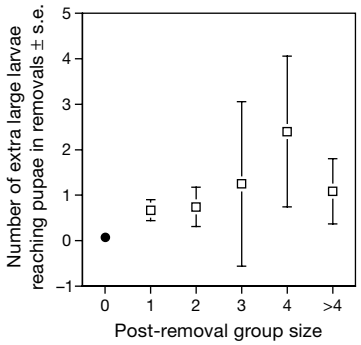

Figure 3 Number of extra large larvae reared through to the pupal stage on removal nests compared with controls (±1 standard error). The filled circle represents our estimate for lone-foundress nests when the foundress dies.

& J.F., unpublished data) suggests that helping is favoured unless lone foundresses can invest at 1.7 times the rate of helpers. Our data (Fig. 1) suggest that helpers and lone females invest at approximately the same rates.

Insurance-based benefits of helping may be larger in insects than in vertebrates, which have relatively low adult mortality rates[3,4]. Our experiment suggests that an AFRs advantage contributes to the maintenance of helping in *L. flavolineata*. The pattern of investment preservation is obviously adaptive in that it is the older, more valuable extra brood that are reared through to maturity. Additional insurance-based benefits[3,4,6,8] are also likely to operate in *L. flavolineata* and other eusocial[20] and communal[21] insect systems, and similar advantages probably applied at the origin of eusociality[3,6]. □

Methods

Our main experiment included all of the active *L. flavolineata* nests in four culverts (sites) that carried streams under a 4-km stretch of road surrounded by forest between Raub and Bukit Fraser in Selangor State, peninsular Malaysia. Starting 17 June 1998, we had individually marked all adult nest residents and identified dominants as the females most often present on nests during regular censuses[11–13]. We allocated nests randomly to removal or control treatments after blocking for site, total brood number and group size (2–9 females)[12]. We carried out wasp removals on 9 July. We captured all residents on all nests before dawn[12]; we then released them all except for 1–2 helpers from each removal nest. By mapping the contents of all cells in every nest just before the removals, we followed the fate of each brood item during subsequent brood censuses every 4–9 days until 9 September. We recognized three brood development stages: eggs/small larvae, large larvae and pupae. We defined large larvae as larvae that filled the full widths of their cells. We focused on whether brood that were initially at one stage successfully reached the next stage. We had to omit a few nests from the analysis because of brood-mapping errors. We calculated mean brood weights from the wet weights of the 324 individual brood in 16 collected nests. Transplanted vacant nests contained 7.7 ± 0.3 brood of all stages, including an average of 1.3 large larvae. Nests were taken without their resident wasps from a site 5 km away and attached[13] among the active nests in our four main sites.

Data analysis

We used general linear modelling in the GLIM statistical package assuming Poisson or normal errors as appropriate[22]. In each analysis we first fitted potential explanatory variables (site, group size, treatment) and their pairwise interactions. Starting with the interactions, we then subtracted terms from the model until further removals led to significant ($P < 0.05$) increases in deviance, as assessed from tabulated values of F with normal errors or χ^2 with Poisson errors[22]. We report significance levels for terms when adding them last to this minimal adequate model. When there was significant over-dispersion using Poisson errors, we re-scaled the model using Pearson's χ^2/d.f. (where d.f. is degrees of freedom)[22]. Means ± standard errors are reported.

We assumed that foraging effort was proportional to percentage time spent off the nest. To look for possible changes in foraging after the removals, we used the number of wasps off the nest during the foraging periods of 7–8 July versus 12–14 July (9–11 censuses each), excluding data for wasps that were removed. We analysed adult mortality rates using a mark–release–recapture model[12] with data from night censuses every four days until 9 September. For brood development time analyses, the y-variable for each nest was the number of brood reaching a given developmental stage, with ln(total number of days taken to reach that stage from the previous stage) as an offset[22]. We separately examined development times from egg to large larva, large larva to pupa, and pupa to adult emergence. We excluded from the development time analyses any nests that failed or were taken over by foreign wasps between the helper removals and the end of our monitoring. In the analysis of Fig. 2, the same effects remain significant if we exclude nests that failed between helper removals and the point at which more than 90% of the initial brood stage concerned had reached the next developmental stage. We avoided pseudoreplication by using nests as data points in all analyses except adult mortality.

