Identification of neuronal injury and survival mRNA pathways after spreading depolarization

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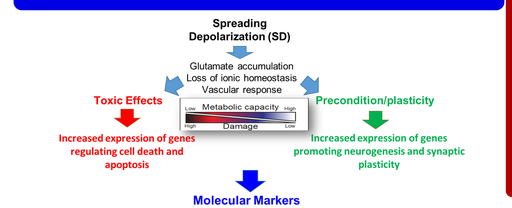
BACKGROUND

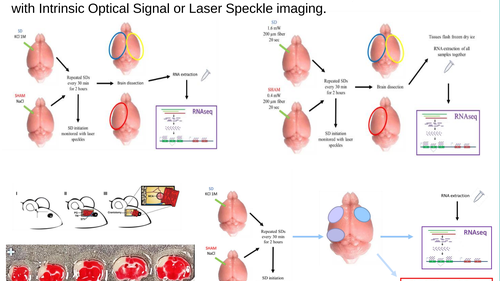
- Spreading depolarization (SD) is a slowly propagating wave of profound depolarization that sweeps through cortical tissue. SD is not harmful to healthy brain tissue, but can cause irrecoverable injury to metabolically compromised tissue, and thus, causing expansion of acute brain injuries.
- Several genes have been previously found increased after SDs in healthy brain tissue:
  - **BDNF, c-Fos and ARC** (Kokaia et al 1993; Kariko et al 1998; Rangel at al 2001; Dietrich et al, 2000; Kaido et al 2012)
  - **TNF-α, IL-1β** and 6 (Takizawa 2019)
  - **COX2 and BCL-2** (Kaido et al 2012)
- Increases in **BDNF** expression have been implicated in ischemic preconditioning (Yanamoto et al 2004).
- Previous studies have also shown activation of **synaptic plasticity** (Sadowska et al 2021) and adult **neurogenesis** following SD (Urbach et al 2017).
- **The present study aimed to perform an extensive analysis to identify a more complete range of biological pathways modified by SD in healthy and injured brain tissues, using RNA-seq**.

HYPOTHESIS



METHODS

SDs were induced repetitively (4 SDs at 30 min intervals) in both healthy mice or in a model of stroke (dMCAO). Two hours after onset of the initial SD, cortical slices were collected and/or total RNA was extracted, total cortical RNA was extracted and subjected to Illumina paired-end RNA-seq to identify differentially expressed (DE) genes (fold change >1.25, p value <0.05). SDs were confirmed with Intrinsic Optical Signal or Laser Speckle imaging.



Stroke was induced in mice through dMCAO, subsequently 4 SDs were induced. Brain slices were collected and subjected to spatial sequencing (GeoMX) to determine Differential Gene Expression (DGE)

RESULTS

After a series of 4 SDs in healthy anesthetized mice:

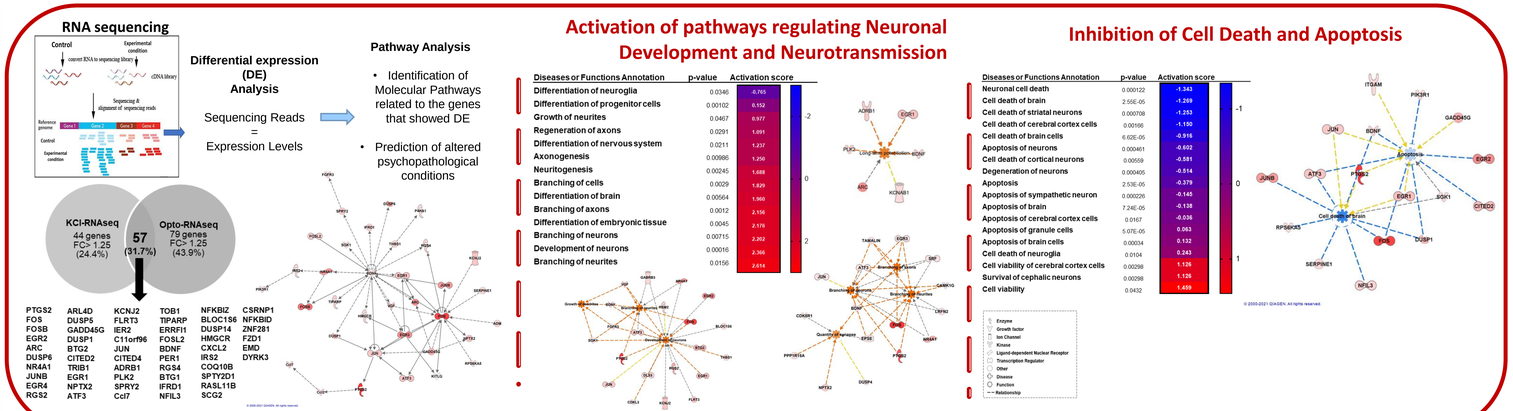
We identified 101 significantly upregulated genes after KCl-induced SDs and 136 after optogenetic SD induction; of these 57 genes (31.7%) where commonly expressed.

