Manganese-Enhanced MRI Reveals Structural and Functional Changes in the Cortex of Bassoon Mutant Mice

Manganese-enhanced magnetic resonance imaging (ME-MRI) was used to analyze the brain architecture in mice lacking the functional presynaptic active zone protein Bassoon. Anatomical characterization revealed a significant increase in the total brain volume in Bassoon mutants as compared with wild-type mice, which is mainly caused by changes in cortex and hippocampus volume. The measured enlargement in cortical volume coincides with an altered Mn²⁺ distribution within cortical layers as visualized by T₁-weighted magnetic resonance imaging. Two days after manganese application, the cortex of Bassoon mutant mice appeared more laminated in ME-MRI, with an enhanced accumulation of manganese in deep, central, and superficial cortical cell layers. Whereas morphologically the cortical lamination is not affected by the absence of a functional Bassoon, an altered basal activation pattern was found in the cortex of the mutant mice both by metabolic labeling with [¹⁸⁶⁴⁺]-2-deoxyglucose and histochemical detection of the potassium analogue thallium uptake. Consequently, the results indicate that the absence of the functional presynaptic protein Bassoon causes disturbance in the formation of normal basal cortical activation patterns and thereby in the functional cortical architecture. Furthermore, this study shows that ME-MRI can become a valuable tool for a structural characterization of genetically modified mice.

Keywords: 2-deoxyglucose, hippocampus, thallium autoradiography, volumetry

Introduction

Neurotransmitter release is a strictly controlled membrane-trafficking process at specific sites of the presynaptic plasma membrane, the so-called active zones. At the ultrastructural level, the active zone appears as an electron-dense region beneath the presynaptic membrane, which biochemically corresponds to a network of cytoskeletal and associated proteins: the cytomatrix at the active zone (CAZ) (Dresbach and others 2001). Components of the CAZ are thought to mediate and control docking, priming, and fusion of synaptic vesicles with the presynaptic membrane. Although a number of components, including Bassoon, Piccolo/Acornin, Munc13-1, Rab3-interacting molecule (RIM1), CAZ-associated structural protein (CAST), and ELKS-Rab6-interacting protein-CAST (ERC) have already been identified (Ziv and Garner 2004), the complete composition and function of the CAZ are still under investigation. To study the role of Bassoon in the organization of various steps of the synaptic vesicle cycle and in the assembly of the presynaptic apparatus, a mouse mutant lacking the central region of the protein was generated (Altrock and others 2003). Bassoon mutant mice are characterized by a reduction in the normal synaptic transmission. Accordingly, a subset of excitatory synapses was found to be ultrastructurally normal but functionally inactive. Nonetheless, Bassoon mutant mice suffer from repeated rapidly generalizing seizures, indicating a general imbalance of excitatory and inhibitory neurotransmitter systems at the systemic level (Altrock and others 2003). The fact that a generalized hyperactivity occurs, although a subset of excitatory synapses is inactivated, rather points to a changed neuronal activation pattern than to a general decrease in the overall activity. To elucidate potential Bassoon-related alterations in the general basal activation pattern, we employed manganese-enhanced magnetic resonance imaging (ME-MRI) as a screening tool to compare Bassoon mutant mice with wild-type littermates.

In ME-MRI, signal intensities depend on the uptake and accumulation of the contrast agent manganese in neurons. Manganese ions can enter neurons through several ways: 1) via transferrin binding and transferrin-receptor-mediated internalization, 2) via divalent metal ion transporters, and 3) via L-type voltage-gated Ca²⁺ channels (Takeda 2003; Pautler 2004). Consequently, brain regions with a high basal neuronal activity should also be characterized by increased signal intensity in a T₁-weighted image. So far, ME-MRI has served as a valuable tool to visualize small morphological structures in the mouse or rat brain (Natt and others 2002; Aoki and others 2004), to detect activated brain areas after olfactory or acoustic stimulation (Pautler and Koretsky 2002; Yu and others 2005), and to trace functionally related brain areas in mice (Watanabe and others 2001, 2004; Pautler and others 2003), rats (Allegrini and Wiessner 2003; Leergaard and others 2003), or songbirds (Van der Linden and others 2002; Tindemans and others 2003). Here, we report that ME-MRI can also be employed to detect structural and functional differences in cortex of genetically modified mice.