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Visual behaviour mediated by retinal projections directed to the auditory pathway

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An unresolved issue in cortical development concerns the relative contributions of intrinsic and extrinsic factors to the functional specification of different cortical areas[1–4]. Ferrets in which retinal projections are redirected neonatally to the auditory thalamus[5]

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have visually responsive cells in auditory thalamus and cortex, form a retinotopic map in auditory cortex and have visual receptive field properties in auditory cortex that are typical of cells in visual cortex[5–8]. Here we report that this cross-modal projection and its representation in auditory cortex can mediate visual behaviour. When light stimuli are presented in the portion of the visual field that is 'seen' only by this projection, 'rewired' ferrets respond as though they perceive the stimuli to be visual rather than auditory. Thus the perceptual modality of a neocortical region is instructed to a significant extent by its extrinsic inputs. In addition, gratings of different spatial frequencies can be discriminated by the rewired pathway, although the grating acuity is lower than that of the normal visual pathway.

The relationship between sensory inputs to cortex and the functional capacity of a sensory projection has commonly been examined using deprivation protocols. In the visual pathway, for example, the development of visual function is correlated with the maturation of inputs to visual cortex[9,10]; visual deprivation early in life alters the anatomical patterning and physiological effectiveness of these projections, with concomitant reductions in visual ability[11,12]. However, a more fundamental issue, whether or how sensory inputs specify the perceptual modality of visual cortex, remains unexplored. Evidence from congenitally blind humans indicates the involvement of visual cortex in non-visual tasks (refs 13, 14; see also 15, 16), but the pathways that may mediate such plasticity are unknown. We have tested whether sensory inputs can shape the perceptual modality of a cortical area by studying ferrets in which visual projections are directed to the auditory pathway, thereby providing inputs of one sensory modality to thalamic and cortical targets that normally process a different modality.

The anatomical pathway and physiological consequences of this cross-modal projection have been well characterized[17]. Projections from the retina to the medial geniculate nucleus (MGN), the principal auditory thalamic nucleus, can be induced by extensively deafferenting the MGN in neonatal ferrets (Fig. 1a): ablating the brachium of the inferior colliculus (BIC) removes ipsilateral inputs to the MGN, whereas ablating the superior colliculus (SC) down to the deep layers removes the contralateral inputs[18]. Primary auditory cortex (Al) of rewired ferrets, which receives visual information through the intact thalamocortical pathway from the MGN, develops many functional features that are uniquely characteristic of visual cortex: cells in rewired Al display visual properties such as orientation selectivity and direction selectivity[7], and they encode a two-dimensional map of visual space[6] and of orientation-selective cells[19]. We investigated whether activation of the crossmodal projection evokes visual or auditory percepts in the behaving ferret (experiment 1), and, if the projection mediates visual behaviour, whether its visual acuity is comparable to normal (experiment 2).

We directed retinal axons to the left MGN in neonatal ferrets (Fig. 1a), providing visual information to auditory cortex in the left hemisphere. After rearing the animals to adulthood, we trained and subsequently tested them on specific behavioural tasks. In experiment 1, four rewired ferrets (animals R1–R4) were trained to make one response to a visual stimulus and a different response to an auditory stimulus. The ferrets were trained to stand stationary on a start platform with their head facing forward to initiate an auditory or visual stimulus (Fig. 1b). Ferrets received a reward (a few drops of water or juice) at a spout on the left following a sound stimulus or at a spout on the right for a light stimulus. Animals were trained extensively, up to asymptotic limits of accuracy (Fig. 2a–c), with a variety of auditory stimuli presented centrally and with light presented only in the left monocular visual field; that is, the portion of the visual field seen only by the monocular portion of the non-rewired visual pathway in the right hemisphere. (Training with light only in the left field was important to ensure that the rewired projection remained untrained.) Following training, we tested the animals' responses to lights presented in the central and right visual fields; in these trials, animals were rewarded at either spout—that is, they received a reward even when they went to the incorrect spout. Subsequently, we lesioned the remaining visual thalamic nuclei in the rewired (left) hemisphere—the lateral geniculate nucleus (LGN) and lateral posterior nucleus (LP)—with ibotenic acid, leaving the retino–MGN projection as the only pathway for retinofugal information in the left hemisphere. After a period of recovery, the rewired visual projection was tested by presenting light in the right visual field and noting the reward spout to which animals went. Again, animals were rewarded at both visual and auditory spouts, to avoid selective training of one response over the other. In a final phase of the experiment, A1 and adjacent cortex were ablated; after recovery, the ferrets were tested again with light in the right field.

