

An investigation of the role of auditory cortex in sound localization using muscimol-releasing Elvax

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Abstract

Lesion studies suggest that primary auditory cortex (A1) is required for accurate sound localization by carnivores and primates. In order to elucidate further its role in spatial hearing, we examined the behavioural consequences of reversibly inactivating ferret A1 over long periods, using Elvax implants releasing the GABA_A receptor agonist muscimol. Sub-dural polymer placements were shown to deliver relatively constant levels of muscimol to underlying cortex for >5 months. The measured diffusion of muscimol beneath and around the implant was limited to 1 mm. Cortical silencing was assessed electrophysiologically in both auditory and visual cortices. This exhibited rapid onset and was reversed within a few hours of implant removal. Inactivation of cortical neurons extended to all layers for implants lasting up to 6 weeks and throughout at least layers I–IV for longer placements, whereas thalamic activity in layer IV appeared to be unaffected. Blockade of cortical neurons in the deeper layers was restricted to ≤500 μm from the edge of the implant, but was usually more widespread in the superficial layers. In contrast, drug-free Elvax implants had little discernible effect on the responses of the underlying cortical neurons. Bilateral implants of muscimol–Elvax over A1 produced significant deficits in the localization of brief sounds in horizontal space and particularly a reduced ability to discriminate between anterior and posterior sound sources. The performance of these ferrets gradually improved over the period in which the Elvax was in place and attained that of control animals following its removal. Although similar in nature, these deficits were less pronounced than those caused by cortical lesions and suggest a specific role for A1 in resolving the spatial ambiguities inherent in auditory localization cues.

Introduction

Until the advent of functional imaging techniques, lesion studies formed the basis for our knowledge of structure–function relationships in the cerebral cortex. Lesion studies remain of great value not only in helping to understand the consequences of brain damage in humans, but also for the study of normal brain function. However, lesions are irreversible and have widespread effects, damaging any area that shares connections with the lesion site.

In animal models, various methods for reversibly silencing brain areas have been examined (Lomber, 1999). Temporary cryogenic inactivation can be achieved during both behavioural testing and electrophysiological recording (Lomber, 2002; Lomber *et al.*, 2002), but this technique is not appropriate for chronic studies and requires the animal to be tethered. More persistent blockade can be achieved by infusion of drugs via a stereotaxically implanted cannula attached to an osmotic minipump, but drug delivery is restricted to a single, focal site. A less invasive and more extensive method of delivering drugs is via surgical placement of sheets of the sustained-release polymer, Elvax (Langer & Folkman, 1976; Smith

et al., 1995; Prusky & Ramoa, 1999). Although Elvax, loaded with a variety of agents, has been widely used to modify the developing nervous system (e.g. Cline & Constantine-Paton, 1989; Chiaia *et al.*, 1992; Schlaggar *et al.*, 1993; Schnupp *et al.*, 1995; Sernagor & Grzywacz, 1996; Rhoades *et al.*, 1998; Dagnew *et al.*, 2003), it has not so far been used for behavioural studies.

For use in behavioural experiments, cortical inactivation by drug-loaded Elvax should: (i) be prolonged; (ii) be complete; (iii) be localized; and (iv) be reversible. Cortical neurons can be silenced by Na⁺ channel blockers such as tetrodotoxin or local anaesthetics, but these agents will also inactivate axons *en passant*. For this reason, we chose to inactivate the cortex at the synaptic level by incorporating the γ -aminobutyric acid (GABA)_A receptor agonist muscimol into Elvax. Acute infusion of muscimol into visual cortex silences cortical neurons whilst leaving thalamic inputs intact (Chapman *et al.*, 1991). In pharmacological and electrophysiological studies on the cortex of adult ferrets, we show that muscimol–Elvax fulfils all four of the above criteria.

We have used muscimol–Elvax to investigate the role of the primary auditory cortex (A1) in sound localization. In humans, temporal lobe damage that encompasses the auditory cortex leads to impaired spatial localization (Clarke *et al.*, 2000, 2002; Zatorre & Penhune, 2001), but there is considerable debate about the relative contributions to this

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important task of A1 and other cortical fields that may have more specialized roles in auditory space perception (Rauschecker & Tian, 2000). Localization deficits have been reported following cortical lesions in carnivores and primates (Neff *et al.*, 1956, 1975; Heffner & Masterton, 1975; Heffner, 1978; Jenkins & Masterton, 1982; Jenkins & Merzenich, 1984; Kavanagh & Kelly, 1987; Heffner & Heffner, 1990; Heffner, 1997), but the type of deficit observed depends on several factors, including the extent of the lesion and the precise nature of the task. The ability to inactivate cortex locally and reversibly should prove very useful for identifying its role in sound localization.

Materials and methods

All experiments involving the use of animals were approved by the local ethical review committee and carried out under licence from the UK Home Office. The number of animals used in each stage of this study is shown in Table 1.

Preparation of muscimol-containing Elvax

Beads of the ethylene-vinyl acetate copolymer Elvax 40-W (also known as Elvax 40P; a gift from Du Pont, UK) were first washed in order to remove traces of the antioxidant butylhydroxy-toluene. Beads were stirred at room temperature for 24 h each in five changes of 95% alcohol followed by a concentration gradient of five changes to 100% alcohol. The beads were filtered, dried and stored in the dark at room temperature. Muscimol (Tocris Cookson, Langford, UK) was incorporated into Elvax by a procedure adapted from Smith *et al.* (1995). Washed Elvax beads (0.5 g) were dissolved in dichloromethane (Sigma-Aldrich, Poole, UK) at room temperature by vortex mixing to give a 10% w/v solution. A 100- μ L sample of either double-distilled water (for control implants) or an aqueous solution of muscimol was added to the Elvax/dichloromethane solution. Aqueous muscimol solutions were produced by dissolving muscimol in one equivalent of 10 M NaOH_(aq) and making-up the volume to 100 μ L with double-distilled water. Muscimol 'concentrations' in Elvax are taken to be the concentration in the Elvax/dichloromethane/aqueous suspension; thus, addition of 100 μ L of 3.75 M muscimol_(aq) gave rise to '75 mM muscimol' Elvax. In order to visualize the polymer slices for implantation and recovery, 50 μ L of 5% Fast Green_(aq) (Allied Chemical, Morristown, NJ, USA) was also added to the Elvax mixture, which was then vortex mixed for 10 min. The resulting suspension was immediately poured in a slow, steady stream onto a horizontal glass plate resting on dry ice. After 20 min, the Elvax sheet was peeled off the plate with cold forceps and freeze-dried overnight (-60°C , 10^{-4} atm). The dry Elvax pancake (approximately 2 mm deep) was

glued to a rubber bung with cyanoacrylate adhesive and sectioned on a Vibratome at 200 μ m. Sections were stored on filter paper at 4°C prior to implantation.

Trace amounts of [^3H]muscimol (19.6 Ci/mmol, 1 μ Ci/ μ L; NEN Life Science Products, Boston, MA, USA) were included in muscimol-Elvax preparations to monitor drug release. The dilution factor of the incorporated radiolabel ranged from 1 : 50 000 (for release profile studies) to 1 : 10 000 (for *in vivo* diffusion studies). An aliquot of [^3H]muscimol was added to a weighed sample of muscimol in a microcone vial, the contents frozen and the vial freeze-dried (i.e. addition of 735 μ Ci of [^3H]muscimol to 0.375 mmoles muscimol gave a 1 : 10 000 dilution). After freeze-drying, the contents were dissolved as described above.

Measurement of muscimol release

Prior to use, the dimensions of Elvax sheets were measured using a computer-assisted image analyser (Seescan, Cambridge, UK) connected to a Wild dissection microscope. To determine the release characteristics of the incorporated muscimol, Elvax sheets were incubated in 0.01 M phosphate-buffered saline (PBS; pH 7.4, 0.5 mL, 37°C) and the bathing solution changed every 24 h. The amount of radioactivity released into PBS during each 24-h period was determined by liquid scintillation counting. Release measurements were made both on fresh Elvax sheets, to determine the *in vitro* release profile, and also on Elvax sheets that had been removed from *in vivo* placements after varying times, in order to estimate the *in vivo* release behaviour of the sheets.

Implantation of Elvax slices

Trimming Elvax slices to the desired size for implantation leaves cut edges that give rise to a 'burst' of drug release, so slices were rinsed for 24 h in PBS (37°C) immediately prior to surgery. All implantations were made in adult ferrets (Marshall Farms, NJ, USA), anaesthetized by i.m. injection of Saffan (0.3% Alphadolone acetate, 0.9% Alphaxalone; 2 mL/kg; Mallinckrodt Veterinary, Uxbridge, UK). After making a craniotomy, a slit was made in the dura away from the region of interest. The Elvax sheet was carefully slipped through the durotomy and moved into position over the appropriate cortical area, with the length of the Elvax sheet running medio-laterally. Elvax placements were made either over the central visual field representation in primary visual cortex (V1, for diffusion and electrophysiological studies) or over the apex of the ectosylvian gyrus in A1 (for electrophysiological and behavioural studies). For electrophysiological studies, a small hole (2 mm diameter) was bored in the Elvax sheet to facilitate subsequent recording and this hole was re-plugged before implantation. The bone-flap was replaced, the scalp sutured and the animal dosed with post-operative analgesic (20 μ g/kg i.m. Vetergesic; Alstoe Animal Health, Melton Mowbray, UK) and antibiotic (0.1 mL i.m. Penbritic; Beecham, Brentford, UK).

