tuning characteristics on a par with those of the associated MURFs. In more mature animals, the sharply tuned MURFs were found to be a reflection of sharply tuned SURFs. However, we cannot entirely exclude the possibility that topographically inaccurate, although spatially tuned SURFs, do make up the MURF, although we feel that this is an unlikely scenario in the light of previous work.

Concern has been raised in other studies that exposure to AP5 produces reductions in spontaneous activity in visual cortical cells (Fox et al. 1989). By reducing spontaneous activity, evoked signals would tend to become more pronounced, that is, the neural signals/noise ratio would be increased. If this was the case, smaller evoked responses away from the best position of the MURF would become unmasked by the reduced noise levels, thereby broadening the receptive field. This would be an indirect/nonspecific effect of the drug on the receptive field shape. However, this was found not to be the case in any of the Elvax-implanted groups. Analysis of spectral power densities showed that the background activity of the drug-exposed groups (CNQX data not available) were not significantly different from the control-Elvax group (nonparametric ANOVA, P > 0.05; see Table 1). The control values returned the smallest mean value, with the drug-exposed groups showing slightly, but not significantly, elevated background levels. The power density measure used here reflects a combination of both the number of evoked spikes and their amplitude. From this information, the spike number and amplitude cannot be differentiated. Although this is perhaps not the ideal method, it was the only option available to us for evaluation of spontaneous activity. Therefore it can be concluded that concerns over changes in background spontaneous activity, at least at the time of recording, are unnecessary for these data. We cannot confirm that this was the case throughout the entire exposure/developmental period; the presence of the drugs may have influenced spontaneous activity before, but not during the recording session. It should be noted that this analysis applies to the deep layer multunit recordings. Because no measurements were taken of activity in the superficial layers, it is unknown whether the drugs are having any effects on the spontaneous activity of cells in this region.

**RESULTS**

**Comparison groups: normal, visual cortex removed, and dark-reared animals**

For purposes of comparison and contrast with the data obtained in the present study, results obtained in earlier studies on age-matched normal guinea pigs and on guinea pigs subjected to developmental removal of visual cortex before 20 DAB (Withington et al. 1991), guinea pigs aged 280 DAB (subjected to dark-rearing from 250 DAB) (Withington et al. 1994), and guinea pigs dark-reared from birth (Withington-Wray et al. 1990b) also are included. Animals subjected to developmental visual cortex removal are included because the surgical procedures used were very similar to those used in the implant studies. Animals aged >280 DAB from the study of Withington et al. (1994) are included as an age-matched control group for the adult AP5-Elvax implants. Response parameter values from all of these different groups are illustrated in Table 2. The distribution of grades allocated to each MURF from these groups is shown in Fig. 1. Information on the topographical organization and various other MURF indices also are given in Table 2. The topography of the MURFs from these four comparison groups is illustrated in Fig. 2.

Taken together, the topography correlation coefficients and the small response parameter values show that the normal, visual cortex removed and 280 DAB animal groups fulfill the criteria for the presence of an auditory space map. In contrast, dark-reared animals show no topographical organization of peak response angle and the tuning parameter values are very large; i.e., these animals do not exhibit a map of auditory space in the SC.

**Elvax-implanted animals**

**SC SPATIAL TUNING PARAMETER VALUES.** The mean values for the spatial tuning parameters obtained from the polar plots (see Fig. 3) of MURFs recorded in the various Elvax-implanted groups are shown in Table 2. The control-Elvax implanted group demonstrated tuning parameter values that on the whole were not statistically significantly different from the values seen in normal (unoperated) animals. The Q75 angle values for the control-Elvax group did reach statistical significance compared with the normal values (ANOVA, P < 0.05), but were in an equivalent range. Thus it can be said that the MURFs recorded from the control-Elvax implanted animals had a similar sharpness of spatial auditory tuning as is seen in normal animals. These values were significantly smaller than equivalent values obtained from animals dark-reared from birth, which demonstrate a complete lack of discrete spatial tuning.