Material and Methods

Animals

Mice lacking a functional Bassoon (Bsn⁺/Exi⁻/⁵) were generated as described previously (Altrock and others 2003) and identified by polymerase chain reaction; age-matched wild-type littermates were used as controls. All experiments were performed with 2-month-old mice.

Anatomical Magnetic Resonance Imaging

Mice were anesthetized with 1.0-1.5% isoflurane (in 70:30 N₂O:O₂, v/v) and fixed using a head holder with bite bars to reduce motion artifacts. Magnetic resonance imaging (MRI) experiments were performed on a Bruker Biospec 47/20 scanner at 4.7 T (free bore of 20 cm) equipped with a BGA 12 (200 mT/m) gradient system. A 25-mm Litzcage small animal imaging system (DotyScientific Inc., Colombus, SC) was used for radiofrequency excitation and signal reception. Eight horizontal or sagittal T₂-weighted spin echo images were obtained simultaneously using a rapid acquisition relaxation enhanced (RARE) sequence (Hennig
and others 1986) with the following parameters: repetition time (TR) = 2000 ms, echo time (TE) = 15 ms, slice thickness = 800 μm, field of view (FOV) = 30 × 30 mm, matrix = 256 × 256, RARE factor 8, number of acquisitions = 8. The total scanning time was 9 min.

**Manganese-Enhanced Magnetic Resonance Imaging**

Two days before MRI measurement, animals were injected subcutaneously with an aqueous solution containing 1 μmol/g (body weight) MnCl₂ (e.g., 200 μl of a 100 mM MnCl₂ solution for a 20-g mouse, which is equivalent to 125 mg/kg). The used concentration is a compromise between manganese toxicity and the fact that the relaxation rates, which define the signal intensity differences, are proportional to effective local concentration. In recent ME-MRI experiments, concentrations up to 175 mg/kg were used with only minor and temporary side effects (Silva and others 2004). A 3-dimensional (3D) data set of T₁-weighted images was obtained using a modified driven equilibrium Fourier transform (MDEFT) pulse sequence with the following parameters: TR 21.18 ms, TE 4.00 ms, flip angle 15°, FOV 30 × 30 × 20 mm, matrix 256 × 256 × 64 (yielding a nominal in-plane resolution of 117 × 117 μm) and a nominal slice thickness of 312.5 μm, 10 averages; the total scanning time was 93 min.

**Thallium and Manganese Autometallography**

The potassium analogue thallium is taken up by electrically active neurons and is revealed in histological brain sections by autometallography. The method was performed as described previously (Goldschmidt and others 2004). Mice were injected intraperitoneally (i.p.) with 200 μl of a 2% aqueous solution of thallium acetate. After 15 min, mice were anesthetized with 100 μl of a mixture of 50 μl ketamine (50 mg/ml) and 50 μl xylazine. After onset of anesthesia, mice were perfused transcardially with sodium sulfide and glutaraldehyde. Two different solutions were used for perfusion, solution A consisting of 0.325% Na₂S in 100 mM phosphate buffer, pH 7.4, and solution B consisting of 0.16% Na₂S and 3% glutaraldehyde in 100 mM phosphate buffer at pH 7.4. Care was taken to perfuse only a strictly limited amount of solution A. A total volume of 8 ml of solution A was perfused during the initial 25 s followed without interrupting by solution B. Solution B was perfused for 10 min. Mice were perfused at a flow rate of 20 ml/min for the first 3 min and then reduced to 10 ml/min.

After perfusion, brains were removed and cryoprotected for 48 h in 30% sucrose in 100 mM phosphate buffer, pH 7.4. Subsequently, brains were frozen in 2-methylbutane (cooled to ~60 °C in liquid nitrogen) and stored at ~80 °C. Sections (25-μm thick) were cut on a Leica cryostat and mounted on Superfrost™ glass slides. Neighboring sections were treated for 5, 15, or 30 min with a 0.1% aqueous solution of the chelating agent sodium diethyldithiocarbamate, washed in H₂O, treated for 5, 15, or 30 min with a 0.1% aqueous solution of the chelating agent sodium diethyldithiocarbamate, washed in H₂O, treated with 0.1 N HCl for 30 min, washed again in H₂O, and finally air-dried. Sections were developed for 150 min in a solution of hydroquinone, citrate buffer, and silver lactate in gum arabic.

