Age-Dependent Impairment of Somatosensory Response in the Amyloid Precursor Protein 23 Transgenic Mouse Model of Alzheimer's Disease

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Quantitative functional magnetic resonance imaging was applied to characterize brain function in amyloid precursor protein 23 (APP23) transgenic mice, which reproduce the neuropathological alterations associated with Alzheimer's disease. Electrical stimulation of the paw led to cerebral blood volume increases in the contralateral somatosensory cortex. In APP23 mice this hemodynamic response decreased with increasing age of the animal and with increasing stimulus amplitude as compared with wild-type animals. The age-dependent dysfunction in APP23 mice may be attributed in part to a compromised cerebrovascular reactivity. Quantitative functional brain mapping that uses standardized sensory inputs should allow for assessment of disease progression and therapy response (e.g., passive immunization against β -amyloid) in patients also.

Key words: Alzheimer's disease; amyloid precursor protein; APP; functional magnetic resonance imaging; fMRI; relative cerebral blood volume; transgenic mice; somatosensory cortex; peripheral stimulation; hindpaw; transcutaneous blood gas monitoring

Introduction

The proliferation of genetically engineered mouse strains, in particular models of human neuropathological disorders, has stimulated the development of methods to assay mouse neuronal function noninvasively. We previously have applied the cerebral blood volume–functional magnetic resonance imaging (CBV–fMRI) method by using a global pharmacological stimulation to a transgenic mouse model (amyloid precursor protein 23, APP23) that reproduces neuropathological changes associated with Alzheimer's disease (AD) (Sturchler-Pierrat et al., 1997). The observed reduced cerebral hemodynamic response in cortical and subcortical brain regions to the GABA_A antagonist bicuculline in aged transgenic mice when compared with age-matched wild-type littermates was attributable in part to a compromised cerebrovascular reactivity as revealed by the affected vasodilatory response to acetazolamide (Mueggler et al., 2002). APP23 transgenic mice express high levels of amyloid plaques with a core of β -amyloid (A β). These deposits start to appear at an age of 6 months predominantly in the neocortex and hippocampus. Their number and size increase with age; at 24 months they occupy substantial regions of the cortex (including the somatosensory area S1), hippocampus, and thalamus. In addition to these parenchymal amyloid plaques, significant A β accumulation also

is observed around cerebral vessels despite the fact that the APP transgene is expressed under the control of a neuronal promoter (Calhoun et al., 1999; Winkler et al., 2001). This cerebral amyloid angiopathy (CAA) and associated pathologies (e.g., microhemorrhages) in APP23 mice resemble pathomorphological alterations in AD patients (Probst et al., 1991; Staufenbiel et al., 1997). Cognitive performance of APP23 mice declines with age, suggesting a link to the pathomorphological and pathophysiological changes that have been described (Kelly et al., 2003).

fMRI probes secondary changes associated with neuronal activation such as regional changes in CBV (Belliveau et al., 1990), cerebral blood flow (CBF) (Detre et al., 1992; Kwong et al., 1992; Williams et al., 1993), and blood oxygenation (BOLD: blood oxygenation level-dependent contrast mechanism) (Ogawa et al., 1990) and has emerged as a powerful tool for investigating functional organization in the mammalian brain (Ogawa et al., 1998). Intravascular paramagnetic contrast agents that maintain a steady-state blood concentration permit the measurements of relative CBV values with greatly improved temporal resolution relative to the bolus tracking technique (Belliveau et al., 1991). This CBV–fMRI method has been applied successfully in rat studies that use various sensory stimulation paradigms such as electrical stimulation of the forepaw (Kennan et al., 1998; Mandeville et al., 1998; van Bruggen et al., 1998; Mandeville and Marota, 1999; Marota et al., 1999; Reese et al., 2000; Sauter et al., 2002). Sensory forepaw stimulation leads to distinct activation in the appropriate contralateral somatosensory cortex representing the forepaw.

