# Transcriptome Analysis of Motor Cortex Network Responses in Spontaneously-recovered Stroke Mice

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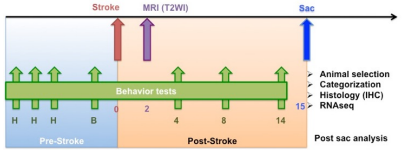
## Introduction

The molecular response following brain ischemia is very complex, and the mechanisms underlying spontaneous recovery are unclear. This study investigates the molecular mechanisms driving spontaneous recovery after unilateral stroke using RNA sequencing (RNAseq) transcriptome analysis of the ipsi-lesional and the contra-lesional primary motor cortex (iM1 and cM1).

## Methods

Unilateral ischemic stroke was induced in C57bl6 adult male mice (n=75) by an intraluminal MCA suture occlusion (30min). To monitor post-stroke spontaneous recovery without any treatment, horizontal rotating- and vertical stationary-beam test, and neurological score were evaluated. Infarcts were visualized by T2WI at post-stroke day (PD) 2 using 7-Tesla MR scanner or histology at PD15. Total RNA was extracted from primary motor cortices from both hemispheres. RNAseq (Hiseq4000, Illumina) transcriptome, and pathway analysis (IPA, QIAGEN) were performed.

### Experimental Timeline




Mice were pre-trained on the behavior tests prior to collecting baseline data. After stroke on Day0, infarct imaging, behavior tests were performed. All mice were sacrificed at Day15 and processed for the post-sacrifice analysis.

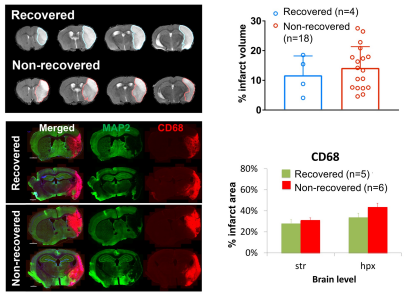
### Animal inclusion/exclusion criteria


All stroke mice included for analysis survived, exhibited similar pre-stroke behavior, post-stroke day 4 deficits based on their rotating beam performance, and cortico-striatal infarct, as verified by MRI or histology (n=22, 11).

## Results (highlights)

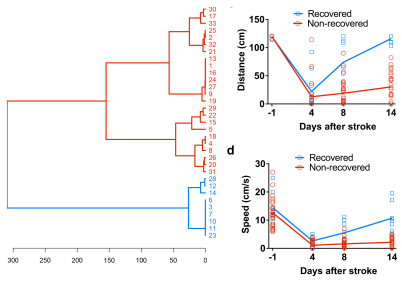
We observed 1) a spontaneous recovery of function in mice after stroke without any treatment. 2) stroke mice with comparable lesion size and location can exhibit different recovery outcome. 3) distinct transcriptome changes of M1 between recovery outcome difference.

### Lesion size was similar in recovered and non-recovered mice




Representative T2WI (PD2) and Histology (PD15) from both groups showed similar cortico-striatal infarct. Quantitative data expressed as mean ± sem.

### Stroke mice exhibit different spontaneous recovery outcome.

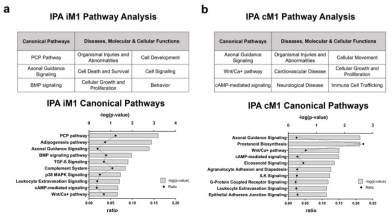



Hierarchical clustering of the rotating beam test (D14 distance) demonstrated 2 distinct clusters in 33 analyzed animals. Gap-statistics confirmed the number of clusters. Behavior data were graphed based on the cluster separation by recovered and non-recovered animals (n=9 and 24).

## Conclusions

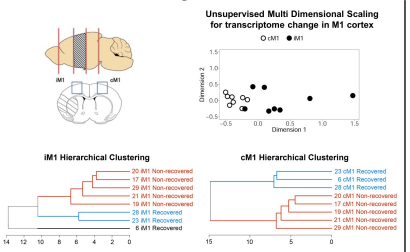
Our study demonstrates that stroke mice with similar cerebral stroke lesion can exhibit significant differences in their behavioral recovery outcome. RNAseq transcriptome analysis revealed that molecular response in contralesional primary motor cortex but not in ipsilesional one has distinct feature relating recovery outcome. These results suggest that molecular-network response in cM1 may be critical for spontaneous recovery after stroke. Thus, axonal guidance signaling and Wnt/Ca+ signaling (neuroplasticity-related) as well as cAMP-mediated signaling (neuroinflamatory or cerebral blood flow an metabloism related) in cM1 may be associated with spontaneous resovery after stroke

### Top IPA-knowldge based canonical pathways and Diseases/Bio-functions involved by DEGs either in iM1 or cM1 in recovered stroke mice




Pathway analysis for differentially expressed genes (DEGs) in recovered stroke animals either in iM1 or cM1 showed multiple endogenous pathways related to recovery, including neuroplasticity -, neuroinflammatory- and crebral blood flow and metabolism-related molecular network-responses.

### Cluster analysis of transcriptome changes in M1 cortices demonstrated distinct profile in cM1 separated by recovery outcome




Result shows the transcriptme profiles from 8 animals (recovered, 3; non-recovered, 5) of both M1 (Upper right) or either (lower panels) of iM1 or cM1. Dendrograms showed clear recovery outcome dependent separation in cM1 gene expression profile but not in iM1, suggesting that molecular-network response in cM1 may be critical for spontaneous recovery after stroke.