For the histological detection of manganese uptake, the same procedure was performed, except that the mice were injected subcutaneously with MnCl₂, as described for ME-MRI.

[^14]Cl⁻-2-Deoxyglucose Incorporation

Mice were injected i.p. with 20 μCi of [¹⁴C] -2-deoxyglucose (Amersham, Amersham, UK) in 200 μl sterile saline and placed in a small cage for 45 min. The animals were decapitated, and the brains were dissected out and frozen in liquid nitrogen and stored at ~80 °C. Frozen brains were serially sectioned at 40 μm in a cryostat at ~18 °C. Coronal section were mounted on coverslips, rapidly dried at 50 °C, and placed in contact with Kodak Ekta NB X-ray film for 2 weeks.

**Data Processing and Statistics**

Signal intensities and volumes were measured using the public domain Java-based image processing and analysis program ImageJ (http://rsb.info.nih.gov/ij/). For volumetric analysis, structures recognizable in an ME-MRI were segmented manually in each section. To avoid a possible biased segmentation, the mice were coded so that the genotype was unknown during the volumetric analysis. Volumes obtained by 2 individuals were found to be within 3% of each other. Data are expressed as mean ± standard deviation, and statistical significance of differences between controls and Bsn.ΔEx4/5 mice was evaluated using the Student’s t-test for independent samples. Differences of P < 0.05 were considered significant. For delineation of manganese distribution within the cortex, cortical layers were first straightened by an ImageJ-based algorithm (Kocsis and others 1991), and then average signal intensities in each layer were measured. Signal intensities in each cortical layer were expressed as ratio to the mean signal intensity of the putamen (set to 100%). This structure was used because no clear difference in the manganese concentration between wild-type and Bassoon mutant mice was found by manganese autometallography (Mn-AMG).

**Results**

An anatomical overview of the brain of 2-month-old Bassoon mutant mice without manganese enhancement obtained by T₁-weighted MRI revealed no distinctive features, such as localized anomalous signal intensities, obvious distortions of certain structures, or ventricle enlargements (Fig. 1). To determine the total brain volume as well as the volume of various brain structures, a T₁-weighted 3D MDEFT imaging sequence was applied. To increase the contrast, a solution containing 1 μmol/g (body weight) MnCl₂ was injected subcutaneously 2 days before the measurement. With its good resolution, the MDEFT protocol allows for the detection and segmentation of a variety of brain structures (Fig. 2). In Table 1, the total brain volume and the volume of different brain structures are summarized.

The absence of functional Bassoon causes a significant increase in total brain volume from 455.9 ± 20.7 mm³ in wild-type mice to 515.8 ± 20.4 mm³ in Bsn.ΔEx4/5 mice (+13.1%, n = 7, P < 0.0005). Segmentation and calculation of the volume of different structures revealed that the increase in total brain volume is mainly caused by an enlarged cortical volume, which increases by approximately 36 mm³ (+24.2%, P < 0.001), and thereby accounts for about 61% of the observed average increase in total brain volume. Pursuant to the enlarge cortex volume, the cortex thickness in Bassoon mutant mice is increased. The thickness of the somatosensory cortex in Bassoon mutant mice is 1.23 ± 0.06 mm and thereby significantly thicker than the somatosensory cortex of control mice (1.11 ± 0.08 mm; P < 0.01). In addition to an increase in cortical volume, a highly significant increase in hippocampal volume (+31.1%, P < 0.001) was observed in Bassoon mutant mice. In contrast, no significant differences in the volume of the cerebellum, brain stem, olfactory bulb, or thalamus were detectable (Table 1).