The tuning parameter values obtained from animals implanted with Elvax-containing neurotransmitter-blocking drugs showed a very different pattern compared with normal and control-Elvax-implanted animals. Each group’s tuning parameter values were significantly larger than those seen in the normal and control-Elvax-implanted animals. That is, animals implanted with a drug either did not develop or, in the case of the adult AP5-Elvax implanted animals, lost the discrete auditory spatial tuning of MURFs recorded from the deep layers of the SC. These responses resembled those seen in animals dark-reared from birth.
which do not develop a map of auditory space (Fig. 1). These distributions graphical organization. The data indicated that recording lo-

regression analysis of the peak angle and collicular position data. ANOVA of these data yields the

TABLE 1. Comparison of spontaneous neural activity in different experimental groups

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Normal Control</th>
<th>Control</th>
<th>Young AP5</th>
<th>Adult AP5</th>
<th>Atropine</th>
<th>Bic/Sac</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>53</td>
<td>4</td>
<td>4</td>
<td>8</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mean implant age, DAB</td>
<td>295</td>
<td>34</td>
<td>28</td>
<td>48</td>
<td>36</td>
<td>34</td>
</tr>
<tr>
<td>Mean SVC angle, deg</td>
<td>129 ± 4</td>
<td>149 ± 125</td>
<td>125 ± 10</td>
<td>221 ± 10*</td>
<td>204 ± 14*</td>
<td>209 ± 12*</td>
</tr>
<tr>
<td>Q75 angle, deg</td>
<td>53 ± 3</td>
<td>61 ± 10</td>
<td>51 ± 8</td>
<td>218 ± 8*</td>
<td>80 ± 8*1</td>
<td>113 ± 12*</td>
</tr>
<tr>
<td>Response area, arbitrary units</td>
<td>137 ± 4</td>
<td>167 ± 16</td>
<td>161 ± 11</td>
<td>242 ± 11*</td>
<td>169 ± 11</td>
<td>220 ± 14*</td>
</tr>
<tr>
<td>Percent appropriate peaks</td>
<td>100</td>
<td>91</td>
<td>100</td>
<td>67</td>
<td>94</td>
<td>56</td>
</tr>
<tr>
<td>Percent tuned</td>
<td>69 n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>75</td>
<td>44</td>
<td>47</td>
</tr>
<tr>
<td>Percent broad</td>
<td>15 n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>11</td>
<td>38</td>
<td>29</td>
</tr>
<tr>
<td>Percent bilateral</td>
<td>14 n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>14</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Percent omnidirectional</td>
<td>2 n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>0</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Values are means ± SE obtained for the spectral power density measurements \( (10^{-11} \text{ V}^2) \) obtained from the various experimental groups [6-cyano-7-nitroquinolinic acid, 2,3-dione-ethylene-vinyl acetate polymer (Elvax) data not available] under conditions of no auditory stimulus (i.e., background, spontaneous neural activity). It can be seen that none of the drug-Elvax groups showed significantly different levels of spontaneous activity when compared with the control-Elvax group [analysis of variance (ANOVA), \( p > 0.05 \)], at least at the time of recording. Overall ANOVA results: \( p = 0.3243 \), NS, AP5, 5-aminophosphonopentanoic acid; Bic/Sac, bicuculline methiodide/2-hydroxysaclofen; NS, not significant.

TUNING PARAMETER GRADE DISTRIBUTIONS. The distribution of tuning parameter grades calculated for the Elvax-implanted animals is shown in Fig. 4. The control-Elvax-implanted animals (Fig. 4A) demonstrated a high proportion of MURFs with grades I and II (i.e., MURFs with tuning parameter values less than the mean + 1 SD based on data from normal animals), with low numbers of MURFs having higher grades. This distribution also was seen in other groups of animals that develop a normal map of auditory space in the SC (see Fig. 1, A–C).

The data obtained for the drug-Elvax-implanted groups were in contrast to those seen in the various control groups. There was a general shift in the distributions toward a predominance of higher grade values (Fig. 4, B–F). This is a reflection of the elevation in mean tuning parameter values also seen in these groups (Table 2). These distributions resembled more closely that seen in dark-reared animals, which do not develop a map of auditory space (Fig. 1D).

The shift of grades was not as pronounced in the bicuculline methiodide/2-hydroxysaclofen (Bic/Sac) animals (Fig. 4E), with a smaller shift to grades III and V compared with the atropine-Elvax-implanted animals. However, the overall distribution was altered compared with the control values, i.e., shifted toward higher grades (see Figs. 1A, 4A, and 4E).

TOPOGRAPHY CORRELATION COEFFICIENTS. Linear regression analysis of plots of MURF peak angle against SC recording locations were used to compare the topographical organization of the position in the auditory field eliciting the maximum SC response. To compare topographical organization between groups, it is necessary to bear in mind the graphic data shown in Figs. 2 and 5 and portions of the numerical data given in Table 2.

Control-Elvax-implanted animals showed good topographical organization. The data indicated that recording locations in the rostral regions of the SC produced maximal responses to frontal space, with a progression caudally along the SC, such that middle SC responded best to lateral space.

