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Acceleration of visually cued conditioned fear through the auditory pathway

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Defensive responses elicited by sensory experiences are critical for survival. Mice acquire a conditioned fear response rapidly to an auditory cue but slowly to a visual cue, a difference in learned behavior that is likely to be mediated by direct projections to the lateral amygdala from the auditory thalamus but mainly indirect ones from the visual thalamus. Here, we show that acquisition of visually cued conditioned fear is accelerated in 'rewired' mice that have retinal projections routed to the auditory thalamus. Visual stimuli induce expression of the immediate early gene *Fos* (also known as *c-fos*) in the auditory thalamus and the lateral amygdala in rewired mice, similar to the way auditory stimuli do in control mice. Thus, the rewired auditory pathway conveys visual information and mediates rapid activity-dependent plasticity in central structures that influence learned behavior.

The contribution of sensory inputs to the function of a central target has traditionally been investigated through deprivation or lesion experiments in which central sensory structures are temporarily or permanently deprived of their inputs[1–8]. In these experiments, the role of inputs is inferred from the resulting loss of function. An alternative experimental approach to investigating the functional contribution of inputs involves gain of function, in which an existing structure is driven by novel inputs and acquires a new or enhanced role. Experiments in rewired ferrets, in which retinal projections are directed to the medial geniculate nucleus (MGN) at birth, indicate that the auditory thalamocortical pathway conveys visual information that is interpreted as vision[9]. Retinal projections to the MGN activate the auditory thalamus and subsequently the auditory cortex, causing these structures to acquire, through development, a new function that can be used to detect visual stimuli. It remains unknown, however, whether or not novel inputs to existing pathways and structures can also induce, in adulthood, functional plasticity capable of mediating learned behavior. We have now examined, in rewired mice, whether visual activation of the auditory thalamus and subsequently the lateral amygdala (LA) is able to mediate a learned response—rapid acquisition of cued fear conditioning behavior—that is characteristic of the auditory pathway but not of the visual pathway.

The pairing of a discrete auditory cue with a mild foot shock in mice quickly induces conditioned fear after as few as one tone-shock pairing[10,11], and subsequent cue presentations in a novel context elicit a defensive freezing response. In contrast, a discrete visual cue is less effective, requiring many more light-shock pairings to elicit a defensive response to the light alone[12]. This behavioral difference may reflect differences in the underlying fear conditioning pathways associated with the two sensory modalities. Dense direct connections from the medial division of the MGN and the posterior intralaminar nucleus of the thalamus to the LA are thought to be crucial for auditory-cued conditioned fear responses (**Fig. 1**)[11,13,14]. An indirect thalamo-cortical-amygdala pathway from the ventral and medial divisions of the MGN via auditory cortex to perirhinal cortex also conveys information to the amygdala[11,15]. However, lesions of the auditory cortex do not affect the magnitude or duration of freezing responses after fear conditioning[16]. In addition, single unit recordings suggest that this cortical pathway shows slower learning-induced changes than the direct thalamo-amygdala pathway, and hence is unlikely to be the principal auditory-cued conditioned fear pathway[17,18]. In contrast to the direct auditory pathway from the MGN to the LA, visual inputs primarily reach the amygdala through indirect pathways (ref. 19, but see ref. 14). Visually cued conditioned fear is thought to be mediated by projections from the lateral geniculate nucleus (LGN) to visual cortical areas V1/V2 to visual association area TE2/perirhinal cortex (PR) to the amygdala (**Fig. 1**), or by projections from the lateral posterior nucleus of the thalamus (LP) to V2/TE2/PR to the amygdala[19].

Retinal ganglion cell axons can be induced to innervate the MGN in mice[20], ferrets[21–25] and hamsters[26–29] by surgical deafferentation of the MGN in neonatal animals (see Methods, below, and **Supplementary Fig. 1** online). Upstream connections of the MGN to central structures, including the primary auditory cortex (A1)[21] and the amygdala (**Fig. 1**), remain intact. A1 in rewired ferrets develops visual response features similar to primary visual cortex (V1)[21–23,25] such as direction- and orientation-selective responses[23], an orderly retinotopic map[22] and a map of orientation-selective cells[25]. Furthermore, rewired ferrets (with the normal retino-LGN projections removed) are able to respond to visual stimuli and resolve gratings of varying spatial frequency with the retino-MGN-A1 pathway[9]. Rewired mice provide an opportunity to investigate the role of novel sensory inputs in the rapid acquisition of a conditioned behavior. If the pathway from the retina to the MGN to the LA conveys

