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Longitudinal Monitoring of Motor Neuron Circuitry in FALS Rats Using in vivo phMRI

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Abstract

Amyotrophic lateral sclerosis (ALS) presents challenges for diagnosis and objective monitoring of disease progression. We demonstrate, using pharmacologic MRI, that alterations in motor circuitry can be characterized using a passive stimulus in a rat model of familial ALS (FALS) as a function of symptom progression. Pre-symptomatic FALS rats had a pattern of activation to amphetamine indistinguishable from wild-type controls. In contrast, symptomatic rats showed significantly decreased response in sensorimotor cortex and increased response in M2 motor cortex, caudate/putamen, and thalamus. These results are similar to findings in humans of altered response to motor tasks in ALS. It may be plausible to use a passive amphetamine challenge as a biomarker to assess progression of the disease and efficacy of potential treatments.

Keywords

amyotrophic lateral sclerosis; transgenic rat; hSOD1 G93A; pharmacologic MRI; motor circuitry; amphetamine

Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating disease with rapid degeneration of motor neurons in brain stem and spinal cord. Multiple pathways [1] are involved in motor neuron death in ALS including aberrant folding of superoxide dismutase and glutamate excitotoxicity [2]. Most cases are of unknown etiology. Therefore, diagnosing the disease can be a considerable challenge and it is difficult to exclude other motor neuron diseases such that final clinical diagnosis can often take several months [3]. Due to the rapid progression of ALS (average life expectancy from diagnosis is 2-5 years) early diagnosis is imperative as it can allow for earlier treatment that may protect the motor neurons [4].

Non-invasive neuroimaging tools may provide improved means for making early objective diagnoses, as well as aid in assessing etiologic mechanisms. Functional magnetic resonance imaging (fMRI) is a tool that can map out patterns of motor circuitry and motor functions using

the coupling of neuronal activity with associated changes in regional blood flow. A number of studies of ALS patients have examined the reorganization of motor circuitry as a consequence of the illness [5-7]. However, monitoring motor circuitry in ALS patients is difficult due to the inability of patients to perform even simple motor tasks at later stages. This necessitates coming up with a means of adjusting motor tasks for the ability of the subjects to perform the studies. Further, it can often preclude meaningful longitudinal comparisons since the motor abilities change so much during disease progression. We therefore sought a means to assess motor circuitry using a passive challenge in a rat model of FALS. Prior data in rats demonstrated that amphetamine activates many components of the sensorimotor system, and further that it provides a sensitive measure of loss of motor function in Parkinson's disease (PD) models [8,9]. Further, when challenged with amphetamine rats show a pronounced motor stimulus as measured by many different tests including locomotion and electrophysiology of striatal and motor neurons [10]. Amphetamine can also facilitate motor recovery after sensorimotor contusion [11] reflecting glutamatergic and dopaminergic interactions in the sensorimotor system [12]. Thus, we utilized pharmacologic magnetic resonance imaging (phMRI) of a passive amphetamine challenge for longitudinal study of motor cortex/dopaminergic circuitry in FALS rats. Evidence in other diseases, such as multiple sclerosis or Alzheimer's Disease, suggests MRI can provide a number of markers that can be used to shrink population sizes, decrease measurement times for following therapeutic interventions and increase statistical power in clinical trials [13,14]. Therefore development of the passive pharmacologic challenge as a potential marker for motor neuron dysfunction in ALS may lead to a useful clinical tool.

Methods

Animals

Male Sprague-Dawley rats expressing SOD1 G93A [15] and wild-type (WT) littermates were purchased from Taconic (Hudson, NY). All animals were scanned under 1-1.5% halothane anesthesia (mixed in N₂O/O₂) wrapped with a circulating water blanket maintained at 38°C. The tail vein was catheterized for contrast agent and amphetamine injection. We studied a total of seven animals. Three animals were followed longitudinally in the pre-symptomatic stage before any overt signs of motor impairment and then until development of partial hindlimb paralysis defined by the ability to use the forelimbs for locomotion while the hindlimbs were fully or partially paralyzed. To control for possible sensitization effects of amphetamine an additional two pre-symptomatic animals and two post-symptomatic animals were studied, both of whom were injected with amphetamine only once. The mean pre-symptomatic age was age 85±28 days and post-symptomatic age was 131±13 days. All procedures were approved by the Massachusetts General Hospital subcommittee on research animal care.

