Increased deep grey-matter functional connectivity of post-stroke hNSC implanted ipsilesional putamen

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Although stroke results in focal brain tissue loss, remote effects consequent to the disruption of structural and functional networks are recognised. Resting-state fcMRI has the ability to image subjects with a broad range of impairments following stroke without the interpretive confound of variable task performance seen in task-based paradigms. Significant proportion of stroke survivors has permanent residual disability, indicating limited endogenous recovery capacity. In rat middle cerebral artery occlusion models (MCAo), CTX0E03 hNSCs (clonally derived from human foetal cortical epithelium) injected to the striatum adjacent to the infarct 4 weeks after MCAo showed improvement in behavioural outcome measures along with histological evidence of increased host angiogenesis and neurogenesis.[1] We examined the effects of intra-cerebral hNSC implantation in the putamen on functional networks assessed by longitudinal resting state functional connectivity and explored associations with clinical measures.

Patients for this study were from the PISCES trial[2] a phase-1, open-label study of intra-cerebral stereotactic implantation of CTX0E03 hNSCs to the ipsilateral putamen in chronic ischaemic stroke patients. Eleven patients (NIHSS≥6, mRS=2-4) with a first ischaemic stroke 6 months to 5 years previously were recruited. Cells were implanted under general anaesthesia and patients followed up over 2 years. Assessments covered neurological impairment (NIHSS), disability (mRS), spasticity (modified Ashworth scale) and activities of daily living (Barthel Index).

Brain MRI was performed on 3 occasions, baseline (1-month prior), M1 (1-month after) and M12 (1-year after implantation) on a 3T GE Signa-Excite-HDxt scanner. Acquisition parameters included T1W (Time to Echo (TE) 8.5ms, Time to repetition (TR) 2500ms, Inversion time (TI) 920ms), T1W IR-FSPGR 3-dimensional (TE 1.5ms, TR 7.2ms, TI 500ms, FOV=24 mm^2, flip angle=12^0), T2W PROP Fast Spin Echo (TR 5000ms,TE 109.2ms), T2* gradient echo (TE 22ms, TR 670ms, flip angle 10°) and T2W FLAIR (TE 140ms, TR 10000ms, TI 2250ms, slice thickness 5mm, slice gap 1.5mm) sequences. Five minute (96 volumes) resting state functional scans were acquired with a gradient echo, echo-planar sequence sensitive to blood-oxygenation-level-dependent (BOLD) contrast (TR=3000ms, TE=30ms, 29 interleaved 5 mm slices, FOV=24 mm^2, matrix 128x128, flip angle=80^0) during which patients remained awake with eyes closed.

Pre-processing steps have been previously published.[3] Using Analyze 10, structural images were used for manual segmentation of infarcts, and masks created. Segmentations were reviewed by two neurologists. Similarly, ipsilesional and normal putamina were segmented and regions-of-interest (ROIs) created. In the first seed based approach, whole brain voxel-wise resting state functional connectivity maps were computed for each putamen by extracting the BOLD time course and computing the correlation coefficients (Pearson r). Coefficients were converted to a normal distribution by Fischer's *z* transform thereby generating z(r) maps. *Z*-score maps were combined across subjects by using a fixed-effects analysis. Some scans were flipped so all lesional hemispheres were on the right and normal on the left, before further statistics. In the second approach, pair-wise functional connectivity between 6 ROIs including 2 in the normal hemisphere (thalamus and caudate) and 4 in the infarct hemisphere (caudate, putamen and parietal cortex) were extracted before further statistics.

Paired t tests were used between 3 time-points. Group-based z-score maps were corrected for multiple comparisons at a significance of $P<0.05$ ($z=2.5$, cluster size=53 voxels). Correlations, exploring change in clinical scores and change in voxel-wise connectivity between the 3 time-points, were tested. For the pair-wise functional connectivity correlations, Cohen's d was calculated for every pair.

Seven eligible patients (3 visits with analysable MRI data) were included. Four patients were excluded (one refused month1 functional scan, one could not complete baseline functional sequences due to back pain and 2 had poor quality month12 scans). Mean infarct volume 36ml (range 6.5 to 68) included 5 cortical and 2 sub-cortical infarcts (four right and 3 left hemispheric). At month1 after implantation of hNSCs, compared to baseline, the group FC map showed increased connectivity with bilateral caudate and contralateral thalamus (Figure: A, z=6, z=0) and reduced connectivity with ipsilateral parietal lobe (Figure: A, z=45, z=51). At month12 compared to baseline, only decreased connectivity was observed with the contralateral parietal cortex. There was no significance observed between month1 and month12. The connectivity of the normal putamen showed no statistical significance at any time points.

Mean modified-Ashworth arm sub-scores for the 7 subjects at baseline were 17.3 (range3-30) which reduced at month1 to 14.5 (1-25) and were stable at month12 at 14.3 (1-27). The change in arm spasticity scores at month1 correlated (r=0.52) with change in functional connectivity (Figure: B1, B2). The other clinical scores NIHSS, BI, summated arm and leg Ashworth scores showed no correlation with functional connectivity in this group of patients. Other clinical analyses have been published.[2] Among the 6 ROIs included in the pair-wise combinational analysis, 6/15 pairs of ROIs (Figure: C) showed a median 0.37 (range 0.07–0.55) increase in effect size r of functional connectivity measured at month1 compared to baseline.