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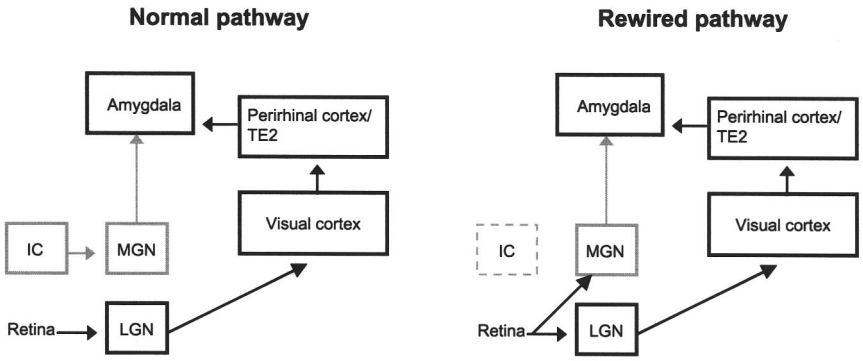

Figure 1 Simplified fear conditioning pathways in normal and rewired mice. Left, schematic of the principal visual (black) and auditory (gray) cued conditioned fear pathways in normal mice; right, schematic of the rewired visual (black) cued conditioned pathway. The IC (shown as a dotted box) was lesioned bilaterally in neonatal mice to induce retinal projections to the MGN. IC, inferior colliculus; LGN, lateral geniculate nucleus; MGN, medial geniculate nucleus.

visual information that is capable of mediating cue-induced fear, then it will elicit the same rapid freezing responses with a visual cue that are normally observed only with an auditory cue. Here, we show that acquisition of visually cued conditioned fear is indeed accelerated in rewired mice. In addition, visual stimuli induce expression of c-fos in the auditory thalamus and the LA in rewired mice, similar to the way auditory stimuli do in control mice.

RESULTS

Visually cued fear is accelerated in rewired mice

Adult sham lesion and rewired mice underwent three sessions of fear conditioning with either a visual or an auditory cue, and behavioral testing after each session. The experimental groups (**Table 1**) were: sham lesion (control) mice conditioned by light (Sham (L)), rewired mice conditioned by light (Rewired (L)), sham lesion mice conditioned by tone (Sham (T)) and rewired mice conditioned by tone (Rewired (T)). The cued testing behavior of each group is shown after either one (**Fig. 2a**,**b**) or three fear conditioning sessions (**Fig. 2c**,**d**). Consistent with previous studies, after one session of fear conditioning, light-conditioned sham lesion mice ($n = 15$) did not freeze significantly more during the cue presentation compared with the habituation period (**Fig. 2a**). Light-conditioned rewired mice, however, froze significantly more during the cue presentation after only one session of fear conditioning ($n = 15$, $P < 0.01$, paired t-test), as did tone-conditioned sham lesion and rewired mice ($n = 14$, $P < 0.001$ and $n = 14$, $P < 0.001$ respectively, paired t-tests).

Both the sham lesion and rewired groups of light-conditioned mice showed an initial decrease in freezing during the 30 s after the onset of the light cue (**Fig. 2b**,**d**), reflecting an initial orienting behavior towards the stimulus. The light stimulus was very different from the lighting they experienced in the home cage environment, and the novelty of this stimulus may have provoked the orienting behavior; however, the behavior did not persist beyond the initial 30 s after light onset.