MRI

MRI experiments were run as previously described at 9.4T (Bruker, Billerica, MA) using IRON (Increased Relaxivity for Optimized Neuroimaging) method to sensitize images to cerebral blood volume (CBV) changes [9,16]. Four to five pre-MION (monocrystalline iron oxide nanoparticles) images were collected followed by MION injection (25 mg/kg i.v.). Post-MION images were collected for at least 20 min and then amphetamine (2.5 mg/kg, i.v.; Sigma, St. Louis, MO) was injected during continuous gradient echo imaging (TR/TE = 600/10ms, FOV = 2.7cm with 256×128 in plane resolution, 0.75mm slice × 16 slices). For CBV the transverse relaxation rate (R_2^*) was measured on a pixel by pixel basis using the standard formulation for gradient echo signal, $R_2^*(t) = -\ln(S(t)/S(0))/TE$, where TE is the echo time and S(0) is the average value of the signal before MION. Data acquired after injection of MION were converted to percent change in functional CBV (fCBV (V)) using the change in transverse relaxation rate relative to the pre-contrast baseline: $fCBV = V(t)/V(0) = R(t)/R(0) - 1$. Then, the average CBV change over 30 minutes was calculated for each ROI for comparisons between

brain regions and groups. For display purposes, maps of fCBV were made by fitting the fCBV time series to a gamma function [19] and thresholded by correction for the number of pixels in the image.

Image Co-registration and Statistical Comparisons

All images were registered onto the same standard brain template for subsequent averaging across animals as previously described [17]. The data were resliced to a thickness of 0.5 mm. The template images are consistent with the stereotactic coordinate system atlas from Paxinos [18]. Regions of interest (ROIs) were drawn following those described in the Paxinos Atlas. Those analyzed are displayed in Fig. 1A.

Statistical analysis was performed using a one-way ANOVA for group differences (WT, pre-symptomatic, post-symptomatic) for each brain region then this was corrected for multiple comparisons using a Tukey HSD correction post-hoc. We further performed a repeated measures ANOVA for the effects of symptom state on brain regional CBV changes in the three rats that were scanned longitudinally.

Results

A typical response to amphetamine produces a pattern that involves anterior somatosensory cortex (strongest in S1), cingulate, motor cortex (M1 and M2), caudate/putamen (CPu), and thalamus. The regions of interest utilized in this study, along with a representative map of amphetamine activation in a WT animal is shown in Fig. 1. The pre-symptomatic animals had a response to amphetamine that was statistically indistinguishable from the WT animals (Fig. 2A). We performed a power analysis that showed that with the effect sizes and standard deviations seen in the pre-symptomatic and WT animals that we would need between 89-200 animals (depending upon brain region) to have 90% power to see a difference between the WT and pre-symptomatic animals. In contrast, the symptomatic animals showed several significant changes in the maps including decreased response in sensorimotor cortex (S1 decreased by 40%) and large increases in M2 motor cortex (+90%), CPu (+70%), thalamus (+115%) and insula (+80%) (Fig. 2B). Time courses of the percentage fCBV changes from S1, M2 and CPu are shown in Fig. 3A. The shape of curves describing time course for S1 and CPu appear to be similar though their peak heights are changed between pre-symptomatic and symptomatic FALS animals (Fig. 3A). Interestingly, in M2 pre-motor cortex there was an increase in both the peak fCBV value and the full-width half-maximum (FWHM) of the fCBV time course from about 12-15 min in pre-symptomatic to about 35-45 min in the symptomatic FALS animals (Fig. 3B).

In order to control for the possibility that we were simply observing amphetamine sensitization we compared the three animals that were studied longitudinally to two additional symptomatic animals that had received just the single dose of amphetamine. The average fCBV in S1, M2 and CPu in the longitudinal (two doses) vs. cross-sectional (one dose) respectively were 11.6% vs. 10.6% (S1), 18.6% vs. 15.3% (M2) and 23.6% vs. 22.6% (CPu). All of these values are quite different from the pre-symptomatic values (Fig. 3B) and are not different from one another. Further, repeated measures ANOVA of just the three longitudinal animals yielded significance for CPu and M2, thalamus and insula.

Discussion

The rat model of familial amyotrophic lateral sclerosis with overexpression of mutant human superoxide dismutase 1 shows a profound loss of motor function that is rapidly progressive. The pathology starts with vacuolation and loss of lower motor neurons and eventually progresses from loss of hind-limb function to all four limbs [19]. Loss of motor function can