Estimating the path length of muscimol diffusion

Adult ferrets that had previously received striate cortical implants of muscimol-Elvax, containing the highest concentration of tritiated muscimol, were overdosed with Sagatal (sodium pentobarbitone, 60 mg/mL; Rhône Mérieux, Harlow, UK) and decapitated. The brains were rapidly removed and frozen on dry ice. Two approaches were employed to determine the extent of spread of muscimol from the implant. In the first, brains were sectioned at 20 μ m in a plane perpendicular to the Elvax implant and the sections rapidly dried onto poly-L-lysine-coated slides on a warming plate. The slides were apposed to [^3H]Hyperfilm (Amersham, Little Chalfont, UK) for between 3 and 6 months, together with tritium brain paste standards

TABLE 1. Number of animals used for each experimental procedure

Experiment	Number of animals
Electrophysiology	
Muscimol-Elvax over A1	2
Muscimol-Elvax over V1	8
Drug-free Elvax over V1	2
Normal controls for V1 recordings	4
Auditory localization behaviour	
Bilateral muscimol-Elvax over A1	3
Unilateral muscimol-Elvax over A1	3
Unilateral drug-free Elvax over A1	2
Normal controls	4

The muscimol release from the Elvax sheets removed from most of these animals was subsequently measured *in vitro*. A1, primary auditory cortex; V1, primary visual cortex.

for calibration of the film response. After film development, the sections were counterstained with thionin and the spatial disposition of muscimol around the implant site measured from the autoradiographs by computer-assisted optical densitometry. In the second method, the frozen brain tissue was blocked by making four cuts at the edges of the rectangular Elvax sheet, perpendicular to the plane of the implant, so that only the tissue directly below the implant remained. The resulting block was sectioned, Elvax side uppermost, at 25 μm in a cryostat, and the sections collected in groups of four in cold scintillation vials. Scintillation counting then gave a measure of the amount of radioactivity in each 100- μm bin of tissue below the implant.

Electrophysiological recordings

A total of 16 adult pigmented ferrets (0.6–1.8 kg) were used in recording experiments, of which 12 received either unilateral or bilateral Elvax implants (10 received implants containing 75 mM muscimol and two received drug-free implants) and four were unoperated controls (see Table 1). Details of the duration of Elvax treatment are given in the Results. Animals were anaesthetized by i.m. injection of Saffan (as above) and then received injections of atropine sulphate (60 μg , i.p.; Martindale Pharmaceuticals, Romford, UK) and Dopram-V (doxapram hydrochloride, 3 mg, i.m.; Fort Dodge Animal Health, Southampton, UK). After intubation of the trachea and catheterization of the radial vein, anaesthesia was maintained by i.v. injection of Saffan (0.1 mL, as required).

Two of the ferrets used for recording had previously received bilateral muscimol–Elvax implants over A1. The temporal muscle was deflected and a minimal headholder attached to the skull, so that the animal could be supported from behind. The skull was removed from the previous craniotomies and the dura reflected in order to expose the Elvax sheets and surrounding cortex. Anaesthesia was switched to a continuous i.v. infusion of Ketaset (ketamine hydrochloride, 2.5 mg/kg/h; Fort Dodge Animal Health) and Domitor (medetomidine hydrochloride, 15 mg/kg/h; Pfizer, Sandwich, UK) and the animal was transferred to a table positioned at the centre of an anechoic chamber. The animal's EEG and heart rate were monitored throughout and its core temperature maintained at $\sim 38^\circ\text{C}$.

Surface-normal penetrations were made into A1 by unplugging the holes that had been made in the Elvax prior to implantation, as well as into adjacent areas of cortex. Single- and multi-unit recordings were made using glass-coated tungsten microelectrodes with a 5 or 10 μm exposed tip and Brainware software (Tucker-Davis Technologies, Alachua, FL, USA). Broadband noise bursts or pure tones were presented at a range of sound levels from a Kef T27 loudspeaker that was positioned at a distance of 0.65 m from the animal's head and in the anterior hemifield contralateral to the side of the cortex from which the recordings were made.

In animals with unilateral Elvax implants over V1 ($n = 10$), the skull overlying the occipital lobe was thinned in order to visualize the position of the Elvax sheet. Troughs were drilled to allow recordings either at known distances from the lateral edge of the Elvax sheet or over the hole bored in the centre of the Elvax sheet. In some cases, a large trough was drilled to one side of the Elvax to allow its later removal, followed by recordings in cortex previously underlying the Elvax. Once this surgery was complete, the animals were paralysed by i.v. infusion of Flaxedil (gallamine tri-ethiodide, 10 mg/kg/h; Sigma, Poole, UK) and artificially respired with a mixture of 75% N_2O and 25% O_2 , containing 0.5–1.0% halothane (Murat & Housmans, 1988). The end-tidal CO_2 concentration was maintained at approximately 4% by adjustment of stroke volume (typically 10–12 mL) and rate (typically 35–40 strokes per minute). The adequacy of the anaesthesia was assessed by continuous examination of the EEG (high-amplitude, low-

frequency waves of 4–6 Hz) and by testing that heart rate was unaffected by pinching the footpad.

Visual stimuli were presented through the contralateral eye, which was protected with a clear zero-power contact lens after topical application of atropine sulphate (1%) to paralyse accommodation and dilate the pupil. The receptive fields of neurons were first mapped on a tangent screen using hand-held stimuli. Using flashed or moving edges and bars, it was possible to categorize most of the neurons quite clearly. In order to quantify the orientation preference of a single unit, sinusoidal gratings were presented on a raster display (subtending an area of 28.5° by 21.5°) controlled by a Cambridge Research Systems VSG 2/4 graphics card (Rochester, UK), allowing the monochrome stimuli to be specified to a resolution of 256 grey-levels. The stimulus display comprised 800×600 pixels, so that each pixel subtended 0.036° of arc. The frame rate was 100 Hz and the display had a space-averaged mean luminance of 95 cd/m^2 .

At the end of recording experiments, animals were overdosed with Sagatal and perfused through the heart with PBS followed by 4% paraformaldehyde in 0.1 M phosphate buffer. Frozen sections (50 μm) of cerebral cortex were cut parasagittally and then stained with Cresyl violet to allow reconstruction of the electrode tracks and calibration of the depth measurements taken during the recordings.

Sound localization studies

The ability to localize sounds in the horizontal plane was tested in a total of 12 adult ferrets (see Table 1). Preliminary studies were carried out in five animals, which received, under Saffan anaesthesia, unilateral Elvax implants over left A1. These animals had been extensively tested on auditory localization tasks prior to Elvax implantation and therefore provided their own control data. In three cases, 75 mM muscimol–Elvax was implanted, while the other two animals received drug-free Elvax implants. As above, 200- μm -thick slices were used, which measured approximately 4 mm by 7 mm in size. The slices were placed over the crown of the ectosylvian gyrus in an attempt to cover the full extent of area A1. In order to avoid damaging the underlying neural tissue, the location of A1 was not measured electrophysiologically in these animals, but was estimated on the basis of optical imaging studies carried out in this laboratory (Versnel *et al.*, 2002; Nelken *et al.*, 2004) and recording studies published by other groups (Phillips *et al.*, 1988; Shamma *et al.*, 1993). The Elvax was positioned in order to avoid the rostromedial part of the gyrus, where the anterior auditory field is located (Shamma *et al.*, 1993).

Most of the behavioural studies reported in this paper were carried out on three other adult ferrets, which received bilateral implants of muscimol–Elvax. These animals were previously untested, having received the minimum amount of training required to acquaint them with the mechanics of the task. No sounds were presented during this preoperative period of shaping. A further group of four normal ferrets were trained and tested in identical fashion, in order to serve as controls for the ferrets with bilateral Elvax implants. The implanted ferrets were allowed to recover for 5–7 days before behavioural testing was recommenced.

Full details of the testing chamber and of the psychophysical procedure used in this spatial identification task are described in Parsons *et al.* (1999). Briefly, water-deprived animals were trained to initiate a trial by standing on a platform and licking a water-spout at the centre of a circular testing arena (radius 75 cm), which was enclosed in a double-walled sound attenuated room. This procedure ensured that the animal's head was in the same orientation from trial to trial. The contact made by the animal between the central spout and the platform triggered the presentation of a single burst of broadband noise (0–30 kHz) from one of 12 peripheral speakers (Audax mid range,

YN43W; Audax UK, Ambleston, UK) equally spaced around 360° of the azimuthal plane at constant elevation. Each speaker was selected at random and its output flattened digitally using finite impulse response filters. A separate water-spout was located next to each of the loudspeakers.

Following the presentation of the stimulus, the animal left the central platform and approached the source of the sound. The accuracy and latency of the initial head movement made by the animals was measured by tracking the position of a reflective strip attached to the animal's head using a vertically mounted infra-red sensitive camera and video contrast detection device (HVS Image, Harlow, UK). A water reward was delivered if the ferret licked the spout adjacent to the speaker from which the noise had been played. The water flow through each spout was controlled by a solenoid valve (Flo-control, Valaeder Pneumatics, Cambridge, UK). Following an incorrect response, up to three correction trials were employed, in which the stimulus was presented from the same speaker, after which continuous noise was presented at that location. Correct responses were rewarded on these correction trials, but were not used to assess localization performance. Noise bursts ranging in duration from 2 s to 40 ms were presented as a descending series during a testing run, which typically took 12–14 days to complete. These runs normally included at least 25 trials for each speaker and for each of the tested noise durations. The sound pressure level at the animal's head was adjusted for each speaker to 70 dB SPL. In order to reduce the possibility of the ferrets being able to identify particular speakers using loudness cues, we varied the sound level randomly from trial to trial in five steps from either 66–74 dB SPL or 56–84 dB SPL. After the end of a testing run, the animals were allowed *ad libitum* access to water and wet food in their home cages for at least 4–5 days before being tested again.

Following the completion of testing in these behavioural tasks, the animals with muscimol–Elvax were anaesthetized with Saffan so that the implants could be removed. At this time, no obvious change in the position of the implant over the ectosylvian gyrus could be detected and, in most cases, there was no sign of any dura growth beneath the implant. Beginning approximately 1 week after surgery, the animals were again tested in the sound localization task. They were then

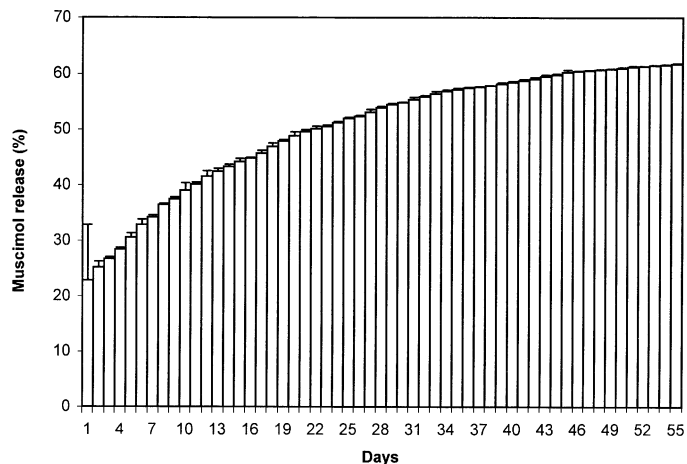


FIG. 1. *In vitro* muscimol release profile. Cumulative *in vitro* release into PBS at 37 °C expressed as a percentage of the total amount of muscimol contained in 200- μ m-thick Elvax slices ($n=4$). Release was measured daily, with the bathing solutions being changed every 24 h.

overdosed with Sagatal, decapitated and the brains removed. The muscimol–Elvax sheets were kept hydrated and transferred to PBS at 37 °C for daily measurement of muscimol release level, as described above.