After three sessions of fear conditioning, light-conditioned sham lesion mice froze significantly more during the cue presentation than during the habituation period ($n = 12$, $P < 0.05$, paired t-test; **Fig. 2c**), as did light-conditioned rewired mice and tone-conditioned sham lesion and rewired mice ($n = 12$ per group, $P < 0.05$, $P < 0.01$ and $P < 0.01$ respectively, paired t-tests). More generally, a three-way repeated-measures ANOVA showed a significant effect of lesion type ($F = 4.5$, $P < 0.05$), time (habituation versus cue; $F = 81.0$, $P < 0.001$) and session ($F = 6.5$, $P < 0.01$). There were also significant interactions between lesion type and time ($F = 25.2$, $P < 0.01$), lesion type, cue type and session ($F = 10.2$, $P < 0.01$) and lesion type, cue type and time ($F = 3.9$, $P = 0.05$). Separate two-way repeated-measures ANOVAs were run on the data collected after either one or three fear conditioning sessions. The ANOVA on the data collected after one fear conditioning session showed a significant effect of group ($F = 2.7$, $P = 0.05$) and time (habituation versus cue; $F = 87.1$, $P < 0.001$) as well as a significant interaction between group and time ($F = 26.9$, $P < 0.001$). The ANOVA run on the data collected after three fear conditioning sessions showed a significant effect of time (habituation versus cue; $F = 53.39$, $P < 0.001$), but no effect of group ($F = 1.29$, $P = 0.29$) and no interaction between group and time ($F = 0.57$, $P = 0.64$). The observed difference between groups was specific to cued conditioned fear responses; no significant differences were observed between groups for contextual conditioned fear responses (data not shown).

Table 1 Experimental groups and the number of mice in each group

Main group	Session 1 (c-fos)	Session 3
Sham (L)	15 (3)	12
Rewired (L)	15 (3)	12
Sham (T)	14 (2)	12
Rewired (T)	14 (2)	12
Total	58 (10)	48

Animals used for c-fos experiments are shown in brackets.

Freezing responses plateau after fear acquisition

After only one session of fear conditioning, freezing during cue presentation reached a plateau for the tone-conditioned mice and the light-conditioned rewired mice ($P > 0.5$, t-test, comparing sessions one and three for each group; **Fig. 3**). In contrast, there was a significant difference between the freezing behavior of light-conditioned sham lesion mice after one and three sessions of fear conditioning ($P < 0.05$, t-test). After one session of fear conditioning, the light-conditioned sham lesion mice froze significantly less during the cue presentation period than the other groups ($P < 0.05$, $P < 0.01$ and $P < 0.01$ respectively, t-tests). The amount of time the light-conditioned sham lesion group spent freezing during the cue presentation period rose steadily across sessions and achieved the level of the other groups after three sessions of fear conditioning (**Fig. 3**). After three sessions of fear conditioning, there was no difference between groups ($n = 12$ per group) in the amount of time spent freezing during the cue presentation period. A previous fear conditioning study in rats found that a tone evoked more conditioned freezing than a light, and that this effect was not overcome by overtraining[30]. Our data indicate that with increased conditioning sessions, light-conditioned rewired mice spend as much time freezing during the cue presentation period as tone-conditioned mice.

The freezing behavior of tone-conditioned rewired mice was similar to that of sham lesion mice, indicating that they have residual hearing.

