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Functional MRI and neural responses in a rat model of Alzheimer's disease

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Abstract

Based on the hypothesis that brain plaques and tangles can affect cortical functions in Alzheimer's disease (AD) and thus modify functional activity, we investigated functional responses in an AD rat model (called the Samaritan Alzheimer's rat achieved by ventricular infusion of amyloid peptide) and age-matched healthy control. High-field functional magnetic resonance imaging (fMRI) and extracellular neural activity measurements were applied to characterize sensory-evoked responses. Electrical stimulation of the forepaw led to BOLD and neural responses in the contralateral somatosensory cortex and thalamus. In AD brain we noted much smaller BOLD activation patterns in the somatosensory cortex (i.e., about 50% less activated voxels compared to normal brain). While magnitudes of BOLD and neural responses in the cerebral cortex were markedly attenuated in AD rats compared to normal rats (by about 50%), the dynamic coupling between the BOLD and neural responses in the cerebral cortex, as assessed by transfer function analysis, remained unaltered between the groups. However thalamic BOLD and neural responses were unaltered in AD brain compared to controls. Thus cortical responses in the AD model were indeed diminished compared to controls, but the thalamic responses in the AD and control rats were quite similar. Therefore these results suggest that Alzheimer's disease may affect cortical function more than subcortical function, which may have implications for interpreting altered human brain functional responses in fMRI studies of Alzheimer's disease.

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Keywords

cognitive dementia; thalamocortical responses; neurovascular coupling; aging; energy metabolism; neuroimaging

Introduction

Alzheimer's disease (AD) is a neurodegenerative disease categorized by progressive loss of memory and other cognitive functions. Histology of AD is characterized by increased levels of amyloid β peptide (A β) in plaques and neurofibrillary tangles within the brain, which in turn are associated with neuronal damage (Hardy and Selkoe, 2002; Tanzi et al., 2004) and vascular reactivity impairment (Cantin et al., 2011; Huang and Muck, 2012; Iadecola, 2004; Paris et al., 2004). Brain imaging studies provide a basis for exploring structural anatomy and function of healthy aging brain and progressive dementia (Aanerud et al., 2012; Ances et al., 2009; Buckner et al., 2000; D'Esposito et al., 2003). AD-related pathophysiology in patients with established AD has been reported using magnetic resonance imaging (MRI) and positron emission tomography (PET) approaches (Jack, 2012; Nordberg et al., 2010; Petrella et al., 2003; Sperling, 2011). Prior applications of functional MRI (fMRI), a non-invasive technique for studying brain function with superb spatiotemporal resolution in both humans and animals has shown that the blood-oxygenation level dependent (BOLD) response in AD brains is quite different from healthy non-aging brain. These recent fMRI studies in AD subjects report reduced hemodynamic responses during sensory stimulation (Fleisher et al., 2009; Jack et al., 2009; Johnson et al., 2000; Small et al., 2011). Since fMRI does not measure neural activity directly, interpreting the magnitude of BOLD response in AD brain is more difficult due to the complexity of the fMRI contrast mechanism. Therefore additional neural measurements can substantially help to interpret the functional BOLD responses in AD brain.

Recently developed animal AD models provide an opportunity to investigate brain function in a manner that would be difficult to study in humans. MRI studies using transgenic mouse AD model have focused on studying structural (Jack et al., 2004; Jack et al., 2005; Poduslo et al., 2002) and functional (Beckmann et al., 2003; Mueggler et al., 2003) abnormalities. Iron containing plaques (a hallmark of AD) have been detected in different cortical and subcortical (thalamus, hippocampus) regions (Braakman et al., 2006; Vanhoutte et al., 2005; Wengenack et al., 2011). Different models can be used to study the two basic types of AD, the rare familial (10% prevalence) and the more common sporadic form. The relatively unknown etiology of sporadic AD based mainly on the amyloid and tau hypotheses postulated that A β amyloid deposits or tau protein abnormalities initiate the disease. Unfortunately transgenic mouse models do not completely address the onset and progression of sporadic AD. None of the mouse models recapitulate all aspects of human AD, but several lines do develop robust AD-like pathology, including A β containing plaques surrounded by phospho-tau containing dystrophic neurites, synaptic damage, and age-related learning and memory deficits (McGowan et al., 2006). Recently, rat AD models have been developed for studies involving neuroimaging, electrophysiology, neurobehavioral testing (Abbott, 2004; Benedikz et al., 2009; Liu et al., 2008). As a model of human AD, the rat models may offer many advantages over the mouse model. Large volumes of in vivo experimental data are available on rats and they are physiologically more resilient than mice for long duration in vivo studies. Moreover, in many cases, the rat's physiology corresponds to the human condition well (Hyder et al., 2013). Furthermore, in studies of cognition and memory, the physiological systems in the rat involved in learning and memory have been extensively studied.