TABLE 2. Spatial tuning properties of MURFs obtained from the SC of Elvax-implanted and comparison groups of guinea pigs

<table>
<thead>
<tr>
<th>Animal Group</th>
<th>Normal Control</th>
<th>VC Removed</th>
<th>280 DAB</th>
<th>Dark-Reared</th>
<th>Control-Elvax</th>
<th>Young AP5-Elvax</th>
<th>Adult AP5-Elvax</th>
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<td>197 ± 13*</td>
<td>229 ± 9*</td>
<td></td>
</tr>
<tr>
<td>Q75 angle, deg</td>
<td>53 ± 3</td>
<td>61 ± 10</td>
<td>51 ± 8</td>
<td>218 ± 8*</td>
<td>80 ± 8*1</td>
<td>113 ± 12*</td>
<td>114 ± 10*</td>
<td>128 ± 9*</td>
<td>121 ± 12*</td>
<td>139 ± 10*</td>
</tr>
<tr>
<td>Response area, arbitrary units</td>
<td>137 ± 4</td>
<td>167 ± 16</td>
<td>161 ± 11</td>
<td>242 ± 11*</td>
<td>169 ± 11</td>
<td>220 ± 14*</td>
<td>244 ± 8*</td>
<td>252 ± 12*</td>
<td>234 ± 15*</td>
<td>266 ± 13*</td>
</tr>
<tr>
<td>Percent appropriate peaks</td>
<td>100</td>
<td>91</td>
<td>100</td>
<td>67</td>
<td>94</td>
<td>56</td>
<td>47</td>
<td>53</td>
<td>87</td>
<td>39</td>
</tr>
<tr>
<td>Percent tuned</td>
<td>69 n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>75</td>
<td>44</td>
<td>47</td>
<td>42</td>
<td>53</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Percent broad</td>
<td>15 n/a</td>
<td>n/a</td>
<td>n/a</td>
<td>11</td>
<td>38</td>
<td>29</td>
<td>35</td>
<td>26</td>
<td>47</td>
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<td>Percent bilateral</td>
<td>14 n/a</td>
<td>n/a</td>
<td>n/a</td>
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<td>12</td>
<td>24</td>
<td>20</td>
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<td>Percent omnidirectional</td>
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<td>n/a</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>8</td>
<td></td>
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</table>

VC Removed indicates the group of visual cortex ablated guinea pigs. 280 DAB, the animals reared in the dark for a 4 week period beginning at 250 days after birth (DAB). Dark-reared, those animals reared in the dark from birth. Tuning parameter values (Q50 and Q75 angle and Response Area) are shown as means ± SE. Data are included to indicate the percentages of multiresonance fields (MURFs) with appropriate peak angles, tuned, broad, bileded, and omnidirectional response profiles, respectively. n/a, indicates data not available. The correlation coefficient, \( r \), was obtained from linear regression analysis of the peak angle and collicular position data. ANOVA of these data yields the \( P \) value shown. The regression analysis was performed a second time on the data from Elvax-implanted animals, in this case, using the secondary peak of any bileded MURF responses in each group (instead of the primary peak position). * Significant difference from normal control values. † Significant difference from values recorded in animals dark-reared from birth (\( P < 0.05 \), ANOVA).
and caudal SC responded best to rear regions of the azimuthal auditory hemifield (Fig. 5A). This pattern also was seen in the normal, visual cortex removed and 280 DAB animals (Fig. 1, A–C). The data from these groups showed high correlation coefficients, indicating good front-back organization of peak angle along the length of the SC (Table 2). This is supported further by the proportions of peak angles that fell in a region of space appropriate to a normal map of auditory space, also shown in Table 2. By definition, 100% of peaks in normal animals are in the correct location.

In the other comparison groups and in the control-Elvax-implanted animals, >90% of MURF peak angles fall into the region predicted from the normal map. The data shown for the drug-Elvax-implanted groups did not show good topographical organization. Overall, there was a high degree of scatter of the data (Fig. 5, B–F), which produced low correlation coefficients for these groups (Table 2). For the majority of groups, the ANOVA test on the regression data indicated that the data were not significantly different from a flat line, that is, no topographical organization of the peak angles. For the AP5-, atropine-, and CNQX-Elvax groups, many of the peak angles fell outside a position appropriate for a normal auditory space map (only 39–56% of peak angles fell into the range predicted by the auditory space map; Table 2). These results resembled those seen in dark-reared animals (Fig. 2D and Table 2).

In contrast to the other drug exposed groups, the peak angles of the MURFs recorded from the Bic/Sac-Elvax-implanted animals did show some degree of topographical organization within the SC (see Fig. 5E). The slope of the data was found to be significant (ANOVA, \( P = 0.0002 \)) with a correlation coefficient of 0.573. This value, although significant, is rather low compared with the control groups. There was a larger degree of scatter of the data around the regression line, thus reducing the value of the correlation coefficient. This scatter is brought about by the increased size of the MURFs producing a smearing of the peak angle around the maximum response location. This group demonstrated 87% of MURFs with a peak in a position predicted by the normal map, a figure approaching that seen in the comparison and control groups (Table 2).