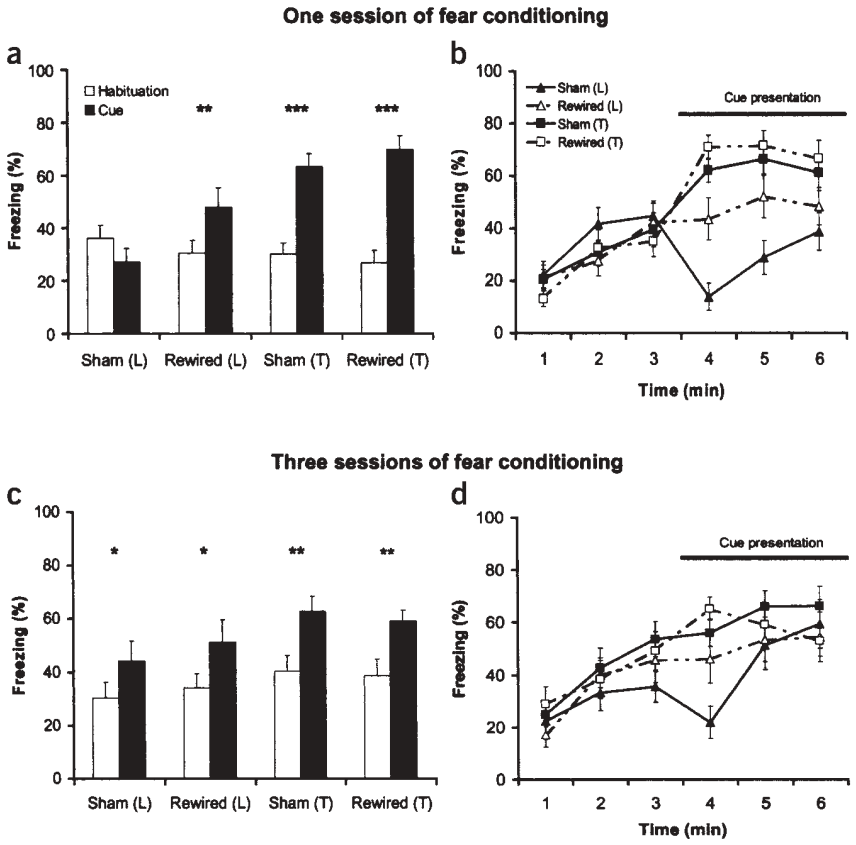

Figure 2 Cued testing behavior in normal and rewired mice. (**a**,**c**) The mean freezing per group during the habituation (white bar) and cue presentation (black bar) periods of the cued testing session after one or three sessions of fear conditioning, respectively (significant paired *t*-tests, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Error bars, s.e.m. L, light conditioned; T, tone conditioned. (**b**,**d**) The mean freezing per minute after one or three sessions of fear conditioning, respectively. Light conditioned sham (▲), rewired (△), tone conditioned sham (■) and rewired (□) groups are shown. The black line represents the duration of the cue presentation. Error bars, s.e.m.

This could be explained by partial sparing of the inferior colliculus (IC) during the neonatal ablation surgery. Retrograde labeling of IC remnants after tracer injections in the MGN is observed in rewired ferrets[31], and such projections can provide sufficient auditory input to the MGN to support relatively robust conditioned fear responses. An additional group of rewired mice ($n = 2$), with extensive IC lesions that extended into the superior colliculus (SC; see **Supplementary Table 1** online), did not show tone-conditioned fear responses but did show accelerated visually cued conditioned fear after only one session of fear conditioning. This indicates that the IC is necessary for tone-cued but not for visually cued fear responses.

c-fos expression correlates with fear responses

After one session of fear conditioning, c-fos immunohistochemistry was performed on brains harvested 30 min after behavioral testing to examine the pathways and structures that were activated. Sections through the amygdala, cortex and thalamus were stained for expression of the immediate early gene *Fos*, an indirect marker of neural activity (**Fig. 4a–c,e**). After one session of fear conditioning, both sham lesion ($n = 3$) and rewired light-conditioned mice ($n = 3$) had high c-fos expression in the basolateral nucleus of the amygdala, but the light-conditioned rewired mice had higher c-fos expression in the LA (**Fig. 4a**), as did tone-conditioned sham lesion mice ($n = 2$, **Fig. 4b**). The basolateral nucleus is believed to encode the emotional component of memories formed during fear conditioning[32], and thus its activation is expected for all groups. The background level of staining in the amygdala was normalized relative to a control region, the primary somatosensory cortex (S1), in the same animals (**Fig. 4c**). A quantitative comparison of the scaled number of c-fos-labeled cells in the LA across groups indicated that c-fos expression was significantly higher for all groups that showed cued fear responses after one session of conditioning than for the light-conditioned sham lesion group, which did not ($P < 0.01$, *t*-test; **Fig. 4d**). This is consistent with the hypothesis that activation of the LA is responsible for cued fear conditioning, and suggests that visual activation of the MGN-LA pathway mediates the rapid visually cued conditioned fear responses observed in rewired mice.