Results

Release characteristics of muscimol–Elvax sheets

Daily monitoring of the release rate from 200- μ m sheets of 75 mM muscimol–Elvax under 'infinite sink' conditions revealed that the sheets released muscimol continuously *in vitro* for about 60 days. Approximately 25% of the muscimol incorporated in a 200- μ m sheet was released in a 'burst' within the first 24 h, immediately after which the release rate fell dramatically, as shown by the cumulative release profile (Fig. 1). Release levels then decreased smoothly and sheets

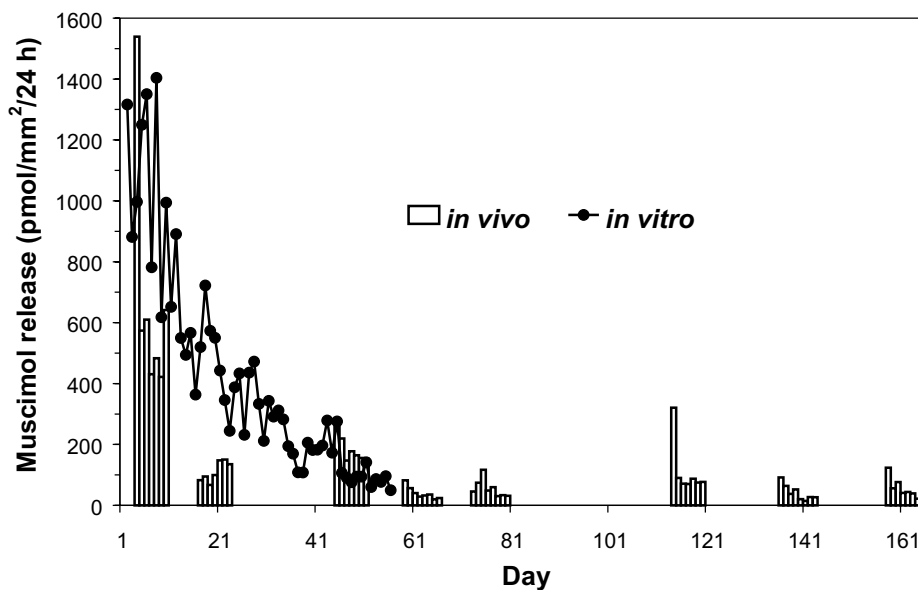


FIG. 2. Comparison of *in vivo* and *in vitro* muscimol release. Daily measurements of muscimol release from 200- μ m-thick Elvax slices into PBS at 37 °C. The graph compares release levels from eight different slices that had been removed from *in vivo* placements after varying times (bars) with those from control slices that were maintained in PBS throughout (filled circles; $n=4$; same slices as in Fig. 1). In all cases, the PBS was changed every 24 h.

were exhausted once they had released about 65% of their contents (Fig. 1).

In contrast to this purely *in vitro* release profile, daily monitoring of release levels from Elvax sheets that had been implanted over cortex for varying lengths of time suggested that a different pattern of release was occurring *in vivo*. When sheets removed from *in vivo* situations after only a few weeks were monitored to reveal their daily *in vitro* release levels, they showed lower release levels than predicted from the previously obtained *in vitro* release profile. However, it appeared that *in vivo* release was maintained for much longer than 60 days. Daily measurement of *in vitro* release levels immediately following removal of Elvax sheets from *in vivo* cortical placements revealed an even level of muscimol release (110 pmol/mm²/day) for implants lasting between 17 and 166 days (Fig. 2). Thus, *in vivo* release levels seem to be maintained at an even level for much longer than predicted by the purely *in vitro* release profile.

In separate experiments, Elvax sheets that had been implanted for periods in excess of 120 days were assayed for the amount of incorporated muscimol remaining. Immediately following removal, the sheets were dissolved in dichloromethane, the muscimol removed by aqueous extraction, and the amount of radioactivity present measured by liquid scintillation counting. In all cases, at least 40% of the incorporated muscimol remained, indicating that the sheets had not yet reached the exhaustion level of 65% depletion previously measured *in vitro*.

Diffusion path length of released muscimol

Measurements of the extent of muscimol diffusion from implanted slices were made by incorporating high concentrations of [³H]muscimol during the polymer preparation. Autoradiographs of sections derived from brains that had received Elvax implants for periods lasting from 1 to 30 days showed that the released radiolabel maintained a tight focus around the site of the implant (Fig. 3A). Maximum spread was seen below the centre of the implant, with radiolabel being detected up to about 700–800 μm from the pial surface. Comparison of the autoradiograph with the Nissl stain of the section from which it was generated shows that this distance approximately corresponds to the bottom of cortical layer IV (Fig. 3B). The lateral spread of radiolabel also extended to about 700–800 μm from the edges of the Elvax implant. A very similar picture of drug distribution was obtained by scintillation counting 100-μm bins of tissue taken from below the implant by cryostat sectioning of frozen tissue. Using this method, radioactivity levels were also found to be significantly above background for approximately 1 mm from the implant (Fig. 3C).

Blockade of cortical activity by muscimol–Elvax

The effectiveness of muscimol–Elvax in blocking cortical activity was assessed in a series of electrophysiological recording experiments. Because we were interested in the effects of selectively inactivating A1 on auditory localization, we recorded from two animals in which muscimol–Elvax had been implanted over this region of the cortex. In one case, the recordings were carried out 3 weeks after implantation and, in the other, at 5 weeks following Elvax placement. A total of seven electrode penetrations were made through small holes in the implants. Virtually no neural activity was encountered in any of these tracks over a distance of >2 mm from the cortical surface (Fig. 4). Histological reconstruction of the electrode tracks revealed that the acoustically evoked activity observed near the end of one of the penetrations made in the ferret recorded at 3 weeks after implantation was located in the white matter (WM) beneath A1 (Fig. 4A, third column).

Electrode penetrations were also made in the cortex adjacent to the implant, which encountered progressively more neural activity as the

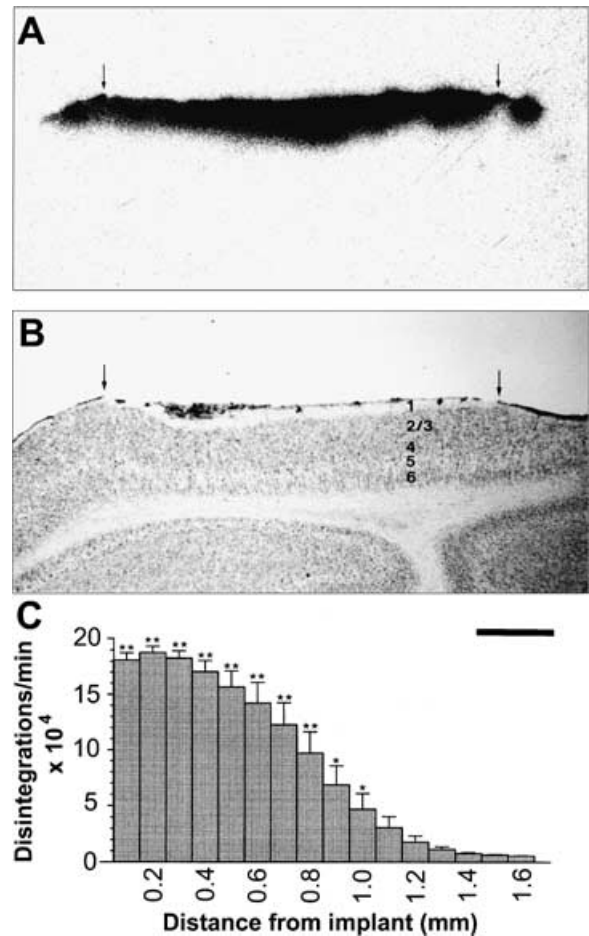


FIG. 3. Diffusion path length of released muscimol. [³H]Muscimol-containing Elvax was implanted subdurally over ferret visual cortex for 2 weeks. (A) In the autoradiograph, dark areas mark the extent of the spread of radiolabel. The arrows indicate the edges of the region covered by the Elvax sheet. (B) Arrows mark the position of the Elvax slice on the corresponding Nissl-stained parasagittal section of cortex from below the implant. The cortical layering is indicated. Scale bar: 1 mm. (C) Scintillation counting of tissue sections cut parallel to the plane of the Elvax reveal the concentration of radiolabel in each 100-μm bin below the implant ($n=3$). Asterisks above bars on the histogram indicate levels significantly above background (** $P < 0.01$; * $P < 0.05$).

distance from the lateral edge of the Elvax sheet was increased (Fig. 4). In one electrode penetration made orthogonal to the cortical surface at a distance of 200 μm from the edge of the implant, we found that the majority of cortical neurons were silenced (Fig. 4B). However, at 400–500 μm from the implant, both spontaneous and sound-evoked activity were encountered in the deeper layers and below and, at a distance of 1000 μm, neural activity was present at nearly all recording sites in three out of four electrode penetrations (Fig. 4A and B).

Blockade of cortical activity shows rapid onset and reversibility

The data shown in Fig. 4 suggest that muscimol–Elvax does effectively silence the underlying cortical tissue. However, in order to provide a better quantification of the time course and spatial extent of cortical activity blockade, we carried out additional recordings from ferret visual cortex. This is because V1 neurons tend to produce stronger responses than those in A1, while their orientation selectivity means that they can be more readily distinguished from thalamic afferents.

