Translating functional connectivity after stroke: fMRI detects comparable network changes in mice and humans

*Stefan J. Blaschke MD1,2,3\*, Lukas Hensel MD1,3\*, Anuka Minassian PhD2, Susan Vlachakis MVD1,2, Caroline Tscherpel MD1,3, Sabine U. Vay MD1, Monika Rabenstein MD1,2, Michael Schroeter MD1,2,3, Gereon R. Fink MD1,3, Mathias Hoehn PhD2,3, Christian Grefkes MD PhD1,2,3#, Maria A. Rueger MD PhD1,2,3#*

1 University of Cologne, Faculty of Medicine and University Hospital, Department of Neurology, Cologne, Germany

2 In-vivo-NMR Laboratory,Max Planck Institute for Metabolism Research, Cologne, Germany

3 Cognitive Neuroscience Section, Institute of Neuroscience and Medicine (INM-3), Research Centre Juelich, Juelich, Germany

\*both first authors contributed equally to this work

#both senior authors contributed equally to this work

**Corresponding author:**

Maria Adele Rueger, M.D., Ph.D.

Department of Neurology, University Hospital of Cologne

Kerpener Strasse 62; 50924 Cologne, Germany

Phone: +49-221-478-32028; Fax: +49-221-478-89143

Email: maria.rueger@uk-koeln.de

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# Abstract

Background and Purpose

The “translational roadblock” has long impeded the implementation of experimental therapeutic approaches for stroke into clinical routine. Considerable interspecies differences, e.g., in brain anatomy and function, render comparisons between rodents and humans tricky, especially concerning brain reorganization and recovery of function. We tested whether stroke-evoked changes in neural networks follow similar patterns in mice and patients using a systems-level perspective.

Methods  
We acquired resting-state functional magnetic resonance imaging (rs-fMRI) data during the early post-stroke phase in a sample of human patients and compared the observed network changes with data from two mouse stroke models, i.e., photothrombosis (PT) and distal middle cerebral artery occlusion (MCAO). Importantly, data were subjected to the same processing steps, allowing a direct comparison of global network changes using graph theory.

Results  
We found that network parameters computed for both mouse models of stroke and humans follow a similar pattern in the post-acute stroke phase. Parameters indicating the global communication structure’s facilitation, such as small worldness and characteristic path length, were similarly changed in humans and mice in the first days after stroke. Additionally, *small worldness* correlated with concurrent motor impairment in humans. Longitudinal observation in the subacute phase revealed a negative correlation between initial *small worldness* and motor recovery in mice.

Conclusions  
We show that network measures based on rs-fMRI data after stroke obtained in mice and humans share notable features. The observed network alterations could serve as therapeutic readout parameters for future translational studies in stroke research.

# Non-standard Abbreviations and Acronyms

rs-fMRI resting-state functional magnetic resonance imaging

PT photothrombosis

dMCAo distal middle cerebral artery occlusion

CC normalized mean clustering coefficient

CPL normalized characteristic path length

# Introduction

A “translational roadblock” has long impeded the implementation of experimental therapeutic approaches for stroke into the clinical routine.1 These shortcomings are mainly attributed to insufficient or inapplicable experimental models used to generate preclinical data.1, 2 Moreover, readouts of therapeutic efficacy often differ significantly between animal models of stroke and clinical studies, leading to a stepwise efficacy decline and raising the question of transferability of experimental study results.3

One significant shortcoming of preclinical research is its almost exclusive focus on molecular and cellular processes. Cerebral ischemia, however, also triggers effects at the systems level that are strongly linked to neurological impairment and recovery of function.4 Additionally, a mouse brain dramatically differs from that of a stroke patient: A mouse brain has no gyri and sulci, a relatively small white matter - grey matter ratio, and a fundamentally different functional organization. Furthermore, even in severe initial deficits following stroke, mice can recover relatively fast, while in humans, a severe deficit is a robust predictor for poor recovery.5 This raises whether the mouse brain is suited to serve as a systems-level model for stroke reorganization in human patients.

We and others have previously suggested noninvasive *in vivo* imaging to generate suitable readouts that may help to close the translational gap between experimental models and patient studies.2, 6, 7 Since it is applicable both in humans as well as mice, rs-fMRI constitutes a promising approach to obtain comparable functional readouts of brain activity across species. When examining functional connectivity in both humans and rodents after ischemic stroke, previous studies have consistently found connectivity alterations even in remote brain areas connected to the lesioned area, including interhemispheric connections of sensorimotor networks.8-10 Longitudinal behavioral- and fMRI-data assessments suggest that network alterations are related to motor deficits in humans 11, 12 and rodents.8 That is, failure to reestablish connectivity within motor regions11 or aberrant recruitment of contralateral motor areas12 is associated with poor motor recovery. On the same note, Wang et al. described network alterations inside the motor execution network within the first year after stroke as assessed by global and local graph parameters and demonstrated that the network parameters could predict the recovery degree after stroke.10 Likewise, longitudinal neuroimaging in rats for up to 70 days after stroke revealed distinct network patterns corresponding to severity of motor impairment. 13 While the synopsis of previous studies provides promising evidence for applying rs-fMRI in translational stroke research, it should be noted that all these studies were conducted independently, varying in the timing of data acquisition, statistical methods, and region of interest selection. Hence, it remains to be demonstrated that rodent models reflect the network alterations observed after stroke in humans and whether those network alterations correlate with clinical parameters of stroke severity.

Therefore, we used rs-fMRI to compare stroke-related network changes in humans and rodents suffering from motor deficits due to a first-ever stroke. Using a uniform methodological approach to assess network changes, we could compare different network parameters between species directly. We hypothesized that (i) experimental mouse models of stroke generate analogous alterations in functional neuronal networks as observed in a human stroke and that (ii) these alterations can be reliably characterized and quantified by rs-fMRI across species using the same imaging protocols, resulting in concordant alterations of global network parameters. Consequently, we compared rs-fMRI data obtained using two well-characterized mouse models of cortical stroke, i.e., photothrombosis (PT) and distal middle cerebral artery occlusion (dMCAo), with rs-fMRI data of a group of patients in the early phase after ischemic stroke.

