Recovery of Locomotor Function after Experimental Stroke Correlated with Enhanced Neuronal Integrity after Amphetamine Treatment

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Introduction

Amphetamine (AM) treatment has been shown to enhance behavioral recovery and increase the expression of proteins related to axonal growth and synaptogenesis in stroke rats [1]. Using diffusion tensor imaging (DTI), a recent study demonstrated an increase of fractional anisotropy (FA) in perilesional areas after AM treatment, suggesting tissue reorganization in these areas [2]. However, the relationship between the cerebral reorganization and behavioral recovery after AM treatment remains obscure. The goal of this study was to elucidate whether the restoration or enhancement of structural integrity after AM treatment is associated with behavioral recovery. Specifically, we investigated whether the increase of FA in perilesional areas after AM treatment would predict behavioral improvement in an elevated body swing (BS) test that has been reliably used to assess neurological behaviors in rats with ischemia stroke [3].

Materials and methods

Animal preparation. A total of sixteen male Sprague-Dawley rats were included in this study. A bilateral common carotids were ligated with nontraumatic arterial clips first and the right middle cerebral artery (MCA) was ligated with a 10-O suture to generate focal infarction in the cerebral cortex. The ligature and clips were then removed after 90-min ischemia to allow reperfusion. All animals received either AM (2 mg/kg intraperitoneal, n=8) or vehicle (10 % DMSO in saline, n=8) injections every three days until 24 days after stroke.

MRI scans. Animals were subjected to serial MRI measurements at 2, 10 and 25 days, respectively, after stroke using a Brucker 9.4T animal MRI scanner. Rats were imaged using DTI, under isoflurane anesthesia (3% for induction and 1.8% for maintenance) in air / O_2 (80:20). DTI were acquired with a spin echo single-shot echo planar imaging sequence, 19 slices with 1mm thickness, FOV = 3.2 cm^2, matrix size = 96x96, TR / TE = 9500 / 38 ms. Thirty diffusion-weighted images along independent orientations (b=1000 s/mm^2) and 1 baseline image (b=0) were acquired for each slice, and the acquisition was repeated three times to improve signal-to-noise ratio. The total acquisition time was approximately 40 min.

Image analyses. Fractional anisotropy (FA) maps were derived using dTV software (University of Tokyo Hospital, Tokyo, Japan). As the lesion size may be a confounding factor interfering with the treatment effects of AM, we divided the rats into the treated and control groups according to similar lesion size measured from the T2-weighted imaging acquired on day 2. A region-of-interest (ROI) was manually drawn within the perilesional hyperintense region on each of the lesion-containing slice shown on the FA map due to its relatively clear boundary. For comparison, an additional ROI was placed in the area of contralateral white matter. After normalizing signal from the perilesional region to the contralteral white matter, the normalized signals (FA ratio, rFA) were compared between the AM-treated and vehicle-treated groups on day 2, 10 and 25 using two-way ANOVA (2 groups by three follow-up time points). A p value < 0.05 was considered statistically significant.

Behavioral assessment. Since AM itself can also alter behavior response, we used an elevated body asymmetry test to reduce pharmacological ambiguity in scoring neurological symptoms for stroke animals after repeated AM administration [2]. Body asymmetry was analyzed using an elevated BS test on day 3 and 18. Rats were examined for lateral movements/turning when their bodies were suspended 20 cm above the testing table by lifting their tails. The frequency of initial turning of the head or upper body contralateral to the ischemic side was counted in 20 consecutive trials. The maximum impairment in body asymmetry in stroke animals is 20 contralateral turns/20 trials. In normal rats, the average body asymmetry is 10 contralateral turns/20 trials (i.e. the animals turn in each direction with equal frequency). The relationship between the rFA changes (difference of rFA between day 2 and 25, $rFA_{25\text{-}2}$) and behavior recovery (difference of BS scores between day 3 and 18, $BS_{18\text{-}3}$) was evaluated using the Pearson correlation coefficient.