The rate of onset of cortical blockade was determined by making acute placements of 75 mM muscimol–Elvax onto V1 during electro-

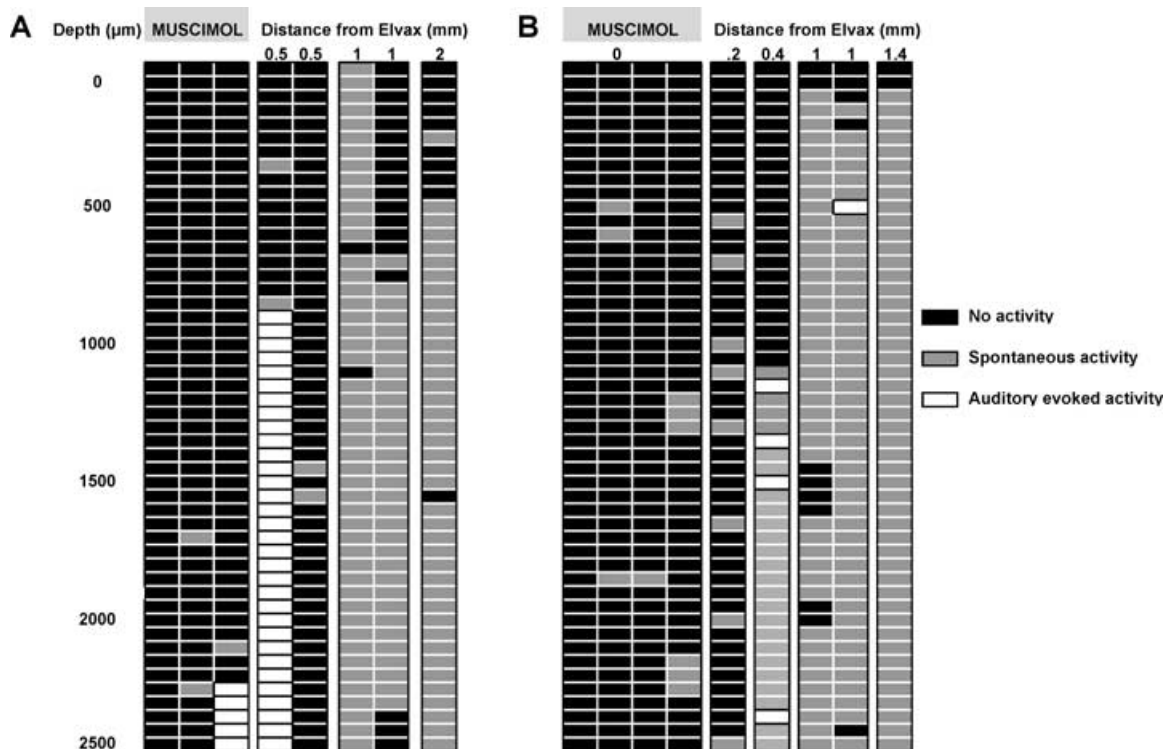


FIG. 4. Blockade of cortical activity at (A) 3 and (B) 5 weeks after application of a sheet of muscimol–Elvax over A1. Surface-normal electrode penetrations were made through a small hole in the centre of the Elvax (represented by the grey bar at the top of each panel; this hole was re-plugged until the recordings were made) or at the distances indicated from the lateral edge of the Elvax sheet. Each column represents a different electrode penetration, in which recordings were made at 50 μm intervals, and the boxes indicate the depths at which no activity, spontaneous activity or sound-evoked activity were recorded, as indicated by the key to the side of the figure. The penetrations made closest to the edge of the Elvax sheet also sampled auditory regions of the ectosylvian gyrus, whereas those further away entered non-auditory areas caudal to the supra-sylvian sulcus.

physiological recording experiments ($n = 2$). Recordings were made in the same region of V1 before Elvax placement, while the Elvax was in position, and then again after its removal. As expected, orientation-selective cortical units were present throughout striate cortex prior to placement (Fig. 5A). We began recording again 2 h after the Elvax was introduced by inserting the electrode through a 2 mm diameter hole in the centre of the sheet. These recordings then continued for several hours. No visually driven activity could be elicited until the electrode reached layer IV, as confirmed by subsequent histological examination of the recording sites. Single units recorded within layer IV had high spontaneous activity and were not orientation selective (Fig. 5B), which suggested that these were thalamic afferents (Chapman *et al.*, 1991). No visually responsive units were encountered in the infragranular layers.

The muscimol blockade was reversible. Following removal of the Elvax, orientation-selective neurons were found throughout the depth of cortex (Fig. 5C). The time of reversal varied according to the duration for which the Elvax sheet had been in place. In the case of the acute studies shown in Fig. 5, it took 9 h before orientation-selective neurons were recorded. However, in the longest placements (16 weeks, $n = 2$), normal responses were recorded in previously blocked cortex within 2 h of Elvax removal.

Sustained and localized blockade of cortical activity

In order to measure the area of V1 over which cortical activity was blocked, we again made penetrations either through a hole in the centre of the Elvax sheet or at varying distances from the lateral edges. The recording electrode was advanced in 100- μm steps, and orientation tuning of single unit or multiunit activity was assessed qualitatively

using bars of light projected onto a tangent screen. Neuronal activity recorded in the region of the Elvax implant is illustrated schematically in Fig. 6, where white boxes indicate locations (100- μm bins) at which orientation-tuned cortical neurons were recorded and black boxes indicate those at which no orientation-tuned cortical neurons could be found. The white crosses indicate where units interpreted to be non-oriented thalamic afferents were recorded, as defined by high levels of spontaneous activity and by their location in layer IV (see Fig. 5B).

In unoperated ferrets, cortical orientation-selective units were found at all depths throughout all cortical layers (Fig. 6A; $n = 4$). This pattern of activity appeared to be mostly unaffected by the placement of control, drug-free Elvax sheets (Fig. 6B), although the lack of orientated units in the most superficial part of cortex suggests that there may be a slight mechanical effect on the cells closest to the implant. In contrast, placements of 75 mM muscimol–Elvax produced profound reductions in the number of active cortical units that could be recorded below the Elvax implants (Fig. 6C–F). In order to monitor the temporal characteristics of muscimol blockade, recordings were performed at 0, 2, 6 and 16 weeks after Elvax placement through small holes in the centre of the implants. In line with the results from A1 (Fig. 4), the complete cortical activity blockade observed within 2 h of Elvax placement in acute experiments (Fig. 6C) was maintained at 2 and 6 weeks (Fig. 6D and E). In these experiments, the only isolated units recorded below the Elvax sheet had the visual driving characteristics of geniculate afferents and were located in layer IV. In the longest implants, the depth of blockade of cortical neurons in one of the two animals tested had reduced to about 700 μm , covering the middle granular and superficial layers, whereas, in the other animal, blockade was complete throughout the depth of cortex (Fig. 6F). Oriented visual

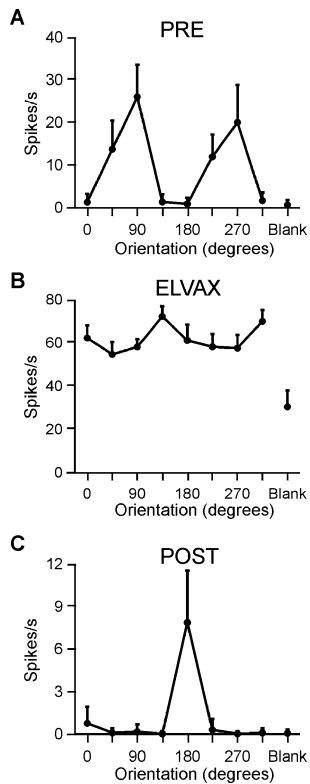


Fig. 5. Blockade of cortical activity shows rapid onset and reversibility. Electrophysiological recordings of three different single-unit responses from the same region of V1 to drifting sinusoidal gratings of different orientations. These recordings were made before the placement of a sheet of muscimol–Elvax (A), through a small hole in the centre of the Elvax 4 h after it was placed over visual cortex (B), and 9 h after the muscimol–Elvax had been removed (C). Tuning curves are constructed from 30 cycles of the grating presented at eight different orientations; error bars represent standard deviations. Note the different scales used on the ordinate for these units and that the unselective unit recorded whilst the muscimol–Elvax was in place (B) had a much higher rate of spontaneous activity than the orientation-selective units recorded before the Elvax was introduced (A) or after it was removed (C).

responses returned after subsequent removal of the Elvax, so the results in Fig. 6F reflect true blockade not an induced pathology.

The lateral extent of functional blockade was investigated at 2, 6 and 16 weeks after muscimol–Elvax implantation. With 2-week implants, normal responses were recorded in the superficial layers at 1500 μm from the implant's edge and, at 500 μm away, only the superficial layers were blocked (Fig. 6D). With time, the blockade appeared to become more restricted to the region of the implant so that, by 6 weeks after implantation, normal orientation-selective cells were recorded at most sites in the superficial layers within 500 μm of the edge of the Elvax sheet (Fig. 6E). The physiology of the two animals that had had implants in place for 16 weeks was a little more variable (Fig. 6F). In the animal showing weaker blockade under the Elvax, cortical activity in the penetration closest to the implant was essentially normal, although that 1000 μm from the edge did have regions where there was no visual driving. In the other animal, there was some silencing of the upper layers up to 1500 μm from the Elvax.

These data, which are consistent with the recordings from A1 (Fig. 4), indicate that the extent of cortical inactivation adjacent to the muscimol–Elvax sheet was variable. In some cases, neural responses were observed in the supragranular layers at 500 μm from the edge of the Elvax sheet, whereas, in others, blockade of activity in

this region appeared to extend out to 1500 μm away. However, we consistently found that blockade of activity in the deeper cortical layers was restricted to ≤ 500 μm from the edge of the implant.

Effects on sound localization of inactivating auditory cortex Deficits in sound localization following bilateral blockade of A1

The behavioural consequences of reversibly inactivating A1 were investigated by implanting muscimol–Elvax bilaterally in adult ferrets. A group of normal, control ferrets and the animals that had received bilateral muscimol–Elvax implants were trained in an identical manner and tested over a range of stimulus durations (1000, 500, 200, 100 and 40 ms). The pooled distribution of responses made by different animals to 40-ms noise bursts presented at each of the 12 speaker locations is shown for the control and muscimol–Elvax groups in Fig. 7A and B. These data, which were obtained during the first testing run, show that the animals in which A1 had been inactivated (Fig. 7B) made more and larger errors than the controls (Fig. 7A), although most of their responses were directed to the appropriate side of space. The muscimol–Elvax group performed less well than the controls over the full range of locations tested, as shown by the distribution of mean percentage correct scores (Fig. 7C and D).