# Materials and Methods

# Data availability

The data that support the findings of this study and all custom-written MATLAB codes are available from the corresponding author upon reasonable request.

Humans Participants

Resting-state fMRI data of thirteen first-ever acute ischemic stroke patients, with unilateral hand deficit, admitted to the Department of Neurology at the University Hospital of Cologne within < 10 days from onset, were compared to 13 healthy controls, matched for age, gender, and head movement within the MRI scanner (Table 1). All participants provided informed written consent before inclusion. The local ethics committee had approved the study, which followed the Declaration of Helsinki. Details are described in the Data Supplement.

Animal stroke models

All animal procedures followed the German Laws for Animal Protection and were approved by the local animal care committee and local governmental authorities (Landesamt für Natur, Umwelt und Verbraucherschutz North Rhine-Westphalia, LANUV; AZ 84-02.04.2013.A068, 84-02.04.2014.A370). The data were obtained from two separate, ongoing stroke studies and retrospectively chosen to compare early network changes after experimental stroke.14

Experimental stroke was induced on the right hemisphere. Seventeen homozygous male NMRI-Fox1nu/Fox-1nu mice with Tyrc albino background were used to conduct the dMCAo,15 while photothrombosis was induced in 14 male C57BL/6JRj mice.16 Details are described in the Data Supplement.

MRI data acquisition and processing

In humans, magnetic resonance images were acquired on a 3-Tesla MAGNETOM PRISMA scanner equipped with a 64-channel head coil (Siemens AG, Germany). Imaging in mice (before stroke induction, three days and 14 days thereafter) was conducted using a small-animal 9.4 T horizontal MRI system (BioSpec; Bruker BioSpin, Ettlingen, Germany) with a 20 cm bore diameter and actively shielded gradient coils (BGA12S2, 600 mT/m; Bruker BioSpin) with a 1H quadrature cryo-genic surface coil (CryoProbe, Bruker BioSpin) described previously.17 To acquire resting-state fMRI data, gradient echo-planar imaging (EPI) was used in both species (Supplementary Table 1).

Further data preprocessing was conducted using SPM8 ([www.fil.ion.ucl.ac.uk/spm](http://www.fil.ion.ucl.ac.uk/spm)) for human data, while rodent data were processed using a recently developed atlas-based imaging data analysis pipeline.18 Details on MRI acquisition, preprocessing, and graph analyses are described in the Data Supplement.

Statistical analyses

Statistical analyses were performed using the SPSS 25 software (IBM, Armonk, NY) and Microsoft Excel (Microsoft, Redmond, WA). To assess the alteration of graph parameters, a delta score compared to control and, in the case of longitudinal data in mice, compared to baseline scores before experimental stroke induction, was computed. Details are described in the Data Supplement.

# Results

Sample

The animal sample consisted of mice subjected to two different experimental models of cortical stroke, i.e., dMCAo (Fig 1A) or photothrombosis (Fig 1B). The human sample consisted of 13 first-ever stroke patients with hand motor deficits, scanned within the first nine days after stroke (*M* = 3.8 days, *SD* = 2.98), with the strongest lesion overlap in the temporoparietal cortex (Fig 1C). The extent of ischemic lesions in % of affected brain tissue was similar in humans and mice, consistent with minor stroke (Fig 1D), without significant differences in relative lesion size between the mouse models and the human sample (M = 1.66 %, SD = 2.5, p = 0.93 vs. PT, p = 0.07 vs. dMCAo). Otherwise, PT (M = 0.54 % of the brain, SD = 0.24) produced significantly smaller infarcts of the sensorimotor cortex than dMCAo (M = 1.3 %, SD = 0.56, X²(2) = 7.64, p = 0.039, KWT), while primarily affecting the forepaw function in mice, leading to an initial decrease of over 50% in the compound sensorimotor neuroscore three days after stroke compared to individual baseline function (*M* = 51.6, SD = 9.55; Fig 1E). As typically observed in rodents, neurological deficits spontaneously improved within a few weeks (X² = 23.01, p < 0.001; Friedman test), with significant recovery as early as 14 days after stroke (M = 32.6, SD = 10.4, p = 0.021; Fig 1E). Likewise, we recently reported that mice after dMCAo displayed an impairment of sensorimotor function as assessed by the rotarod test during the first week after stroke, followed by complete recovery.15 Stroke patients presented with mild to moderate symptoms, predominantly affecting the upper extremity, with a mean score on the National Institutes of Health Stroke Scale (NIHSS) of 1.67 (+/- 2.46). Since patients were screened within the first days after stroke, some patients spontaneously improved hand motor function during screening. Importantly, all patients showed unilateral hand motor deficits at the time of the fMRI session, showing at least mild paresis or deficits in fine motor skills, represented by a decrease relative grip strength (stroke group M = 0.84, SD = 0.2, healthy controls M = 0.95, SD = 0.17) and in finger tapping frequency (stroke group M = 3.9 /s, SD = 1.2 /s, healthy controls M = 4.9, SD = 0.5). All stroke patients recovered from their deficits within three to six months (NIHSS M = 0.3, SD = 0.48, t(21) = 2.4, p = 0.024; Fig 1F).

Resting-state networks

In stroke patients within the first nine days after stroke (Fig 2A) and both mouse models 14 days after stroke (Figs 2B and C), respectively, there was a pronounced increase in functional connectivity (fc) strength as compared to the respective control groups. In both species, an increase in resting-state connectivity was found for sensorimotor regions and other areas, including frontal and cerebellar regions. Hence, both humans and rodents featured similar neural network responses following a stroke, with a general increase of connectivity early after stroke. This increase in connectivity was most strikingly not limited to interconnected regions (modules) in the healthy brain but extended across the entire connectome.