Expression patterns of c-fos in visual and auditory structures of the different groups of mice supported this hypothesis. In light-conditioned mice, one session of fear conditioning increased c-fos expression in the LGN and V1 of both sham lesion and rewired mice, as well as in the MGN of rewired mice, but not in the MGN of sham lesion mice (**Fig. 4e** and **Table 2**). In contrast, in tone-conditioned mice, there was relatively little c-fos expression in the LGN and V1, but increased c-fos expression within the MGN (**Fig. 4e** and **Table 2**). That is, the rapid acquisition of a fear response in tone-conditioned mice or in light-conditioned rewired mice is accompanied by activation of the MGN and LA. Lack of acquisition of the response after one conditioning session in light-conditioned sham lesion mice is accompanied by little activation of the MGN or LA. The LGN and V1 are activated by light in both sham lesion and

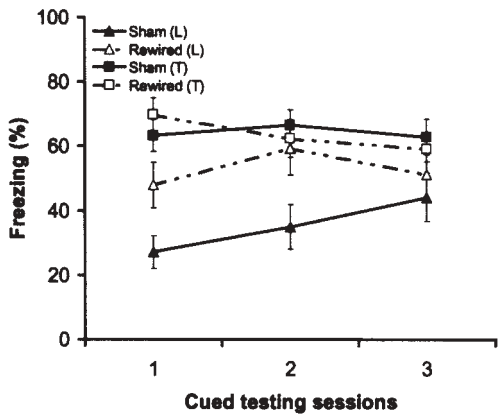

Figure 3 Cued freezing across sessions. The mean freezing for each group during the cue presentation period across cued testing sessions is shown for light-conditioned sham (▲) and rewired (△) and tone-conditioned sham (■) and rewired (□) groups. Error bars denote s.e.m.

