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Manganese-Enhanced MRI of the Mouse Auditory Pathway

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Functional mapping of the lateral lemniscus and the superior olivary complex as part of the auditory pathway was accomplished for the first time in mice in vivo using manganese-enhanced MRI (2.35T, 3D FLASH, 117 μm isotropic resolution). These and other auditory centers in the brainstem presented with pronounced signal enhancements after systemic administration of manganese chloride when animals were exposed to acoustic stimuli for 48 hr, but not when kept in a quiet environment. The results indicate an activation-dependent accumulation of manganese in the neural circuit composed of the cochlear nucleus, the superior olivary complex, the lateral lemniscus, and the inferior colliculus. The marked enhancement of the lateral lemniscus suggests that the stimulus-related accumulation of manganese reflects not only a regional uptake from extracellular fluid but also a concurrent delivery by axonal transport within the auditory system. Magn Reson Med 60:210–212, 2008. © 2008 Wiley-Liss, Inc.

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Manganese-enhanced MRI is increasingly used for functional characterization of a wide variety of neural systems in animal brain (for a review, see Ref. (1)). More specifically, a recent study mapped sound-evoked activity in the inferior colliculus of young mice during development (2). Because the inferior colliculus is only one (early) gate to the pathways processing auditory information in the mammal brain, the purpose of this study was to examine whether evoked activity can be observed in other structures of the auditory system, for example, by exposing the animals to intense acoustic stimuli for a long period of time. According to anatomical data, the auditory pathway is composed of the cochlea, cochlear nucleus, superior olivary complex, lateral lemniscus, inferior colliculus, medial geniculate body, and auditory cortex (3). A schematic outline of the connectivity of these centers is sketched in Fig. 1 for input from the cochlea of one side (4). Hence, the primary aim was a more extensive functional mapping and morphological delineation of the auditory system in adult mice in vivo with use of local MRI signal enhancements that are due to the activation-dependent accumulation of manganese (Mn^{2+}) ions.

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MATERIALS AND METHODS

Animals

Eight female mice (NMRI, 8–12 weeks old, 28–38 g) were studied in accordance with German animal protection laws after approval by the responsible governmental authority. Animals received manganese chloride (0.5 mmol/kg body weight; Sigma, Taufkirchen, Germany) dissolved in distilled water via subcutaneous injection. They were returned to a chamber with unlimited access to food and water. For a period of 48 hr before MRI, four mice were kept in a quiet environment, while four animals were exposed to acoustic stimuli generated by an ultrasound wave generator (Conrad Electronic, Germany). The ultrasonic stimuli involved noise bursts within a frequency range of 25–65 kHz and an acoustic pressure of about 110 dB SPL.

The relatively long duration of the acoustic stimulation used here was chosen because pilot experiments demonstrated a less pronounced signal enhancement in all auditory areas for shorter exposures (e.g., 24 hr). On the other hand, because long-term exposure to high-pressure noise might induce seizures, animals were tightly monitored during the stimulation period. No obvious abnormal behavior was seen in either group.

MRI

For MRI the mice were anesthetized and placed in the magnet as previously described (5,6). All measurements were carried out at 2.35T using a 4.7/400 mm magnet (Magnex Scientific, Abingdon, UK) equipped with 200 mT m^{-1} gradients (Bruker Biospin MRI GmbH, Ettlingen, Germany). RF excitation and signal reception was accomplished with use of a Helmholtz coil (inner diameter 100 mm) and an elliptical surface coil (inner diameter 20 × 14 mm), respectively. T_1-weighted 3D MRI datasets (RF-spoiled 3D FLASH, TR/TE = 17/7.6 ms, flip angle 25°, field of view 15 × 30 × 30 mm^3, matrix 128 × 256 × 256, 5 averages, measuring time 93 min) were acquired at 117 μm isotropic resolution.