We obtained complete sets of data from three of the four rewired

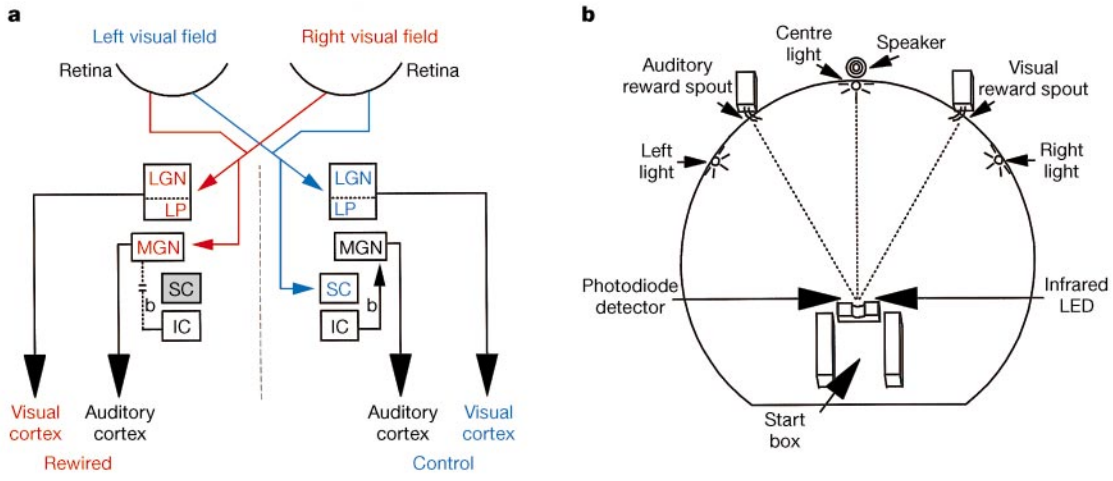

Figure 1 The behavioural role of retinal projections routed to the auditory pathway. **a**, Pathway from the retina to the visual thalamus, including the lateral geniculate nucleus (LGN) and the lateral posterior nucleus (LP), and to the superior colliculus (SC) in the control hemisphere (right); and to the LGN/LP and medial geniculate nucleus (MGN) in the rewired hemisphere (left). The SC and adjacent brachium (b) of the inferior colliculus (IC) were ablated neonatally in the left hemisphere. Visual projections in each hemisphere represent the contralateral visual field. **b**, Apparatus for experiment 1. Dashed lines denote the borders of the left and right monocular fields and the direction of central gaze. Animals were rewarded at the right spout after a light in the left monocular field, and at the left spout after a sound from a central speaker. Subsequently, their responses to light in the centre or the right monocular field were tested. Animals initiated trials by standing in the start box with their muzzle between an infrared LED and a photodiode detector.

ferrets (animals R1, R2 and R3; Fig. 2a–c). Following the LGN/LP lesions, these animals consistently responded as though they perceived the light stimulus presented to the rewired projection to be visual ($P \ll 0.01$, Student's t-test, comparing responses to the right field light at the visual and auditory reward spouts, respectively, in each animal; $P > 0.1$ comparing post-LGN/LP lesion right light responses with pre-lesion right, left or centre light responses in each animal). The timing of the responses to the right light were within the normal range of response times for all other visual and auditory stimuli. The proportion of false starts and delayed responses (see Methods) in the post-LGN/LP lesion condition was not significantly different following the right field light from that following sound stimuli or light stimuli at other locations, or stimuli during the pre-LGN/LP lesion condition ($P > 0.1$ for each comparison). To control for the possibility that the ferrets' visual responses were influenced by stimulus location rather than stimulus modality, a block of trials in each ferret was done with the auditory speaker positioned in the right monocular field (but out of sight). All ferrets maintained > 90% response accuracy for this auditory stimulus location, which was comparable to their performance with a centrally positioned speaker.

As a key control for whether the behavioural responses to light stimuli in the right field were mediated by the rewired visual projection, the three rewired animals subsequently underwent ablation of auditory cortex and were then tested again. All animals showed a significant reduction in their responses at the visual reward spout to the right field light ($P < 0.01$, Student's t-test, comparing right light post-A1 lesion responses with post-LGN/LP lesion responses in each animal). Indeed, responses at the visual spout dropped to near-chance levels, indicating that ferrets went to either reward spout at random in this two-choice protocol. One ferret (animal R2) showed an increase in the proportion of aborted trials resulting from delayed responses. There was little change in the responses to other stimuli (sound, left light or centre light) in the post-A1 lesion condition. These results are consistent with the conclusion that the rewired ferrets interpreted light stimuli in the right monocular field as visual (or at least as more visual-like than auditory-like), and became functionally blind in the right field after the A1 lesion.