The purpose of the present study was to assess the functional response in distinct areas in the somatosensory cortex of transgenic APP23 mice at different ages by using the CBV–fMRI method. The hypothesis was that the AD-like pathology would

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provoke functional deficits that could be assessed quantitatively by using a standardized sensory stimulation paradigm and fMRI readouts. This might constitute a sensitive tool to phenotype functionally the transgenic models of AD pathology and monitor the response to therapy in preclinical trials (e.g., after passive immunization against β -amyloid peptide) (Pfeifer et al., 2002). Critical factors in fMRI are sensitivity because of the high demands of spatial and temporal resolution and physiological control of the animals, because variation in blood gases may affect the observed fMRI signal. To minimize confounding effects because of variable physiological parameters, we have applied a recently developed study protocol that allows for mechanical ventilation of mice and continuous monitoring of their blood gases during an fMRI experiment (Mueggler et al., 2001).

Materials and Methods

Animals. The generation of APP23 mice expressing human mutated APP₇₅₁ (Swedish mutation) has been described in detail (Sturchler-Pierrat et al., 1997). FMRI studies have been performed by using APP23 mice and control littermates at the ages of 5.7 ± 0.8 months (5.8 ± 0.7 for controls), 13.1 ± 0.8 months (12.9 ± 0.5), and 24.8 ± 1 months (24.9 ± 1). The weight of the animals ranged from 27 to 44 gm for wild-type and 24 to 38 gm for the APP23 mice. For fMRI feasibility studies Hanlbm/NMRI mice (22–36 gm) were used.

Animal preparation. All animal experiments were performed in strict adherence to the Swiss Law for Animal Protection. Animals were anesthetized with an initial dose of 2% isoflurane in an air/oxygen (2:1) mixture, endotracheally intubated with a tube made from polyethylene (PE; inner diameter/outer diameter, 0.4/0.8 mm), and actively ventilated by using a ventilator for small animals (KTR 3, Alfes Electronics, Biel-Benken, Switzerland). Respiration rate was 100–120 breaths/min with an inspiration/expiration ratio of 1:4. For the subsequent intravenous infusions the tail vein was cannulated with a 30-gauge needle (Microlance 3, 0.3×13). The animals were positioned in the magnet by using a cradle made from Plexiglas. Isoflurane levels were reduced to 1.1%, and the animals were paralyzed with 10 mg/kg, i.v., gallamine triethiodide (Alldrich, Milwaukee, WI) in saline (3 mg/ml). Body temperature was maintained at $36.5 \pm 1^\circ\text{C}$ by using warm air, regulated by a rectal temperature probe (DM 852, Ellab, Copenhagen, Denmark). Blood carbon dioxide values were monitored during the experiment with a transcutaneous monitoring device (TCM3, Radiometer, Copenhagen, Denmark) via an experimental protocol described previously (Mueggler et al., 2001). For somatosensory stimulation, pairs of small needle electrodes were implanted subcutaneously in each hindpaw. The distance between the electrodes was 6 mm.

Magnetic resonance imaging. Experiments were performed on a Biospec system equipped with a PharmaScan 70/16 magnet (Bruker Medical System, Ettlingen, Germany) operating at 7 T. The RF probe was a bird-cage resonator of 28 mm inner diameter. A single coronal slice, including the somatosensory cortices, was recorded in the fMRI studies with the use of a fast spin-echo sequence (RARE: rapid acquisition with relaxation enhancement) (Hennig et al., 1986) with an effective echo time of 80 msec (repetition time/echo time, 1135/6.7 msec; RARE factor, 32). For some of the feasibility studies a horizontal slice (-2.94 inferior relative to the Bregma) was recorded as well. The field-of-view was $\text{FOV} = 25.6 \times 25.6 \text{ mm}^2$, the matrix dimension 128^2 , and the slice thickness 1 mm. With four averages the acquisition time amounted to 21 sec per image.

Functional MRI. At 20 min after the intravenous administration of 50 mg/kg Endorem (ferumoxides, 11.2 mg Fe/ml; Laboratoire Guerbet, Roissy, France) the fMRI experiment was initiated. Electrical stimulation was performed by using constant current pulses with 0.5 msec pulse length at a frequency of 1.5 Hz. In the feasibility studies the paradigm was composed of a resting period of 105 sec (5 images) followed by a stimulation period of 105 sec. This basic module was repeated three times with progressive current strengths of 0.5, 1, and 2 mA.