In the current study we used a non-transgenic rat AD model known as the FAB rat or the Samaritan Alzheimer's Rat from Taconic, which has shown to develop plaques, tangles, and even neuronal loss – found in post mortem human AD brain (Lecanu et al., 2006; Lecanu et al., 2010). The FAB rat requires a much shorter time (just 4 weeks) to disease onset stage compared to some transgenic AD models. Therefore the FAB rat may enable research directions like drug target screening and even testing with higher throughput than would be possible with a mouse AD model. The FAB rat seems to mimic the onset and progression of sporadic AD that does not have genetic link, allowing researchers to study a disease state that accounts for 95% of all Alzheimer's cases. Based on the hypothesis that plaques and tangles found throughout the cerebral cortex in the AD brain can affect not only memory and cognitive processes but also cortical and subcortical function (i.e., alter the neurovascular coupling in AD brain vs. the normal brain), we conducted high-field fMRI and electrophysiology studies to compare functional differences in the FAB rat and age and species matched controls, where both fMRI and neural activity measurements were concurrently conducted in the same subjects.

Methods

Animal preparation

All procedures were performed in accordance with protocols approved by the Yale University Institutional Animal Care and Use Committee and in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Experiments were conducted on artificially ventilated (70% N₂O / 30% O₂; Harvard Apparatus, Inspira) adult male Long Evans (250–350 g, n=5; Charles River, Wilmington, MA) and Samaritan Alzheimer's or FAB rats (250–350g, n=5; Taconic Farms Inc., Germantown, NY). Alzheimer's symptoms were induced in the FAB rat at Taconic by slow release of ferrous sulfate heptahydrate, L-Buthionine-(S,R)-sulfoximine and Beta-Amyloid (1–42) peptide into the lateral ventricle of the brain, also known as FAB surgical procedure (see www.taconic.com; surgery model# FAB) and then later (i.e., 4 weeks after initial injection) delivered to Yale University. Over the course of 4 weeks slow ventricular infusion, the animal develops memory impairment accompanied by increased hyperphosphorylated Tau protein levels in the CSF. In addition, FAB-infused animals display thioflavin-S-positive amyloid deposits, neuronal loss and gliosis (Lecanu et al., 2006). During these procedures a cannula was implanted through the skull (DV = –3.4 mm, ML = +1.4 mm and AP = –0.92 caudal to bregma) and connected to an osmotic pump (Alzet, Cupertino, CA) in the subcutaneous pocket. These rats were housed with normal control rats of same age and body weight in the same room till the experimental day. Before the MRI experiments, both the magnetic Alzet pump and the cannula were carefully removed.

Throughout the surgical phase of experiment, rats were initially anesthetized with isoflurane (2–3%), which was discontinued after tracheostomy and anesthesia was maintained with α -chloralose. A femoral artery and vein were cannulated (PE-50 and PE-10) for monitoring physiological parameters (pCO₂, pO₂, pH, blood pressure). To prevent any movements during the fMRI or electrophysiology experiments and to facilitate artificial breathing, rats were paralyzed by intravenous injections of 0.5 mg/kg d-tubocurarine chloride (Sigma-Aldrich) and supplemental 0.25mg/kg every hour. An intraperitoneal catheter was used for administration of α -chloralose (~40 mg/kg/h). Ventilation parameters were adjusted to maintain normal physiology. Adequate analgesia was ensured by monitoring the pain response in blood pressure to an automated electrical tail-pinch every 15 minutes (5 mA, 0.3 ms, 10 Hz, 1 s), except during the experimental scan when forepaw stimuli were given to the rat. The animal's core temperature was monitored and maintained at 37±0.5°C with a rectal

probe and homoeothermic blankets, respectively. After the fMRI experiment the same rat was subjected to localized electrophysiology experiments.

fMRI measurements

The fMRI data were obtained on a modified 9.4T system with Varian (Agilent Technologies, Santa Clara, CA) spectrometer using a custom-built ^1H surface coil. During fMRI recordings, the rat was positioned prone in a specially designed plastic holder in such manner that a surface coil (diameter, 1.4 cm) would be at the top of the rat's head. Five contiguous coronal slices were selected to cover somatosensory forepaw and thalamic regions (which were located in different slices). The magnetic field homogeneity was optimized by localized shimming to yield typical water spectrum line-width of less than 20 Hz across the slices. Details of fMRI measurements are discussed elsewhere (Sanganahalli et al., 2009a; Sanganahalli et al., 2009b). Briefly, BOLD signal was acquired with echo-planar imaging (EPI) with sequential sampling (Hyder et al., 1995) using gradient-echo contrast with repetition and echo times of 1000 and 16 ms, respectively (Kida et al., 2004). The field-of-view was 2.56 cm in both axes with in-plane matrix of 64×64 and slice thickness of 2 mm resulting voxel size of $400 \times 400 \times 2000 \mu\text{m}^3$. Sixteen dummy scans were carried out before fMRI data acquisition began. The anatomical images were obtained using gradient-echo or fast spin-echo contrast sequences in 128×128 matrix and field-of-view of 2.56 cm.