Percentage correct scores averaged across speaker location are presented at each stimulus duration in Fig. 8A. Overall, the ferrets achieved lower scores as the duration of the stimulus was reduced ($F_{4,20} = 40.939$, $P < 0.001$). Combining data across different durations showed no difference between the groups ($F_{1,5} = 1.828$, $P = 0.234$), but there was a significant interaction between stimulus duration and group ($F_{4,20} = 5.176$, $P = 0.005$). A difference in performance between the two groups of animals became apparent when the stimulus duration was reduced to 100 ms, although pairwise comparisons revealed that this was significant at 40 ms only ($P < 0.05$).

As in previous auditory localization studies (e.g. Carlile *et al.*, 1999; Parsons *et al.*, 1999), we calculated the mean unsigned errors at each stimulus location, after excluding those trials in which ferrets made front–back confusion errors (see below). These changed in a complementary way to the percentage correct scores, in that they increased in magnitude with decreasing stimulus duration ($F_{4,20} = 19.917$, $P < 0.001$). Although there was no overall difference between the two groups ($F_{1,5} = 3.694$, $P = 0.113$), a significant interaction between group and stimulus duration was observed ($F_{4,20} = 5.020$, $P = 0.006$), and subsequent pairwise comparisons revealed significant group differences at both 1000 ms and 40 ms ($P < 0.05$).

Unlike previous multiple target tests of sound localization, which have been restricted to the frontal hemifield (e.g. Jenkins & Masterton, 1982; Jenkins & Merzenich, 1984), our 360° speaker array allowed us to measure the incidence of front–back confusion errors. These errors are defined as mislocalization of sounds originating from the frontal sound field into the ipsilateral posterior field and *vice versa*. The percentage of front–back errors made at each duration in the first testing run are shown in Fig. 8B. Reducing the stimulus duration led to a significant increase in the proportion of front–back errors ($F_{4,20} = 26.625$, $P < 0.001$). Across all durations, we observed no difference between the groups ($F_{1,5} = 2.354$, $P = 0.186$), but there was a significant interaction between duration and group ($F_{4,20} = 6.229$, $P = 0.002$). As with the other measures of performance, the bilateral-Elvax ferrets made significantly more front–back errors than the normals with 40-ms noise bursts ($P < 0.05$).

In contrast to the effects of muscimol–Elvax on the accuracy of approach-to-target responses, we observed no deficit in the initial head-orienting response made by these animals following presentation of the stimulus. Head-orienting errors were defined as the angular

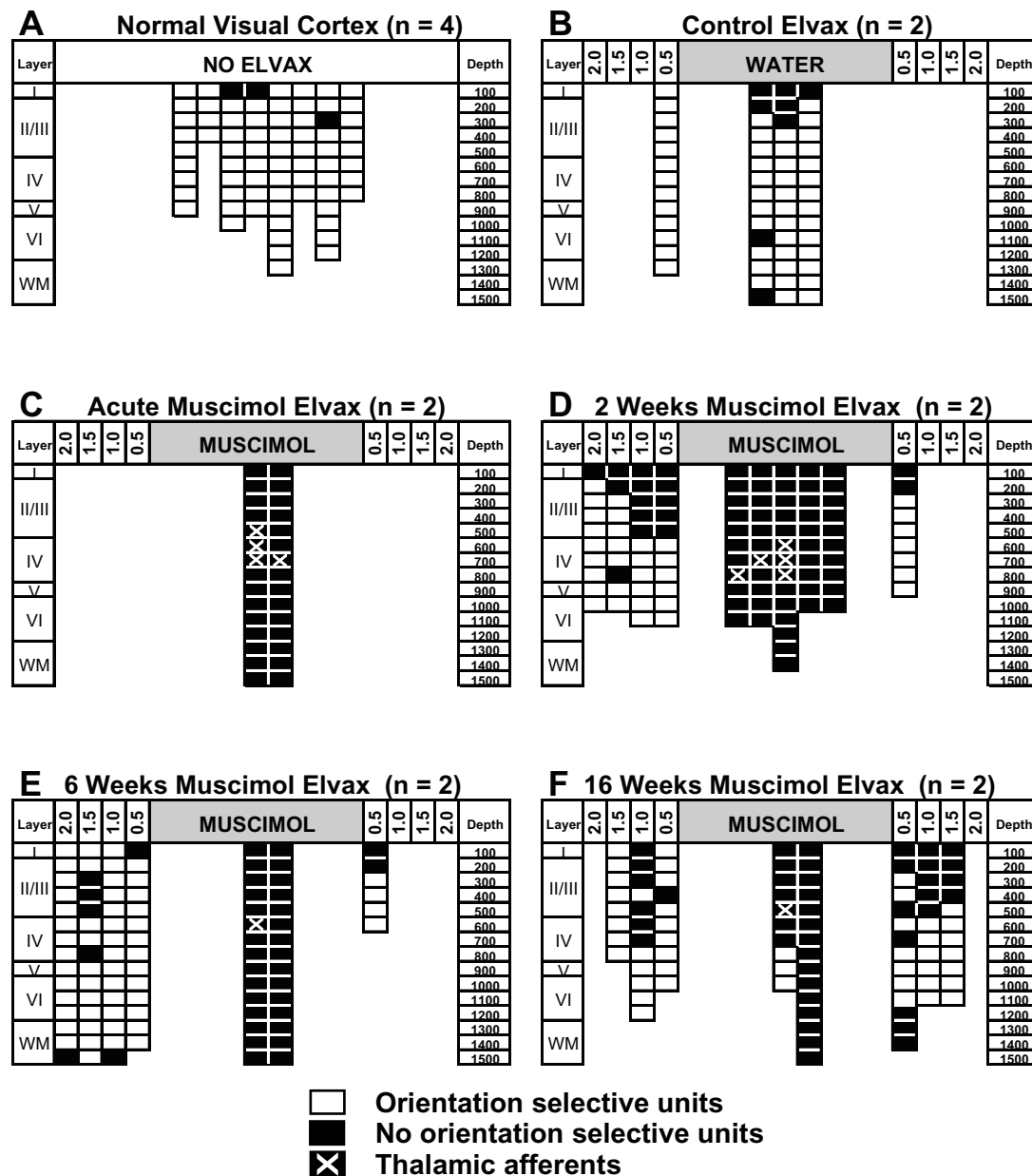


FIG. 6. Profile charts of functional cortical blockade in primary visual cortex (VI) by 75 mM muscimol-Elvax. Each panel (A–F) shows the activity profile for a number of surface-normal electrode penetrations (in total 39 penetrations in 14 animals). The electrode was advanced in 100- μ m steps and single- or multi-unit activity was examined at each site; the orientation tuning of the neuronal activity was assessed qualitatively. White boxes depict where orientation-selective cortical cells were recorded and black boxes indicate where no cortical neurons could be recorded. At some locations, neuronal activity was recorded but the responses were characteristic of geniculate neurons: high spontaneous activity and no orientation selectivity. These locations are indicated by the presence of a white cross in a black box. The depth and corresponding cortical layer (determined from histological reconstruction of the electrode penetrations) of each recording site are shown on the vertical axis. (A) Data from untreated visual cortex. (B–F) Data from animals with Elvax implants. The central grey region at the top of these panels corresponds to the Elvax sheet. Recordings were made beneath the Elvax by advancing the electrode through a hole in the sheet made prior to implantation (this hole was re-plugged until the recordings were made). In most animals, a single penetration was made through the Elvax, although in one of the two cases shown in (B) and both cases shown in (D) two such penetrations were made. Additional penetrations were made lateral to the edge of the Elvax sheet (B, D–F) and the distance (in mm) from the edge is indicated. Penetrations to the left and right of the Elvax come from different animals. The cortical blockade is compared for untreated cortex (A), for cortex acutely implanted with control Elvax containing only vehicle solution (B), and for cortex implanted with muscimol-Elvax for durations of 0 (acute), 2, 6 and 16 weeks (C–F, respectively). WM, white matter.

difference between the head bearing as the animals started to move off the platform and the direction of the sound source. The magnitude of the errors made by muscimol-Elvax ferrets ($32 \pm 19^\circ$; mean \pm SD) in response to 40-ms noise bursts was no different from that measured for the control animals ($29 \pm 18^\circ$; $F_{3,51} = 0.338$, $P = 0.798$). The average head-orienting latency for the muscimol-Elvax ferrets was

137 ± 35 ms, indicating that the observed differences in localization accuracy when the ferrets approached the sound source were restricted to stimulus durations that were too short for head movements to occur. There was some indication that cortical inactivation reduced the head-orienting latency (control values 183 ± 49 ms; $F_{3,47} = 5.336$, $P = 0.003$). However, interpretation of this result is difficult because,