In addition to internal controls in the three rewired ferrets, we examined three other kinds of control animal. First, to assess whether retinal projections other than those to LGN, LP and SC could mediate the visual response in normal animals, one normal ferret (animal N1) was trained identically to the rewired ferrets and tested with light in the right monocular field before and after a lesion of the LGN/LP and SC in the left hemisphere (Fig. 2d; a second animal, N2, had a spared SC and was not considered further). After the lesion, the animal's performance in response to the right field light was close to chance, denoting functional blindness in this part of the visual field. A fundamental difference between this normal ferret and the rewired ferrets was that the latter had their left SC and BIC lesioned neonatally (whereas their left LGN/LP was lesioned in adulthood); right field light stimuli activated the rewired retinal projection to the auditory pathway and elicited behaviour consistent with a visual percept. However, in similar fashion in the normal and rewired ferrets, lesioning the target of visual projections (subcortical visual structures or A1, respectively) led to functional blindness in the right visual field.

Second, to assess whether the representation of the right light in the rewired hemisphere was similar to that of the left light in the control hemisphere, one rewired ferret (animal R5) was trained only with light stimuli in the left monocular field (and received no training with sound stimuli). Subsequently, LGN/LP in its left hemisphere was lesioned and it was tested with light in the right monocular field. It responded with >95% accuracy at the visual reward spout, indicating that the right field light was perceived as being similar to the left light.

Third, to assess whether the representation of the right light was separable from that of the sound stimulus, one rewired ferret (animal R6) was trained only with auditory stimuli. After its

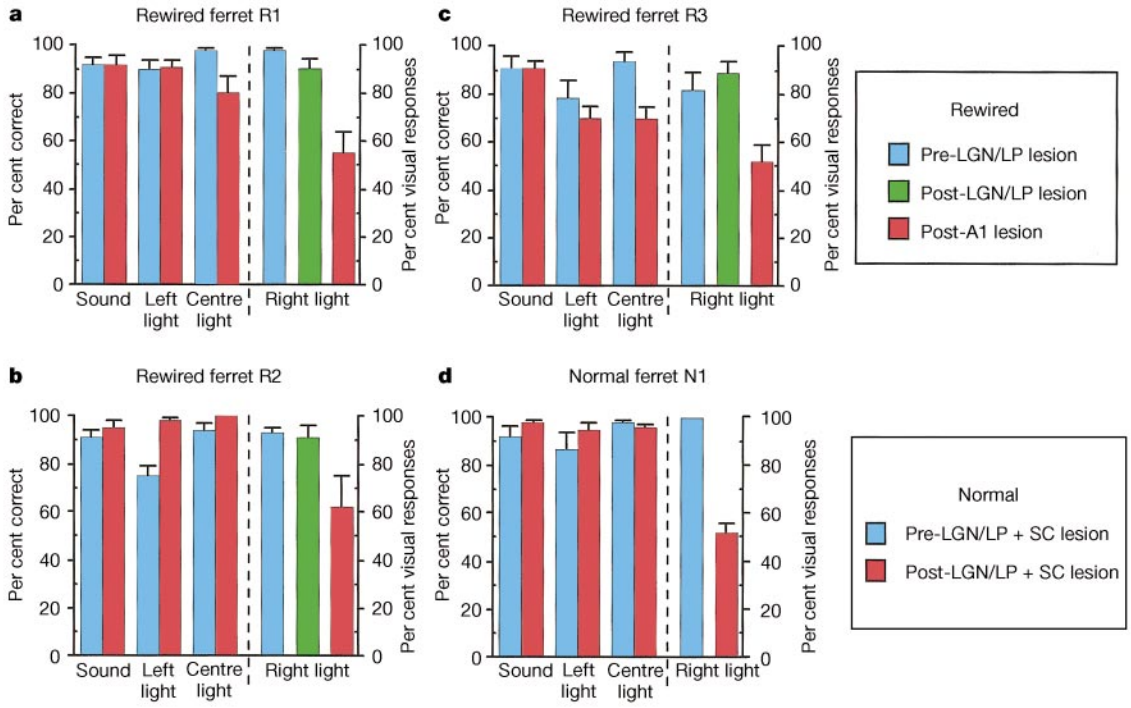

Figure 2 Responses of rewired and normal ferrets to sound and light stimuli. **a–c**, Responses of rewired ferrets to the various stimuli under three separate conditions: after training with sound and left light stimuli, before the left LGN/LP lesion (blue bars); after the left LGN/LP lesion (green bar; only the response to the right light is shown); and after the left A1 lesion (red bars). Response bars (mean ± s.d.) depict performance in the final 10–19 days in the pre-LGN/LP lesion condition, and in the first 10–18 days in the post-LGN/LP lesion and post-A1 lesion conditions. **d**, Responses of normal ferret N1 to the stimuli before a lesion of the left LGN/LP and SC (blue bars) and after (red bars). Response bars depict performance in the final 9 days in the pre-LGN/LP + SC lesion condition, and in the first 12 days in the post-LGN/LP + SC lesion condition.