Neural activity measurements

After completing the fMRI measurements (3–4 hours) rats were rapidly moved to the electrophysiology set up for the neural activity measurements. The rats were mounted on a stereotaxic frame (David Kopf Instruments, Tujunga, CA) placed on a vibration-free table inside a Faraday cage. First the scalp and the *galea aponeurotica* were removed and then small burr holes were drilled for insertion of high impedance tungsten microelectrodes (2–4 M Ω ; FHC, Bowdoinham, ME) to measure neural electrical signals. With reference to bregma and the sagittal midline plane, electrodes were placed in the following coordinates: somatosensory forelimb (S1_{FL}) cortex [1 mm anterior, 4.4 mm lateral, ~ 1 mm ventrodorsal]; ventral posterior lateral (VPL) nucleus of the thalamus [3 mm lateral and 3 mm posterior to bregma, ~ 5 mm ventrodorsal]. Neural activity in the form of multi unit activity (MUA) and local field potentials (LFP) were simultaneously recorded with Spike2 software (CED, Cambridge, UK). Electrophysiological signals obtained were digitized at 20 kHz and actively filtered to LFP and MUA signals (Krohn-Hite Corp., Brockton, MA) by splitting the electrical signals into low (< 150 Hz) and high frequency (0.4–10 kHz) bands, respectively, using Butterworth filters (24 dB/oct attenuation). After completion of the electrical measurements rats were intracardially perfused with physiological NaCl solution and 4% cold paraformaldehyde (PFA) in 0.01 M phosphate buffered saline (PBS) at pH of 7.4. After perfusion the brain was harvested maintaining integrity and stored in 4% PFA in PBS at 4 °C. Later these brains confirmed the location of the microelectrodes in S1_{FL} and VPL. The whole procedure lasted less than 8 hours from the start of the experiment till the euthanasia procedure.

Stimulation paradigm

Two subcutaneously placed copper needles were inserted into the contralateral forepaw (between the second and fourth digits) and all snout whiskers were shaved to avoid contaminating somatosensory signals. All stimulus presentation was controlled by a μ -1401 analog-to-digital converter unit (CED, Cambridge, UK) running custom-written script to provide 0.3 ms duration pulses with 2 mA amplitude and 3 Hz frequency by an isolation unit (WPI, Sarasota, FL) for 30 s duration. We used 3 Hz stimulus frequency since we observed robust responses at S1_{FL} under α -chloralose anesthesia (Herman et al., 2009; Sanganahalli

et al., 2009a; Sanganahalli et al., 2009b). The protocol consisted of a series of trials. Each trial consisted of single block design: 30 s rest and 30 s stimulation followed by 60 s rest. We used similar stimulation protocol for both fMRI and neural measurements.

Data analysis

fMRI—All fMRI data were subjected to a translational movement criterion using a center-of-mass analysis (Chahboune et al., 2007). After masking of non-brain tissues by thresholding each image within a series, the masked raw images were converted into binary maps (i.e., brain vs. background). Removal of image intensity information (i.e., binary maps) assured that the analysis was not biased by stimulation-induced movement artifacts. For each binary map in the series two center-of-mass values were calculated, one for each in-plane direction. If either center-of-mass value in a series deviated by more than $\frac{1}{4}$ of a pixel, the entire dataset was discarded from further analysis. A data set which did not pass the movement analysis step were not analyzed further. We calculated the relative responses for every individual trial (i.e. the percentage change of response compared to the pre-stimulus state) and calculated t-maps to identify the localization of functional responses (see Statistical Analysis section below) [R2.6].

Neurophysiology—Spike activity was extracted from MUA time series with wavelet spike sorting and superparamagnetic clustering algorithms (Quiroga et al., 2004). Data sections of 2 minute length (i.e., a block design paradigm of 30 s rest, 30 s stimulation, 60 s rest; see above) were selected for analysis. Spiking frequency was calculated in 1 s bins, and subsequently averaged in the selected time series. We applied two types of analysis to describe the neural responses. First, we calculated the time series of responses with respect to the block stimulation paradigm, similar to the fMRI data [R2.1]. The MUA activity was estimated using the root-mean-square (RMS) calculation (Sanganahalli et al., 2009b). The raw data were squared, averaged over 1 s bins, and then the square root was calculated. The mean RMS was estimated for every data block. Second, we checked the neural responses related to every single stimulus within the block design [R2.1]. Peristimulus time histograms (PSTH) were created as the cumulative number of identified spikes from the stimulation period of MUA time series in 1 ms bins, from stimulus to stimulus (i.e., every 333 ms). Spike numbers were averaged for 90 trials (i.e., 3 stimuli/s $\times$ 30 s = 90 stimuli). For better visualization, we used histograms with 50 ms before and 150 ms after the stimulus (i.e., reflected by time point at 0 s) because no change of spike counts was observed after 150 ms. The large peaks at 0 s were the stimulus artifacts. Statistical significance between control and treated conditions were tested using a one-way ANOVA with post-hoc Tukey's HSD test, where $P < 0.05$ was considered significant.