In the present study, manganese was used for 2 reasons: 1) to enhance the contrast in T₁-weighted images for an easier segmentation and 2) to gain information about the putative basal neuronal activation pattern. Whereas the overall incorporation of manganese did not differ considerably between control and Bassoon mutant mice, the distribution within the cortex was clearly altered (Fig. 3). Both in horizontal and coronal sections, the cortex of the mutants appeared more structured than that of wild types. Consequently, different cortical layers became easily distinguishable. An increased concentration of manganese was found in deep, central, and superficial cortical layer (Figs 3 and 4). In addition, the pирiform as well as the agranular insular cortex became also recognizable in cortical sections of Bassoon mutant mice (Fig. 3).

To identify in greater detail the cortical layers that correspond to the observed signal enhancement, we performed an Mn-AMG after ME-MRI. In contrast to ME-MRI, Mn-AMG visualizes only manganese that precipitates in the presence of sodium sulfide, so that especially tightly protein-bound manganese was
not stained. Comparable with our ME-MRI findings, we also detected in Bassoon mutant mice a clearly higher labeling within deep cortical layers, that is, cortical layers V and VI. However, in contrast to ME-MRI, a defined labeling of layer IV cells was only observed in control mice (Fig. 5). Interestingly, this staining occurred mainly in somatosensory areas, such as primary auditory, visual, and barrel field cortex. Based on these results, we conclude that the signal intensity changes within the cortex of Bassoon mutant mice observed by ME-MRI require a considerable increase in total manganese concentration and that the appearance of central and superficial layers in ME-MRI depends very likely on the increased presence of tightly protein-bound manganese.

Next we checked if the observed differences in manganese accumulation in cortical layers correlate with an altered basal neuronal activation pattern or are the result of a disturbed cortical architecture. A Nissl staining was used to visualize the general cortex structure. As discernable in Figure 6, the different cortical layers in Bassoon mutant mice are similarly recognizable as in wild-type mice, indicating that the gross anatomy of the cortex is not affected by the absence of a functional Bassoon. Although the general architecture of the cortex is indistinguishable between wild-type and Bassoon mutant mice, the cortex of Bassoon mutant mice appeared to be thicker in histological sections. This finding was confirmed by an independent MRI-based analysis (Fig. 4, Table 1), which circumvents any possible tissue shrinkage or distortion that occurs unpreventable during histological processing. Therefore, we conclude that the altered manganese distribution within the cortex of Bassoon mutant mice reflects not simply a changed cortical lamination but comes along with an enlargement of this structure.

To get information about the presence of a putative altered cortical activity in Bassoon mutant mice, we performed both a thallium autoradiography (Tl-AMG) (Goldschmidt and others 2004) and a [14C]-2-deoxyglucose autoradiography. Tl-AMG is based on the tight coupling of neuronal activity and potassium uptake or, in this case, on the uptake of the potassium analogue thallium. In contrast to ME-MRI where manganese (which is
used as a calcium analogue) uptake and accumulation were measured over a time span of 48 h, the thallium accumulation within cells was measured already 15 min after injection; therefore, it reflects on a more defined way the ongoing neuronal activity. The comparison of thallium distribution within the cortex of control and Bassoon mutant mice indicated the presence of an altered basal neuronal activation pattern in the cortex of the mutant mice (Fig. 7). Compared with wild-type mice the neuropil in cortical layer II/III is stronger stained in Bassoon mutant mice, suggesting a higher synaptic activity in this region under resting condition, whereas in wild-type mice, thallium uptake was found predominantly in layer IV cells, the thalamic input layer. Again, the staining of layer IV cells occurred predominantly in somatosensory areas and is in a good agreement with the observed results of the Mn-AMG. The 2nd way to map cortical activity was to measure the incorporation of $^{14}$C-2-deoxyglucose, which is based on the tight coupling of energy metabolism and neuronal activity (Sokoloff 1991). Although with this method no laminar-specific labeling was detectable, clear differences in cortical areas became obvious (Fig. 8). In control mice, a strong labeling was found especially in somatosensory areas, which is in accordance with the concomitant staining of layer IV cells by Mn-AMG and Tl-AMG. Whereas compared with control mice, the metabolic activity in Bassoon mutant mice was clearly reduced within somatosensory areas an increased activity was found in the cingulate cortex. Based on both activity maps (Tl-AMG and $^{14}$C-2-deoxyglucose incorporation), it seems obvious that manganese distribution within the cortex as seen by ME-MRI
Discussion

The principal findings of this study are as follows. 1) Absence of a functional presynaptic protein Bassoon in mice causes an enlarged brain size, which is mainly caused by an increased cortex and hippocampus volume. 2) The observed increase in cortex size in Bassoon mutant mice is paralleled by an altered manganese uptake and accumulation within cortical layers. 3) The increase in cortex thickness and altered manganese uptake are not associated with obvious changes in cortical lamination. 4) The basal neuronal activation pattern within the cortex of Bassoon mutant mice differs from wild-type mice. 5) Regional differences in the ongoing cortical activity are not reflected in ME-MRI.