LGN/LP was lesioned, it was tested with light in the right monocular field. In 100% of these trials, it turned towards the light but did not move from the start position or go to the auditory reward spout, indicating that the light was perceived as being distinct from sound.

Together, these results show that the rewired visual projection to the auditory thalamus and cortex can mediate functional responses to visual stimuli. In experiment 2 we tested whether the visual acuity of the projection is comparable to that of the normal visual pathway. Two rewired ferrets (animals R6 and R7) which had received lesions of LGN/LP on the side of the rewired projection (the left hemisphere) and a normal ferret (animal N3) were tested for grating acuity in their left and right monocular fields (Fig. 3a). Ferrets were trained to go to a reward spout adjacent to a central light as their default response (see Methods) and to a reward spout adjacent to a display monitor when a grating was visible.

The contrast–response functions of rewired ferrets, obtained for the left and right monocular fields (seen by the control and rewired hemispheres, respectively), show very different contrast sensitivities in the two visual fields. In animal R6, the lowest spatial frequency presented in the left visual field, 0.25 cycle per degree, elicited a 50% correct response level at about 0.06 contrast (Fig. 3b). This spatial frequency was not visible up to a contrast level of 0.22 when presented in the right visual field. However, a lower spatial frequency of 0.18 cycle per degree elicited a non-zero (but less than 50%) response at 0.22 contrast. For all animals, responses to a matrix of stimulus parameters (spatial frequency and contrasts) were compared with responses to equiluminant control screens, and responses to gratings that were significantly different from control ($P < 0.05$, Student's t-test) were noted (Fig. 3c). The data show that the grating acuity of rewired ferrets is comparable to normal in the left monocular field, but substantially lower in the right monocular field (Fig. 3d shows the acuities at 0.15 contrast). In a variation of the experiment shown here, we used a centrally placed monitor with a split screen to define grating acuity in the left and right halves of the central, binocular field. The data (not shown) again indicate a difference between performance in the two halves: at a contrast of 0.15, for example, a grating of 1 cycle per degree could be detected in either half by the normal ferret and in the left half by the two rewired ferrets, while only a grating of 0.25 cycle per degree could be detected in the right half by the rewired ferrets. Thus, the contrast sensitivity and spatial resolution of the rewired projection is lower than that of the normal visual pathway.