Image analysis. At 20 min after bolus administration the plasma concentration of Endorem has reached a steady state (measured plasma

half-life in mice, 3.5 hr). Hence the amount of tracer in cerebral voxels, which can be calculated as:

$$-\ln\{\bar{S}_0/S_{\text{pre}}\},$$

where S_{pre} and $\bar{S}_0$ denote the signal intensity before and after administration of the contrast agent, directly reflects relative CBV values, CBV_{rel} (Belliveau et al., 1990; Boxermann et al., 1995). Activation-induced changes of CBV_{rel} in percentage of prestimulation values ($\Delta\text{CBV}_{\%}$) therefore can be computed according to:

$$\Delta\text{CBV}_{\%}(t) = \frac{\Delta\text{CBV}_{\text{rel}}(t)}{\text{CBV}_{\text{rel}}} \cdot 100 = \frac{\ln\{S(t)/\bar{S}_0\}}{\ln\{\bar{S}_0/S_{\text{pre}}\}} \cdot 100,$$

with $S(t)$ signal intensity at time t (e.g., during electrical stimulation). To depict the activated area for quantitative analysis of fMRI, statistical activation maps were calculated on the basis of a correlation of the T_2 -weighted time series with the given stimulation design on a pixel-by-pixel basis. A correlation coefficient of $r = 0.3$ was used as a threshold value, and the minimum size of the region-of-interest (ROI) was set to 10 pixels. For each animal $\Delta\text{CBV}_{\%}$ values were calculated in the resulting stimulated area on the contralateral side to the stimulated hindpaw and in two user-defined ROIs (ipsilateral somatosensory cortex and a control ROI). Data analysis was performed by using in-house-developed imaging analysis software (Biomap v3.1). Data are given as the mean $\pm$ SEM. Multiple comparisons were evaluated by ANOVA. Probability values of <0.05 were considered statistically significant.

Results

Localization of activated brain area

Before the experiments in APP23 transgenic mice, feasibility studies examining functional activation during electrical stimulation of the hindpaw were performed in normal mice. Electrical stimulation of the right hindpaw, using the repetitive block design with increasing current amplitudes (0.5, 1, 2 mA), led to statistically significant signal changes in the contralateral somatosensory cortex (Fig. 1a). The activation map, displayed as a colored overlay on a high-resolution anatomical image, identifies ROIs of >10 pixels with a correlation coefficient of $r > 0.3$. The activated region in the primary somatosensory cortex (R5) is consistent with the known topographic representation of the murine hindlimb region (S1HL). Activation of this cortical area in response to electrical hindpaw stimulation was observed in all of the mice that were studied ($n = 6$). Minor fMRI signal changes also have been observed at the level of the hippocampus.

Quantitative analysis of activation-induced CBV changes

For quantitative analyses of the fMRI response three ROIs have been defined (Fig. 1b): (1) the activated ROI comprising the S1HL region contralateral to the stimulated hindpaw as obtained from the statistical map (Fig. 1a), (2) the equivalent topographical location on the ipsilateral side, and (3) a ROI comprising ipsilateral hippocampal structures used as a signal reference. In the contralateral S1HL area transient signal attenuations of 4 and 6% have been observed, depending on the amplitude of electrical current applied, which translate into corresponding increases in cortical CBV values (Fig. 1c). For each stimulation period the CBV increased within the first images after onset, peaked at ~ 60 sec, and declined at the end of stimulation period. Maximal $\Delta\text{CBV}_{\%}$ amplitudes for the individual periods were 11 ± 1 , 13.6 ± 2 , and $17.7 \pm 2.4\%$ for electrical current strengths of 0.5, 1, and 2 mA, respectively, i.e., CBV changes in the activated S1HL areas increased with increasing current amplitude. A significant correlation between maximal $\Delta\text{CBV}_{\%}$ values and current strength was observed ($r = 0.64$; $p = 0.004$).

No activation was observed in the S1HL region ipsilateral to

Figure 1. *a*, Statistically derived ROIs with a correlation coefficient threshold of 0.3 and a minimum cluster size of 10 pixels, superimposed on a horizontal high-resolution image. Intracerebral ROI (R5) is confined to cortical representation of the S1 region, and minor activations (R6, R7) also are observed at the level of hippocampus (approximately -3 mm posterior relative to Bregma). *b*, ROIs used for quantitative signal analysis in normal mice overlaid on a high-resolution image: the activated area (ROI) comprising the S1HL region *contralateral* to the stimulated hindpaw as obtained from the statistical analysis, the corresponding ROI on the *ipsilateral* side, and a third area comprising ipsilateral hippocampal structures serving as a signal reference (control ROI). *c*, Temporal profiles of $\Delta\text{CBV}\%$ for the three ROIs defined in *b*. $\Delta\text{CBV}\%$ responses for the different stimulation periods (indicated by bars) increased with progressive current amplitude. In-between stimulation blocks (off-periods) $\Delta\text{CBV}\%$ reached values of the order of 2–4% over baseline. Data are given as the mean $\pm$ SEM.