Patients with motor deficits demonstrate interhemispheric connectivity disruption compared to controls and stroke patients with no motor impairment, which is re-established in spontaneously recovered patients. [4] This 'first-in-man' trial had no a-priori hypothesis for the NSCs' effects on connectivity. Mechanisms of action include cell differentiation, paracrine or trophic effects, potential improved white-matter integrity, stimulation of endogenous neuro or angiogenesis. The natural history of connectivity in chronic stroke is unclear. Park et al.,[5] observed, in a longitudinal study, that preservation of connectivity between ipsilesional motor cortex and contralesional thalamus positively correlated with motor improvement (Fugl-Meyer scores),6months after stroke. Restoration of connectivity between ipsilesional putamen and contralesional thalami may have similar implications that could be used as a recovery imaging bio-marker. Limitations to our study includes, small patient numbers (n=7), unblinded, no control group, short duration (5 minutes) resting state data with considerable head movement, thereby at-risk of considerable confounding factors that impede robust imaging inference. Although longitudinal is better than cross-sectional data, lack of significant effect at month 12 raises possibility of reported results to-be chance alone or an un-sustained treatment effect. Control group selection is challenging due to invasive protocol and primarily safety, stage of research. Imaging biomarkers offer valuable insights into the biological effects of stem cells. Further work, in larger patient cohort is required.

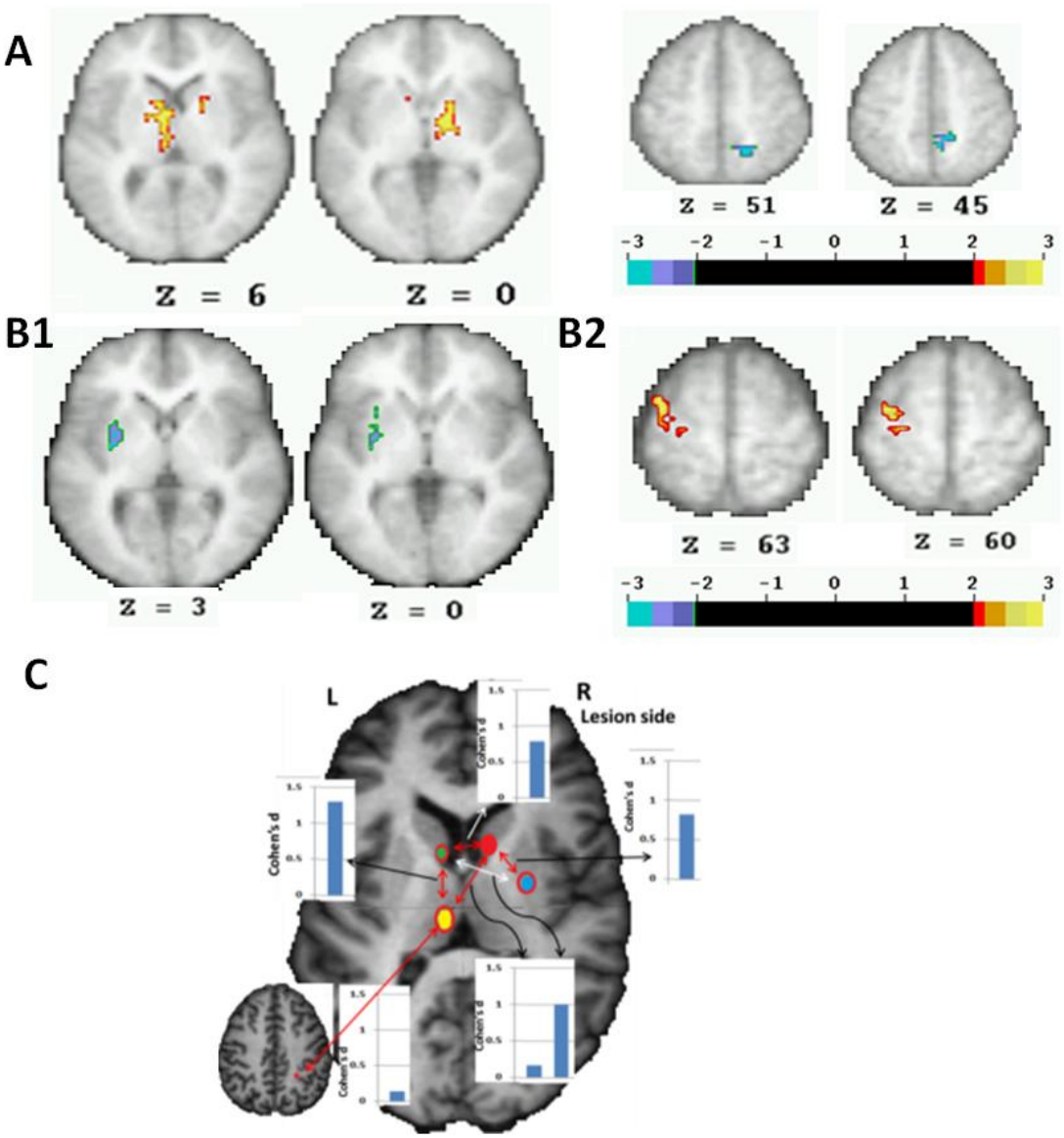

Figure: **A**. Group functional connectivity map of lesional putamen at month 1 compared to baseline. Statistically significant ($p<0.05$; $z=2.5$; minimum voxels 53) and multiple comparisons corrected. Lesional side is on the right hand side. **B1**. Group map of negative correlation with contralateral putamen and **B2**.Group map of positive correlation with prefrontal motor cortex between change in functional connectivity and change in modified Ashworth arm score. **C**. Effect size of positive change in correlation coefficients at month 1 compared to baseline. Red = Caudate (Lesional side), Blue = Putamen (Lesional side), Green = Caudate (Normal side), Yellow = Thalamus (Normal side).

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Ethics Approval: National Research Ethics Committee (Formerly Gene Therapy Advisory Committee, United Kingdom)

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