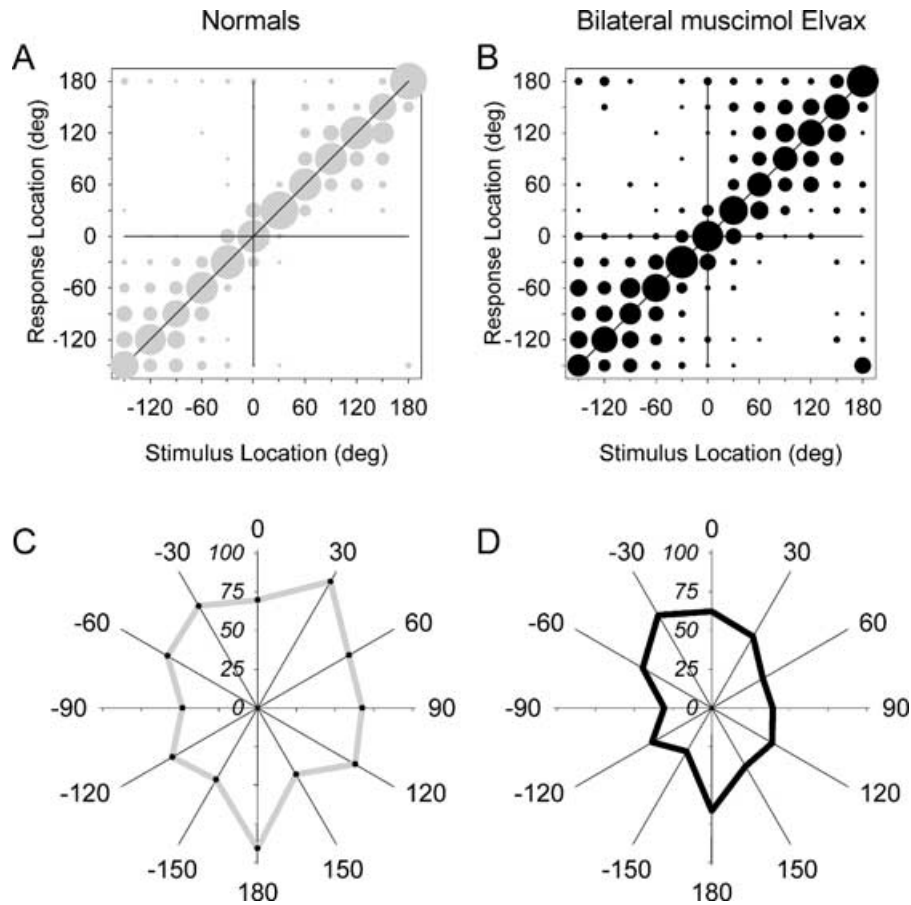


Fig. 7. Effects of bilateral implantation of 75 mM muscimol–Elvax over A1 on the ability of ferrets to localize 40-ms noise bursts presented pseudo-randomly from 1 of 12 equally spaced (30°) speakers in the horizontal plane. (A and B) Stimulus–response plots showing the combined data from four control ferrets (A) and three ferrets approximately 10 days after the Elvax was implanted (B). These plots illustrate the distribution of responses (ordinate) as a function of stimulus location (abscissa). The size of the dots indicates, for a given speaker angle, the proportion of responses made to different reward spout locations. Correct responses are those that fall on the diagonal line, whereas all other responses represent errors of different magnitude. (C and D) Mean percentage correct scores at each speaker location for each group. Muscimol–Elvax-implanted ferrets performed poorly compared with normal ferrets: they made fewer correct responses, larger errors and more front–back errors (see text).

after the Elvax was removed, the head-orienting latency in the experimental group was only slightly, but not significantly, increased (160 ± 36 ms).

Persistence and reversibility of auditory localization impairment

Because the initial deficits in performance following bilateral muscimol–Elvax implantation were most apparent with the briefest sounds used, we examined the persistence of these effects at 40 ms only. The percentage correct scores and percentage of front–back errors are shown for individual animals on each of the eight testing runs in Figs 9A and B, and 10A and B, respectively, while the mean values for each group are presented in Figs 9C and 10C, respectively. With subsequent testing, the ability of the animals to localize 40-ms noise bursts showed some improvement whilst the Elvax was in place (Figs 9A and C, and 10A and C), although they persisted in making more front–back errors than the normal controls (Fig. 10C). This improvement in performance over the 80-day period during which the animals were tested with the Elvax in place does not seem to be a practice effect, as the performance of the normal group remained at a constant level over the entire testing period (Figs 9B and C, and 10B and C). Following removal of the Elvax, the auditory localization performance of the ferrets stabilized and was indistinguishable from that of the normal controls.

The percentage correct scores of the normal and muscimol–Elvax ferrets were arcsine transformed to correct for homogeneity of variance (Winer, 1971) and analysed using a repeated-measures ANOVA. The between-subjects factor was group (Normal or Elvax) and the repeated measure was the testing run. There was no overall effect of group ($F_{1,5} = 0.711$, $P = 0.438$), but there was an overall effect of testing run ($F_{7,35} = 2.617$, $P = 0.028$), indicating that there was a general trend for the animals' performance to improve over the period of testing. Clearly, most of this improvement was due to the Elvax animals (see Fig. 9A and C) and this is shown statistically by a significant interaction between group and testing run ($F_{7,35} = 3.432$, $P = 0.007$). Significant differences in the percentage correct responses were found between the two groups in the first two testing runs only (Fig. 9C; pairwise comparisons, $P < 0.05$).

As expected, an analysis of the mean unsigned error magnitude revealed a similar profile to that of the percentage correct responses. Combining data from the eight testing runs showed no between-group difference ($F_{1,5} = 0.848$, $P = 0.399$). There was, however, a significant effect of testing run ($F_{7,35} = 3.820$, $P = 0.004$), indicating that the magnitude of the localization errors declined over the course of testing. Most of this improvement can be attributed to the implanted ferrets. The significant interaction between the testing run and group ($F_{7,35} = 5.276$, $P < 0.001$), combined with pairwise comparisons

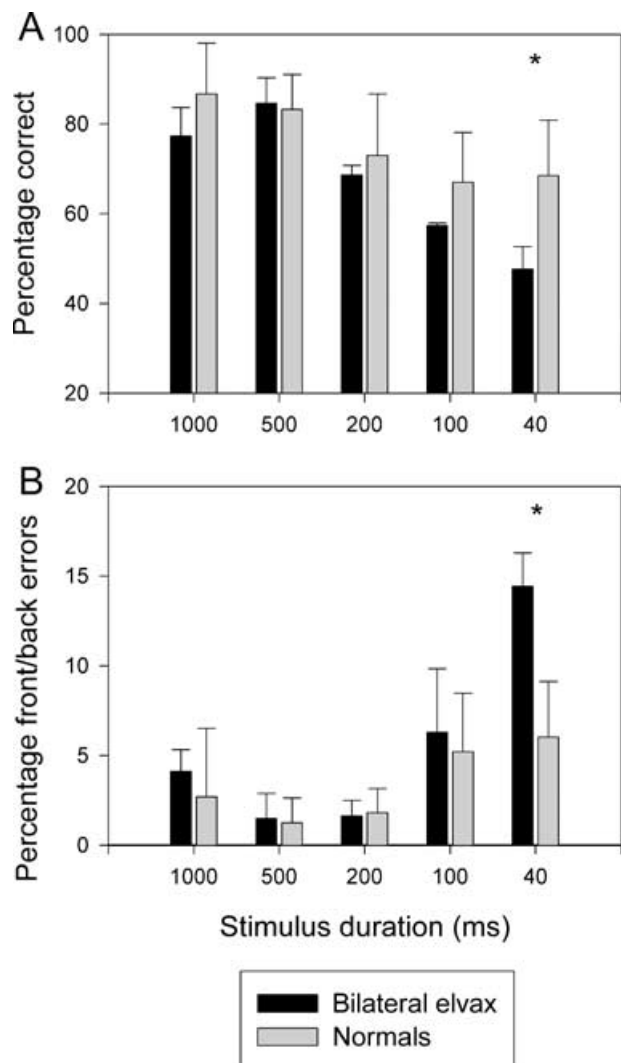


FIG. 8. Duration-dependent effects on auditory localization. (A) Mean (\pm SD) percentage scores for three ferrets that had received bilateral muscimol-Elvax implants and for a group of four normal control animals that were trained and tested in identical fashion. (B) Mean percentage (\pm SD) of front-back errors for these groups. Noise bursts of different durations were used, as indicated on the abscissa of each panel. The data from the animals that had received Elvax implants were obtained during the second week following implantation.

between the groups for each testing run, revealed that the ferrets that received bilateral muscimol-Elvax implants made significantly larger errors than the normal controls for the first testing run ($P < 0.05$), after which there was no difference in error magnitude between the two groups.

A clearer difference between the normal ferrets and the animals that had received bilateral implants of muscimol-Elvax was found by examination of front-back confusion errors. Each of the animals in which A1 had been inactivated persistently made more front-back errors than the controls while the Elvax was in place, but not after it had been removed (Fig. 10). A repeated-measures ANOVA showed that, overall, the difference between the two groups was significant ($F_{1,5} = 23.007$, $P = 0.005$). There was no overall effect of testing run ($F_{7,35} = 2.039$, $P = 0.077$), but there was a significant interaction between the groups and the testing runs ($F_{7,5} = 3.661$, $P = 0.005$). In contrast to the other measures of localization performance, pairwise comparisons revealed significant group differences for all four runs in

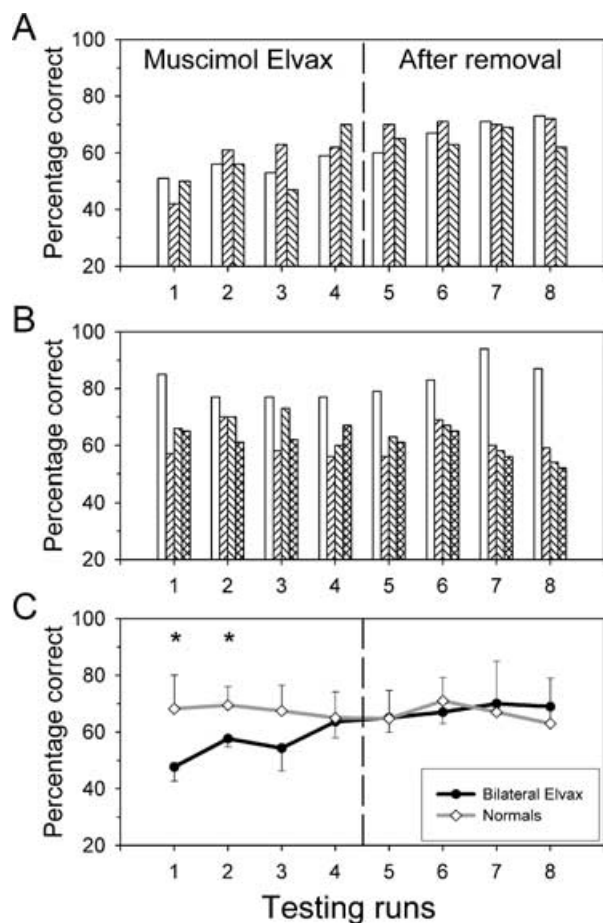


FIG. 9. Persistence and reversibility of auditory localization impairments in animals in which muscimol-Elvax had been implanted bilaterally on A1. (A) Percentage correct scores (averaged across all 12 speaker locations) for each ferret with muscimol-Elvax implants ($n = 3$) in response to 40-ms noise bursts. Data from different animals are distinguished by the presence or absence of hatching in the histogram bars. The first four testing runs were carried out with the Elvax in place. The data in runs 1–3 were obtained at approximately 14-day intervals, and those in run 4 after a further 39 days. Testing runs 5–8 were carried out at similar intervals after the Elvax had been removed. The dashed vertical line represents the point at which the Elvax was removed. (B) Percentage correct scores (averaged across all 12 speaker locations) for four normal ferrets trained and tested in the same way as the muscimol-Elvax implanted ferrets. (C) Mean \pm SD percentage correct scores for the muscimol-Elvax and normal control groups shown in (A) and (B), respectively. The asterisks indicate testing runs in which significant differences between the two groups were found. Bilateral A1 inactivation led to significantly lower scores during the first two testing runs.

which the Elvax was in place ($P < 0.05$). However, no differences were found between the two groups after the Elvax was removed.