For quantitative evaluations the signal-to-noise ratio (SNR, defined as the mean MRI signal intensity of a brain region divided by the standard deviation of the noise) was determined using software supplied by the manufacturer. The analysis followed a previously described strategy (5,6). Briefly, anatomical cross-sections were obtained by multiplanar reconstructions from the original 3D MRI datasets. In close accordance with resolved anatomical structures, standardized regions of interest (ROIs) were selected in the inferior colliculus, the lateral lemniscus, the superior olivary complex, the cochlear nucleus, and the cerebral cortex re-

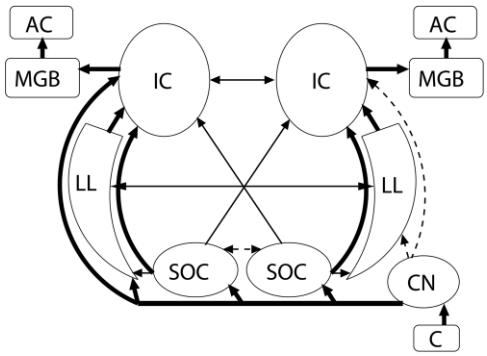

FIG. 1. Schematic outline of the auditory system. The ascending pathway from the cochlea of one side to the main auditory centers comprises major (thick lines), moderate (thin), and minor projections (dashed). AC = auditory cortex, C = cochlea, CN = cochlear nucleus, IC = inferior colliculus, LL = lateral lemniscus, MGB = medial geniculate body, SOC = superior olivary complex.

mote from the auditory cortex. The caudal surface of the inferior colliculus was chosen as a reference plane following the histological convention for the mouse auditory system (3).

RESULTS

As reported previously (1,2,5), systemic administration of manganese led to a generalized signal enhancement in the brain of all mice. While there were similar enhancements for the two groups (quiet vs. stimulation) in the forebrain, the brainstem revealed marked differences. First of all, strong differential—that is, stimulus-related—enhancements were observed in the ventral part of the inferior colliculus, which were most prominent about 1.5 mm lateral from the midline, as shown in Fig. 2a. Below the inferior colliculus, as shown in coronal sections in Fig. 2b, even more pronounced stimulus-related enhancements were observed as a curved band. In agreement with histology (3), this enhanced structure represents the lateral lemniscus. The band can be tracked further ventrally until it ends in another enhanced structure located on the ventral surface of the pons, as shown in Fig. 2c. It represents the superior olivary complex (3). Lastly, in Fig. 2d the cochlear nucleus can be more clearly delineated in stimulated animals in comparison to mice kept in quiet conditions. These qualitative observations in all animals of either group were confirmed by a quantitative evaluation of selected ROIs summarized in Table 1. While there is no difference in SNR between groups in nonauditory cerebral cortex, the SNR of various auditory structures in stimulated mice is statistically significantly higher than in animals kept in a quiet environment.

DISCUSSION

The present work demonstrates morphological and functional characteristics of the lateral lemniscus and the superior olivary complex in the mouse brain in vivo in an unprecedented way. These structures are important components of the auditory pathway, which hitherto have only been described by histology, that is, in brain slices postmortem (3). In electrophysiology, these structures have been much less intensively studied than the inferior colliculus, which is located just beneath the skull and therefore offers convenient experimental access. Manganese-enhanced MRI now offers an alternative in vivo approach which is expected to add complementary information to histology and electrophysiology when performing functional investigations of mouse models with disorders or mutations affecting the auditory pathway.

In agreement with manganese-enhanced MRI of developing mice (2), the present results demonstrate an MRI signal enhancement of the inferior colliculus due to the local accumulation of Mn^{2+} ions. In addition, however, the data reveal a stimulus-related enhancement in the lateral lemniscus and the superior olivary complex. Apart from the use of adult animals, this new finding may be ascribed to the long exposure to intense acoustic stimuli. When the cochlea is stimulated, neurons in the cochlear nucleus are excited via the spiral ganglion cells. Subsequently, the excitation expands to the neurons in the connected supe-