Statistical Analysis—We applied statistical hypothesis tests at two different levels of the analysis: 1. Trial based identification of functional responses of the BOLD data. 2. Group level comparisons between control and AD rats, both for cortical and subcortical responses of BOLD and MUA signals. For the identification of functional responses we applied Student's *t*-test comparison within each trial, where we compared 30 s rest and 30 s stimulation data for every voxel of the recorded slices to obtain the t-map of the responses. We calculated the probability (p-value) from the t-statistic of Student's *t*-test where the activated voxels were defined on the basis of $p \leq 0.001$. Then the activation map was overlaid on the corresponding anatomical image. Then we generated time courses of BOLD responses from S1_{FL} layer 4 and VPL by interrogating the activated voxels located in these regions. Finally, group averages were calculated for the BOLD responses from similar data collected for all rats in each group. Then responses were time averaged from 30 s to 60 s, which represented the entire stimulation duration. These averaged BOLD responses were

subjected to descriptive statistics and to calculate statistical differences between groups [R2.4] [R2.5] [R2.6].

Before the hypothesis test we checked the scattering of data in each group to find out whether the group data was homoscedastic or heteroscedastic. Since the heteroscedastic data has biased standard error, a special test should be applied for hypothesis testing (i.e., the control and AD conditions are different or not). We performed F-test of equality of variances with a null hypothesis that the data were homoscedastic. The data sets were considered heteroscedastic if $p < 0.05$. When the result showed homoscedastic data, the statistical significance between control and AD rats were tested using a one-way ANOVA with post-hoc Tukey's test, where $p < 0.05$ was considered significant. When the result showed heteroscedastic data, we applied t-test with unequal variance as a hypothesis test [R2.1].

Transfer function calculation—Transfer functions were calculated to mathematically characterize the neurovascular coupling. We applied the gamma variate function (GVF) as the transfer function (Sanganahalli et al., 2009b). Since the deconvolution methods are uncertain we convolved the GVF on neural signals (MUA) to simulate a potential BOLD signal output and applied a least square minimum search algorithm (in detail see (Herman et al., 2009; Sanganahalli et al., 2009b)) to find those parameters of GVF which give the best fit of simulated signals on measured BOLD signals. The goodness of fit between measured and simulated data was calculated as square of Pearson correlation coefficient (r^2). The cortical data provided well-defined fits, but since the low signal to noise of thalamic recording the thalamic transfer function calculation was unreliable. Two important parameters of the GVF, the time to peak and α (the exponential rise and decay of the function) defined the dynamic characteristic of the neurovascular coupling, while a third parameter (amplitude of the transfer function) depended on the temporal resolution of the neural data, therefore it had to be adjusted for the control vs. AD rat measurements.

Results

Forepaw stimulation-evoked functional responses were measured in the rat AD model and compared with normal rats of the same age and strain using BOLD and extracellular neural activity measurements. During the course of experiment the systemic physiological changes in blood pressure, core body temperature, pH, pO_2 , and pCO_2 were in the physiological range in both AD and normal control groups. The average experimental parameters (blood pressure, pH, pCO_2 , pO_2) of control and AD rats are given in Table 1.

Reduced cortical and conserved thalamic BOLD response in AD rats

Evoked BOLD responses in control and AD rats are shown in Fig. 1. Stimulation of the forepaw led to localized and significant BOLD activations in the contralateral $S1_{FL}$. Both in control and AD rats, good reproducibility of the BOLD responses was observed in the same subject across trials (each trial: 30 s rest, 30 s stimulation, 60 s rest) (Figs. 1A and 1B) as well as across subjects (Figs. 1C and 1D) [R2.2]. No significant activations were observed in the ipsilateral $S1_{FL}$ region. The magnitude of the $S1_{FL}$ BOLD response was decreased in AD rats by about 50 % of the response from control rats. We also found decrease in spatial area of $S1_{FL}$ BOLD response in AD rats (81 ± 14 pixels) as compared to control rats (140 ± 16 pixels). We further quantified the temporal dynamics of BOLD response in AD vs. control rats. The interrogated time course of BOLD response for the activated pixels at $S1_{FL}$ region for normal control (red trace) and AD (blue trace) rats are shown in Figs. 2A–C. The F-test of equality of variances showed that the cortical BOLD data were homoscedastic ($p=0.14$) [R2.1]. Mean $\pm$ standard deviation of BOLD responses of control rats were