To further investigate these alterations of functional networks, graph-theoretical analyses were performed. Graph parameters were computed over the full range of density thresholds to quantify the increase in connectivity strength, and mean values of a density range from 0.1 to 0.3 were used for comparison (Fig 3A). Within this range, there was a significant increase in node strength between stroke patients and healthy controls (*t*(24) = 2.64, *q* = 0.028, Fig 3B). Similar to the human stroke group, there was a significant increase in node strength after PT (*t*(18) = 3.83, *q* = 0.005), as well as after dMCAo (*t(20)* = 3.86, *q* = 0.005, Fig 3B).

We next computed *small worldness* as an index of global network function, reflecting a network organization with locally robust clusters, while distances between any pair of nodes are relatively small despite the network size. All datasets showed small-world topology with CC/CPL > 1. In the human sample, stroke led to a significant increase in *small worldness* of almost 30% compared to age-matched healthy controls (*t*(24) = 2.58, *q* = 0.029*,* Fig 3C). A concordant increase in *small worldness* was observed after PT (73%; t(18) = 3.74, q = 0.005) as well as after dMCAo (38%; t(20) = 2.48, q = 0.029) as compared to respective baselines and controls (Fig 3C). For further analyses of small worldness over the full density range, please see Supplemental Fig. 3.

This increase in *small worldness* was driven by both a reduction of *characteristic path length* (Fig 4A) and an increase of the *clustering coefficient* (Fig 4B). Both effects were most pronounced in the mouse models but could also be observed in human stroke patients. However, in the human stroke sample, differences in *small worldness* were mainly attributed to a decrease in *characteristic path length* by about 18% in stroke patients compared to healthy controls (*t*(24) = -2.61, *q* = 0.029, Fig 4A), while the *clustering coefficient* did not change significantly (*t*(24) = 1.71, *q* = 0.11, Fig 4B). In both animal models, concordant but even more pronounced effects were observed. The decrease of *characteristic path length* compared to baseline was stronger when induced by PT compared to controls ( t(18) = -3.88, q = 0.005), than following dMCAo compared to controls (t(20) = -2.23, q = 0.042, Fig 4A). In contrast, the increase in the *clustering coefficient* compared to baseline was greater after dMCAo compared to controls (*t*(20) = 3.36, *q* = 0.006) than after PT compared to controls (*t*(18) = 2.20, *q* = 0.046, Fig 4B).

To further compare the driving effects of stroke on *small worldness* across species, the normalized decrease in *characteristic path length* was correlated to the normalized increase in the *clustering coefficient* (Fig 4C). In both mice and humans, the increase in *small worldness* after stroke was predominantly explained by a decrease in *characteristic path length*, with the delta of *characteristic path length* 20% larger than the delta of the *clustering coefficient* in humans (*t*(24) = 4.62, q < 0.001), and similar changes occurring in mice subjected to PT (19%; *t*(16) = 2.20, q = 0.043) or dMCAo (13%; *t(*26) = 3.50, *q* = 0.002, Fig 4D), respectively.

To quantify the similarities across species, we used the intra-class correlation coefficient to evaluate group averages, yielding high similarity of *small worldness* (0.85, q = 0.001) and node strength (0.83, q = 0.002). Furthermore, thepercentage of common responses (PCR) indicated that the distribution of stroke groups was considerably more similar across species in the case of mean node strength (mean PCR = 80.43%) and *small worldness* (mean PCR = 78.08 %), compared to stroke groups and respective controls (node strength = 42.2 %; small worldness = 50.24%).

Relationship with the clinical deficit

We next sought to elucidate the functional relevance of global network changes after stroke and assessed graph parameters’ relationship to stroke-induced motor deficits. Correlating the initial patient’s compound motor performance to their network organization as reflected by *small worldness* showed a negative correlation, with higher *small worldness* being associated with reduced motor performance early after stroke (r = -0.56, p = 0.048, Fig 5A). In contrast, the mice’s initial motor performance showed no significant correlation with network parameters (Fig 5B).

Besides, correlations between motor recovery in mice after PT, measured as the difference in the compound motor function score (neuroscore) between day 21 and day three, showed a strong negative correlation with *small worldness* three days after stroke (r = -0.82, p = 0.004, Fig 5C). However, there was no correlation between recovery scores and graph-theoretical parameters in humans, possibly owing to the small between-patients variance with a substantial fraction of patients with rather small deficits leading to ceiling effects.

# Discussion

Preclinical models of stroke are essential for investigating the pathophysiology underlying cerebral ischemia and developing novel therapeutic approaches. Nonetheless, despite a wealth of promising results from preclinical models, most clinical trials have failed to reproduce those beneficial effects19. Our study suggests that it might be possible to mitigate the translational roadblock in stroke research by choosing (i) a preclinical model optimally matched to the patient collective and (ii) neuroimaging-based readouts producing meaningful data on the systems-level that are functionally relevant to both mice and men.

Ischemic insults have successfully been modeled in rodents, showing various molecular and pathophysiological characteristics, as observed in human stroke. In the photothrombosis model, a defined and highly reproducible photochemical thrombosis of terminal arterial vessels is induced. Another robust model of ischemic cortical lesions is generated by occlusion of the distal part of the middle cerebral artery (dMCAo) via cauterization. These two models were used in the current study and reflected essential aspects of minor human strokes, with similar relative lesion sizes compared to the human brain. Although individual studies regarding post-stroke adaptations on the network level exist in both mice and humans, comparability between experimental models and the clinical course has not been systematically investigated to date.