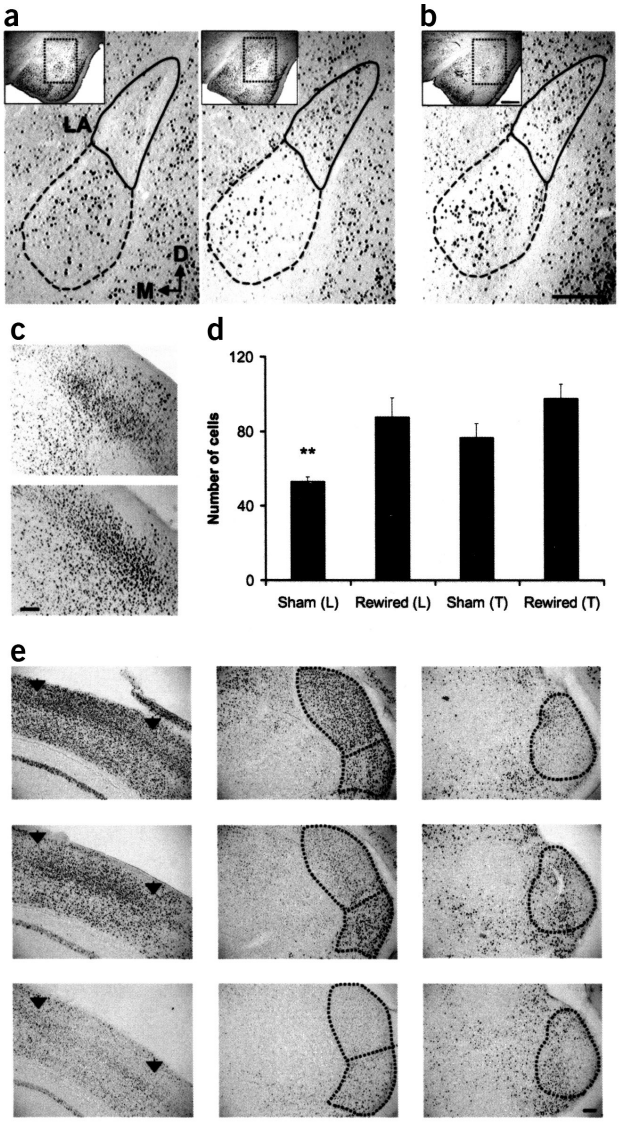

Figure 4 c-fos expression after one session of fear conditioning (**a**–**e**). (**a**) 50 μm coronal sections at ×10 magnification at the same level of the amygdala for a light conditioned sham lesion (left) and a light conditioned rewired mouse (right). The lateral amygdala (LA) is contained within the solid lined region. The basolateral nucleus is indicated by the dotted line. D, dorsal; M, medial. Insets, the same sections at ×4 magnification. (**b**) 50 μm coronal section through the amygdala of a tone conditioned sham lesion mouse at ×10 magnification. Inset, the same section at ×4 magnification. Scale bar, 0.5 mm; applies to both **a** and **b**. (**c**) 50 μm coronal sections through S1 in the same light conditioned sham lesion (top) and rewired mouse (bottom) shown in **a**. Scale bar, 0.1 mm. (**d**) The scaled mean number of c-fos-labeled cells per group; error bars, s.e.m. (** $P < 0.01$, *t*-test). L, light conditioned; T, tone conditioned. (**e**) 50 μm coronal sections through: left column, primary visual cortex (V1); middle column, the lateral geniculate nucleus (LGN); right column, the medial geniculate nucleus (MGN). Sections are taken from: top row, a light conditioned sham lesion mouse; middle row, a light conditioned rewired mouse; bottom row, a tone conditioned sham lesion mouse. The arrowheads in the left column delineate the extent of V1; the dotted lines in the middle column contain the LGN, including the dorsal LGN at the top and the ventral LGN below; dotted lines in the right column outline the MGN, including the dorsal, ventral and medial divisions. Scale bar at bottom right, 0.1 mm.

rewired mice, but this activation is unrelated to the rapid acquisition of a cued fear response.

Rewired pathway underlies accelerated visually cued fear

Because the visual pathway through the LGN to visual cortex is intact in rewired mice, the net visual drive to the amygdala might be higher in rewired mice than in sham lesion mice. To examine the possibility that the observed acceleration in visually cued fear in rewired mice is caused by an increase in visual projections to the amygdala rather than by specific involvement of the MGN-amygdala pathway, in a separate group of mice we enhanced retinal projections to the amygdala without engaging the MGN (see **Supplementary Note** and **Supplementary Table 1** online). Mice with lesions of only the SC have greater retinal innervation of LP without retinal innervation of the MGN ($n = 5$; see **Supplementary Fig. 2** online). Thus, the direct LP-amygdala pathway in these mice[14,19] provides enhanced visual drive to the amygdala compared with normal. However, the SC lesion mice did not show an acceleration in the rate of acquisition of visually cued conditioned fear ($P > 0.1$, *t*-test, comparing sham lesion mice, $n = 15$, and SC lesion mice, $n = 5$, after one session of fear conditioning). Therefore, simply an increase in visual drive to the amygdala cannot explain the observed acceleration in visually cued conditioned fear in rewired mice. In addition, these results suggest that the rewired retinal projections to the MGN, not neonatal superior collicular lesions *per se*, are necessary for the acceleration in visually cued conditioned fear responses.

DISCUSSION

Our results show a gain of behavioral function in rewired mice, which acquire an association between a visual stimulus and a fearful stimulus more rapidly than control mice. The finding that acquisition of a conditioned fear response to a visual cue is accelerated in rewired mice is consistent with the evidence that the MGN-amygdala pathway is activated by vision in these mice (although an indirect MGN-cortex-amygdala pathway may also influence the responses). In contrast, the normal visual pathway through visual thalamus to cortex is intact in rewired mice, is activated to a similar extent in rewired and sham lesion mice, and is unlikely to have a role in the observed acceleration in visually cued fear responses.

Cued fear and sensory activation of the amygdala

Appropriate sensory activation of the MGN-amygdala pathway may access targeted neurons within the LA that form the association between the conditioned stimulus (CS) and unconditioned stimulus (US). Notably, evoked field potentials in the LA are correlated with the presence of contingency information that identifies the CS as a danger signal, as well as with the extent to which conditioned animals make use of this information[33]. Specifically, there is an increase in the slope and amplitude of evoked field potentials in the LA in response to paired but not unpaired auditory CS-shock US information[33]. Certain patterns of sensory activity, when paired with a stimulus signaling danger, may be necessary to make the CS-US association, and even a visually driven signal with properties such as short latency and high frequency may be comparable enough to audition to elicit a defensive response. The observed acceleration in visually cued freezing in rewired mice indicates that visual driving of the MGN sufficiently influences the subsequent structures associated with conditioned fear. This implies that the responses do not necessarily require activation by auditory stimuli, instead perhaps allowing other relevant sensory stimulation of the MGN-amygdala pathway to elicit defensive freezing.

Table 2 c-fos expression in V1, LGN and MGN of the three groups of mice

	V1	LGN	MGN
Sham (L)	+++	++	–
Rewired (L)	+++	++	++
Sham/rewired (T)	+	–	++

–, +, ++, +++ represent increasing levels of c-fos expression as compared with the other groups.

