Single-synapse analysis of synaptic remodeling in the post-stroke rodent brain

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Introduction

Functional recovery after stroke is thought to involve structural plasticity in circuits adjacent to the lesion (1). Deciphering these changes at the synaptic level is key to understanding the re-organization of the synaptic circuitry. Here we describe the use of array tomography (2,3), a new high-resolution imaging method, to determine the composition of glutamate and GABA synapses in the post-stroke mouse brain. Electrophysiology was done to determine if these quantitative changes resulted in functional changes.

General Methods

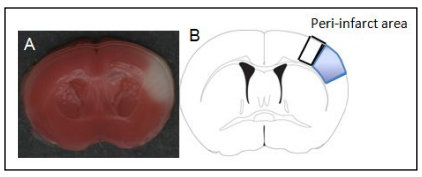


Fig. 1: dMCAO model
in 12-week-old C57BL/6J male mice The box in (B) indicates the peri-infarct tissue taken for array tomography. Whole-cell patch clamp recordings were performed in neocortical brain slices to evaluate synaptic properties of neocortical pyramidal neurons in the peri-infarct cortex.

Array Tomography

Tissue Preparation: 300 µm brain sections were cut, tissue dissected from the peri-infarct cortex , dehydrated inethanol, embedded in LR-white resin, enabling ribbons of 70nm thick sections to be cut and subsequently stained and imaged for synapses.

Synapse Type	Antibody	Antigen Localization	Vendor
All synapses	Synapsin I	Pre-synaptic	Millipore
Glutamatergic	VGluT1	Pre-synaptic	Millipore
	VGluT2	Pre-synaptic	Millipore
	PSD-95	Post-synaptic	NeuroMab
GABAergic	GAD	Pre-synaptic	Abcam
	VGAT	Pre-synaptic	Synaptic Systems
	GABAARα1	Post-synaptic	NeuroMab

Table 1: Synaptic antibodies
(immunofluorescence staining).

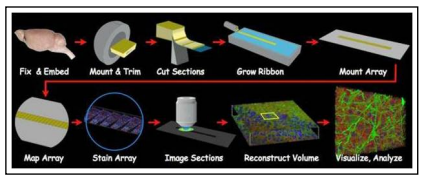


Fig. 2: Schematic representation of the array tomography method
Array tomography improves image resolution in the Z-plane by cutting ribbons, or 'arrays', of serial ultra-thin (70 nm) sections, , and then digitally recombining these image stacks to form an image of the tissue. The array can be repeatedly eluted, restained, and fluorescently imaged.

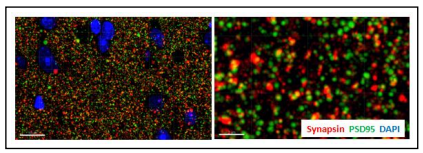


Fig. 3: Reconstructing tissue from image stacks using Fiji

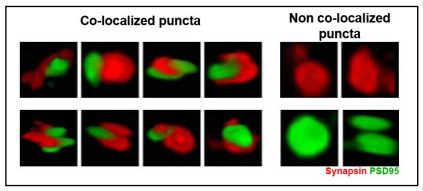


Fig. 4: High resolution images of synapses.

•Glutamatergic synapses: colocalization of synapsin Ⅰ and PSD-95 with either: (post)
①VGluT1 ②VGluT1+2 ③VGluT2
•GABAergic synapses: colocalization of synapsin Ⅰ, GAD, VGAT, and GABAAR (post)

Table 2. Definition of synapse sub-types
We used a synaptogram with a machine learning based algorithm, that is a way to visualize multi-channel volumetric image data.

Results

A. Array Tomography

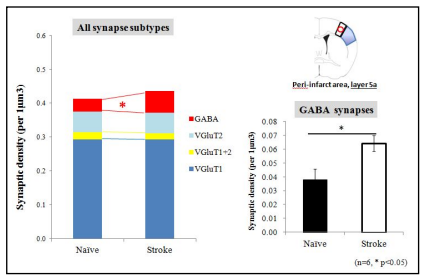


Fig. 5: Synaptic density in layer 5, 1wk after stroke, peri-infarct area
The density of GABAergic synapses was increased (n=6, *p<0.05).