The topographical organization of the MURFs was reexamined, taking into consideration the secondary peak of bilobed responses. In this case, the regression analysis was repeated, using the secondary peak instead of the primary peak for those MURFs classified as bilobed. These scatter plots of the topography found in the various Elvax-implanted groups, including the bilobed secondary peaks in given in Fig. 6. The associated regression analysis data are given in Table 2. In the control-Elvax group, it can be seen that (Fig. 6A) when using the secondary lobe peak, the topographical organization is somewhat disrupted, although not to a large extent. Of the five bilobed MURFs, the secondary peaks of four of them are effectively a front-back reversal of the primary peak (a typical feature seen in bilobed responses). Including secondary peaks in the analysis of the organization of responses from the other drug-Elvax-implanted groups does not show any dramatic differences to the analysis of all primary peaks (Fig. 5). The results from young AP5-Elvax-implanted animals indicate a slight improvement in MURF organization if secondary peaks

FIG. 1. Distributions of tuning parameter grades for the animal groups used for comparisons [shown as a percentage of the total number of multiunit receptive fields (MURFs) in each group]. A: normal controls. B: visual cortex removed. C: 280 days after birth (DAB). D: dark-reared from birth. Each tuning parameter is illustrated by different shading, as indicated.

FIG. 2. Topographical organization of MURFs in the comparison groups. Plots of collicular recording position against peak response angle are shown for normal controls (A), visual cortex removed (B), 280 DAB (C), and dark-reared from birth (D). Correlation coefficient, \( r \), and the result of analysis of variance (ANOVA) of the regression data are shown on each graph.
FIG. 3. Representative polar plots obtained from the experimental groups included in this study. Data shown are arranged in columns: A, control-ethylene-vinyl acetate copolymer (Elvax-40W polymer); B, young aminophosphonopentanoic acid (AP5)-Elvax; C, adult AP5-Elvax; D, atropine-Elvax; E, bicuculline methiodide / 2-hydroxysaclofen (Bic/Sac)-Elvax; and F, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX)-Elvax. Bottom: dorsal views of the superior colliculus (SC) from the various group; ●, rostrocaudal recording locations. Scale and orientation of the colliculus is indicated on the bars; r, rostral; c, caudal; m, medial; l, lateral (the length of the scale bar represents 2 mm in the guinea pig colliculus). Three rows of polar plots are shown. Row 1: polar plots of auditory spatial MURFs recorded from the rostral region of the SC. Row 2: central region. Row 3: caudal region of the SC. Scale bar labeled R represents the radius of the polar plot, i.e., the normalized maximum multiunit response amplitude.


are used (Fig. 5B). However, these results are still insufficient to imply the presence of a map of auditory space in the SC of these animals, especially as the properties of the tuning parameters of these MURFs are so different to the control group. Of note are the secondary peaks of some of the bilobed MURFs from atropine-exposed animals. Of the eight bilobed responses, three demonstrated a secondary peak within the rear ipsilateral hemisphere to the colliculus recorded from, a feature not seen in any of the other groups. The mean angular difference between the primary and secondary peaks of the bilobed MURFs from the various groups was found to be 115°. Of the 32 bilobed MURFs recorded, 20 demonstrated and primary-secondary peak angular difference of ≈90°, sufficient to produce a shift in the response into the opposite hemisphere.

INDIVIDUAL MURF RESPONSE PROFILES. Individual MURFs were allocated a response profile category, as described in METHODS. The control-Elvax-implanted animals showed similar percentages of the four classes of response profiles as was seen in normal-control animals; in full, more than two-thirds of the MURFs were classed as tuned, with very few MURFs classed as omnidirectional. The drug-implanted groups demonstrated much lower percentages of MURFs with a Q50 angle of less than the mean ± 1 SD (that is, tuned). Also higher proportions of MURFs showed no portion of their receptive field responding with a <50% of maximum amplitude (that is, omnidirectional) than were seen for the control groups. The drug-implanted animals demonstrated more MURFs with broad response profiles compared with the control groups, although the proportions of bilobed MURFs was found to be similar in all groups and the controls. These data are given in numerical form in Table 2. The pattern of profiles obtained in the drug-implanted animals further illustrates the deterioration in auditory spatial tuning of these MURFs.