In summary, these data show that chronic, bilateral application of muscimol to A1 produced a marked impairment in the localization of brief sounds. The most pronounced deficit exhibited by these animals was a reduced ability to discriminate between anterior and posterior sound sources. The persistence of this deficit compared with other measures of performance suggests that the significantly higher incidence of front-back confusions was not simply a consequence of an overall impairment in auditory localization. To confirm this, we compared the incidence of errors that were of equal magnitude, but not in the appropriate direction to be classified as a front-back error. In contrast to the proportion of front-back errors, we found no difference between the normal and Elvax groups ($F_{1,5} = 2.933$, $P = 0.147$). Thus,

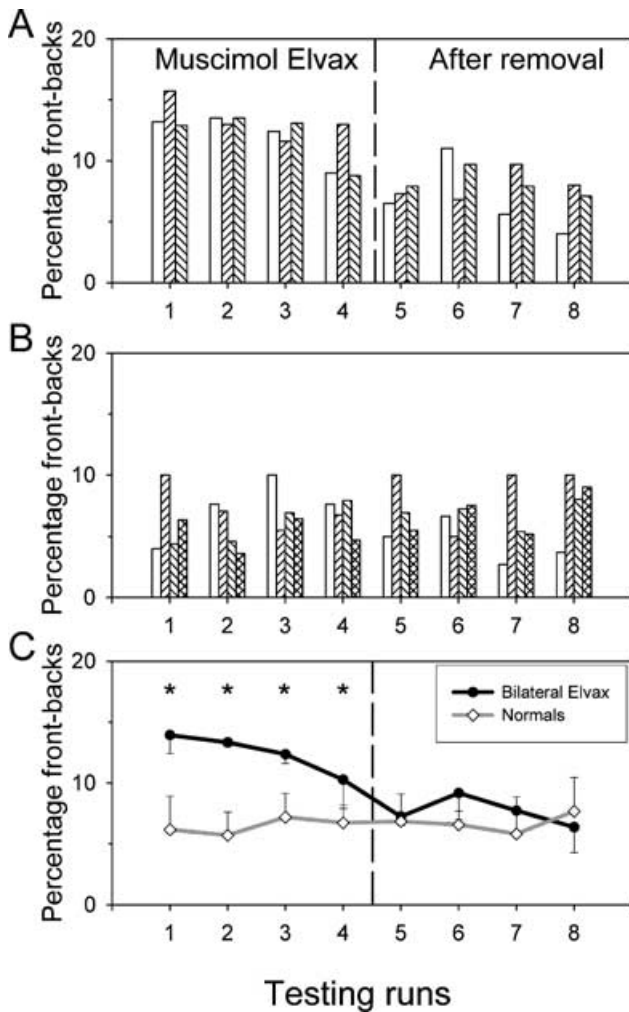


FIG. 10. Persistence and reversibility of front-back confusion errors made by ferrets in which muscimol-Elvax had been implanted bilaterally on A1. (A) Percentage of trials in which front-back errors were made by each animal with bilateral Elvax implants ($n = 3$) as a function of testing run. The stimuli were 40-ms noise bursts. Other details are as in Fig. 9. (B) Percentage of trials in which front-back errors were made by each of the four normal ferrets. (C) Mean percentage (\pm SD) of front-back errors for the muscimol-Elvax and normal ferrets shown in (A) and (B), respectively. Significantly more front-back errors were made in all testing runs when A1 was inactivated.

it would appear that inactivation of A1 specifically degrades the ability of ferrets to distinguish between sound locations on either side of the interaural axis, although this deficit gradually declined while the Elvax was in place and was no longer apparent following its removal.

Drug-free Elvax does not alter auditory localization performance

As described above, drug-free Elvax sheets had a negligible effect on cortical activity (Fig. 6B). We examined the behavioural consequences of placing control Elvax on the left A1 in two ferrets. We found no difference in mean percentage correct responses between the left and right hemifields (paired t -test: $T_3 = 0.515$, $P = 0.642$; two-tailed test), nor was there a significant left-right difference in the magnitudes of the mean unsigned error (Wilcoxon signed rank test, $z = 0.365$, $P = 0.715$, two-tailed test). The percentage of front-back errors made by the control-Elvax animals fell within the range of normal, unoperated ferrets, and also showed no difference between the left and right hemifields ($T_3 = 0.212$, $P = 0.846$).

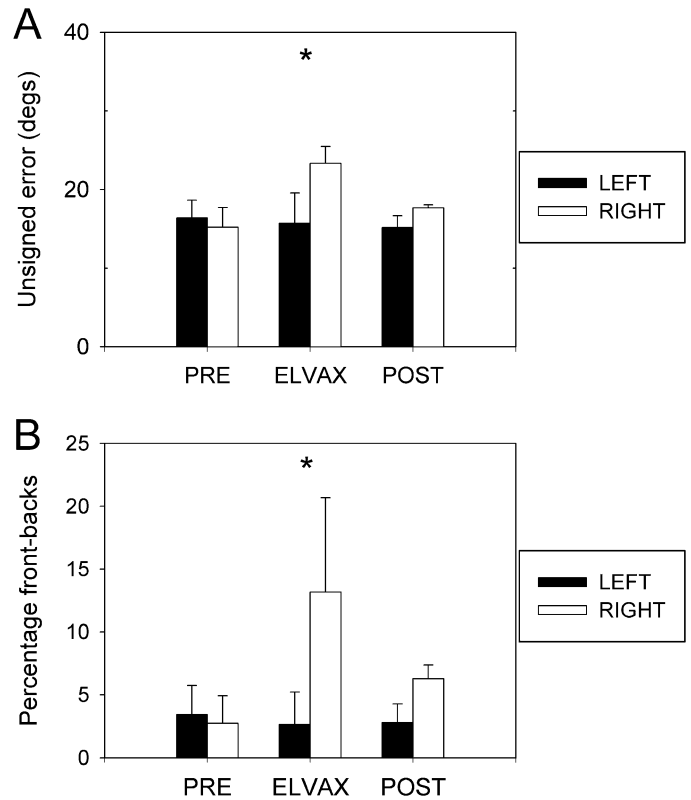


FIG. 11. Effect of unilateral implantation of muscimol-Elvax over left A1 on the ability of ferrets ($n = 3$) to localize 40-ms noise bursts presented from one of 12 equally spaced loudspeakers in the horizontal plane. (A) Bar charts showing the magnitude of the mean unsigned errors (\pm SD) in the left and right sound fields. Because of the similarity in the effects of cortical inactivation, the data from the three animals have been combined. Prior to muscimol-Elvax (PRE), there was no difference in localization performance between the two sides. Following unilateral muscimol-Elvax application (ELVAX), larger errors were made in the sound field contralateral to the side of the Elvax implant. After the muscimol-Elvax was removed (POST), performance returned to preimplantation control levels. (B) Percentage of front-back errors measured on each side for the same testing runs. A significant difference in performance between the two sides was observed only when muscimol-Elvax was applied to A1.

In contrast, unilateral application of muscimol-Elvax to A1 in three ferrets led to a reversible impairment in their ability to localize sounds in the contralateral hemifield. In this case, the animals were trained and tested prior to Elvax implantation and made errors that were almost identical in size in the left and right hemifields (Fig. 11A). The ipsilateral error magnitudes were unchanged after Elvax implantation (modified Wilcoxon rank-sum test with correction for ties; $P > 0.05$ in each case, two-tailed test), whereas those on the contralateral side increased significantly in two of the animals ($P < 0.008$ in both cases). We also observed a greater incidence of front-back errors in the hemifield contralateral to the muscimol-Elvax implant (Fig. 11B). A repeated-measures ANOVA showed that the main effect of side of testing was significant ($F_{1,6} = 12.498$, $P = 0.012$), as was the interaction between testing side and run ($F_{2,6} = 6.416$, $P = 0.032$). Pairwise comparisons made with Bonferroni corrections subsequently revealed that the percentage of front-back errors differed significantly between the two hemifields only when muscimol-Elvax was in place over A1 ($P < 0.05$). The contralateral deficits observed in these animals were very similar to those initially exhibited at all sound directions by the ferrets that received bilateral muscimol-Elvax implants, suggesting that the amount of training received prior to cortical inactivation did not influence the way in which muscimol affected their performance.

Discussion

We have demonstrated that subdural implants of 200- μm -thick sheets of Elvax releasing the GABA_A receptor agonist muscimol produce a long-lasting, local and reversible inactivation of cortex. This inactivation is restricted to the area around the implant and does not affect thalamo-cortical afferents. When implanted over ferret A1, muscimol-releasing Elvax results in a significant but reversible deficit in auditory localization.

Muscimol release from the Elvax

Because it has been reported to produce more persistent inactivation, muscimol tends to be used rather than GABA itself in experiments in which a long-lasting blockade of neuronal activity is required (reviewed by Martin & Ghez, 1999). Depending on the amount applied, the resulting inactivation can last for several hours following intracranial microinjections or iontophoresis of muscimol (Hikosaka & Wurtz, 1985; Grieve & Sillito, 1991; Martin & Ghez, 1993). Despite the difference in our method of application, this accords with our own finding that neuronal activity returns within a few hours of removing the Elvax.