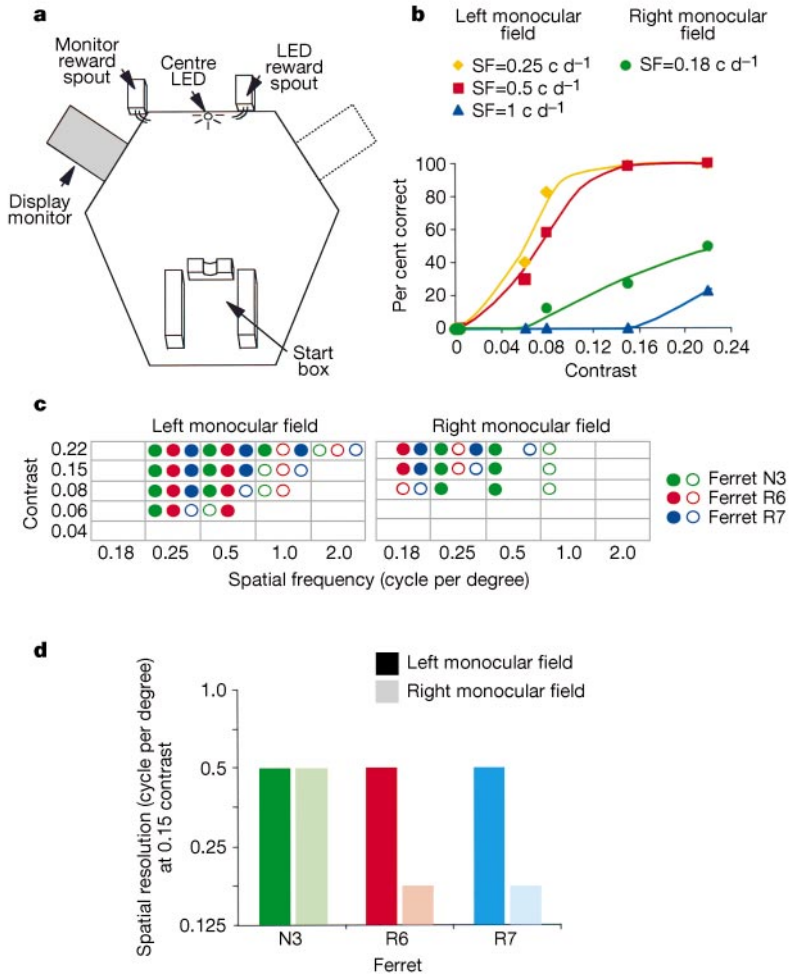

Figure 3 Grating acuity in rewired and normal ferrets. **a**, Apparatus for experiment 2. Grating or no-grating stimuli were presented on a display monitor placed either in the left or the right monocular field. Ferrets received a juice reward at a central spout when a centre LED was presented, and at a monitor reward spout when a grating was presented. **b**, Contrast–response functions for a rewired ferret (R6), showing the fraction of correct responses against stimulus contrast for a range of spatial frequencies (0.25–1 cycle per degree (c d^{-1})) presented in the left monocular field, and for one spatial frequency (0.18 cycle per degree) presented in the right monocular field. Higher spatial frequencies tested did not elicit responses in either field. Each curve is a psychometric function of the form $x^k/(2^k + x^k)$. **c**, Response matrix for stimuli presented to two rewired ferrets and a normal ferret. Filled circles, responses significantly above chance ($P < 0.05$, Student's t-test, comparing grating and no-grating conditions); open circles, responses indistinguishable from chance. Whereas the responses in the normal animal are roughly similar in the left and right monocular fields, responses in rewired ferrets are similar to normal in the left monocular field but occur at lower spatial frequency and higher contrast in the right monocular field. **d**, A subset of data from **c**, showing the highest resolvable spatial frequency (spatial resolution) at 0.15 contrast for the three animals.

The retinal input to MGN in rewired animals arises to a significant extent from retinal W-cells[8], which may explain the reduced visual acuity of the rewired pathway compared with the X- and Y-cell-dominated normal retinogeniculate pathway[20]. More generally, these data show the profound capacity for behavioural plasticity in even primary sensory areas of the neocortex. How might a visual pathway to auditory cortex enable visual behaviour? Because the rewiring lesions were done within a day after birth and ferrets were behaviourally tested only when they reached maturity, rewired ferrets had many months of visual experience during which stimuli in the contralateral visual field could simultaneously drive the rewired projection and the normal retinogeniculate projection. Our animals were not dark-reared or visually deprived postnatally (which would in turn constrain vision[11,12]), and it is possible that the rewired projection is instructed or entrained by activity in the normal visual projection. However, the pathway by which such training might occur is unclear: the cortico–cortical connections of rewired A1 remain essentially the same as in normal animals[21] (but see refs 19,22,23), and new connections do not develop between early visual and auditory cortical areas, at least, in rewired ferrets.

Although the precise quality of the light-induced sensation in rewired animals remains unknown, one possibility is that all projections central to the MGN in the rewired hemisphere, including connections to motor pathways, come to be visually driven[24]. In principle, such connections would allow visually driven activation of auditory cortex to lead to a 'visual' response. Alternatively, auditory cortex might develop new connections that allow access to specific visuomotor pathways and generate a 'visual' response. Regardless of mechanism, however, our results show that, during development, sensory afferents can instruct their cortical target as to its eventual function. □

Methods

Ten ferrets (seven rewired and three normal) were used. All procedures were done under protocols approved by MIT's Institutional Animal Care and Use Committee and in accordance with NIH guidelines. Ferret kits from timed-pregnant mothers (Marshall Farms) received lesions in the left hemisphere within one day after birth, inducing retinal projections to the MGN[5,18]. The BIC was lesioned and the SC ablated; visual cortex was partially ablated to reduce the size of the LGN. We reared animals to adulthood before using them for further experiments.

Experiment 1

Ferrets were water deprived overnight, then had free access to water for 3–4 h each day after behavioural testing was completed. Ferrets were trained to hold their heads stationary and facing forward, with their muzzle positioned between an infrared light emitting diode (LED) and a photodiode detector (Fig. 1b). The offset of this beam for 300–500 ms (the exact time was randomly determined) triggered the presentation of a visual or auditory stimulus for 300 ms. Trials on which ferrets left the start position before stimulus onset (false starts), or arrived at the reward spout more than 2 s after stimulus onset (delayed responses), were automatically aborted and were not rewarded. Reward spouts were positioned 25° to each side of the midline. Visual stimuli consisted of red LEDs positioned 55° to the left of midline (the binocular field in ferrets extends 30° to each side of the midline[25]), straight ahead (0°) or about 55° (distributed in different blocks of trials between 45 and 65° to ensure coverage of the retino–MGN projection) to the right of midline. The left and right LEDs were sufficiently far from the midline that head or eye movements could not cause one stimulus to 'leak' into the opposite visual field. In addition, only the LED tips were left exposed and the entire apparatus was painted matt black to avoid reflections and reduce light spread. Auditory stimuli consisted of either white noise or pure tones of frequency ranging from 30 Hz to 3 kHz. (The ability of animals to generalize their auditory response to novel auditory stimuli was demonstrated anecdotally in several ferrets when unintended random noises during the stimulus onset period caused the animals, in every instance, to go to the auditory spout.) The speaker for the auditory stimuli, which was not visible to the ferrets, was positioned at 0°, except for one block of control trials (see text) when it was positioned at 55° in the right field.