the stimulated paw as well as in the reference ROI. During the course of the experiment the mean PtcCO_2 value changed from 33.2 ± 2 to 36.5 ± 0.7 mmHg ($n = 6$). There was no statistically significant difference between PtcCO_2 values during stimulation and resting periods.

Impaired functional response in aged APP23 mice

For fMRI studies in transgenic and wild-type animals a coronal image orientation (center position, 0.94 mm posterior relative to Bregma) including the somatosensory cortex has been selected. Pixels displaying significant changes in signal intensity after electrical stimulation of the right hindpaw as derived from statistical data analysis ($r > 0.3$; pixel size, >10) are confined to the contralateral S1HL region for both control (Fig. 2*a*) and APP23 mice (Fig. 2*b*), respectively. Figure 3 shows the temporal profile of CBV_{rel} changes in S1HL for 6-, 13-, and 25-month-old control and APP23 animals. Analysis of maximal amplitudes for 6-month-old control littermates ($n = 7$) revealed ΔCBV values for the individual periods of 8.4 ± 2 , 11.5 ± 2 and $17.6 \pm 2\%$ of baseline values for current strengths of 0.5, 1, and 2 mA, respectively (Fig. 3*a*). The corresponding values for 13-month-old ($n = 6$; Fig. 3*b*) and 25-month-old control animals ($n = 4$; Fig. 3*c*) were 6.7 ± 1 , 10.7 ± 1 , $15.2 \pm 2\%$ and 7.6 ± 2 , 12.6 ± 2 , $14.6 \pm 2\%$, respectively. There was no significant dependence of the fMRI response on the age of the wild-type animals.

In young (6 months; $n = 8$) and matured (13 months; $n = 7$) APP23 mice $\Delta\text{CBV}\%$ values were identical within error limits to those of age-matched controls for current amplitudes of 0.5 and 1 mA. At 2 mA stimulation the CBV response in transgenic mice was slightly weaker as compared with wild-type animals, yet these decreases were not statistically significant (Fig. 3*a,b*).

In aged (25 months, $n = 8$) APP23 mice the CBV did not increase with increasing stimulation current, with maximal

Figure 2. Statistically derived ROIs (correlation coefficient threshold of 0.3 and a minimum cluster size of 10 voxels) overlaid on coronal spin-echo images (0.96 mm posterior relative to the Bregma) for a 25-month-old control (*a*) and an age-matched APP23 mouse (*b*). The ROIs are consistent with the known topographic location of the murine hindlimb region within the primary somatosensory cortex S1.

$\Delta\text{CBV}\%$ values of 7.9 ± 1 , 8.0 ± 1 , and $6.7 \pm 1\%$ for electrical current strengths of 0.5, 1, and 2 mA, respectively (Fig. 3*c*). The on-line monitoring of transcutaneously measured PtcCO_2 values in 25-month-old animals (Fig. 3*d*) revealed a slight increase in PtcCO_2 of the order of 3.5 mmHg for both groups. No significant differences in PtcCO_2 between 25-month-old control (39 ± 3 mmHg) and APP23 mice (38.4 ± 2 mmHg) or between stimulation and resting periods have been detected during the experiments. Similarly, no significant differences were found between control and APP23 at 6 months of age (35.7 ± 3 and 35.5 ± 2 mmHg) and 13 months of age (29 ± 3 and 30 ± 3 mmHg).

The CBV response of APP23 mice, expressed in relation to the wild-type response (Fig. 4), depends on both the age of the animals ($p = 0.35$, $r = 0.21$; $p = 0.005$, $r = 0.58$; and $p = 0.001$, $r = 0.64$ for 0.5, 1, and 2 mA, respectively) and on the amplitude of the stimulation current ($p = 0.73$, $r = 0.07$; $p = 0.37$, $r = 0.2$; and $p < 0.0001$, $r = 0.68$ for ages of 6, 13, and 25 months, respectively). Individual comparison of maximal $\Delta\text{CBV}\%$ amplitudes revealed significant differences for the 2 mA current strength between control and APP23 mice at 25 months of age ($p < 0.029$) and between 6- and 25-month-old APP23 mice ($p < 0.003$; ANOVA, Tukey's test).

