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Cortical Hemodynamics and Motor Recovery After Motor Cortical Infarcts

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Cortical Hemodynamics and Motor Recovery After Cortical Infarcts

by

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Abstract

Cortical Hemodynamics and Motor Recovery After Cortical Infarcts

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Stroke is the leading cause of disability and the fourth leading cause of death in the United States. Of those that survive a stroke, many are left with long term functional motor impairments. Spontaneous recovery of motor function occurs after a stroke and the reorganization of spared neural tissue is a contributing factor. To study motor recovery following a stroke, rodent models have been especially useful because experimental manipulations can be paired with controlled infarcts to understand physiologically relevant changes. For example, stroke to the sensory-motor cortex (SMC) in mice produces functional motor impairments which are dependent on the reorganization of the remaining cortex. Ironically, after about 20 years of research on the reorganization of the peri-lesion following cortical ischemia, there has been a lack of focus on the neuro-vascular changes as they relate to functional outcome after stroke. The central hypothesis of this report is that spontaneous vascular remodeling contributes to behavioral recovery and cortical reorganization following ischemic insult.

To investigate the relationship between blood flow recovery and improvement of motor function after an ischemic insult, we developed a mouse model of upper extremity impairment after a stroke that can be repeatedly imaged *in vivo*. Specifically, 14 C57/BL6 mice either received photo-thrombotic cortical lesions (n=7) or vehicle procedures (n=7), were allowed 3 days to recover, and then received forelimb function probes using the pasta matrix reaching task (PMRT), an assay for skilled forelimb function, in tandem with the imaging of cortical blood flow using multi-exposure speckle imaging (MESI) at Days 3, 5, 10, and 20.

Results indicate that the mice that received injections with Rose Bengal displayed significantly decreased performance on the PMRT and a significantly reduced amount of cortical blood flow compared to both their baseline performance and the control group. Skilled forelimb performance following the ischemic lesion correlated strongly with stroke severity (as indexed by cortical blood flow in the lesion core 2 hours following lesion induction). Additionally, the re-establishment of cortical blood flow to the infarct core precedes the recovery of motor performance, indicating potential importance for the re-establishment of blood flow to support the adaptive plasticity required for motor recovery.

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Introduction

Stroke is the leading cause of longterm disability in the United States (American Heart Association, 2013) and, of those that survive, about 77% exhibit upper extremity dysfunction (Lawrence et al., 2001). No drugs currently exist to promote functional motor recovery after a stroke and treatment is largely focused on rehabilitation. Spontaneous recovery of motor function occurs after a stroke and the reorganization of spared tissue is a contributing factor (Levy et al. 2001; Liepert et al., 2000; Nudo, 2003). Animal models of chronic stroke have been shown to be informative for human stroke and rodent models of stroke have been especially useful because ischemic lesions to the SMC result in upper extremity dysfunction (Bouet et al., 2007; Farr et al., 2002; Tennant, Jones, 2009).

After ischemic SMC insults, there is an initial process of cell death in the cortex followed by neural regenerative events in surviving tissue (Nudo, 2007). The latter can occur robustly in the adjacent or peri-infarct cortex and contribute to functional recovery (Biernaskie, Corbett, 2007; Castro-Almancos, Borrel, 1995). For example, axonal sprouting and the formation of new synapses in the adjacent tissue after a stroke are linked with functional improvements (Biernaskie, Corbett, 2007; Castro-Almancos, Borrel, 1995). Skilled forelimb training of the paretic forelimb after a motor cortical infarct promotes these neural changes in peri-infarct cortex and improves motor function in rodents compared to spontaneous recovery (Biernaskie, Corbett, 2007; Biernaskie, Chernenko, Corbett, 2004). Furthermore, this training has been shown to alter the organization of movement representations in motor cortex as indexed by intra-cortical microstimulation (ICMS) (Castro-Almancos, Borrel, 1995). The neuroanatomical

changes which underlie this reorganization have yet to be fully elucidated, especially the potential role that vascular changes play. Ironically, given that stroke is a traumatic vascular event (American Heart Association, 2013), little has been done to investigate the extent to which vascular changes contribute to this cortical reorganization and to functional improvements. That is, reduced blood flow follows ischemic injury (American Heart Association, 2013), but cortical reorganization is neural activity-dependent (Liepert et al., 2000; Nudo, 2003) and thus likely to be dependent on adequate restoration of blood flow.