Results

Figure 1 shows the rFA changes for animals treated with AM or vehicle after 25 days of stroke. Using a two-way ANOVA, we found that rFA was significantly enhanced by AM treatment ($P<0.05$, Figure 2) or follow-up time ($P<0.05$). There was a significant interaction between treatment and follow-up time ($P<0.05$). Animals treated with AM also showed a significant improvement of body asymmetry ($BS_{18\text{-}3}$ = -5.9±1.8) as compared with control animals ($BS_{18\text{-}3}$ = -2.4±3.6, $P<0.05$). Moreover, our data indicated a correlation between the changes of behavioral recovery ($BS_{18\text{-}3}$) and changes of rFA ($rFA_{25\text{-}2}$) in the AM treated animals (Figure 3, R=-0.79, P=0.02). We did not find any significant correlation between the behavioral recovery and rFA changes in the control animals.

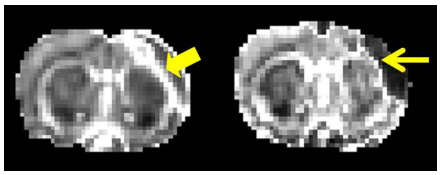

Fig 1. Representative coronal FA maps of an animal from the amphetamine-treated group (left) and an animal from the vehicle-treated group on day 25 (right). Animal from the amphetamine group demonstrated significantly higher FA values in perilesional areas (arrowhead) as compared to the vehicle group (arrow).

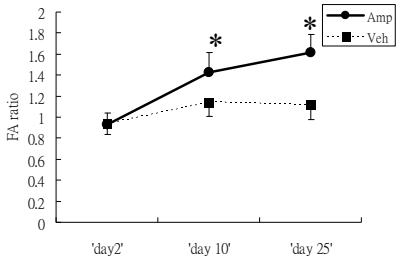

Fig 2. Time course changes of normalized FA in animals with ischemia. Amp: amphetamine-treated; Veh: vehicle-treated. *$P<0.05$ for Amp vs. Veh.

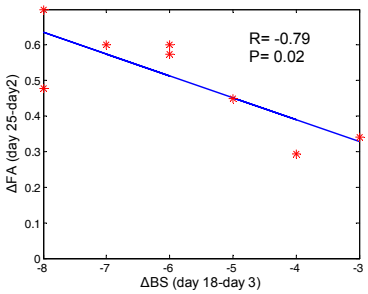

Fig 3. Correlations between $rFA_{25\text{-}2}$ (y axis) and $BS_{18\text{-}3}$ (x axis) for animals treated with amphetamine. A significant correlation was found with correlation coefficient R = -0.79 and $P = 0.02$.

Discussion and conclusions

In the present study, we demonstrated a correspondence between the temporal changes of FA in perilesional areas and locomotor function in stroke rats treated with AM, suggesting that the structural integrity may be used as an imaging biomarker to predict locomotor function recovery. Restitution of lost function and reorganization of brain structure after stroke have been demonstrated in several animal and human studies, especially for reinstatement of activity in ipsilesional regions, which has been frequently observed at later stages [4]. Our finding provides an evidence to support that AM enhances neuronal intetrigy in peri-lesioned tissue, which may contribute to the recovery of neurological function after ischemic stroke. The poststroke increase in locomotor recovery may be brought by remodeling of neuronal elements after AM treatment. Improved insights into the relationship between behavioral recovery and reorganization of brain structure may provide a valuable tool to monitor therapeutic effects for clinical interventions.

References: [1] Stroemer RP, et al., Stroke 1998; 29: 2381-2395. [2] Liu H, et al., Neuroimage 2011;56: 280-9. [3] Barlongan CV, et al., NeuroReport 1998 ; 9: 3615-21. [4] van Meer MP, et al., Neuroscience 2010; 30:3964-72.