DISCUSSION

Neurotransmitter systems within the superior colliculus

Excitatory amino acids and their receptors are found in the SC, implicating glutamatergic systems in SC function at
least in the superficial layers (for example, Chalmers and McCulloch 1991a,b; Sakurai et al. 1990). Several studies imply that glutamatergic transmission is the major mediator of visual inputs to the superficial SC, both from the cortex and retina (Binns and Salt 1994, 1995; Chalmers and McCulloch 1991b; Okada 1993; Sakurai et al. 1990). Muscarinic cholinergic receptor binding sites were found in the rat SC, at high levels in the Stratum griseum superficiale (sgs), intermediate levels in the S. griseum intermediate (sgi) and lower levels in the deeper layers (Tan and Harvey 1989). Despite the abundance of cholinergic receptors, excitatory amino acids are believed to be the transmitter substance for retinal input to the SC. Cholinergic receptors appear to play a role as a neuromodulator in the transmission of afferent visual information to the SC. GABA systems in the mammalian SC have been extensively reviewed by Mize (1992). GABAA and GABAB subtypes are found predominantly in the superficial layers (Bowery et al. 1984; Palacios et al. 1981; Skangiel-Kramska et al. 1986).

Development of the superficial layer visual representation

Although visual responses from the superficial layers of the SC of the guinea pigs in the Elvax-implanted groups were not investigated in any detail, such responses were found to be present in all animals included in this study. These responses were elicited easily using a light source stimulus and showed habituation to repeated stimuli. Schnupp et al. (1995) described normal visual topography in the ferret SC after exposure to NMDA-receptor antagonists. In both the guinea pig and ferret, the visual map in the superficial SC is already mature at the time of implantation of the polymer, at >2 wk old. Therefore it is suggested that a visual map would be found in the superficial SC of the Elvax-implanted guinea pigs. During earlier development, NMDA receptors are involved in the normal development of connections to and terminations within the superficial layers of the SC. The results of several studies (for example, Cline and Constantine-Paton 1989; Hofer et al. 1994; Simon et al. 1992) support the involvement of NMDA receptor antagonists in the developing visual system and indicate that the NMDA receptor plays important roles in the developing SC in particular.

Glutamate receptor involvement in auditory space map development

NON-NMDA GLUTamate RECEPTORS. Glutamate is thought to be the major neurotransmitter underlying retinal and corti-
cal input to the superficial layers of the SC. These systems are mediated primarily by non-NMDA glutamate receptors with only a small component of superficial layers cellular responses generated by the NMDA receptors (Binnns and Salt 1994). Therefore exposure to CNQX potentially blocks a large amount of the visual synaptic input to the SC from the retina. The ipsilateral cortico-tectal pathway, lesioned by aspiration of the primary visual cortex during the initial implant surgery, does not appear to play a vital role in the development of the auditory spatial representation in the SC (control-Elvax implants in this study) (see also Withington et al. 1991). The visual input to the superficial SC of the Elvax-implanted guinea pigs originates from the contralateral retina (Hess 1960). Because visual responses still could be elicited easily in the superficial SC of the CNQX exposed animals after retinal stimulation, it would appear that the CNQX was not blocking all of the retinal input. It may be that the concentration of CNQX in the SC was insufficient to fully block the receptors or that the effect of the drug had worn off by the time of mapping. Perhaps in the guinea pig, other transmitter systems also are involved in retinotectal transmission.

The effects of CNQX seen in this study could be produced by a number of mechanisms: first by obstruction of retinotectal transmission, CNQX removes some visual synaptic input to the SC or reduces input such that only subthreshold stimulation is achieved in collicular cells. The presence of CNQX in the SC results in reduced postsynaptic activity across the SC neural network produced by visual inputs. By interfering with afferent visual inputs, the instructive role of vision in auditory space map development is disturbed, preventing the normal emergence of the auditory space map.

The involvement of NMDA receptors in auditory space map maturation implies that activity-dependent mechanisms are functioning within the SC during the development of the auditory space map. The phenomenon of long-term potentiation (LTP) often is associated with the NMDA receptor. However, there are examples of NMDA-receptor-independent LTP. In the mammalian CNS, such mechanisms have been found in the visual cortex and hippocampus (reviewed by Johnston et al. 1992) and are mediated through non-NMDA receptors. The effects of CNQX on auditory space map development may imply that there is a form of NMDA-receptor-independent LTP in operation in the superficial layers of the SC.

Third, the effects produced by CNQX during development could also be brought about by an effect on NMDA-receptor-mediated LTP mechanisms. CNQX could prevent some of the large depolarization of postsynaptic cells produced by non-NMDA glutamate receptor occupation. If this prevents the release of the Mg$^{2+}$ block of NMDA receptors, the induction of long-term changes in cellular activity also would be prevented. In effect, CNQX may be mimicking the effects of NMDA-receptor antagonists.