Previous clinical neuroimaging studies on patients more heavily affected by stroke primarily reported a reduction of interhemispheric connections, observed for both activation tasks20 and at rest.9, 11 Most interesting, Park et al. found a rather delayed increase in connectivity within the late subacute to chronic stage.9 Recovery of function was associated with normalization of functional connectivity.12 In contrast, previous studies reported an increase in functional connectivity within contralesional regions or non-affected subnetworks.21-23 Studies on severely affected stroke patients with subcortical lesions described both *clustering coefficient* and *characteristic path length* to decrease during recovery, suggesting a more random network topology10 or *characteristic path length* to increase.24 When exclusively investigating thalamic lesions with sensory deficits, no alterations of global network parameters were detected within the first week.25

Within mouse stroke models, only a small number of studies investigated functional connectivity. In proximal MCA occlusion, which produces larger infarcts compared to brain size, including subcortical structures, van Meer and colleagues observed conflicting results about changes in *characteristic path length* when subcortical structures were affected by the ischemic lesion.13 Moreover, lesions containing subcortical and cortical regions showed a pronounced increase in small world topology, global *clustering coefficient*, and *characteristic path length*.13 Likewise, our recent study on this severe stroke model impacting subcortical structures showed a pronounced decrease of connectivity, also concerning the opposite hemisphere.17 Other studies using this model observed a decrease in task-based activation,26 or impaired interhemispheric functional connectivity of the primary motor cortex.8, 17 Strikingly, the present study is the first to investigate the effects of cortical infarcts using rs-fMRI. Importantly, our results converge with electrophysiological data following PT, which described widespread disinhibition and hyperexcitability remote from the lesion itself.27

We here investigated a relatively homogenous patient sample with minor (predominantly cortical) stroke. We did not see any reduced interhemispheric connectivity but rather a widespread increase in connectivity within the first days after stroke onset. Overall, our findings indicate an increase in global communication structure across the network, likely to facilitate information transfer between distant regions of the brain.28

To assess global alterations on the network level, graph theory depicts an approach increasingly used in neuroscience to characterize network properties and compare networks from different backgrounds. Relatively few studies so far explored parameters based on graph theory to describe global network affection after stroke. We here report a comparable increase of network parameters across species, evidenced by an increase in mean node strength and small-world topology in the early subacute phase after a minor stroke. Both parameters of integration, captured by *characteristic path length,* and segregation, captured by *mean clustering coefficient,* were altered in our mouse models, with the predominance of reduction in *characteristic path length*. Most interestingly, relationships between changes in global integration and segregation were similar between mice and humans. *Small worldness* – a combination of the topological parameters of high clustering with low characteristic path length29 - is altered after human stroke30, and some neuropsychiatric disorders such as schizophrenia31 or Alzheimer’s disease32. It was suggested that a small world topology provides the basis for efficient information processing33, and allows for segregation of information clusters, leading to increased network resilience.34 Otherwise, longer-range communication was proposed to go along with higher metabolic demand.35 As such, the increased integration and segregation measures described in our study is comparable with other disturbances of the cerebral communication structure, e.g., after mild to moderate traumatic brain injury36, 37 or multiple sclerosis,38 resulting in compensatory network upregulation. Likewise, Hillary et al. describe an increased long-range communication structure, with a gradual drift to decrease in characteristic path length and increase in clustering coefficient in the late subacute phase after moderate to severe traumatic brain injury.39 Comparable to network adaptations after stroke, these findings seem to depend on the extent of disconnection, e.g., by diffuse axonal injury or the affection of subcortical hub nodes.

Several additional factors need to be considered when comparing the mentioned changes in functional connectivity across trials. Foremost, our study focused on minor cortical stroke with mild to moderate motor deficits and good recovery. This differentiates our approach from other published findings observed in more severely affected patients or more severe stroke models, showing decreased connectivity and more random network topology, resulting in disconnection syndromes.40 Additionally, data suggest that in both mice and humans, ischemic lesions in predominantly cortical localization induce specific alterations in functional networks distinct from those caused by lesions involving subcortical structures. The most parsimonious explanation is that cortical lesions reduce the characteristic path length, reflecting increased information transfer, an effect canceled out by co-existing subcortical lesions affecting long-distance tracts. This distinction is crucial for choosing the appropriate rodent model in preclinical stroke research and generating translatable results.

Only a few studies have investigated network changes in the early subacute stage of stroke.41 However, the timing of the investigation and the analysis of dynamic changes are crucial to extract comparable results about functional network alterations. For example, the increase in neuronal excitability already decreases after the first week after photothrombotic stroke in rats.42 In contrast, in humans, interhemispheric connectivity decreases at least two weeks after acute stroke,43 while little is known about the first few days post-stroke. We here show an increase in functional connectivity in a group of mildly affected patients. However, the described alterations might be temporarily confined or even obscured in patients with more severe network disruptions and more significant functional deficits. Furthermore, data processing steps used, i.e., global signal regression, physiological nuisance regression or segmentation, and the normalization, are crucial to interpreting functional connectivity data, rendering comparisons across trials or species difficult. Additionally, computation of graph parameters depends on other preferences and assumptions, e.g., brain parcellation methods or the thresholding mode, warranting detailed consideration and precautions when comparing results across trials. Accordingly, we assessed functional connectivity using similar processing steps across species to improve the comparability of our findings.

Besides, we found *small worldness* to correlate with the functional deficit in humans. In line with previous studies demonstrating the predictive value of functional connectivity after ischemic lesions,44, 45 our data suggest that increases in *small worldness* are linked to a more pronounced functional deficit in minor stroke patients in the early subacute phase. Additionally, we found that motor recovery in mice is inversely related to an early increase in *small worldness*. Given the hypothesis that predominantly cortical lesions are more likely to spare critical white matter tracts connecting network hubs, our results of increased network connectivity converge with analyses in the context of other neuropsychiatric diseases of network disruption, e.g., traumatic brain injury or multiple sclerosis,39 pointing to a compensatory reaction of the network. Small world topology is often considered a beneficial property of brain function,34 an assumption that may not necessarily be valid for patients throughout their recovery. Small worldness was found to increase early after stroke15 and decrease again over time12, a phenomenon hypothesized to reflect initial mechanisms of neural reorganization, including excessive rewiring and hyperconnectivity during the first days after stroke15. The present study results support the idea that high levels of small worldness may serve as a marker of network damage or at least indicates a somewhat diminished network reserve early after stroke. Altogether, further research is necessary to confirm these results and investigate the direction of influence between behavior and network parameters.