Discussion

In control mice unilateral electrical stimulation of the hindpaw led to CBV increases in the contralateral somatosensory cortical area S1HL. Quantitative assessment of the fMRI signals revealed that this CBV response correlated with the current amplitude that was applied. This observation is in line with results that used Doppler flowmetry in normal rats showing that the local CBF response to forepaw stimulation is related to the intensity of the stimulus (Silva et al., 1999). A similar correlation has been found

Figure 3. Time course of $\Delta\text{CBV}\%$ calculated from ROIs (defined in Fig. 2) for 6-month-old (a), 13-month-old (b), and 25-month-old (c) APP23 mice and age-matched control littermates. The bars at the bottom of the panels represent the stimulation design (repetitive stimulation periods of 105 sec with progressive current amplitudes of 0.5, 1, and 2 mA). Data are given as the mean $\pm$ SEM. All control littermates show an increased $\Delta\text{CBV}\%$ response with increasing current amplitude. An analogous response pattern is observed in 6- and 13-month-old APP23 mice, with a tendentially reduced response for the highest current amplitude (2 mA). In 25-month-old APP23 mice, however, no CBV dependence on current amplitude is found; in fact, values at 2 mA were decreased as compared with lower current strengths. d, On-line monitoring of blood carbon dioxide of 25-month-old control and age-matched APP23 mice. Averaged PtcCO_2 values (in mmHg) during the fMRI experiments for control (39.1 ± 3) and APP23 mice (38.4 ± 2) did not show any alterations during electrical stimulation of the hindpaw.

between the BOLD-fMRI signal in the primary somatosensory cortex and the stimulation current during median nerve stimulation (Backes et al., 2000). Increases in stimulus intensity cause higher frequencies in the afferent fibers and cause more fibers to become activated.

Figure 4. Maximal $\Delta\text{CBV}\%$ response of APP23 mice, relative to the corresponding wild-type values. Data are obtained from statistically derived ROI on contralateral side to the stimulated hindpaw of 6-, 13-, and 25-month-old control and APP23 mice for current amplitudes of 0.5, 1, and 2 mA, respectively. The response decreases with both increasing age of the animals and increase in the amplitude of the stimulation current. Individual comparison between the groups revealed significant differences between control and APP23 mice only at an age of 25 months for the 2 mA current strength ($p < 0.029$) and between 6- and 25-month-old APP23 mice ($p < 0.003$; ANOVA, Tukey's test).

APP23 mice overexpressing human APP exhibit elevated brain levels of A β and develop neuropathological features such as A β deposits (referred to as neuritic plaques) (Sturchler-Pierrat et al., 1997; Sturchler-Pierrat and Staufenbiel, 2000). These pathomorphological alterations are associated with behavioral (cognitive) deficits (Kelly et al., 2003).

Such functional impairments can be assessed objectively and quantitatively by using fMRI in conjunction with a standardized stimulation paradigm, such as sensory electrical stimulation, with well controlled current amplitudes. Consistent with the results obtained in normal mice, the fMRI response in wild-type littermates of ages 6, 13, and 25 months increased linearly with the current strength over a range of 0.5–2 mA. In contrast, the hemodynamic response in the S1HL region of APP23 mice declined as a function of age and of the amplitude of the stimulation current as compared with the littermates. Although this trend was significant on the basis of regression analysis, individual comparisons between transgenic and wild-type animals revealed significantly different ΔCBV values only at an age of 25 months and for a current amplitude of 2 mA. A slightly, but not significantly reduced, CBV response at a current strength of 2 mA has been measured in 6- and 13-month-old APP23 mice. No difference in PtcCO_2 values between transgenic and control animals has been measured. This rules out the possibility that the observed differences are attributable to hyper- or hypocapnia.