While the presence of these events is well established, the time course of blood flow recovery has not been explored in tandem with post-ischemic motor functional changes. Recent advances in imaging techniques have allowed for the cortex of animals, especially mice, to be imaged repeatedly with microscopic resolution (Mostany, Portera-Cailliau, 2008; Yang et al., 2010; Holmaat et al., 2009; Svoboda et al., 1997; Tomita et al., 2005). We used *in vivo* multi-exposure speckle imaging (MESI) (Kazmi et al., 2013) to follow the temporal recovery of cortical blood flow after an ischemic injury. In mice with cranial windows, a photothrombotic infarct was created in the forelimb area of SMC, which is established to result in upper extremity (forelimb) dysfunction (Farr, Whishaw, 2002; Tennant, Jones, 2009). In combination with imaging, the pasta matrix reaching task (PMRT), a sensitive measurement of forelimb motor function, was used to correlate behavioral outcome with blood flow changes over time within the same animals (Tennant, Jones, 2009).

The primary goal of this project was to determine the temporal relationship between neurovascular remodeling in the SMC and functional behavioral improvements following stroke. This study addressed this shortcoming by using behavioral manipulations with *in vivo* imaging to determine the relationship between motor improvements and recovery of blood flow in peri-infarct cortex. By observing the time course of cortical blood flow restoration in tandem with the recovery of skilled forelimb function following a unilateral infarct to the SMC, we aimed to establish the relationship between neuro-anatomically relevant recovery of blood flow with improvements in motor function. In so doing, this model will improve the direction of experimental models of chronic stroke and inform clinical treatment approaches.

Activity-Dependent Plasticity Following Stroke

Upper extremity impairments in both humans and animal models can be improved by focused training with the paretic forelimb (Nudo, RJ, 2003; Biernaskie, J, Chernenko, G, Corbett, D, 2004; Nudo et al., 1996). In rodent models, both spontaneous improvements and those that are induced by rehabilitative training are linked to the plasticity which occurs in peri-infarct cortex (Nudo, RJ, 2007; Biernaskie, J, Chernenko, G, Corbett, D, 2004; Nudo et al., 1996). The development of *in vivo* imaging techniques and transgenic mouse lines has increased the resolution at which these plastic responses can be studied spatially and temporally (Mostany, R, Portera-Cailliau, C, 2008; Yang et al., 2010; Holmaat et al., 2009; Svoboda et al., 1997; Tomita et al., 2005; Kazmi et al., 2013). For example, the visualization of dendritic spine changes in the peri-infarct cortex using 2-photon microscopy has revealed that there is a dramatic reorganization with both

dendrites and blood vessels radiating away from the lesion core after a stroke (Brown et al., 2007). The reperfusion of the infarct core has also been linked with recovery of dendritic spine densities in the late phases of recovery (Mostany et al., 2010). However, the neuroanatomical and temporal specificity of these responses have not been studied as they relate to improvements in motor function after a stroke.