Animals were deliberately overtrained to associate the right reward spout with light presented in the left monocular field and the left spout with sound. In the first phase of the experiment, after training with the left light and sound was complete, responses to light in the centre and right fields were tested. Stimuli were presented in random order, and the block of trials presented in each daily session was designed to contain an equal number of auditory and visual stimuli. The number of light stimulus presentations was evenly divided between the three portions of the visual field. In the second phase of the experiment, we lesioned the LGN and LP in the left thalamus (the side of the rewired retinal projection) under direct visual control. A craniotomy and durotomy was performed, and the cortex was gently reflected rostrally. Several small injections of ibotenic acid (0.4 μl each, 10 μg μl^{-1} in saline) were evenly spaced in the LGN and LP. After about 2 weeks of recovery, animals were tested daily as in the first phase until at least 30 trials with the right field light stimulus were completed. In the third and final phase, A1 in the left hemisphere was ablated by cautery; after recovery, animals were again tested in the same way. Control normal ferrets received ibotenic acid lesions of the left LGN and LP and heat ablation of the left SC. At the completion of each experiment, the brain was processed histologically. The location and extent of all lesions were confirmed in Nissl-stained sections.

Experiment 2

Ferrets were rewired in the left hemisphere within one day after birth, and in adulthood received lesions of LGN and LP in the same hemisphere. They were water deprived overnight, and trained to initiate each trial by maintaining their head stationary and facing forward as in experiment 1 (Fig. 3a). Equiluminant grating and no-grating stimuli were presented on a monitor (Tektronix 620 with P31 phosphor) with a square screen that subtended 10° of visual angle and extended from 40 to 50° in the left or right monocular field. Onset of one of three stimuli (grating, no-grating or a central LED) was triggered when proper head position was maintained for 300–500 ms. Stimuli were displayed for 500 ms, or were terminated earlier if the head moved and the infrared LED–photodiode beam was re-established. Ferrets received a reward at a spout adjacent to the monitor if a grating was presented, or at a central spout if a central light was presented. They were not rewarded for the no-grating condition, which was used to determine chance performance. We deliberately overtrained ferrets to develop a bias toward the central spout by presenting twice as many central light stimuli as grating or no-grating stimuli. Thus ferrets approached the monitor spout only when they saw a grating, and this behaviour could be compared statistically against performance in the no-grating condition.

In a separate task, acuity in the central (binocular) field was tested using only two stimuli: grating and no-grating. These stimuli were presented simultaneously on a centrally placed monitor in split-screen fashion. Ferrets were rewarded at the spout only on the side of the grating. Equal numbers of trials with left or right gratings were presented. In both tasks, the spatial frequency of sine-wave gratings (drifting at 1 Hz) was varied in octave steps. Contrast was maintained below monitor saturation and increased in octave steps of a control signal; maximum (L_{max}) and minimum (L_{min}) light intensity values were measured directly from the screen and actual grating contrast calculated as $(L_{max}-L_{min})/(L_{max}+L_{min})$.

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Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex *in vivo*

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Do changes in neuronal structure underlie cortical plasticity[1,2]? Here we used time-lapse two-photon microscopy[3,4] of pyramidal neurons in layer 2/3 of developing rat barrel cortex[5] to image the structural dynamics of dendritic spines and filopodia. We found that these protrusions were highly motile: spines and filopodia appeared, disappeared or changed shape over tens of minutes. To test whether sensory experience drives this motility we trimmed whiskers one to three days before imaging. Sensory deprivation markedly (~40%) reduced protrusive motility in deprived regions of the barrel cortex during a critical period around postnatal days (P)11–13, but had no effect in younger (P8–10) or older (P14–16) animals. Unexpectedly, whisker trimming did not change the density, length or shape of spines and filopodia. However, sensory deprivation during the critical period degraded the tuning of layer 2/3 receptive fields. Thus sensory experience drives structural plasticity in dendrites, which may underlie the reorganization of neural circuits.