Although the rate of acquisition of the defensive freezing response is comparable for vision and audition in the rewired group, there is also a notable difference. The relative duration of freezing elicited by the visual cue is lower than that elicited by the auditory cue after one session of fear conditioning (**Figs. 2** and **3**). This lower duration of freezing may reflect specific differences in amygdala drive, related, perhaps, to the spatiotemporal characteristics of activation by vision compared to audition.

Role of afferents and targets in learned behavior

The results of this study show that existing pathways can convey novel information to central structures, and that this information is capable of mediating learned behavior. Other studies have shown gain of behavioral function owing to alterations in postsynaptic target neurons, particularly in the hippocampus[34], but there are few model systems in which gain of function owing to changes in inputs has been examined. Experiments in rewired ferrets have suggested that visual inputs to auditory thalamus and cortex convey information that is interpreted as vision[9], although the quality of the evoked sensation remained unknown and the experiments required additional lesions to isolate the rewired pathway from the retina to the auditory cortex. Rewired mice learn a conditioned response faster than normal mice, by using novel sensory inputs (due to vision) to drive a pathway (from the MGN to the amygdala) that accesses existing networks and outputs (of the amygdala).

The role of afferents and target structures in the specification of behavioral function may differ in development and adulthood. In rewired ferrets, retinal inputs to the MGN instruct the perceptual role of the auditory thalamus and cortex during development, such that visual stimulation of these structures supports vision[9]. In rewired mice, retinal inputs to the MGN elicit a fear response to a conditioned visual stimulus similar to that of normal mice to a conditioned tone stimulus: that is, visual inputs to the auditory thalamus elicit a target-mediated behavior. The precise mechanism and locus of the rapid acquisition of visually cued fear in rewired mice remains unresolved. A parsimonious explanation is that retinal inputs activate the same MGN cells that normally project to the LA, and provide appropriate drive to rapidly induce conditioned fear. More generally, this implies that novel inputs to existing targets are likely to evoke target derived function when the learning takes place in adulthood, similar to phantom limb sensations[7]. Nonetheless, associative matching of afferents and targets in the adult brain, as shown here, can also have a role in the developing brain, as a means by which input activity shapes the function of target structures[35,36].

Our findings have relevance for the development of emotional responses and for their evolution. A current view is that emotions are closely related to the internal state of the organism and engage structures related to the representation and regulation of this state[37]. Imaging studies in humans suggest that the midbrain, insula and secondary somatosensory cortex are activated during the recall of fear memories[37] and that activity in the amygdala is increased during fear conditioning[38,39]. In addition, patients with amygdala damage have impaired recognition of fearful facial expressions[40] and deficits in fear conditioning[41]. The set of processing circuits that detect and respond to danger in the sensory environment reflect this link between fear, an organism's internal state, and external stimuli. For example, reciprocal connections between the amygdala and the processing regions of each sensory modality may facilitate the rapid detection and processing of danger in the external environment, whereas the outputs of the amygdala are thought to provide the neural basis for the physical response[11]. In this view, rapid acquisition of visually cued fear in rewired mice provides a powerful demonstration that visual inputs to the auditory pathway and the amygdala influence learned behavior, illustrating a mechanism that potentially enables diverse physical stimuli to elicit a common emotion.

METHODS

We used 58 adult SvEv/129 mice (14.6 ± 4.4 weeks old); 29 of these mice received neonatal brain lesions to direct retinal axons to the medial geniculate nucleus (MGN). The groups were balanced for age and gender. All experiments were performed under protocols approved by MIT's Institutional Animal Care and Use Committee and conformed to NIH guidelines.

Neonatal surgery. Mice born to timed-pregnant mothers bred in the animal colony were anesthetized 1 d after birth by deep hypothermia and divided into two equal groups: a rewired and a sham lesion group. Under microscopic guidance, the rewired group received bilateral lesions of the IC, inducing retinal axons to innervate the deafferented MGN. On completion of the surgery, the skin was sutured and the pups were revived under a heat lamp. The sham lesion group underwent the same surgical procedure without ablation. In additional control experiments (see **Supplementary Note**), a SC lesion group ($n = 5$) underwent the same surgical procedure but received bilateral lesions of the SC only, and an extensive IC lesion group ($n = 2$) received bilateral lesions of the IC that extended into the posterior edge of the SC. All mice were returned to their mothers and reared to adulthood before being used in further experiments.