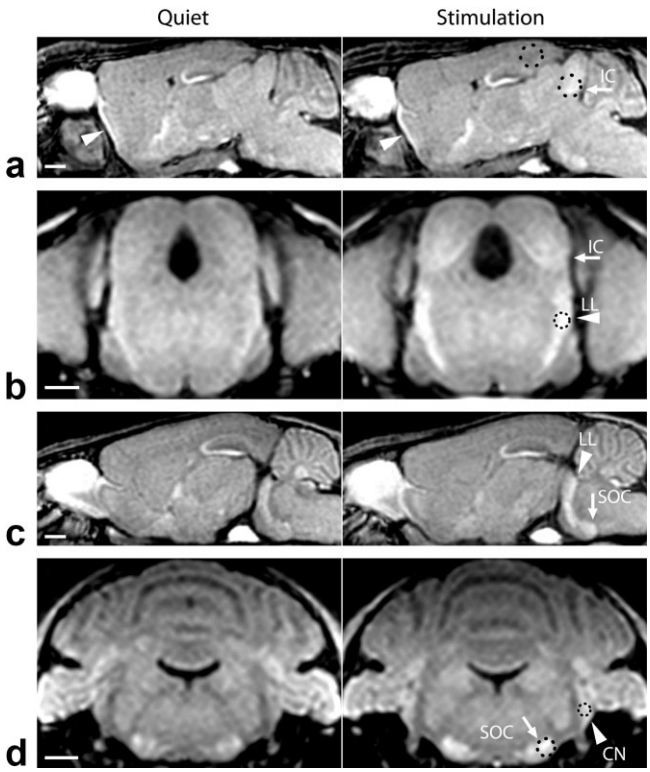

FIG. 2. (Right) Stimulus-related Mn^{2+}-induced MRI signal enhancements in mice exposed to acoustic stimulation in comparison to (left) animals kept in a quiet environment (data from eight different animals, scale bars = 1 mm). The regions-of-interest used for quantitative evaluations are indicated by dotted circles. **a:** Left-hemispheric parasagittal sections 1.5 mm from the midline identify the ventral part of the inferior colliculus (IC, arrow) and demonstrate otherwise similar enhancements of the lateral olfactory tract (arrowhead) and olfactory bulb. **b:** Coronal sections 2.0 mm rostral from the caudal surface of the IC identify the IC (arrow) and lateral lemniscus (LL, arrowhead). **c:** Oblique sagittal sections cutting through the LL identify the LL (arrowhead) and its connection to the superior olivary complex (SOC, arrow) in a curved band. **d:** Coronal sections cutting through the SOC identify the SOC (arrow) and the cochlear nucleus (CN, arrowhead).

Table 1
SNR in Different Brain Regions of Mice Kept in a Quiet Environment (Quiet) and Mice Exposed to Acoustic Stimulation (Stimulation)

	Region	SNR	
		Quiet (n = 4)	Stimulation (n = 4)
Nonauditory cerebral cortex	L	31.5 ± 2.5	31.3 ± 2.7
	R	31.6 ± 2.9	30.9 ± 4.0
Interior colliculus	L	32.9 ± 2.1	38.1 ± 1.8**
	R	33.1 ± 1.8	38.8 ± 2.5*
Lateral lemniscus	L	34.8 ± 1.4	40.9 ± 1.1**
	R	34.7 ± 2.0	41.4 ± 1.4**
Superior olivary complex	L	36.6 ± 0.6	42.0 ± 2.2**
	R	37.4 ± 2.3	41.8 ± 2.6*
Cochlear nucleus	L	34.2 ± 1.1	41.0 ± 0.5**
	R	34.6 ± 1.3	40.5 ± 1.2**

SNR values are given as mean values ± SD averaged across animals; L = left, R = right; *P < 0.05, **P < 0.01 (unpaired t-test vs. Quiet).

rior olivary complex, lateral lemniscus, and inferior colliculus (4). Together, these four auditory centers form the auditory pathway as sketched in Fig. 1. In such continually excited neurons, Mn^{2+} ions are taken up from the extracellular fluid through opened membrane channels much more frequently than in spontaneously active neurons, e.g., neurons in the auditory pathway of mice kept in a quiet chamber. After a period of 48 hr, this functional difference results in higher Mn^{2+} concentrations in activated regions. Other potential factors that may contribute to the spatially heterogeneous signal enhancements summarized in Table 1 (or more precisely, the underlying Mn^{2+} accumulation) include regional differences in cell density and blood supply (1,5). However, both mechanisms are less likely to generate the differential signal enhancements between the two groups of animals that are observed in response to acoustic stimulation versus quiet conditions.

Besides the regional uptake from surrounding extracellular fluid, Mn^{2+} ions may also be delivered by axonal transport from remote tissue (5,7). In mice, the distance between the four auditory centers within one hemisphere as well as to their contralateral counterparts is less than 10 mm. Because Mn^{2+} ions can be transported in axons as fast as 2.8 mm/hr (5), it is highly probable that, after 48 hr, at least part of the MRI signal enhancement stems from manganese delivered from connected sites of the auditory pathway. In the lateral lemniscus, this type of accumulation may be even dominant because neurons in this histologically unique structure are intermingled among axonal fibers from the other auditory centers (3).