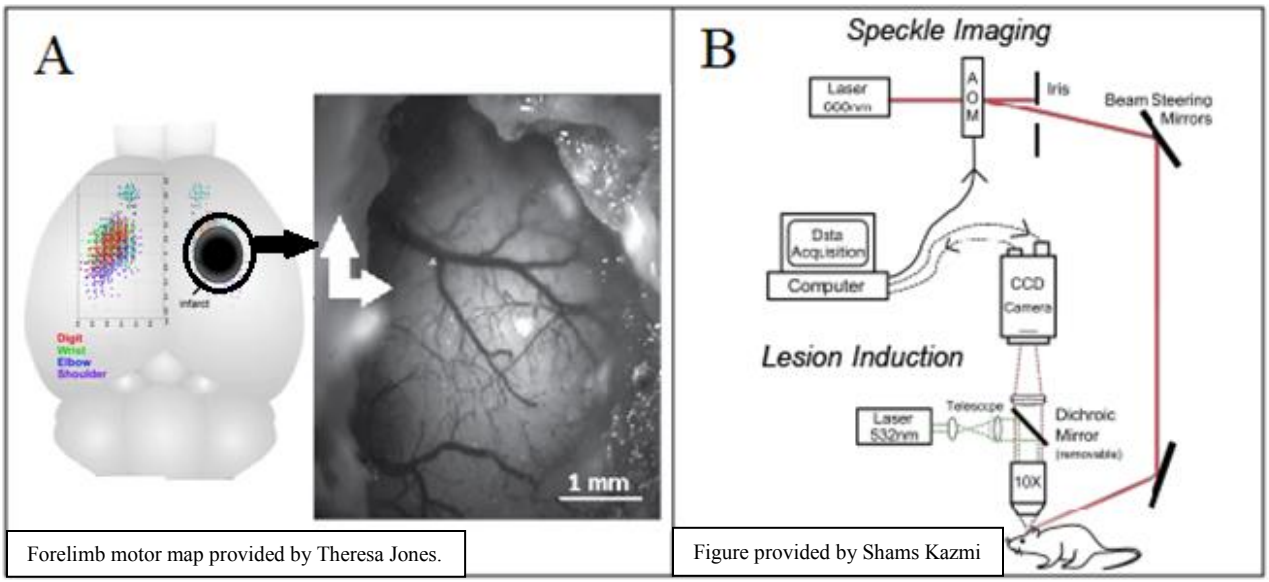
Vascular Dependence of CNS Plasticity

Blood flow is increased in regions of heightened neural activity (i.e., activation-flow coupling) (Ances et al., 1999), and behavioral experiences such as exercise have the capacity to induce angiogenesis (Swain et al., 2003). There can be prolonged reductions in blood flow and vessel densities in cortex after stroke, but ischemic lesions trigger a process of vascular changes including angiogenesis and shunting to collateral blood vessels to restore blood flow to the peri-infarct cortex (Wei et al., 2001). In mice, stroke can instigate the reorganization of cortical blood vessels and induce angiogenesis (Wei et al., 2001). Many of the new vessels generated early after stroke are transient and leaky (Dirnagl, U, Iadecola, C, Moskowitz, MA, 1999). Blood flow recovery over the chronic period is likely to depend on both the creation and stabilization of new vessels. Furthermore, the process of reperfusion has been implicated in restoring the integrity of dendritic spines following a stroke in mice (Mostany et al., 2010). However, the temporal dependence on which this process of reperfusion to the motor cortex has not been studied as it relates to improvements in motor function.

In vivo Imaging and Mouse Models of Stroke

As we were interested in combining *in vivo* imaging (Kazmi et al., 2013) with

sensitive behavioral assays for forelimb motor function (Tennant, KA, Jones, TA, 2009), we used a mouse model of cortical ischemia with a cranial window (Holmaat et al., 2009) installed over the SMC (See Figure 1A). The mouse as a model is ideal for this study as most *in vivo* imaging techniques have been developed using mouse models (namely for their thin skulls and transgenic capabilities) (Mostany, R, Portera-Cailliau, C, 2008; Yang et al., 2010; Holmaat et al., 2009; Svoboda et al., 1997; Tomita et al., 2005; Kazmi et al., 2013). Additionally, focused infarcts to the SMC in rodents has been shown to reliably induce upper extremity impairments (Tennant, KA, Jones, TA, 2009). Photothrombotic lesions (See Figure 1B) were used because this method allows non-invasive infarct induction through cranial windows and minimizes the potential for other aspects of the induction procedure to disrupt vasculature (Brown et al., 2007; Brown et al., 2009). We used a large cranial window installed over the SMC to allow for repeated imaging of cortical blood flow (Kazmi et al., 2013). Creating the lesions over the SMC will also allow us to track motor recovery over time and determine the extent to which the blood flow changes following a stroke are temporally linked with recovery of motor function. To assess forelimb function, the pasta matrix reaching task (PMRT) was used as it is established as both a reliable metric for skilled forelimb function and later as a model of motor rehabilitative training, i.e., it can be used to promote improvements in skilled forelimb function after SMC infarcts.



Forelimb motor map provided by Theresa Jones.