significantly higher than in AD rats (i.e., $4.7 \pm 1.2\%$ vs. $2.2 \pm 0.05\%$; $p < 0.05$) [R2.7]. During the stimulation period BOLD signal increased and peaked within 5–6 s after the stimulus onset and declined at the end of stimulation period. Time constants for rise, decay, and time-to-peak of BOLD responses across control and AD rats are listed in Table 2. There were no statistically significant differences in these time constants ($p < 0.1$) and also time-to-peak values ($p < 0.08$) for both groups [R2.7].

In addition to $S1_{FL}$ BOLD responses, we also observed thalamic activation during forepaw stimulation in both control and AD rats, as shown in Figs. 2D–F. Large parts of various thalamic nuclei were activated including the ventral posterior medial and lateral (i.e., VPL) nuclei in both AD and control rats during the forepaw stimulation (see in Fig. 2E, with the overlaid digitized rat brain atlas (Paxinos and Watson, 1998)). We interrogated for thalamic activation only from VPL because this location is the main sensory input during forepaw activation (Aguilar et al., 2008) and electrical data were obtained from this location (see below). Time course of interrogated BOLD signal from VPL in control and AD rats are shown in Fig. 2D. The mean amplitudes of BOLD activation during 30 s stimulus duration are compared in Fig. 2F. The F-test of equality of variances showed that the thalamic BOLD data were homoscedastic ($p = 0.06$) [R2.1]. Unlike the $S1_{FL}$ responses (see Fig. 2C), we observed similar magnitude of changes in BOLD responses at VPL in both control and AD rats. These results show that sensory responses in the thalamic region were not significantly different ($p = 0.073$) [R2.7] in the AD rat model compared to the control rats. Similar time constants for rise, decay and time to peak of BOLD responses across control and AD rats in the thalamic regions were observed (Table 2).

Compromised neural responses from the cortex in AD rats

Extracellular neural recordings from the $S1_{FL}$ and VPL regions were performed in control and AD rats, as shown in Figs. 3A–D. Evoked neural activities from the $S1_{FL}$ were reduced in AD rats compared to the normal rats. Figs. 3A and 3C show the averaged MUA changes from the $S1_{FL}$ in 5 normal controls (total of 18 trials) and 5 AD rats (total of 17 trials), respectively [R2.2]. The F-test of equality of variances showed that the cortical MUA data were homoscedastic ($p = 0.14$) [R2.1]. A significant decrease ($p < 0.001$) was observed in the fractional change in cortical MUA in AD rats as compared to control rats [R2.7]. The mean changes in MUA in control rats during 30 s stimulation period decreased from 0.26 ± 0.14 to 0.10 ± 0.08 in AD rats (i.e., decreased by 61%). These MUA changes were similar to the BOLD results (Fig. 2), where we observed about 50% decrease in cortical BOLD response in AD rats as compared to control rats. We also observed similar LFP changes in both the groups (data not shown). While magnitudes of both BOLD and neural responses from the cerebral cortex were attenuated similarly in AD rats vs. normal rats (by about 50%), the neurovascular coupling as assessed by transfer function analysis (see Supplementary Fig. 1) remained unaltered between the two groups. In both cases the same transfer function (time-to-peak = 1.15 s, $\alpha = 0.3$) could be used to simulate the BOLD responses (fits for data from control and AD rats were $r^2 = 0.96$ and 0.89 , respectively).

Figs. 3B and 3D show the MUA responses from the VPL in control and AD rats, respectively. The F-test of equality of variances showed that the thalamic MUA data were heteroscedastic ($p = 0.001$) [R2.1]. MUA of control cortical responses were significantly higher than thalamic MUA responses ($p < 0.005$). But no significant differences were found in the magnitude of MUA responses from VPL of AD and control rats ($p = 0.073$) [R2.7]. These VPL results for MUA were consistent with BOLD thalamic responses (Fig. 2D–F). Because the subcortical data were much noisier and low magnitude compared to the cortical data, a thorough transfer function analysis was beyond the scope.