**NMDA GLUTAMATE RECEPTORS.** Neural networks can alter NMDA-receptor function at different stages of development, such that during periods of high levels of plastic change, the NMDA receptor is more excitable, having a slower NMDA-mediated postsynaptic response (Hestrin 1992) and a lower threshold for release of Mg$^{2+}$ block (Kato and Yoshimura 1993), facilitating the ability of the system to generate the change. Later in life, the NMDA receptors favor a more stable situation, being less excitable such that a greater stimulation level is needed to induce change. As well as these properties, the NMDA receptor also seems to play a subtle role in guiding the development of correct neural connections within the SC. Of the many glutamate receptor subtypes in the SC, only NMDA-receptor subunits showed distinct changes in expression throughout development (Hofer and Constantine-Paton 1994). NMDA receptors also are believed to play a role in the developing visual cortex (Fox et al. 1989).

The data presented here show that the NMDA receptor plays an important role during both the development of the SC auditory space map and its maintenance in the adult animal. Chronic blockade of collicular NMDA receptors during the time in and around the “crucial period” for auditory space map development in the guinea pig prevents the normal emergence of the map. This finding correlates with similar results obtained in the ferret (Schnupp et al. 1995). Exposure of the adult (250 ± 280 DAB) guinea pig SC to NMDA-receptor blockade for an equivalent period of time also produces disruption of the space map. This result was not found in the ferret study; adult (110–160 DAB) ferrets exposed to MK801 demonstrated topographical order of auditory spatial responses. Thus there appears to be a species-dependent difference in the susceptibility of the adult SC auditory space map to blockade of NMDA receptors. The different pharmacology of the two agents may provide a source of this difference. The use of MK801 (a noncompetitive blocker requiring channel opening) would allow a certain degree of NMDA receptor activation to occur before blockade could occur, whereas the use of AP5 (a competitive blocker) should prevent even this initial activation of the receptor. Thus the potentially more thorough NMDA-receptor blockade produced by AP5 may be sufficient to disrupt the differences seen in the adult SC.

The involvement of NMDA receptors in auditory space map maturation implies that activity-dependent mechanisms are functioning within the SC, participating in the overall developmental processes leading to space map emergence. From earlier discussion, it is clear that NMDA receptors play vital roles in many aspects of neuronal development and plasticity. By blocking NMDA receptors, activity-driven mechanisms of plasticity are likely to be inhibited, if not completely abolished. Therefore the mammalian SC will be rendered unable to “learn” the correct associations between visual space and auditory space; that is, the instructive role exerted by the visual system on the guidance of correct auditory spatial tuning (for example, Knudsen 1994) in the deeper layers also will be inhibited. The neural locus of peak activity in the visual network would be unable to form permanent changes in its connectivity with the auditory network, resulting in ambiguous and spatially untuned activity in the auditory network. Thus despite receiving coincident auditory and visual activity, the NMDA-receptor blockade prevents the normal synaptic gain (i.e., potentiation), preventing activity-dependent changes becoming initiated.

The effects of the drugs released from the Elvax are likely to be restricted to the superficial layers of the SC. These pharmacologically induced changes must be passed down to the deep layers to affect the development of spatial responses to auditory stimuli. The SC possesses interlaminar anatomic links, providing possible routes for the neural transmission of information between the superficial and deep layers.
(Mooney et al. 1984, 1988, 1992). Blockade of NMDA receptors found on the superficial dendrites of deep layer cells, or the soma of superficial cells with axon collateral projections to the deeper layers, could prevent activity-dependent changes from being transmitted to the deep layer auditory responsive cells.

Thus the appropriate synapse of a widespread auditory afferent to the deep layers would not become strengthened, and inappropriate synapses would not be silenced. The restricted visual receptive fields of the superficial layers would not be properly imposed on deep layer auditory cells, and the broad auditory spatial tuning properties seen in young animals would be retained in the more mature animal. Thus animals exposed to AP5 would not develop the sharply spatially tuned and topographically accurate auditory receptive fields seen in normal animals.

The SC auditory map of guinea pigs aged 250 DAB is no longer susceptible to sensory deprivation of either visual or auditory experience (Withington et al. 1994). This age would appear analogous to other systems where, at the end of a critical period, the particular system could no longer undergo plastic change (for example, Knudsen and Knudsen 1990). However, the finding that the map organization still can be disturbed by the chronic administration of AP5 would imply that the SC retains at least some capability to undergo change in the adult animal. The results from the adult AP5-exposed group were clearly different from the control groups. Ideally, another group of animals aged 250 DAB implanted with control-Elvax polymer for the same period as the adult AP5-Elvax-implanted animals would have been tested. However, recent experiments in our laboratory using other drugs introduced through Elvax in the adult have shown that these animals retain a normal auditory space map (McCrossan et al. 1996). Therefore concerns over the lack of control-implant data or of possible roles of visual cortex at this age would appear to be without foundation.