Consistent with previous studies, top differentially-expressed (DE) genes include the neurotrophic factor BDNF, as well as intermediate early genes ARC, FOS, JUN and EGR. Among other DE genes with significantly increased levels were HOMER1a and c, COX2, NR41, DUSP6, and KCNJ2.

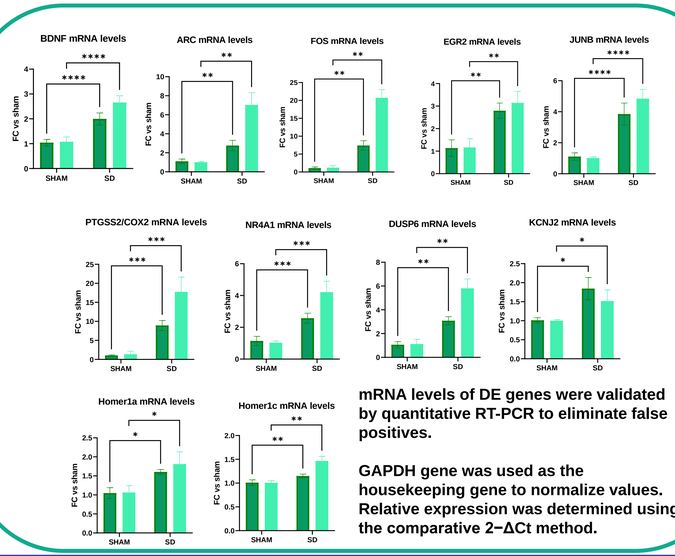
Through pathway analysis we found significant increases in the expression of genes associated with axogenesis, branching of axons, neuritogenesis, dendritic growth, and regeneration of neurites.

Interestingly, we also found a significant decrease in expression in genes associated with cell death, apoptosis and neuronal degeneration.

PATHWAY ANALYSIS



qPCR VALIDATION



mRNA levels of DE genes were validated by quantitative RT-PCR to eliminate false positives.

GAPDH gene was used as the housekeeping gene to normalize values. Relative expression was determined using the comparative 2−ΔCt method.

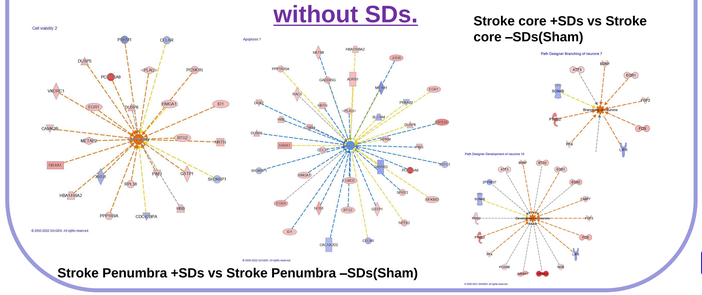
STROKE MODEL

Stroke was induced in mice through dMCAO, subsequently 4 SDs were induced.

RNA was extracted from 3 different areas and subjected to RNAseq to determine DGE in:

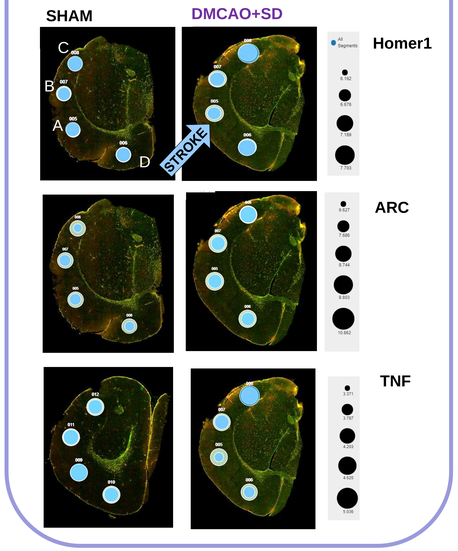
A) Stroke core +SDs vs Stroke core –SDs(Sham)
B) Stroke core +SDs vs Stroke Penumbra +SDs
C) Stroke Penumbra +SDs vs Stroke Penumbra –SDs(Sham)

Pathway analysis showed that repeated SDs induce the activation of protective pathways in the stroke core compared to the stroke penumbra or stroke tissues without SDs.



SPATIAL GENOMICS

Through Spatial Genomics (GeoMX) DGE was determined in 4 different areas: A) STROKE core, B) temporal, C) dorsal and D) remote.



CONCLUSIONS

- **These results indicate that SD in both healthy and injured brains causes relatively rapid upregulation of pathways that could potentially be involved in plasticity or circuit modification.**
- **Additionally, downregulation of pathways involved in neuronal injury could contribute to previous reports of ischemic preconditioning by SD.**
- **These results also identify a range of novel targets that could be used to test whether clusters of SD may enhance plasticity or recovery in surviving peri-infarct tissue, in addition to the well-established role of SD in expansion of the infarct core.**

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SUPPORT

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