One limitation of our study is the relatively small sample size, especially concerning stroke patients in the early subacute phase. Within this sample, cerebral blood flow-relevant arterial stenoses (> 40% NASCET) were excluded by ultrasound, rendering a substantial interference with neurovascular coupling and impact on fc-measurements unlikely.46 Across species, time points of measurements differed slightly, as we found connectivity changes in rodents 14 days after stroke, while measurements in patients were conducted within the first nine days after symptom onset. While human data were compared to controls, rodent data were additionally normalized to individual baseline measurements. Most noteworthy, in contrast to the homogeneous lesion distribution in the experimental stroke models, stroke lesions in the human sample spread considerably but within the range of even larger stroke trials.47 Additionally, hemispheric effects on reorganization patterns might have been obscured by homogenization of the lesioned side in human samples. Likewise, lesion location within the cortex differed between the two mouse models with homogenous lesion distribution. Despite these limitations and differences across species at the spatial and temporal level, we observed similar functional network changes manifest within the early subacute phase after minor - predominantly cortical - stroke, independent of lesion distribution and stroke induction mode. This suggests robustness and underscores the generalizability of the findings. It also strongly encourages further investigations and confirmation in more extensive trials.

In conclusion, this translational study supports the concept of stroke as a network disease,48 while at the same time, it suggests meaningful readout parameters for preclinical rodent models that – for a long time – have neglected the network aspect of stroke. This systems approach opens yet unexplored possibilities for translational studies designed to address the network effects of treatments, including the primary modulation of neuronal networks, such as noninvasive brain stimulation. Furthermore, the underlying mechanisms of network phenomena observed across species are assessable on the rodent’s cellular- and molecular levels, paving the way for meaningful translational stroke research.

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Supplemental Materials

Expanded Materials & Methods

Online Table I, II

Online Figures I to III

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# Literature

1. Dirnagl U, Hakim A, Macleod M, Fisher M, Howells D, Alan SM, Steinberg G, Planas A, Boltze J, Savitz S et al. A concerted appeal for international cooperation in preclinical stroke research. *Stroke; a journal of cerebral circulation*. 2013;44:1754-1760

2. Fisher M, Feuerstein G, Howells DW, Hurn PD, Kent TA, Savitz SI, Lo EH. Update of the stroke therapy academic industry roundtable preclinical recommendations. *Stroke; a journal of cerebral circulation*. 2009;40:2244-2250

3. Schmidt-Pogoda A, Bonberg N, Koecke MHM, Strecker JK, Wellmann J, Bruckmann NM, Beuker C, Schabitz WR, Meuth SG, Wiendl H et al. Why most acute stroke studies are positive in animals but not in patients: A systematic comparison of preclinical, early phase, and phase 3 clinical trials of neuroprotective agents. *Annals of neurology*. 2020;87:40-51

4. Grefkes C, Fink GR. Connectivity-based approaches in stroke and recovery of function. *The Lancet. Neurology*. 2014;13:206-216

5. Hendricks HT, van Limbeek J, Geurts AC, Zwarts MJ. Motor recovery after stroke: A systematic review of the literature. *Archives of physical medicine and rehabilitation*. 2002;83:1629-1637

6. Backes H, Walberer M, Ladwig A, Rueger MA, Neumaier B, Endepols H, Hoehn M, Fink GR, Schroeter M, Graf R. Glucose consumption of inflammatory cells masks metabolic deficits in the brain. *NeuroImage*. 2016;128:54-62

7. Rueger MA, Backes H, Walberer M, Neumaier B, Ullrich R, Simard ML, Emig B, Fink GR, Hoehn M, Graf R et al. Noninvasive imaging of endogenous neural stem cell mobilization in vivo using positron emission tomography. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2010;30:6454-6460

8. van Meer MP, van der Marel K, Wang K, Otte WM, El Bouazati S, Roeling TA, Viergever MA, Berkelbach van der Sprenkel JW, Dijkhuizen RM. Recovery of sensorimotor function after experimental stroke correlates with restoration of resting-state interhemispheric functional connectivity. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2010;30:3964-3972

9. Park CH, Chang WH, Ohn SH, Kim ST, Bang OY, Pascual-Leone A, Kim YH. Longitudinal changes of resting-state functional connectivity during motor recovery after stroke. *Stroke; a journal of cerebral circulation*. 2011;42:1357-1362

10. Wang L, Yu C, Chen H, Qin W, He Y, Fan F, Zhang Y, Wang M, Li K, Zang Y et al. Dynamic functional reorganization of the motor execution network after stroke. *Brain : a journal of neurology*. 2010;133:1224-1238

11. Golestani AM, Tymchuk S, Demchuk A, Goodyear BG. Longitudinal evaluation of resting-state fmri after acute stroke with hemiparesis. *Neurorehabilitation and neural repair*. 2013;27:153-163

12. Rehme AK, Fink GR, von Cramon DY, Grefkes C. The role of the contralesional motor cortex for motor recovery in the early days after stroke assessed with longitudinal fmri. *Cerebral cortex (New York, N.Y. : 1991)*. 2011;21:756-768

13. van Meer MP, Otte WM, van der Marel K, Nijboer CH, Kavelaars A, van der Sprenkel JW, Viergever MA, Dijkhuizen RM. Extent of bilateral neuronal network reorganization and functional recovery in relation to stroke severity. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2012;32:4495-4507

14. Minassian A, Green C, Diedenhofen M, Vogel S, Hess S, Stoeber M, Radmilovic MD, Wiedermann D, Kloppenburg P, Hoehn M. Human neural stem cell induced functional network stabilization after cortical stroke: A longitudinal resting-state fmri study in mice. *Frontiers in cellular neuroscience*. 2020;14:86-86