Volumetric Characterization of the Mouse Brain

The noninvasive determination of total brain size and the volume of different structures is one major advantage for applying MRI methods for structural analysis of mutant mice. Volumetric measurements are performed under in vivo condition within a reasonable time span (≤2h); any potential artifacts during tissue processing, such as shrinkage or distortion, are completely avoided. Furthermore, compared with tedious histological sectioning, MRI allows us to perform

Figure 4. Manganese concentration within deep cortical layers is enhanced in Bassoon mutant mice. (A) To compare the manganese distribution within different cortical layers in wild-type and Bassoon mutant mice, the cortex was straightened using an ImageJ-based algorithm. For the alignment, the cranium (recognizable by the very low signal intensity) was used as the landmark. (B) The straightened cortex (insert) was then used to measure the average signal intensities in each layer. The arrow indicates the localization of the cranium, and the bars indicate the location of the cortex in each plot. (C) This graph shows the average signal intensities calculated from each 7 control (black line) and 7 Bassoon mutant mice (gray line) of the area highlighted by a beige box in (B). The signal intensities were referred to the mean signal intensity of the striatum (100%). Significantly enhanced signal intensity could be observed in deep cortical layers, indicating an enhanced accumulation of manganese especially in these layers (asterisk: *P < 0.05). The bars indicate again the localization of the cortex in control as shown in (B) (dark control mouse, gray Bassoon mutant mice). In the measured area, the determined cortex thickness is significantly higher in Bassoon mutant mice (1.23 ± 0.07 mm) compared with control mice (1.11 ± 0.08 mm, *P < 0.01).
longitudinal analysis in the same animals. Similar to a recent MRI study of perfused mice brains (Kovacevic and others 2004), the variation in total brain size within a group was less than 5% in our study. Hence, even small changes in brain size of less than 10% can be detected with high reliability between different groups. Furthermore, the use of manganese as a contrast agent facilitates the segmentation and allows for volumetric analysis of a number of brain structures, which are normally difficult to distinguish (Aoki and others 2004).

In our study, the observed increase in total brain volume in Bassoon mutants is mainly caused by enlarged cortical and hippocampal structures. Because the body weight of Bassoon mice is not significantly different from wild-type mice (Table 1), the enlarged size of the brain is not caused simply by an enlarged body size of the mutant mice. Although mouse strain-specific neuroanatomical differences are not unusual (Chen and others 2005), a clear change in cortex volume (+24%) as a result of the absence of a single protein is a rare phenotype and might indicate that the normal cortical development critically depends on the presence of Bassoon. To date, a significant increase in total brain size has only been observed in mice overexpressing the insulin-like growth factor I (IGF-I) (Carson and others 1993) in mceph/mceph mice, which is caused by a mutation in the Shaker-like voltage-gated potassium channel (Donahue and others 1996; Petersson and others 2003), and in Fgfr3-mutant mice (Inglis-Broadgate and others 2005). Whereas in IGF-I transgenic mice most brain structures appeared to be affected, the mceph/mceph and mutant Fgfr3 mice showed a limited increase in hippocampal and cortical volume similar to the Bassoon mutant mouse. Most interestingly, in mceph/mceph mice, this was also accompanied with recurrent seizure activities (Donahue and others 1996; Diez and others 2003). The reason for the cortex enlargement in Bassoon mutant mice is still unclear; conceivable is that a permanent increased activity facilitates the vascularization (Swain and others 2003) and by it the cortex volume. Alternatively, absence of a functional Bassoon may also affect the control of growth factor or neurotrophin release. It will be interesting to check in longitudinal studies at what age the main cortex enlargement occurs and to correlate this enlargement with changes in vascularization and growth factor/neurotrophin concentrations.
ME-MRI as a Screening Tool to Detect Differences in the Cortical Neuronal Activity Pattern of Genetically Modified Mice?