lead to cortical reorganization. Imaging techniques such as functional MRI have provided insights in cortical reorganization caused after neuronal loss such as in stroke [20]. There have been a number of fMRI studies of motor function in ALS patients [6,7,21]. These studies showed a pattern of recruitment of additional cortical (pre-motor) and anterior motor areas while performing a motor task concomitant with loss of motor cortex activation [6] and/or somatosensory and sensorimotor cortex [7]. Amphetamine stimulation produces intense locomotor activity when administered to awake rats [10] and, in the magnet, produces a pattern of brain activation that includes many components of the motor system including motor cortex, sensory cortex and basal ganglia [8,9]. The alterations in the patterns of motor system activation seen in the amyotrophic lateral sclerosis patients are similar to the alterations we find in the rat model of amyotrophic lateral sclerosis with amphetamine stimulation after symptoms progress. We found in rats that there is a decrease in somatosensory cortex (S1) with an increase in anterior (especially M2) motor cortex. Further, the large increase we note in the CPu as the animals become symptomatic is consistent with the results of Tessitore et al. in humans [7], where they noted increased putaminal activity with a motor task. Unfortunately, rapid degeneration of motor neurons in ALS patients renders the evaluation of motor tasks progressively more difficult with time and the same task is more difficult for patients than control subjects thus making it harder to differentiate results originating from the pathology of neurodegeneration or from the changing difficulty of the task. Thus the amphetamine provides an alternative means to stimulate the motor system passively.

Two other studies examined passive conditions in amyotrophic lateral sclerosis patients. A PET study showed that cerebral blood flow (CBF) at rest in the primary sensorimotor cortex was significantly lower in ALS patients than in controls [5]. Another study using cutaneous stimulation, as a form of passive stimuli, of the hand and sole to elicit the palmomental response and plantar response in ALS patients showed that patients activated a significantly smaller volume of the contralateral sensorimotor cortex compared to control subjects [21]. These studies, along with ours, implicate the loss of neurons in sensorimotor cortex in ALS patients.

Aside from loss of sensorimotor cortex activity and increased pre-motor cortex (M2) activity, we also found a large increase of response in the caudate/putamen in the symptomatic animals. This finding can potentially be explained in the following manner. There is glutamate input from sensory and motor cortex to striatum and glutamate decreases striatal dopamine D2 receptor (D2R) synthesis in animal studies [22]. Motor cortex densely innervates dorsolateral striatum and amphetamine typically activates brain regions where dopamine receptors are richly expressed including motor cortex and striatum. There is decreased striatal D2R binding *in vivo* in drug naïve sporadic ALS patients [23]. Decreased D2R tone may lead to increased dopamine (DA) release after amphetamine. In a previous study using microdialysis and pHMRI we showed that D2 antagonism increases DA release and fCBV [24]. We have also found that increased DA release, as measured using microdialysis, leads to increased CBV in the caudate/putamen [8,9,24]. Thus a potentiation of DA release as a result of decreased D2 tone may explain the large increase in activation that we find in striatum from symptomatic FALS rats.

Since amphetamine is commonly prescribed for ADHD, and has been used as an adjunct to physical therapy in stroke in humans [25] or cortical injury in rats [11], it may represent an alternative passive stimulus to electrical stimulation of extremities in ALS. Further studies examining relations between pathological changes in both motor neurons and spinal cord and the altered patterns of brain activity will allow for determination of the added value of this tool compared to existing clinical markers.

Conclusions

MRI of a passive motor stimulus elicited by the drug amphetamine produces a pronounced alteration in the pattern of brain activity as a function of the symptomatic state of the FALS rats. The changes noted in the symptomatic stages of the disease are paralleled by similar changes seen using motor tasks using either PET or fMRI in ALS patients which include upregulation of pre-motor areas and loss of activity in primary motor and sensory cortex.

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Fig. 1.

Regions of interest and brain activation pattern after amphetamine administration. A) Overlay of the brain regions analyzed on a standard rat atlas following Paxinos [18]. The data here are presented in 1mm slice intervals. B) Map of statistically significant changes in fCBV in a WT littermate control animal. There is strong activation in sensorimotor cortex (S1) and caudate/putamen (CPu).

Fig. 2.

Changes in the brain response to 2.5mg/kg amphetamine as a function of symptoms in FALS rats. A) Averaged map of statistically significant response to amphetamine in 5 pre-symptomatic FALS rats. The white arrows indicate the sensorimotor cortex. The numbers at the bottom are the distance from bregma. B) Averaged map of 5 symptomatic FALS rats. There is a large increase in CPu, thalamus, insula and M2 motor cortex (white arrows) and a decrease in sensorimotor cortex. The color scale shows positive CBV changes in red-yellow and negative CBV changes in blue-green.

Fig. 3.

Quantitative changes in time courses and fCBV values. A) Averaged temporal pattern of CBV changes in three selected ROIs including S1 (decreased CBV), M2 (increased CBV and FWHM of the time course) and CPu (increased CBV). There were 5 animals per group. B) Regional changes in CBV across groups including WT (n=6), pre-symptomatic (n=5) and symptomatic (n=5). The abbreviations are indicated in Fig. 1. * $p < 0.05$; ** $p < .01$ by ANOVA (see text).