15. Minassian A, Dobrivojevic Radmilovic M, Vogel S, Diedenhofen M, Nelles M, Stoeber M, Wiedermann D, Hoehn M. Cortical tissue loss and major structural reorganization as result of distal middle cerebral artery occlusion in the chronic phase of nude mice. *Scientific reports*. 2019;9:6823

16. Walter HL, van der Maten G, Antunes AR, Wieloch T, Ruscher K. Treatment with amd3100 attenuates the microglial response and improves outcome after experimental stroke. *Journal of neuroinflammation*. 2015;12:24

17. Green C, Minassian A, Vogel S, Diedenhofen M, Beyrau A, Wiedermann D, Hoehn M. Sensorimotor functional and structural networks after intracerebral stem cell grafts in the ischemic mouse brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2018;38:1648-1661

18. Pallast N, Diedenhofen M, Blaschke S, Wieters F, Wiedermann D, Hoehn M, Fink GR, Aswendt M. Processing pipeline for atlas-based imaging data analysis of structural and functional mouse brain mri (aidamri). *Frontiers in neuroinformatics*. 2019;13:42

19. Bosetti F, Koenig James I, Ayata C, Back Stephen A, Becker K, Broderick Joseph P, Carmichael ST, Cho S, Cipolla Marilyn J, Corbett D et al. Translational stroke research. *Stroke; a journal of cerebral circulation*. 2017;48:2632-2637

20. Grefkes C, Nowak DA, Eickhoff SB, Dafotakis M, Kust J, Karbe H, Fink GR. Cortical connectivity after subcortical stroke assessed with functional magnetic resonance imaging. *Annals of neurology*. 2008;63:236-246

21. Chen J, Sun D, Shi Y, Jin W, Wang Y, Xi Q, Ren C. Alterations of static functional connectivity and dynamic functional connectivity in motor execution regions after stroke. *Neuroscience letters*. 2018;686:112-121

22. Chen L, Luo T, Wang K, Zhang Y, Shi D, Lv F, Li Y, Li Y, Li Q, Fang W et al. Effects of thalamic infarction on the structural and functional connectivity of the ipsilesional primary somatosensory cortex. *European radiology*. 2019

23. Dacosta-Aguayo R, Grana M, Savio A, Fernandez-Andujar M, Millan M, Lopez-Cancio E, Caceres C, Bargallo N, Garrido C, Barrios M et al. Prognostic value of changes in resting-state functional connectivity patterns in cognitive recovery after stroke: A 3t fmri pilot study. *Human brain mapping*. 2014;35:3819-3831

24. Zhang J, Zhang Y, Wang L, Sang L, Yang J, Yan R, Li P, Wang J, Qiu M. Disrupted structural and functional connectivity networks in ischemic stroke patients. *Neuroscience*. 2017;364:212-225

25. Chen L, Li C, Zhai J, Wang A, Song Q, Liu Y, Ma R, Han L, Ndasauka Y, Li X et al. Altered resting-state signals in patients with acute stroke in or under the thalamus. *Neuroscience bulletin*. 2016;32:585-590

26. Weber R, Ramos-Cabrer P, Justicia C, Wiedermann D, Strecker C, Sprenger C, Hoehn M. Early prediction of functional recovery after experimental stroke: Functional magnetic resonance imaging, electrophysiology, and behavioral testing in rats. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2008;28:1022-1029

27. Buchkremer-Ratzmann I, Witte OW. Extended brain disinhibition following small photothrombotic lesions in rat frontal cortex. *Neuroreport*. 1997;8:519-522

28. Rubinov M, Sporns O. Complex network measures of brain connectivity: Uses and interpretations. *NeuroImage*. 2010;52:1059-1069

29. Watts DJ, Strogatz SH. Collective dynamics of 'small-world' networks. *Nature*. 1998;393:440-442

30. Caliandro P, Vecchio F, Miraglia F, Reale G, Della Marca G, La Torre G, Lacidogna G, Iacovelli C, Padua L, Bramanti P et al. Small-world characteristics of cortical connectivity changes in acute stroke. *Neurorehabilitation and neural repair*. 2017;31:81-94

31. Bassett DS, Bullmore E, Verchinski BA, Mattay VS, Weinberger DR, Meyer-Lindenberg A. Hierarchical organization of human cortical networks in health and schizophrenia. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2008;28:9239-9248

32. John M, Ikuta T, Ferbinteanu J. Graph analysis of structural brain networks in alzheimer’s disease: Beyond small world properties. *Brain Structure and Function*. 2017;222:923-942

33. Latora V, Marchiori M. Efficient behavior of small-world networks. *Physical review letters*. 2001;87:198701

34. Achard S, Salvador R, Whitcher B, Suckling J, Bullmore E. A resilient, low-frequency, small-world human brain functional network with highly connected association cortical hubs. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2006;26:63-72

35. Kitzbichler MG, Henson RN, Smith ML, Nathan PJ, Bullmore ET. Cognitive effort drives workspace configuration of human brain functional networks. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2011;31:8259-8270

36. Zhou Y. Small world properties changes in mild traumatic brain injury. *Journal of magnetic resonance imaging : JMRI*. 2017;46:518-527

37. Caeyenberghs K, Leemans A, Leunissen I, Michiels K, Swinnen SP. Topological correlations of structural and functional networks in patients with traumatic brain injury. *Frontiers in human neuroscience*. 2013;7:726-726

38. Hillary FG, Roman CA, Venkatesan U, Rajtmajer SM, Bajo R, Castellanos ND. Hyperconnectivity is a fundamental response to neurological disruption. *Neuropsychology*. 2015;29:59-75

39. Hillary FG, Rajtmajer SM, Roman CA, Medaglia JD, Slocomb-Dluzen JE, Calhoun VD, Good DC, Wylie GR. The rich get richer: Brain injury elicits hyperconnectivity in core subnetworks. *PloS one*. 2014;9:e104021