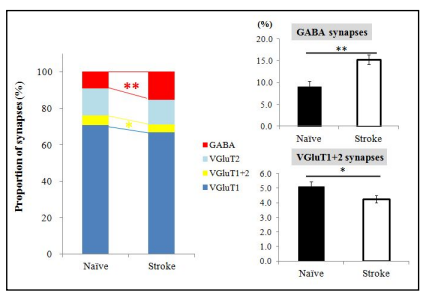


Fig. 6: Proportion of Synapse subtypes in layer 5, 1wk after stroke, peri-infarct area
The percentage of GABAergic synapses was increased (n=6, * p<0.05), with a corresponding decrease in the % of glutamatergic synapses.

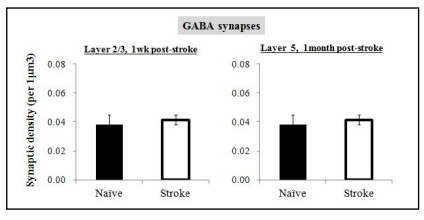
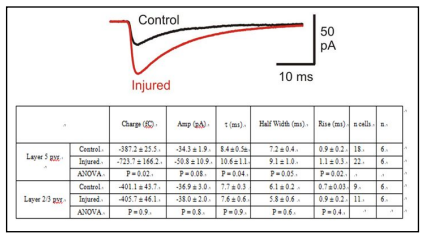


Fig. 7: Density of GABA synapses in layer2/3, 1wk after stroke and layer 5, 1month after stroke,
There was no difference between naive and stroke groups (n=6).

B. Electrophysiology



		Charge (fC)	Amp (pA)	τ (ms)	Half Width (ms)	Rise (ms)	n cells	n
Layer 5 pyr	Control	-387.2 ± 25.5	-34.3 ± 1.9	8.4 ± 0.5a	7.2 ± 0.4	0.9 ± 0.2	18	6
	Injured	-723.7 ± 166.2	-50.8 ± 10.9	10.6 ± 1.1	9.1 ± 1.0	1.1 ± 0.3	22	6
	ANOVA	P = 0.02	P = 0.08	P = 0.04	P = 0.05	P = 0.02		
Layer 2/3 pyr	Control	-403.1 ± 43.7	-36.9 ± 3.0	7.7 ± 0.3	6.1 ± 0.2	0.7 ± 0.03	9	6
	Injured	-405.7 ± 46.1	-38.0 ± 2.0	7.6 ± 0.6	5.8 ± 0.6	0.9 ± 0.2	11	6
	ANOVA	P = 0.9	P = 0.8	P = 0.9	P = 0.6	P = 0.4		

Table 3: Enhanced GABAA receptor-mediated currents in layer 5 were observed in layer 5 but not layer 2/3 peri-infarct cortex at 1 week.

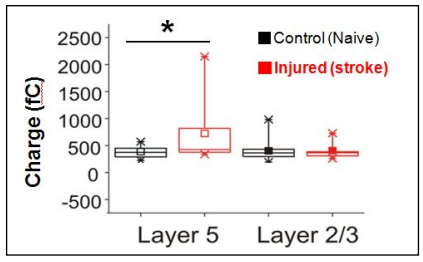


Fig. 8: Layer 5 but not layer 3 pyramidal cell sIPSC charge was enhanced in peri-infarct region. Enhanced IPSCs are specific to the pyramidal neurons, not interneurons.

C. Behavior Test

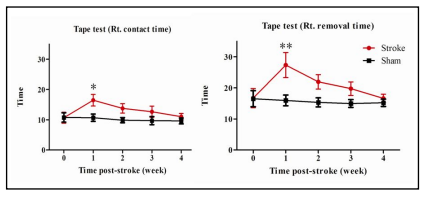


Fig. 9: Adhesive tape test (* p<0.05, ** p<0.01 comparing stroke to naïve animals, n=10). There was a significant difference only at 1week post-stroke. These changes were correlated with changes in GABAA receptors.

Summary

At 1 week post-stroke, an increase in the density and proportion of GABAergic synapses was observed in layer 5 of the peri-infarct cortex. Changes in GABA synapses were transient and returned to basal levels by 1 month. These changes were only found in layer 5 but not layer 2/3. GABAA receptor-mediated currents were enhanced in layer 5 pyramidal neurons at 1 week post-stroke.

Conclusions

Together, our results suggest that stroke leads to an increased expression of functional GABAA receptors in peri-infarct neocortex and that these changes are layer- and cell type-specific.
These changes could underlie a mechanism of post-stroke functional recovery and remapping of surviving circuits.

References

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Acknowledgements

This work was funded by NIH NINDS RO1 NS058784, William Randolph Hearst Foundation, Russell and Elizabeth Siegelman, Bernard Lacroute, and Ronni Lacroute. We thank Stephen Smith for providing images for figures.