A major factor in the interpretation of studies in which specific brain regions are silenced pharmacologically is the extent of drug diffusion from the site of application. Previous studies have used a variety of approaches for comparing the diffusion radius of intracranial microinjections of muscimol with the extent of the resulting inactivation (Martin, 1991; Edeline *et al.*, 2002). It has been reported that the measured spread of muscimol is considerably less than the inactivated area (Martin, 1991). This is consistent with our own data, which show that cortical inactivation was restricted to the depth of cortex below and around the Elvax implant, but nevertheless extended further from the implant than our measured [³H]muscimol diffusion. The limited spread of released muscimol may be the result of local sequestration by GABA_A receptors, while the greater, surrounding area of hypoactivity is presumably a consequence of silencing projecting axons whose cell bodies lie within the diffusion zone. Nevertheless, the region of inactivation around the Elvax was smaller than that measured following intracerebral microinjections of free muscimol (Martin, 1991; Edeline *et al.*, 2002), most likely reflecting the release characteristics of this slow-release polymer. More restricted delivery can also be achieved by incorporating drugs within injected latex nanospheres (Mackliss & Quattrochi, 1991). However, because it can be cut into sheets that are matched in size to a given part of the brain, Elvax would appear to provide the most effective method for producing a well-defined inactivation of entire cortical fields.

There are conflicting reports as to whether the labelled area changes during the first few hours following an intracranial muscimol injection (Hikosaka & Wurtz, 1985; Martin, 1991; Edeline *et al.*, 2002). In our study, the dimensions of the muscimol diffusion zone seemed to be established within 1 day and then remained unchanged for at least 1 month. We found that Elvax sheets that had previously been implanted over the cortex for periods ranging from 17 to 166 days continued to release muscimol at approximately the same daily rate. This contrasts with the release pattern observed for Elvax sheets maintained *in vitro*, which stopped releasing Elvax after nearly 60 days. Differences in the concentration gradient and the direct apposition of the Elvax sheet to the brain on one side and the dura on the other most likely explain the lower release levels observed *in vivo*. This technique therefore has the potential to inactivate restricted areas of the brain over periods of at least 5 months.

Inhibition of cortical activity

Electrophysiological recordings from below and around the site of the implant were used to quantify the spatial extent and persistence of

cortical inhibition. Recordings made directly below the implant from both auditory and visual cortex via a hole in the Elvax revealed a lack of cortical activity throughout the depth of cortex, indicating that blockade was equally efficient at all depths for implants that lasted for several weeks. This finding contrasted with the relatively normal activity found in some electrode penetrations made as close as 500 μm to the lateral border of the Elvax, suggesting that muscimol-induced inactivation extended only a limited distance from the edge of the implant. In other cases, more extensive inactivation of the supragranular layers, which appeared to exceed the lateral spread of muscimol from the Elvax, was observed. Complete blockade of all layers of the underlying cortex was reliably observed up to 6 weeks following implantation, and the data from one of the 16-week animals showed that complete blockade can persist for that long. This is consistent with our finding that Elvax left *in situ* for this length of time was still releasing muscimol.

The electrophysiological data suggest that prolonged blockade does not have a substantial lasting effect on the functional integrity of cortical circuits. Two hours after removing the Elvax from V1 in the 16-week animals, orientation-tuned neurons could be recorded in the upper cortical layers previously blocked by the implant. A fully quantitative study would be needed, however, to determine whether there were any more subtle changes in the properties of cortical neurons.

Behavioural consequences of cortical blockade

In order to assess the behavioural consequences of reversibly inactivating a selected area of the cortex, we chose to examine the effects of applying muscimol–Elvax to A1 on sound localization. Although the functional organization of the auditory cortex is poorly understood compared with that of the visual cortex, it is well established that lesions of A1 lead to deficits in sound localization. More specifically, unilateral lesions in cats (Jenkins & Masterton, 1982; Jenkins & Merzenich, 1984) and ferrets (Kavanagh & Kelly, 1987) produce localization deficits in the contralateral hemifield, whereas bilateral lesions in carnivores and primates impair performance on both sides of the midline (Neff *et al.*, 1956, 1975; Heffner & Masterton, 1975; Heffner, 1978; Kavanagh & Kelly, 1987; Heffner & Heffner, 1990; Heffner, 1997). The localization deficits reported in these studies were most pronounced for single, brief sounds and varied in magnitude with the size of the lesion and the nature of the localization task performed by the animal.

In keeping with these studies, we found that bilateral application of muscimol–Elvax to the region of the ectosylvian gyrus occupied by A1 markedly reduced the accuracy with which ferrets approached the source of the sound at all azimuthal angles tested; whereas unilateral inactivation degraded performance on the contralateral side only. As with the lesion studies, these deficits were most pronounced for brief sounds that are too short for additional dynamic cues to be gained from head motion. In contrast, the accuracy of the initial head-orienting movement executed before the animal started to move toward the sound source in order to obtain a reward was not affected by cortical inactivation. This has also been reported for cats with lesions of the auditory cortex (Thompson & Masterton, 1978), although Beitel & Kaas (1993) did find that cortical lesions produced deficits in sound-evoked head-orienting responses.

The behavioural impairments that we observed following muscimol–Elvax inactivation were apparent for each of the measures used to assess localization performance in our multi-speaker task. However, the most persistent deficit that we observed was a significant increase in the incidence of front–back errors. This suggests that the poor performance is unlikely to be due to an attention problem and provides direct experimental evidence for the suggestion that A1 may play a

specific role in resolving the spatial ambiguities inherent in frequency-specific binaural cues (Kavanagh & Kelly, 1987). The relatively high frequency resolution of A1 neurons may therefore be important for processing the spectral shape information conveyed by monaural pinna cues, which are responsible for resolving front-back confusions (King & Carlile, 1995). This is consistent with reports that the azimuth sensitivity of A1 neurons is shaped, at least in part, by the directional filtering properties of the contralateral ear (Samson *et al.*, 1993; Mrsic-Flogel *et al.*, 2003). As expected from the electrophysiological recordings, all the behavioural changes could be reversed by removal of the Elvax.

Despite these qualitative similarities between the behavioural effects of pharmacological inactivation and lesions of A1, there are two key differences between our results and those previously reported in the lesion studies. Firstly, although we observed a significant impairment in performance, at least during the initial stages of testing, these deficits were smaller than those typically reported following cortical lesions. Secondly, in contrast to the lesion studies where, in most cases, persistent deficits have been reported over the various testing periods used (Heffner & Masterton, 1975; Jenkins & Merzenich, 1984; Kavanagh & Kelly, 1987), the localization deficits produced by muscimol–Elvax implants became less pronounced with subsequent testing.

There are several possible explanations for these differences. Although frequency-specific, spatial deficits have been reported following partial lesions of A1 (Jenkins & Merzenich, 1984), many of the previous behavioural studies were carried out on animals in which the region of cortical damage extended well beyond A1. Indeed, the degree of inability to localize brief sounds seems to correlate well with the magnitude of the lesion (Heffner & Masterton, 1975; Heffner, 1978; Kavanagh & Kelly, 1987). In ferrets, Kavanagh & Kelly (1987) reported that bilateral lesions restricted to A1 produced smaller deficits than those impinging on adjoining areas, although this was not the case with unilateral lesions. The Elvax implants used in this study should have been large enough to cover the full extent of A1 and did not move to any noticeable degree during the period that they were in place. Assuming that A1 was adequately covered, our electrophysiological data suggest that limited regions of the supragranular layers in neighbouring cortical fields may have been silenced as well. On the other hand, we cannot rule out the possibility that incomplete inactivation of A1 may have been responsible for the partial recovery of function observed while the Elvax was in place. Indeed, the transient deficits in performance sometimes reported following A1 lesions have been attributed to incomplete removal of this cortical field (Jenkins & Merzenich, 1984).

Our measurements of the release characteristics of muscimol–Elvax and of its effects on the activity of neurons in A1 and V1 suggest that the majority of cortical neurons beneath the implants should have been silenced throughout the period of behavioural testing. Nevertheless, these recordings provide some support for the possibility that the progressive recovery of sound localization during the period of implantation may have resulted from a gradual wearing off of the effects of muscimol on A1 neurons, as the activity of neurons in the infragranular layers of V1 was more variable at 16 weeks after implantation than at 6 weeks (Fig. 6). This is consistent with the finding that single applications of muscimol via a well implanted over A1 in rats lead to transient deficits in tone detection and frequency discrimination, which recover within 1 day as cortical activity returns (Talwar *et al.*, 2001). If future work confirms a correlation between the layer specificity of the inactivation and the degree of behavioural impairment, this technique may provide a useful way of dissecting the relative contributions of feedback and feedforward pathways in functions attributed to the cortex.

The gradual improvement in the ability of the ferrets to localize sound after several weeks of continuous inactivation of A1 may also reflect a recovery in function involving other brain areas. Such compensatory changes could potentially involve other cortical areas that receive direct thalamic inputs or even subcortical processing at the level of the thalamus or midbrain. It is therefore possible that we would have observed a more profound initial deficit had we been able to measure the ferrets' performance as soon as the Elvax was first applied, instead of allowing them to recover for several days from the surgery.

Because muscimol–Elvax silences cortical neurons without affecting thalamic afferents or presumably fibres of passage, it is likely that the more extensive deficits reported following A1 lesions in part reflect degenerative effects on neurons in other brain areas connected with the lesion site. Indeed, sparing of those neurons may be necessary in order to reveal the full capacity of the brain to compensate for localized inactivation of specific cortical areas. Consequently, lesion studies may have overestimated the role played by A1 in sound localization. This view is supported by studies of the primate auditory cortex, which suggest the existence of parallel processing streams for spatial and object information (Rauschecker & Tian, 2000). In particular, the existence of thalamocortical pathways that bypass A1 provides a potential route by which spatial information may reach other areas of the cortex (Winer, 1992; Rauschecker *et al.*, 1997). Local, long-term and reversible inactivation of cortical neurons may therefore provide a more sensitive measure of the contribution of specific cortical fields to a given behavioural task and of the capacity of other, intact areas to take over this function.

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Abbreviations

A1, primary auditory cortex; GABA, γ -aminobutyric acid; PBS, phosphate-buffered saline; V1, primary visual cortex; WM, white matter.

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