40. Griffis JC, Metcalf NV, Corbetta M, Shulman GL. Structural disconnections explain brain network dysfunction after stroke. *Cell reports*. 2019;28:2527-2540.e2529

41. Chi NF, Ku HL, Chen DY, Tseng YC, Chen CJ, Lin YC, Hsieh YC, Chan L, Chiou HY, Hsu CY et al. Cerebral motor functional connectivity at the acute stage: An outcome predictor of ischemic stroke. *Scientific reports*. 2018;8:16803

42. Schiene K, Bruehl C, Zilles K, Qu M, Hagemann G, Kraemer M, Witte OW. Neuronal hyperexcitability and reduction of gabaa-receptor expression in the surround of cerebral photothrombosis. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 1996;16:906-914

43. Volz LJ, Rehme AK, Michely J, Nettekoven C, Eickhoff SB, Fink GR, Grefkes C. Shaping early reorganization of neural networks promotes motor function after stroke. *Cerebral cortex (New York, N.Y. : 1991)*. 2016;26:2882-2894

44. Puig J, Blasco G, Alberich-Bayarri A, Schlaug G, Deco G, Biarnes C, Navas-Marti M, Rivero M, Gich J, Figueras J et al. Resting-state functional connectivity magnetic resonance imaging and outcome after acute stroke. *Stroke; a journal of cerebral circulation*. 2018;49:2353-2360

45. Siegel JS, Seitzman BA, Ramsey LE, Ortega M, Gordon EM, Dosenbach NUF, Petersen SE, Shulman GL, Corbetta M. Re-emergence of modular brain networks in stroke recovery. *Cortex; a journal devoted to the study of the nervous system and behavior*. 2018;101:44-59

46. Chang TY, Kuan WC, Huang KL, Chang CH, Chang YJ, Wong HF, Lee TH, Liu HL. Heterogeneous cerebral vasoreactivity dynamics in patients with carotid stenosis. *PloS one*. 2013;8:e76072

47. Siegel JS, Ramsey LE, Snyder AZ, Metcalf NV, Chacko RV, Weinberger K, Baldassarre A, Hacker CD, Shulman GL, Corbetta M. Disruptions of network connectivity predict impairment in multiple behavioral domains after stroke. *Proceedings of the National Academy of Sciences of the United States of America*. 2016;113:E4367-4376

48. Guggisberg AG, Koch PJ, Hummel FC, Buetefisch CM. Brain networks and their relevance for stroke rehabilitation. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology*. 2019;130:1098-1124

49. van der Vliet R, Selles RW, Andrinopoulou E-R, Nijland R, Ribbers GM, Frens MA, Meskers C, Kwakkel G. Predicting upper limb motor impairment recovery after stroke: A mixture model. Annals of neurology. 2020;87:383-393

50. Yushkevich PA, Piven J, Hazlett HC, Smith RG, Ho S, Gee JC, Gerig G. User-guided 3d active contour segmentation of anatomical structures: Significantly improved efficiency and reliability. NeuroImage. 2006;31:1116-1128

51. Power JD, Barnes KA, Snyder AZ, Schlaggar BL, Petersen SE. Spurious but systematic correlations in functional connectivity mri networks arise from subject motion. NeuroImage. 2012;59:2142-2154

52. Cordes D, Haughton VM, Arfanakis K, Carew JD, Turski PA, Moritz CH, Quigley MA, Meyerand ME. Frequencies contributing to functional connectivity in the cerebral cortex in "resting-state" data. AJNR. American journal of neuroradiology. 2001;22:1326-1333

53. Kalthoff D, Po C, Wiedermann D, Hoehn M. Reliability and spatial specificity of rat brain sensorimotor functional connectivity networks are superior under sedation compared with general anesthesia. NMR in biomedicine. 2013;26:638-650

54. Liu TT, Nalci A, Falahpour M. The global signal in fmri: Nuisance or information? NeuroImage. 2017;150:213-229

55. Holmes CJ, Hoge R, Collins L, Woods R, Toga AW, Evans AC. Enhancement of mr images using registration for signal averaging. Journal of computer assisted tomography. 1998;22:324-333

56. Ashburner J, Friston KJ. Unified segmentation. NeuroImage. 2005;26:839-851

57. Pallast ND, M., Blaschke S, Wieters F, Wiedermann D, Hoehn M, Fink GR, Aswendt M. A novel atlas-based imaging data analysis pipeline (aida) for structural and functional mri of the mouse brain. Journal Frontiers in Neuroinformatics. under review

58. Hanel PHP, Maio GR, Manstead ASR. A new way to look at the data: Similarities between groups of people are large and important. *Journal of personality and social psychology*. 2019;116:541-562

# Figure Legends

Figure 1: Comparable ischemic lesions in human stroke patients and experimental mouse models

A) Experimental model of distal occlusion of the middle cerebral artery (dMCAo, n=17), heat-maps of individual lesions two weeks after stroke show a core overlap of 100%.

B) Experimental model of photothrombosis (PT, n=13); likewise, fused heat-maps after two weeks demonstrate a core overlap of 100%.

C) Human stroke patients (n=13) with mild to moderate motor deficits predominantly showed a temporoparietal lesion distribution on fused heat-maps. Human lesion location was adjusted by mirroring, if necessary.

D) Comparing lesion sizes, displayed as the percentage of the individual’s whole brain size, revealed only significant differences between PT and dMCAo, while the lesion size of mouse models was not significantly different from the human sample (\*p<0.05).

E) Compound motor score in mice subjected to PT showed a functional impairment to about 50% of baseline values three days after stroke. Over three weeks, motor deficits improved spontaneously with significant recovery as early as day 14 (\*p<0.05; \*\*\*p<0.001).

F) Human patients were mildly to moderately affected by stroke, with a mean score on the National Institutes of Health Stroke Scale (NIHSS) of 1.67. Similar to the mouse model, significant functional recovery occurred during observation (\*p<0.05).