The neuronal spike counts were also calculated as peristimulus time histograms (PSTH) in AD and control rats, which showed the temporal distribution of spikes before and after a stimulus pulse. PSTH in control and AD rats from S1_{FL} and VPL regions were compared during somatosensory forepaw stimulation, as shown in Fig. 4A–D. We observed a significant reduction ($P<0.005$) in the spiking count at S1_{FL} in AD rats as compared to the control rats (Fig. 4A and 4C), whereas the VPL spike count of AD rats were not significantly different ($p=0.07$) from control VPL (Fig. 4B and 4D) [R2.7]. The observed response latencies at S1_{FL} and VPL of control rats are consistent with the previous studies (Aguilar et al., 2008; Armstrong-James and George, 1988). We found that response latency in S1_{FL} of AD rats was significantly delayed as compared to control rats (i.e., 15.0 ± 2.1 ms vs. 10.2 ± 1.6 ms, $p<0.05$). There were no statistically significant differences ($p<0.06$) in the response latency in VPL between control rats and AD rats (i.e., 3.3 ± 0.6 ms vs. 3.5 ± 0.5 ms) [R2.7]. The delay in response latency in AD rats could be due to the presence of plaques affecting signal transmission.

Discussion

In this study we measured BOLD and neural responses in control and AD rats to demonstrate that cortical function is affected more than subcortical function in a nontransgenic rat model of AD. In contrast to AD related cortical functional decrease, the BOLD and neural responses of the thalamic relay station of the somatosensory pathway (i.e., the VPL nucleus) were unaltered. This could be due to differential plaque distribution in cortex and thalamus (Wengenack et al., 2011). In the current rat model the deposition of plaques is the result of slow ventricular infusion, where the thalamus remained plaque free and its response to somatosensory stimuli did not differ from response in control rats. Therefore these results justify our null hypothesis, that the plaques deposited in the cerebral cortex in the AD brain can alter basic somatosensory cortical functions.

fMRI is widely used to map stimulus-induced changes in BOLD response, which is thought to reflect evoked neural activity. But the BOLD signal itself is only a partial measure of neural activity (Barinaga, 1997; Fitzpatrick and Rothman, 1999). Neural activity alters the ratio of oxyhemoglobin to deoxyhemoglobin, which have diamagnetic and paramagnetic properties respectively to affect the BOLD signal. Changes in the oxyhemoglobin to deoxyhemoglobin ratio are influenced by many factors including cerebral blood flow (CBF), cerebral blood volume (CBV) and cerebral metabolic rate of oxygen consumption (CMR_{O2}). Therefore the complexity of BOLD effect renders the interpretation of differences in the magnitude of BOLD responses in AD and control rats quite difficult. In order to exploit more information about the localized neural activity, we measured extracellular signals simultaneously from the S1_{FL} and thalamic VPL region shortly after fMRI experiments were completed in the same rats. Quantitative assessment revealed neural activity changes were correlated with BOLD signal changes in the S1_{FL} and VPL regions (Figs. 2–4). The BOLD activation spread and magnitudes of the BOLD/neural responses in the middle cortical layers of S1_{FL} in AD rats were found to be significantly smaller than the control rats (Figs. 1–4). These results suggest functional deficits in vascular and neural responses in the cerebral cortex of AD rats as compared with control rats of same age and strain.

A previous study using transgenic mouse AD model showed age-dependent impairment in somatosensory CBV response (Mueggler et al., 2003). This study reported decreased CBV response with increasing age of APP23 transgenic mice during hind paw stimulation experiments as compared with wild type animals. Other fMRI studies using APP23 transgenic mice also observed similar compromised hemodynamic response using global pharmacological stimulation (Mueggler et al., 2002) and cerebrovascular abnormalities (Beckmann et al., 2003). Similar to our results in AD rats, the BOLD signal amplitude and

the number of activated voxels within the sensorimotor cortex were decreased with increasing ageing in human studies (Hesselmann et al., 2001; Huettel et al., 2001). Other studies in human subjects have shown that several neurovascular factors are affected in ageing and Alzheimer's disease (de Leon et al., 2001; Fleisher et al., 2009; Lin et al., 2012; Rombouts et al., 2005; Rosengarten et al., 2009; Zhang and Raichle, 2010; Zhang et al., 2010). These results showed differences in BOLD responses in ageing and Alzheimer's disease and also they strongly concluded those differences in imaging results do not necessarily reflect changes in metabolism (Small et al., 2011).