The ability of the brain to change its response properties throughout life is an important aspect of its function, allowing the animal to adapt to and overcome changes in its environment and even changes in bodily functioning. It has been suggested that computational representations of auditory space within the central auditory system should retain a degree of plasticity throughout life, in order that possible changes in the auditory system could be overcome (Merzenich et al. 1984). The finding that the adult guinea pig auditory space map retains the capacity to undergo NMDA-receptor-dependent alteration implies that the map could remain a plastic feature for much of the animals life, allowing the map to adapt to changes in the auditory system, such as presbycusis or damage/injury. NMDA-receptor mechanisms may allow the animal to compensate for changes in sound localization cues, allowing recalibration of the space map, perhaps using visual and somatosensory instruction.

**Muscarnic receptor involvement in auditory space map development**

Cholinergic systems are widespread in the mammalian SC, showing a widespread superficial distribution and a patchwork pattern in the deeper layers. There is much evidence suggesting that acetylcholine plays important roles in neuronal plasticity in various areas of the brain. Two systems studied include the cholinergic modulation of the formation of LTP in the hippocampus (Hopkins and Johnston 1988; Markram and Segal 1990) and ocular dominance related plasticity in the visual cortex (Bear and Singer 1986; Gu and Singer 1993). Cholinergic mechanisms also have been implicated in plastic and developmental changes in the functioning of other neural areas; examples include the vibrissal barrel cortex (Kossut et al. 1993) and sympathetic ganglia (Barbu et al. 1992).

Using the Elvax method for chronic delivery of atropine to the SC, the resultant disruptive effects on auditory space map development may be mediated through several potential mechanisms.

First, as a result of inhibition of cholinergic input from the parabigeminal nucleus (Tan and Harvey 1989) or perhaps the retina (Hess 1960), the SC may be deprived of a vital portion of the visual instructive signal necessary for auditory space map development.

Second, disruption of the functioning of intrinsic cholinergic neurons within the SC (for example, Henderson 1987) may interfere with vital modulatory actions provided by these cells. And third, by altering the influences of acetylcholine on other plasticity mechanisms, such as NMDA-receptor-mediated activity-dependent mechanisms, such systems may not function correctly. Recent unpublished studies using an in vitro preparation of the SC indicate that cholinergic systems participate in modulatory mechanisms affecting synaptic transmission. The muscarinic agonist, carbachol, was found to produce a facilitatory effect on postsynaptic responses in the superficial layers in response to stimulation of afferent fibers (B. Platt, personal communication).

Disruptions to the cholinergic systems of the SC produced by the chronic presence of atropine are sufficient to disturb the developmental mechanisms leading to auditory space map development.

**GABA receptor involvement in auditory space map development**

GABA systems are prevalent in the mammalian SC, as outlined earlier. In the adult nervous system, GABA is accepted widely as the primary inhibitory neurotransmitter. Data are accumulating implicating a role of GABA as an excitatory neurotransmitter in the developing nervous system (Cherubini et al. 1991), and it is believed that the excitatory actions of GABA, receptors early in life may play a trophic role, producing conditions optimal for the correct neuronal morphological development (Ben-Ari et al. 1994). Developmental GABA, receptors may play a similar role in the SC; bicuculline has been shown to inhibit the growth of tectal neurons (Michler 1990). In the ferret SC, GABA, receptors appear to be differentially expressed during the developmental time course of the auditory space map (Baron et al. 1996). In the light of the disruptive effects of chronic GABA-receptor blockade on auditory space map development, GABA, receptors in the SC may be involved in the correct development of neuronal growth and connectivity in the SC.

GABAergic cells form a large proportion of the neuronal population in the more superficial layers of the SC (S. zonale, sgs, S. opticum, and sgi) (Mize 1992) and represent another possible site of the disruptive influences on auditory space map
development produced by blockade of GABA receptors. By reducing the influence of lateral inhibition in the superficial layers of the SC, visual receptive fields are likely to have dramatically different properties, such as altered directional selectivity and broader receptive fields. Thus a spatially discrete visual stimulus may activate a larger region of the superficial SC. This could have drastic effects of the instructive visual influence on auditory space map development. The larger visual receptive fields (broader spread of visual activity) may be passed onto the deeper layer auditory responsive neurons, thereby producing the effects observed in the Bic/Sac-Elvax-implanted animals: broad auditory spatial receptive fields. Such a less well-defined visual map still could confer sufficient information to produce the crude topographical organization of the auditory responses seen in the deeper layers of the Bic/Sac-Elvax-exposed guinea pigs.