In this study, manganese was used to enhance the contrast in a $T_1$-weighted 3D-MRI and to obtain information about possible differences in the basal neuronal activation pattern in Bassoon mutant mice. Bassoon mutant mice are characterized by the appearance of spontaneous epileptic seizures (Altrock and others 2003), implying the existence of a disturbed activation pattern. Changes in neuronal activity can be visualized by functional MRI methods, which rely on the fact that within activated areas an enhanced neuronal activity correlates either with an increase in blood flow/volume or with the oxygenation level of hemoglobin. However, these methods are not suitable to detect putative differences in the basal neuronal activation pattern because they depend on the existence of regional relative signal intensity changes before and during specific stimulation.

Another MRI approach to visualize areas of different brain activity is the use of manganese ions, which enter neurons via voltage-gated calcium channels and subsequently accumulate over time in activated cells (Lin and Koretsky 1997; Pautler and Koretsky 2002). However, it should be emphasized that in addition to neuronal activity, manganese distribution within the brain also depends on a number of other factors, including cell density (both astrocytes and neurons), distance to the plexus choroidesus, and the presence or density of not yet identified metal transporters (Roth and Garrick 2003; Takeda 2003; Aoki and others 2004; Crossgrove and Yokel 2004; Wadghiri and others 2004). Furthermore, manganese is transported along axons and released together with glutamate into the synaptic cleft. Therefore, it can be taken up by the next neuron (Takeda 2003) and, thereupon, will be enriched over time in neuronal tracts that originate in highly manganese-labeled areas. Because we performed ME-MRI 48 h after injection, all these factors may have contributed to the observed manganese distribution.

The major difference in manganese accumulation was found within the cortex of Bassoon mutant mice. The cortex appeared more structured, so that different cortical layers are easily distinguishable (Figs 3 and 4). The highest concentration of manganese was found in deep cortical layers, and also a superficial and a central layer became clearly labeled (Fig. 4). In addition to the observed prominent lamination of the cortex, the piriform cortex became also detectable by ME-MRI in Bassoon mutants. This is of particular interest because the piriform cortex exhibits an unusually strong tendency to generate and propagate epileptic discharges, especially after reduced γ-aminobutyric acid (GABA)$_2$ receptor activity (Löschler and Ebert 1996; Rigas and Castro-Alamancos 2004; Schwabe and others 2004). Whether the piriform cortex is in fact the focus of rapidly generalizing seizures in Bassoon mutant mice cannot be answered unambiguously with our approach as we cannot distinguish whether the observed manganese accumulation within the piriform cortex is the result of recurrent epileptic seizures or if a temporarily increased activity in this area causes the observed generalized epileptic activity. Based on the two basal activity maps (TI-AMG and 2-deoxyglucose incorporation), the piriform cortex of Bassoon mutant mice is at least not characterized by a permanent increased neuronal activity.

Combining ME-MRI with Mn-AMG indicates that at least two different manganese pools are present, a sodium sulfide-precipitable and a sodium sulfide-nonprecipitable fraction. The biological meaning is still elusive and may point to different forms of how manganese accumulates within cells; in addition to a cytosolic localization, manganese is sequestered into the mitochondria (Wedler and others 1989; Hazell 2002) or the Golgi apparatus (Van Baalen and others 2004). Nevertheless, both forms of intracellular localized manganese contributes to the observed signal enhancement in ME-MRI of Bassoon mutant mice, that is, the sulfide-precipitable manganese in deep cortical layers as well as the sulfide-nonprecipitable manganese in superficial and central cortical layers; therefore, it is likely that also different cellular mechanisms are responsible for the observed phenotype in Bassoon mutant mice. For comparing ME-MRI with Mn-AMG, it should also be noted that although in ME-MRI signal intensity changes depend on variations in the concentration of manganese ions, these changes are not necessarily linear because, in contrast to Mn-AMG, different local environment may have also an effect on the signal strength. Because in this study we compared only identical structures between Bassoon mutant and wild-type mice, we could disregard this problem. Future studies measuring the effect of manganese on the spin-lattice relaxation time (so-called $T_1$-relaxation map) will give additional information about the concentration of manganese in each structure.