In our study, the decrease in BOLD response and compromised neural activity in the cerebral cortex indicates that the presence of A β plaques can affect both the hemodynamic and electrical activities without affecting per se the dynamic neurovascular coupling in the cerebral cortex of AD and control rats (see Supplementary Fig. 1). Because A β plaques are found mainly throughout the cerebral cortex, it is important to show that the presence of these plaques can indeed affect somatosensory BOLD and neural responses without affecting the neurovascular coupling. The mechanisms by which the specific brain regions are vulnerable to A β plaque deposition and by which these plaques lead to brain dysfunction are not fully understood (Huang and Mucke, 2012; Parihar and Brewer, 2010; Small et al., 2000). There is some evidence that A β plaques can damage synapses and therefore increase their vulnerability to excitotoxicity to hinder neuropil function (Hynd et al., 2004; Mattson, 1997; Tanovic and Alfaro, 2006). A recent study characterized the A β plaque load in different regions (whisker barrel cortex, cingulate cortex, piriform cortex, striatum, hippocampus) in a transgenic mouse model and concluded neural activity across regions may be vulnerable to plaque deposition (Bero et al., 2012; Bero et al., 2011). In our current AD rat model the distribution of plaques have been shown to be present across different regions including cortex and hippocampus, but not as much in the thalamus (Lecanu et al., 2006). Future MRI studies could be designed to image the A β plaques independently in different brain regions in this AD rat model in conjunction with the functional measurements. Thus while both hemodynamic and neural responses in the cortex are affected in the AD rat, these activities are attenuated in a correlated manner.

In this study, impaired neural excitability, loss of synaptic connectivity, and compromised vascular reactivity because of the A β plaque accumulation in the cortex may have contributed to the decreased neural and hemodynamic responses observed in AD rats. The significant difference in cortical response latency times in AD rats compared to control rats indicated that thalamocortical signaling was affected in the former group. We expect that the imaging correlate of this neural behavior in AD rats manifests in the reduced amplitude of the BOLD response. However the fMRI data also showed that the AD rats had reduced spreading of BOLD activation in the cortex. It is possible that the presence of A β plaques in the cortical region affected certain population of neurons across the span of the cortex and which in turn reduced the spreading of the activity. Therefore the measured neural responses in AD rats represent the activities of working neurons that can still respond to the sensory stimuli, but overall their responses are weaker in magnitude. Future studies with microelectrode arrays could allow the confirmation of the reduced BOLD activation spreading in AD rats.

Since the A β amyloid peptide in AD development has a critical role, its direct injection (in this non-transgenic approach) is believed to be a straightforward method to induce "Alzheimer-like" neuropathology in the rat by producing a resembling microenvironment, which may occur in AD brain. The histopathology of these rat brains indicated the presence of neuritic plaques, tangles, neuronal loss and gliosis, typical features of postmortem AD human brain specimens (Lecanu et al., 2006). Therefore the current FAB rat model is suitable for our combined fMRI and electrophysiology studies. But this kind of successive

fMRI and electrophysiology measurements in a transgenic AD model could prove to be challenging given the fragile nature of mouse physiology, especially in diseased models. However there are some experimental caveats of the current FAB model that should be considered, e.g., chronic infusion of the amyloid peptide, potentially modified sensitivity to anesthetics. The long term (i.e., 4 weeks) intracerebroventricular infusion of amyloid peptide may induce chronic inflammation, and behavioral changes in AD rats. Since the amyloid peptides are distributed in both hemispheres and in our current study we performed our measurements in the opposite hemisphere of the injection side, the potential for local inflammation may not have influenced our results (Fig. 1). Recent studies have shown different sensitivity of inhaled anesthetics in transgenic models of AD (Bianchi et al., 2010). We did not find any difference in anesthetic sensitivity in control and AD rats. Both groups maintained normal physiology during the entire duration of the experimental paradigms (see Table 1). Furthermore level of anesthesia was checked by pain reflexes (i.e., variation in blood pressure) at various time points of the experiment as required by the approved protocol (see Methods). In this study we used control rats of similar strain, age, and bodyweight as the AD rats. While in our current study we did not perform the cognitive tests to assess whether or not the behavior is impaired in FAB rats, previous studies using this model have shown that the cognitive functions (i.e., Morris water maze test) are impaired compared to control rats (Lecanu et al., 2010).

Conclusion

Successive measurements of BOLD and neural responses in the same rats showed correlated reduction in cortical function of AD rats, sparing thalamic activity. While these results of BOLD and MUA suggest correlated functional deficits in vascular and neural responses in AD rats as compared with the control rats of same age and strain, further studies with other measurements of CBV, CBF, and CMR_{O2} (e.g., as in calibrated fMRI) are needed to examine if the neurometabolic couplings are different in AD brain compared to the normal brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

fMRI and Neural activity measurements in Alzheimer rat model by high field fMRI and electrophysiology

Decreased somatosensory cortical fMRI-BOLD and neural responses in Alzheimer rat

Thalamic functional activity was unaltered in Alzheimer rat

Implications for understanding altered brain function in human Alzheimer's disease

Figure 1.

Reproducibility of $S1_{FL}$ BOLD activation maps during contralateral forepaw stimulation (2mA, 3Hz) in the same subject (top row, (A) control rat and (B) AD rat) as well as across three different subjects (bottom row, (C) control rat and (D) AD rat). Inset in the middle shows the axial view of the BOLD activation in a control rat, where the selected 2 mm coronal slice thickness is shown (white lines). All data are from single trial runs where the stimulation period was 30 s in duration. The t maps were generated by comparing the mean signals of a 30 s stimulation epoch vs. a 30 s baseline epoch before the stimulation. The lowest and highest thresholds were $p=0.001$ and $p<0.0001$, respectively [R3.1].