More than 90% of excitatory axodendritic synapses in the mammalian cortex occur on small dendritic appendages called spines[6]. During development the emergence of spiny dendrites is preceded by a period when dendrites are studded with filopodia[7], relatively long (up to 10 μm) actin-rich protrusions which often make several synapses[8]. In the cerebral cortex the presence of dendritic filopodia coincides with an intense burst of synaptogenesis[9,10]. In cultures of developing hippocampus, dendritic filopodia are highly motile[11,12] and initiate contact with axons, leading to synapse formation[13]. Mature spines, on the other hand, are structurally relatively stable[13,14]. These observations support the idea that filopodia actively search for presynaptic partners and might in fact be precursors of mature spines[11–13]. Filopodia[12] and spines[15,16] sprout in response to strong synaptic stimuli that produce long-term potentiation, suggesting that such motility may be an important aspect of activity-dependent synaptic plasticity.

To explore the role of protrusive motility in the plasticity of neural circuits, experiments in the intact brain are necessary. For this purpose we imaged the dynamics of spines and filopodia in the developing primary vibrissa (barrel) cortex[5] of the rat. Modulating the sensory input to the barrel cortex by trimming whiskers changes the response properties of cortical neurons[2,17,18]. This allowed us to examine the effects of the rat's sensory experience on the structure and dynamics of spiny protrusions as a substrate of experience-dependent plasticity.

To label neurons for fluorescence imaging we injected a suspension of Sindbis virus containing the gene for enhanced green fluorescent protein (SIN–EGFP)[12,19] along the medial edge of the barrel cortex. Typically tens to hundreds of neurons, distributed over all cortical layers and over one to three barrels, were infected by the virus. One to two days after infection, EGFP had reached concentrations sufficiently high for imaging. Visualized with a custom-made two-photon laser scanning microscope (2PLSM), infected barrel cortex neurons showed bright EGFP fluorescence that was distributed homogeneously throughout their dendritic and axonal arborizations (Fig. 1a). High-resolution structure could be seen down to the level of dendritic spines and presynaptic terminals (Fig. 1b).

We examined the structures of layer 2/3 pyramidal neurons, as they are within easy reach of our imaging technique (imaging depth < 600 μm)[20] and also because in the adult they show the most pronounced form of experience-dependent plasticity[17,18]. In addition we focused our observations on postnatal day 8 to 18, a period that spans the development of much of the intracortical circuitry[9].

To characterize the dynamics of spiny protrusions *in vivo* we performed time-lapse imaging in anaesthetized rats (Fig. 2A, B). Small image stacks containing a particular dendritic branch were typically collected at 10-min intervals for at least 90 min (Fig. 2Aa, Ba). Motility was quantified by measuring the length of individual protrusions as a function of time (Fig. 2Ab, Bb). Sampling intervals of 10 min were sufficient to capture most protrusive movements (Fig. 2Ab); occasional experiments with more frequent data collection (1 min) showed little additional structural change over shorter timescales (Fig. 2Ab). To describe the structural dynamics for an individual protrusion we use the average change of length per sampling interval (micrometres per 10 min).

Time-lapse imaging revealed that spines and filopodia are highly motile *in vivo* (Fig. 2A–C). They changed length and shape over tens of minutes. In addition to length and shape changes, a significant proportion (2–20%) of protrusions appeared or disappeared during the observation period (Fig. 2A, B). The largest motility was observed in the youngest animals probed (P8–12; Fig. 2C). At these ages dendritic structure was characterized by numerous irregular spiny protrusions, with a relatively large fraction of long filopodia (length >4.5 μm; ~6–7%; Fig. 2D). With increasing age protrusive motility decreased (Fig. 2C). In older animals (P16–19) dendritic structure was characterized by spines typical of mature dendrites (Fig. 2Ba), with relatively few long filopodia (1–2%; Fig. 2D).

Previous *in vitro* studies have established that the protrusive motility of spines and filopodia indicates a rapid rearrangement of synaptic connections and neural circuits[12,13,15,16]. To investigate the role of sensory experience in this plasticity we examined the effects of sensory deprivation on the structure and dynamics of spiny protrusions. Deprivation was induced 1–3 days before imaging by trimming all large whiskers (columns 1–4, α–δ) on one side of the rat's muzzle, contralateral to the injection site. We compared dendritic structure and dynamics under three conditions (Fig. 3a). To assess the effects of deprivation, imaging was performed in control (left, 'in, control') or deprived (middle, 'in, deprived') barrel cortex. To test whether the effects of deprivation are specific to the deprived input, imaging was performed in the trunk, back and head regions of somatosensory cortex[21], <1 mm medial to deprived barrel cortex (right, 'out, deprived'). The locations of

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