

Perspective

Structural integrity and remodeling underlying functional recovery after stroke

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Stroke is the second leading cause of death worldwide with about 50% of survivors being chronically disabled (Donkor, 2018). The behavioral improvement seen in stroke patients in the first weeks after a stroke is contributed by behavioral compensation, reorganization in somatotopic maps and activity in peri-infarct but also distant regions which are connected to the stroke area as supported by animal studies. Spontaneous recovery is related to region-specific changes in recovery-related genes (Ito et al., 2018), growth factor expression, axonal sprouting and dendritic spine turnover (Murphy and Corbett, 2009). These brain plasticity processes follow an intrinsic time-line with a limited period of heightened neuroplasticity for which the behavioral experience is a key modulator. However, experimental studies correlating structural and functional brain network changes during recovery are scarce and it remains controversial which mechanisms, regions and time points are most relevant and thus best suited for translational interventions. In that context, longitudinal *in vivo* imaging will be essential. MRI is particularly suited for repetitive, non-invasive imaging with high spatial resolution. Clinical stroke studies use routinely MRI in a standardized acquisition and post-processing regime for measuring the stroke size and location. In addition, structural connectivity analyses using diffusion MRI (dMRI) in the corticospinal and corticocortical fiber tracts effectively predict motor impairment and improvement, respectively (Koch et al., 2016). Stroke disrupts brain connectivity by primary mechanisms such as cell death and injury in white matter tracts, but also secondary mechanisms such as axonal degeneration which spreads to structurally connected cells (Cao et al., 2020). On the macroscopic level,

dMRI, sensitive to tissue-specific water diffusion properties, offers the unique possibility to quantify microstructural changes, e.g. related to stroke-related processes of cell swelling, cell lysis and demyelination longitudinally on the whole brain level expressed in the dMRI measures of axial, radial, mean diffusivity, and fractional anisotropy. Furthermore, fiber tracking, a more complex post-processing and mathematical modeling of dMRI exploits the preferential diffusivity of protons along the myelin sheets to generate a quantitative measure of fiber tracts between brain regions. Experimental studies in rats and mice only recently evolved due to technical challenges such as the required signal-to-noise and susceptibility-induced image distortions (Hoehn and Aswendt, 2013). Based on pioneering work more than 20 years ago in a mouse model of reversible focal ischemia measuring the acute temporal dynamics of diffusion, so far, only *ex vivo* Diffusion Tensor Imaging (DTI) fiber tracking was applied in stroke mice to compare selected ipsi- vs. contralesional tracts (Granziera et al., 2007).

With the aim to characterize for the first time the lesion size and location-dependent white matter reorganization after cortical stroke in mice over 4 weeks, we applied a comprehensive experimental protocol. Specific care was taken to perform the study under standardized conditions related to the ARRIVE and IMPROVE guidelines, e.g. to ensure that the experimenters were blinded against the experimental groups and that the data acquisition and recording followed standard operating procedures for which we used a custom cloud-based relational database (Pallast et al., 2018). We applied photothrombosis to induce cortical lesions in the sensorimotor cortex, behavioral tests, multiparametric MRI

(acquired at 9.4T), and histology. MRI and microscopy were analyzed using our standardized software pipeline tailored to the specific needs of mouse brain MRI transformed to the Allen Mouse Brain Atlas framework (Pallast et al., 2019). With a unique level of detail, we were able to map the lesion size and location in the acute phase - 1 week after stroke - using high-resolution T2-weighted MRI with an in-plane resolution below 70 μ m. The *in vivo* results were correlated to the lesion size measured on GFAP immunohistochemistry, which was also registered to the mouse brain atlas. With that quantitative mapping, it was possible to identify the regions mostly affected by stroke and to correlate the lesion size and location directly with behavioral measures. We measured the influence of the brain lesion on motor function and spontaneous recovery with the cylinder, grid walk and rotating beam test. These tests were selected as they record natural exploratory behavior of rodents and detect long-term deficit after stroke without the need for long training time or reward. We were able to measure the differences in spontaneous recovery in two experimental groups with “small strokes” mainly in the primary somatosensory cortex and “large strokes” with approximately 2-fold larger lesions and a larger involvement of primary and secondary motor areas. Importantly, the lesion size and location had a strong effect for the impairment after stroke but both groups reached similar scores at 4 weeks after stroke. In order to determine the white matter differences between the small and large strokes we measured the degeneration of the thalamocortical tract using dMRI which increased radial diffusivity, a sign of demyelination, in the ipsilesional thalamus only. In this line, the DTI fiber tracking in the ipsilesional thalamocortical pathway revealed a strongly reduced connectivity in the large stroke group only. Both *in vivo* results were correlated with degeneration-specific accumulation of reactive astrocytes and immune cells in the sensory-motor cortex related part of the thalamus (DORsm). These results are in line with previous descriptions of secondary degeneration

Perspective

of thalamocortical fiber tracts and secondary inflammatory injury in the thalamus after cortical strokes (Cao et al., 2020). In addition, we verified that our DTI fiber tracking for the DORsm as a seed region was in line with the viral tracing data for that region provided by the Allen Mouse Brain Connectivity Atlas. With that robust toolbox, we further compared the structural connectivity in transhemispheric corticocortical and corticothalamic pathways. Interestingly, in both scenarios and independent of stroke size, the structural connectivity was decreased in the acute phase, however, developed differently over time. At the final time point, when small and large strokes were not different in terms of sensorimotor behavior anymore, the structural connectivity from contralesional DORsm to the ischemic cortex regions and primary motor cortex to the ischemic cortex regions reached control (no stroke) levels and increased from day 1 to day 28 after stroke only in the large stroke group, respectively.

Discussion and future directions: There is evidence that the level and location of axonal sprouting depends on the stroke size. Small strokes might solely rely on per-infarct tissue, whereas larger strokes require axonal sprouting in neurons on the contralateral hemisphere (Carmichael, 2016). As postulated before, we could show with *in vivo* DTI that mice with large strokes in contrast to small strokes recruit to a larger extent contralesional brain regions and to balance the structural connectivity back to control (pre-stroke) levels.

A major goal will be to align the structural connectivity changes with changes on the functional level by combining DTI with fMRI, electrophysiology or *in vivo* calcium imaging and 3D histology (Goubran et al., 2019). In order to predict which pathways will be recruited and which function will be remapped for targeted

therapies, connectivity correlations with behavioral outcome need to be accompanied in a more standardized framework, e.g. by applying graph-theoretical whole brain network analysis (Pallast et al., 2020).

Future translational stroke studies need to focus on how to best extract the information in a standardized, unbiased approach in statistically relevant cohorts for multiple stroke models and strokes in different locations. Biomarker studies using multi-parametric MRI can be a relevant approach for predicting key brain areas in the spontaneous recovery process as one element in a large multimodal approach to overcome correlations and gain causality in the biology of functional recovery after stroke.

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