Taken together, the observed activation-dependent enhancements reflect a 48-hr integration of manganese uptake and delivery in the neural circuit formed by neurons in the four auditory centers and their axonal connections. This interpretation is in line with the observation of concurrent enhancements of the olfactory bulb and its major efferent axonal fibers in the same animals and further agrees with previous reports (5). It must be noted, however, that higher auditory centers such as the medial geniculate body or the auditory cortex could not be distinguished from surrounding tissue, although they are connected with the inferior colliculus. This may be explained by an inhomogeneous distribution of Mn^{2+} ions within the extracellular fluid possibly leading to an insufficient supply of Mn^{2+} ions to these centers. In fact, it may be even assumed that after 48 hr a certain amount of Mn^{2+} ions is indeed axonally transported from the inferior colliculus to the medial geniculate body or auditory cortex. However, their MRI visibility which requires the establishment of a local concentration gradient (corresponding to image contrast) is hampered or completely masked by the unspecific accumulation of Mn^{2+} ions in spontaneously active cells in surrounding tissue. Whether contributions from unspecific Mn^{2+} accumulations may be reduced by lower Mn^{2+} dosages or other routes of administration (7–11) remains to be seen in future studies. This also applies to the use of shorter waiting periods and the putative influence of stimulus features such as duration, frequency, and intensity of the acoustic exposure.

CONCLUSIONS

In summary, the present work demonstrates the stimulus-related accumulation of manganese in the neural circuit formed by four auditory centers in the brainstem of adult mice in vivo. In particular, the accumulation and corresponding MRI signal enhancement in the lateral lemniscus suggests a pronounced manganese delivery by axonal transport that complements the activation-dependent uptake from the local extracellular fluid. The present approach by manganese-enhanced MRI emerges as a new tool for studies of the function of the auditory pathway.

REFERENCES

1. Koretsky AP, Silva AC. Manganese-enhanced magnetic resonance imaging (MEMRI). NMR Biomed 2004;17:527–531.
2. Yu X, Wadghiri YZ, Sanes DH, Turnbull DH. In vivo auditory brain mapping in mice with Mn-enhanced MRI. Nat Neurosci 2005;8:961–968.
3. Willard TH, Ryugo DK. Anatomy of the central auditory system. In: Willott JT, editor. The auditory psychobiology of the mouse. Springfield, IL: Charles C. Thomas; 1983. p 201–303.
4. Helfert RH, Snead CR, Altschuler RA. The ascending auditory pathways. In: Altschuler RA, editor. Neurobiology of hearing: the central nervous system. New York: Raven Press; 1991. p 1–25.
5. Watanabe T, Frahm J, Michaelis T. Functional mapping of neural pathways in rodent brain in vivo using manganese-enhanced three-dimensional magnetic resonance imaging. NMR Biomed 2004;17:554–568.
6. Watanabe T, Radulovic J, Spiess J, Natt O, Boretius S, Frahm J, Michaelis T. In vivo 3D MRI staining of the mouse hippocampal system using intracerebral injection of $MnCl_2$. Neuroimage 2004;22:860–867.
7. Pautler RG, Koretsky AP. Tracing odor-induced activation in the olfactory bulbs of mice using manganese-enhanced magnetic resonance imaging. Neuroimage 2002;16:441–448.
8. Kuo YT, Herlihy AH, So PW, Bhakoo KK, Bell JD. In vivo measurements of T1 relaxation times in mouse brain associated with different modes of systemic administration of manganese chloride. J Magn Reson Imaging 2005;21:334–339.
9. Kuo YT, Herlihy AH, So PW, Bell JD. Manganese-enhanced magnetic resonance imaging (MEMRI) without compromise of the blood-brain barrier detects hypothalamic neuronal activity in vivo. NMR Biomed 2006;19:1028–1034.
10. Chaudhri OB, Parkinson JR, Kuo YT, Druce MR, Herlihy AH, Bell JD, Dhillo WS, Stanley SA, Ghatei MA, Bloom SR. Differential hypothalamic neuronal activation following peripheral injection of GLP-1 and oxyntomodulin in mice detected by manganese-enhanced magnetic resonance imaging. Biochem Biophys Res Commun 2006;350:298–306.
11. Yu X, Zou J, Babb JS, Johnson G, Sanes DH, Turnbull DH. Statistical mapping of sound evoked activity in the mouse auditory midbrain using Mn-enhanced MRI. Neuroimage 2008;39:223–230.