Figure 2.

BOLD responses from the contralateral S1_{FL} (A, B, C) and VPL nucleus (D, E, F) during forepaw stimulation in control (red traces and bars) and AD (blue traces and bars) rats. The 30 s stimulation period is shown with the black horizontal bar. Averaged BOLD responses in the control vs. AD rats were statistically different in S1_{FL} (A-C; * $p < 0.05$), but not in the VPL (D-F; $p = 0.073$) [R2.7]. The data represents mean $\pm$ standard deviation of 15 trials in each case (each trial: 30 s rest, 30 s stimulation, 60 s rest) from control ($n = 5$ subjects) and AD ($n = 5$ subjects) rats [R2.2]. The noise levels, estimated from the magnitude of spontaneous fluctuations in the images in the absence of stimuli, were similar in control and AD rats (i.e., within 1.5% in cortex and thalamus) [R2.3].

Figure 3.

Relative MUA changes in control (A and B, red traces) and AD (C and D, blue traces) rats from S1_{FL} and VPL during forepaw stimulation. The stimulation periods are marked by the black horizontal bars. The data represents mean $\pm$ standard deviation of 18 trials from 5 control rats and 17 trials from 5 AD rats (each trial: 30 s rest, 30 s stimulation, 30 s rest) [R2.2]. The MUA data are shown as 1 Hz root means square (RMS) time courses. The MUA responses in control and AD rats were significantly different in S1_{FL} (* $p < 0.005$), but they were not significantly different in VPL ($p = 0.073$) [R2.7].

Figure 4.

Peristimulus time histograms (PSTH) in control (A and B, red traces) and AD rats (C and D, blue traces) rats from S1_{FL} and VPL during forepaw stimulation. The histograms were created as the cumulative number of identified spikes from the stimulation period of MUA time series in 1 ms bins (i.e., from stimulus to stimulus, 333 ms). Spike numbers were average of 19 trials from 5 rats in control rats and 19 trials from 5 AD rats (each trial: 3 stimuli/s * 30s = 90 stimuli) [R2.2]. Histograms are shown for better visualization as 50 ms before and 150 ms after the stimulus (i.e., at 0 s), because there were no increase of spike counts after 150 ms. The large peaks at 0 s represent the stimulus artifact. The PSTH of S1_{FL} were significantly different between control and AD rats (* $p < 0.005$), whereas the PSTH of VPL responses were not significantly different between control and AD rats ($p = 0.07$) similar to the BOLD and MUA data [R2.7].

Table 1

Vital physiological parameters of control and Alzheimer's rats. Data averaged from 5 subjects in each case. (MABP: mean arterial blood pressure; pCO₂ / pO₂: partial pressure of CO₂ / O₂)

Rats	Systemic physiology			
	MABP (mm Hg)	pH	pCO ₂ (mm Hg)	pO ₂ (mm Hg)
Control	104 ± 8	7.34 ± 0.07	34.2 ± 8.4	157 ± 41
Alzheimer's	94 ± 11	7.38 ± 0.05	37.1 ± 5.6	153 ± 20

Table 2

Values of rise time, decay time, and time-to-peak of functional BOLD responses across control and Alzheimer’s rats. All values shown are mean $\pm$ standard deviation (units in seconds). Each time constant of rise and decay, calculated by a single exponential fit, represents the time that response takes to reach $\frac{2}{3}$ of the peak value, both following stimulation onset (i.e., rise) and offset (i.e., decay). The time-to-peak was estimated from the responses reach full maximum value. There were no significant differences in values of rise time, decay time, and time-to-peak across control and Alzheimer’s rats both in the cortical and thalamic region. See Figs. 2A and 2D for details on spatial and dynamic representations of functional responses at cortex and thalamic regions.

BOLD	Cortex (S1 _{FL})			Thalamus (VPL)		
	rise time	decay time	time-to-peak	rise time	decay time	time-to-peak
Control Rats	2.6 $\pm$ 0.3 s	5.3 $\pm$ 1.6 s	6.2 $\pm$ 1.5 s	1.4 $\pm$ 0.5 s	6.3 $\pm$ 3.3 s	4.6 $\pm$ 2.3 s
Alzheimer’s Rats	2.4 $\pm$ 0.4 s	5.3 $\pm$ 1.5 s	5.3 $\pm$ 0.5 s	1.3 $\pm$ 0.5 s	5.4 $\pm$ 2.7 s	3.3 $\pm$ 0.9 s