The exact reasons for the observed differences in manganese uptake and accumulation in Bassoon mice remain uncertain. So far, a correlation of manganese uptake and activity was found
only after a strong and/or long-lasting sensory stimulation (Yu and others 2005). Furthermore, a specific labeling of individual cortical areas by manganese has not been reported. In our study, we did not stimulate the mice with a specific sensory stimulus, but we compared mutant mice that are characterized by recurrent seizure activities with mice that do not suffer from these epileptic seizures. Comparable with studies using a sensory stimulus, we could also detect a different accumulation of manganese, namely, within the cortex. In contrast to studies that utilize external stimuli, we cannot modify the "stimulus" strength—meaning the frequency and duration of the epileptic seizures—because the etiology for these seizures is unknown and we have so far no reliable treatment to prevent the epileptic activities.

In general, there are two mechanisms for the observed altered manganese distribution conceivable, a changed structural anatomical organization of the cortex and an altered neuronal activation pattern. One indication for a structural difference in the cortex of Bassoon mutant mice is the size difference. But although the cortex is thicker in Bassoon mutant mice, the gross structural organization, that is, the laminar structure, is not obviously different. Future studies have to clarify if cell density, cell number, dendritic arborization, or the ratio between neurons and glia correlate with the observed changes in cortex size and subsequently manganese distribution. According to the Nissl staining (Fig. 6), an obvious increase in cell density in deep cortical layers was not observed in mutant mice.

The comparison of activity maps that are based on thallium uptake and $^{13}$C-2-deoxyglucose incorporation points to an altered basal activation pattern within the cortex of Bassoon mutant mice. Both methods revealed a decreased activity in somatosensory areas in the mutant mice. Thus, the thalamic input layer IV is less active in this region as visualized by the uptake of the potassium analogue thallium, which coincides well with a decreased metabolic activity in this entire cortex region, as seen by $^{13}$C-2-deoxyglucose incorporation. This observation is in good agreement with findings that both visual and auditory stimuli are not processed appropriately in Bassoon mutant mice (Dick and others 2003; Khimich and others 2005). However, these regional variations in cortical activity are not reflected by the manganese distribution as seen by ME-MRI, in which the manganese accumulation appeared more or less uniform across the cortex. The only clear match between cortical activities and manganese localization was found by Mn-AMG within layer IV cells in somatosensory areas of control mice. But the concentration of manganese in this area seems less uniform across the cortex. The only clear match between in which the manganese accumulation appeared more or less uniform across the cortex. The only clear match between

Based on these results, we conclude that ME-MRI, although potentially able to detect changes in neuronal activity (see layer IV cells), detects rather long-term consequences of changed cortical activity than the current activity pattern itself. This is not surprising because ME-MRI is performed 48 h after manganese application so that a number of temporarily and spatially varying cortical activation patterns including epileptic episodes may cancel out each other over time. This supports recent findings, demonstrating a clear activity-dependent ME-MRI signal increase within auditory brain stem nuclei but not in the corresponding auditory cortex (Yu and others 2005). Consequently, any interpretation of signal intensity changes in the cortex observed in ME-MRI with respect to neuronal activity may have to be scrutinized with an independent method.

In summary, the presented results demonstrate that ME-MRI can be applied as a new screening tool for "functional phenotyping" of genetically modified mice. First, the detection of a structure-specific volume change can point to the brain region that is primarily affected by the absence or malfunction of a particular protein. Second, present differences in the manganese distribution in one structure are promising indicators that more time-consuming histological studies as well as metabolic ($^{13}$C-2-deoxyglucose) or activity (TI-AMG) mapping are successful in finding structural differences and/or changed basal activation pattern. Such an evidence of changes in the basal activity of the brain may explain why complex brain functions, such as learning and memory formation, are affected in mutant mice. In this context, an ME-MRI-based functional characterization of mutant mice can facilitate our understanding of how the absence of a specific protein can cause disturbances in complex function of the brain as measured in behavioral experiments.

Notes

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References