Figure 2: Across species, functional connectivity increased after minor stroke

A) Human rs-fMRI scans were parcellated by atlas-based registration to the Automated Anatomical Labeling atlas (AAL, 116 nodes) and displayed as delta maps of the group mean of full networks within the first nine days after stroke (Pearson full correlation, Z-transformed). Adjacency matrices were sorted by maximized modularity of control networks based on the Louvain community detection algorithm (gamma = 1.1). Additionally, a histogramm displays changes in the strength of functional connectivity (fc-strength) in the human stroke sample as compared to age-matched healthy controls (Mdn=0.2, Mad=0.07).

B) Rs-fMRI scans of mice subjected to PT 14 days before were parcellated by atlas-based registration to the Allen Mouse Brain Atlas (AMBA, 98 nodes) and displayed as delta maps of the group mean of full networks two weeks after stroke (Pearson full correlation, Z-transformed). Adjacency matrices were sorted by maximized modularity of control networks as in (2A). Additionally, a histogramm displays changes in fc-strength in mice subjected to PT as compared to the individual baseline (Mdn=0.184, Mad=0.08).

C) Rs-fMRI scans of mice subjected to dMCAo 14 days before imaging were parcellated by atlas-based registration to the AMBA and displayed in analogy to (2A) and (2B). Additionally, a histogramm displays Changes in fc-strength in mice subjected to dMCAo as compared to the individual baseline values (Mdn=0.1, Mad=0.06).

For raw matrices of within-group networks, see Supplementary Fig. 2.

Figure 3: Stroke induces an increase in node strength and small worldness in mice and humans

A) Mean node strength (exemplary data shown for human stroke patients) was computed over the full range of density thresholds, and mean values of a density range between 0.1 and 0.3 were chosen for further evaluation.

B) Node strength of each stroke group is displayed as the difference to its corresponding control group. Two weeks after PT, node strength was significantly increased compared to baseline, while there was a similar significant increase in node strength after dMCAo (\*p<0.05; \*\*p<0.01).

C) Boxplots displaying delta values of *small worldness* compared to healthy controls. Stroke increased *small worldness* by almost 30% in humans compared to healthy age-matched controls. Likewise, *small worldness* increased two weeks after PT compared to baseline control, and dMCAo compared to baseline control (\*p<0.05; \*\*p<0.01).

Figure 4: *Small worldness* is increased after stroke across species, driven by a decrease in characteristic path length

A) Increase in *small worldness* induced by a reduction of *characteristic path length* (*CPL)*. In stroke patients, *CPL* decreased by about 18% compared to healthy age-matched controls. In both mouse models, this effect was pronounced, with the most substantial *CPL* decrease observed after PT compared to control. A similar reduction of *CPL*, albeit with a smaller effect size, occurred after dMCAo compared to control (\*p<0.05; \*\*p<0.01).

B) Additionally, an increase of *small worldness* was, in part, induced by the rise in *mean clustering coefficient* (*CC)*. While this effect was not statistically significant in humans, it was present after PT, compared to control, and most pronounced after dMCAo compared to control (\*p<0.05; \*\*p<0.01).

C) The normalized decrease in *CPL* is depicted about the normalized increase in CC. The solid line indicates an equal change of both parameters. The changes in *CPL* provide the most significant distinction between species after stroke.

D) The ratio of reduction in *CPL* to increase in *CC* indicates that in all three datasets, the decrease in *CPL* dominates the increase in *CC,* as the two parameters are associated with the increase in *small worldness*. Decreased *CPL* dominates increased *CC* by about 15% in humans, with comparable proportions in both PT and dMCAo (\*p<0.05; \*\*p<0.01).

Figure 5: Across species, an increase in *small worldness* is associated with more severe functional deficits after stroke

A) In a human stroke, *small worldness* was inversely correlated to motor function assessed by a composite score of ARAT, grip strength, and finger-tapping frequency.

B) In mice after PT, the relationship between *small worldness* and motor function as assessed by a composite motor score (neuroscore in %) was not statistically significant.

C) In mice after PT, motor recovery, measured as improvement in the neuroscore between days 3 and 21, showed an inverse correlation with *small worldness*. Data are displayed as a partial regression plot, showing the partial correlation between *small worldness* and recovery while controlling for the initial motor impairment.

Table 1: characteristics of the human sample of stroke patients

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ID | Gender | Age | Handed-ness | Affected  side | time after  stroke (days) | thrombolysis/ thrombectomy | ARAT Score  (affected arm) | NIHSS | Lesion  (mm³) |
| 1 | M | 62 | r | l | 8 | no/no | 43 | 5 | 62485 |
| 2 | F | 82 | r | r | 5 | no/no | 51 | 4 | 2106 |
| 3 | F | 60 | l | r | 2 | no/no | 57 | 1 | 99252 |
| 4 | M | 54 | r | r | 4 | yes/yes | 54 | 0 | 137207 |
| 5 | M | 68 | r | r | 2 | no/no | 49 | 3 | 11 |
| 6 | M | 83 | r | l | 2 | no/no | 54 | 2 | 1428 |
| 7 | M | 43 | r | l | 1 | yes/yes | 57 | 1 | 52232 |
| 8 | M | 78 | r | r | 5 | no/no | 57 | 0 | 1269 |
| 9 | M | 64 | r | l | 1 | yes/no | 57 | 1 | 1947 |
| 10 | M | 54 | r | l | 1 | no/no | 43 | 4 | 3794 |
| 11 | M | 69 | l | r | 1 | no/no | 57 | 0 | 2653 |
| 12 | M | 59 | r | l | 8 | no/no | 55 | 1 | 1343 |
| 13 | M | 71 | r | r | 9 | yes/yes | 57 | 0 | 29946 |
| Total | 11M/2F | - | 11r/2l | 7r/6l | - | 4/3 | - | - | - |
| Mean  (SD) | - | 65.2  (11.7) | - | - | 3.8  (3.0) | - | 53.2  (5.2) | 1.7  (1.8) | 30436  (44905) |