Fear conditioning. Conditioned fear experiments present an emotionally neutral stimulus (CS) paired with an aversive stimulus (US), after which subsequent exposure to the CS alone elicits a defensive response, such as freezing, that reflects an internal state of fear. This response is expressed to both the CS (cued fear) and the context in which the CS-US pairings occurred (contextual fear). As adults, the mice underwent three consecutive sessions of fear conditioning and behavioral testing. The sessions occurred in two chambers, a 30 × 26 × 30 cm rectangular Plexiglas conditioning chamber housed inside a sound-attenuated chamber and a 35 × 35 × 35 × 40 cm triangular Plexiglas cued testing chamber scented with vanilla extract. The day before the first fear conditioning session, the mouse freely explored the cued testing chamber for 6 min. The next day the mouse freely explored the conditioning chamber for 10 min before experiencing three cue-shock pairings (30 s interstimulus interval). The CS was either auditory (75 dB noise) or visual (four diodes flickering at 1 Hz). The visual cue was presented on two panels located on either side of the chamber for 5 s, coterminating with a mild foot shock (2 s, 0.3 mA) that served as the US. After each fear conditioning session the mouse underwent two behavioral testing sessions. During contextual testing (24 h after conditioning) the mouse was placed in the conditioning chamber and allowed to freely explore for 5 min without incident. During the cued testing session (48 h after conditioning) the mouse was placed in the cued testing chamber, and allowed 3 min of free exploration (habituation) followed by a 3-min presentation of the CS. A ceiling mounted camera recorded the amount of time the mouse spent freezing. Freezing during the cue presentation period was compared with that during the habituation period, and was taken as an indication of cued fear.

Control of the stimuli, data acquisition and analysis were performed automatically using Image FZ software, which is a modified version of the NIH Image program. Images were captured (one frame per second) and for each pair of successive frames, the area (in pixels) the mouse moved was meas-

ured. If this amount was equal to or above threshold (ten pixels), then the mouse was considered 'moving'; otherwise the mouse was considered 'freezing'[42]. Freezing that lasted less than 2 s was not included in our analysis. Statistical analysis was conducted using SPSS and StatView. The data were analyzed by two-tailed paired *t*-tests, two-tailed *t*-tests, or a three-way repeated-measures ANOVA.

c-fos expression. At 30 min after the first cued testing session, mice in each group (see text) were killed (Nembutal, 80 mg/kg) and perfused transcardially with saline followed by fixatives. Their brains were cryoprotected, coronally sectioned (40–50 μm) and immunohistochemically stained for c-fos. Quantification of c-fos-labeled cells in the LA was performed using a three-dimensional counting method[43], which uses stereology to determine the number of cells contained within a tissue volume, in four sections through the LA for each mouse. The number of c-fos-labeled LA cells in each section was scaled by a normalizing factor (label in S1 in each mouse relative to the mean S1 label in all sections in all mice; $n = 40$ sections). To examine activation of brain pathways by visual stimuli, two sections through the MGN, LGN and V1 were quantified for each mouse after one session of fear conditioning. The label was scaled by the normalizing factor above. The relative label observed in these regions is represented as follows: –, the least amount of c-fos labeling, comparable to background; +, moderate c-fos labeling; ++, high c-fos labeling; +++, the most c-fos labeling observed relative to all sections through that region (**Fig. 4e** and **Table 2**).

Retinothalamic projections. Retinothalamic projections were labeled in sham lesion ($n = 6$), rewired ($n = 7$) and bilateral SC lesion mice ($n = 5$), through intraocular injections of the anatomical tracer cholera toxin subunit B (1%) made after the last behavioral testing session. After a survival period of 24–48 h, the mice were killed (Nembutal, 80 mg/kg) and perfused transcardially with saline followed by fixatives. The brains were cryoprotected, coronally sectioned (40–50 μm) and processed using immunohistochemistry.

Note: Supplementary information is available on the Nature Neuroscience website.

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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