GABAergic systems are known to influence LTP in the SC. Slice preparations facilitate detailed investigations of this mechanism of synaptic plasticity. Application of GABA before giving a tetanic stimulation inhibits induction of LTP, whereas application of bicuculline results in facilitation of LTP above normal levels (Hirai et al. 1993). These results suggest that GABAergic neurons within the SC are capable of modifying the induction of LTP in superficial cells. It therefore may be predicted that the chronic blockade of the superficial GABA_A receptors may enhance the induction of LTP in this region. The blockade of presynaptic GABA_B receptors may produce increased excitation of postsynaptic neurons (Seabrook et al. 1990). These factors may contribute to the ability of the SC to develop at least a crude topographical auditory organization in the deeper layers of the SC.

Effects of GABA-mediated inhibition on the barn owl’s auditory system have been investigated. Fujita and Konishi (1991) found that inhibition mediated by GABA receptors produces responses in the core and external nuclei of the inferior colliculus (ICc and ICx), which show increased specificity for interaural time difference. Thus GABAergic inhibition is believed to shape azimuthal auditory spatial receptive fields. This finding raises a potential problem with the Elvax-implant technique in the data presented here. If the GABA antagonists (or indeed the other classes of neurotransmitter blockers used in these studies) can diffuse into the IC, the effects produced in the SC may simply be a reflection of disturbances in this region. However, it is felt that this is a very unlikely possibility; any diffusion into the IC is likely to be minimal. The positioning of the polymer implant meant that only a very small proportion of the ICc and hardly any of the ICx was likely to receive an effective concentration of the drugs. With such a small region of the IC being affected, indeed if any, it is highly unlikely that this would have a detrimental effect on the responses seen in the deep SC. Supporting evidence for this comes from recent work in this laboratory in which discrete lesions of the ICx seemed to produce disruptive effects only in the corresponding projection region in the SC (Thornton and Withington 1996). Thus any effects produced by diffusion of the drugs into the IC may be expected to only affect the region of space coded for by the rostral ICx, i.e., the frontal region of auditory space. This was not seen. The responses obtained from the rostral region of the SC did not appear different from responses in other regions.

The preceding discussion has concentrated on the role of specific neurotransmitter systems during maturation of the SC, involving the development of correct neuronal connectivity and responsivity. It is also feasible that the drugs are inducing their disruptive effects through an action at a different level. As eluded to in the consideration of the effects of CNQX, the drugs may be acting in the SC to simply disrupt the normal sensory functioning within the SC. Several studies have detailed the effects of sensory deprivation of collicular auditory spatial tuning (for example, Binns et al. 1992; King et al. 1988; Knudsen 1985; Withington-Wray et al. 1990a,b,d). Thus we have to consider that exposure of the SC to the neurotransmitter receptor blocking drugs does not have a direct effect on the developmental plasticity of synapses within the colliculus. Instead, the drug may induce a deprivation of sensory input to the collicular neurons resulting in a failure of map maturation.

Conclusions

Our analysis of levels of spontaneous activity was restricted to the time of recording. It is possible that the drugs have caused more dramatic changes in background neuronal activity earlier in the developmental period. Although we cannot completely rule out such potential nonspecific effects of the drugs released from the polymer implants, we feel that such as effect is perhaps unlikely in the view of the data obtained from adult animals implanted with AP5-Elvax. The disruptive effects seen in the adult animal may suggest that the drugs are indeed having a specific effect, as tuning is affected by exposure to NMDA blockade even when developmental changes in neurotransmitter receptor expression have become stabilized.

The developmental emergence of the guinea pig SC map of auditory space appears to very susceptible to disruption of a number of neurotransmitter/neuromodulator systems. Glutamatergic and cholinergic systems must be allowed to function correctly if the map is to develop normally. GABAergic mechanisms seem to play a more subtle role, being involved in the “fine-tuning” of the auditory space map. However, as both GABA_A and GABA_B receptors were blocked simultaneously, we can be less certain as to the role of GABA in auditory space map development. The adult SC appears to retain the ability to undergo functional modification, at least through NMDA-receptor-dependent mechanisms. We cannot, as yet, discount the possibility that treatment of the SC with these drugs may act to impede normal sensory transmission to the colliculus, i.e., that the drugs have no specific action on the developmental plasticity or modification of synapses.

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