In 25-month-old APP23 mice pathomorphological alterations reflecting extensive neurodegenerative processes are observed around compact amyloid deposits. These plaques are surrounded by enlarged (swollen) dystrophic neurites that can be visualized by neurofilament immunolabeling or silver impregnation (Staufenbiel et al., 1997; Sturchler-Pierrat et al., 1997). These dystrophic structures react with APP and synaptophysin antibodies analogously to the corresponding structures in human AD brain tissue. The loss of synapses in the neocortex correlates with the plaque load (Calhoun et al., 1998), predominantly on axons

and their connections and less pronounced on dendrites (Staufenbiel et al., 1997; Phinney et al., 1999). These methods also revealed the distortion of neurites by the deposits. In older animals with heavy plaque load, a local disruption of the laminar cytoarchitecture, which strongly affects neuronal connectivity, is apparent predominantly in hippocampus as well in cortex (Sturchler-Pierrat and Staufenbiel, 2000). It is reasonable to assume that these degenerative changes of the neuroarchitecture of the neuronal system in aged transgenic mice translate into an impaired functional response in the somatosensory cortex, which is involved in the mediating and processing of tactile sense and deep sensibility (primary and secondary somatosensory cortex) (Backes et al., 2000).

The mechanisms by which A β leads to brain dysfunction are not understood fully (Small et al., 2001). Although there is evidence that A β alters neuronal function (Mattson, 1997), recent data suggest that it also causes cerebrovascular dysfunction as derived from studies of endothelial-dependent induced vasodilation, using vasoactive agents (Niwa et al., 2000, 2002). We previously have found compromised CBV responses to the GABA_A antagonist bicuculline in cortical and thalamic structures of aged APP23 mice (Mueggler et al., 2002). This could, at least in part, be attributed to a compromised vascular reactivity, as revealed by the reduced responsiveness to the vasodilator acetazolamide, reflecting the severe CAA described for aged APP23 mice (Calhoun et al., 1999; Winkler et al., 2001). In analogy, the decreasing CBV response after sensory stimulation observed with the increasing age of APP23 mice might reflect progressive CAA, which reduces the ability of the cerebral arteriolar and/or capillary compartment to regulate CBF effectively. This is supported by the observation that, for higher amplitudes of the stimulation current (2 mA), the vasodilation observed in APP23 mice cannot cope with the significantly larger response in wild-type littermates and control mice. Hence in APP23 mice the increased metabolic rate resulting from neuronal activation did not translate into a proportional change in the hemodynamic parameters detected by fMRI. Consistent with this hypothesis is the fact that structural and functional disruption of vascular smooth muscle cells in pial vessels has been observed in 14-month-old mice overexpressing APP (Tg2576), which display a similarly compromised ability to respond appropriately to endothelium-dependent and endothelium-independent vasodilators (Christie et al., 2001). In APP23 mice CAA has not been observed at an age of 8 months (Calhoun et al., 1999) in line with the normal fMRI response in the somatosensory cortex observed in 6-month-old APP23 mice. Similarly, no difference has been observed between the CBV response of 6-month-old APP23 mice and control littermates to acetazolamide (Mueggler et al., 2002). At a progressed stage the deposition of amyloid in cerebral blood vessels leads to a loss of smooth muscle cells and a weakening of the vessel wall in mice (Winkler et al., 2001) and humans (Maeda et al., 1993). Despite the evidence for CAA playing a major role for the explanation of the fMRI observations, it cannot be excluded that the response at the neuronal level is compromised as well prompting a reduced amplitude of associated changes in local CBV. Electrophysiological investigations would be required to verify this hypothesis.

In conclusion, overexpression of human mutated amyloid precursor protein leads to an impaired hemodynamic response in the somatosensory cortical area S1HL of APP23 mice, which can be assessed quantitatively by using fMRI in combination with a standardized sensory stimulus. This functional deficit becomes more severe with increasing age of the animals and with increasing amplitude of the stimulation current. Two factors might con-

tribute to the compromised hemodynamic response: an impaired neuronal excitability because of A β -related neurodegenerative processes, in particular loss of synaptic connectivities, and/or severe CAA compromising the vascular reserve capacity. Related studies that used a vasoactive stimulus favor the latter mechanism (Mueggler et al., 2002). However, further studies, including electrophysiological recordings, are required to elucidate the underlying mechanism. It is conceivable that similar quantitative fMRI approaches that have used a defined stimulation paradigm (i.e., median nerve stimulation) (Spiegel et al., 1999; Arthurs et al., 2000; Del Gratta et al., 2002) could be applied to characterize the severity of disease in AD patients and to monitor the response to therapy.

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