Figure provided by Shams Kazmi

Figure 1. A: Forelimb Motor Map and Cranial window. At 2 months of age, mice will have a cranial window installed over their sensorimotor cortex to allow for lesion induction and repeated imaging using multi-exposure speckle imaging (MESI). **B:** Photothrombotic lesion induction and MESI. Ischemic lesions will be created by injecting Rose Bengal and then focusing a green laser through a 10X objective for 15 minutes. Vehicle controls received injections of saline instead of Rose Bengal. MESI is the imaging modality which enables in vivo measurements of cortical blood flow rates. It's a variation on speckle imaging which enables blood flow rates to be compared between imaging sessions.

Current Project: Exploring the temporal link between restoration of cortical blood flow and spontaneous motor recovery following a stroke.

There is typically some degree of spontaneous recovery over time after stroke. The temporal relationship between restoration of blood flow to peri-infarct tissue and spontaneous recovery has not yet been investigated. To study this, mice received imaging of cortical blood flow at multiple time points before and after subtotal infarcts of the SMC, which result in substantial motor impairments that partially recover over time

(Tennant, KA, Jones, TA, 2009; Kerr et al., 2013). PMRT was used in tandem with MESI to link temporal improvements of motor function with cortical blood flow.

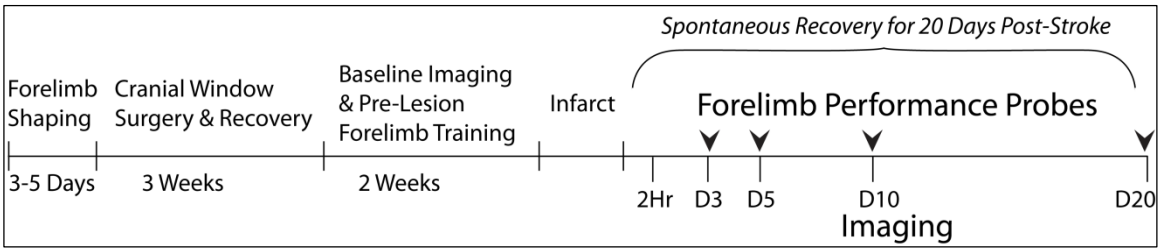


Figure 2: Experimental design. The aim of this experiment was to link the cerebro-vascular changes following stroke with behavioral changes. After determining their preferred for reaching forelimb using the PMRT, mice then had a cranial window installed over the contralateral region of their motor cortex. Following a 3 week recovery period from the cranial window surgery, mice then received 2 weeks of skilled forelimb training using the PMRT. In this 2 week period mice also received 3 baseline imaging sessions using MESI. At the end of this period, mice either received a photothrombotic lesion or vehicle procedures followed by 20 days of spontaneous recovery with behavioral and impaging probes at days 3, 5, 10, and 20. At day 20, mice were perfused and their tissues harvested for post-mortem histology.

We expected that cortical infarcts will result in spontaneous improvements in cortical blood flow that will temporally relate with improvements in skilled forelimb function. We expected blood flow recovery to be more limited in the infarct core and for blood flow recovery to increase spatially moving away from the core. Furthermore, regions with the greatest recovery of blood flow will correspond to more normal vessel densities and to greater surviving forelimb movement representation area. We also expected that the magnitude of recovery of blood flow recovery in surviving forelimb regions is predictive of improvements in skilled forelimb function.

Methods

Design Overview:

Three weeks after window installation, mice were trained with the dominant forelimb on the pasta matrix reaching task (PMRT) until they could retrieve at least 7 pasta pieces (~70% of the maximum) per training session (Tennant, KA, Jones, TA, 2009; Kerr et al., 2013). During this pre-stroke training period, mice will also undergo a series of 3 baseline imaging sessions, spaced at least 3 days apart, to establish basal blood flow rates (Kazmi et al., 2013). After reaching the criteria on the PMRT, a photothrombotic lesion to the caudal forelimb area (CFA) of the motor cortex contralateral to the dominant forelimb will be induced in half the mice, so that the trained forelimb becomes the paretic forelimb.

Subjects

14 male and female mice were group housed (3 or 4 to a cage) on a 12:12 light/dark cycle in the Animal Resource Center. These mice received weekly cage supplement (e.g. bedding, PVC pipes, and wooden toys). At the time of the experiment, mice were placed on a restrictive diet (dropping to no less than 90% of their initial body weight) and kept on this diet for the duration of the experiment. After determining limb dominance for reaching (left or right), all mice had cranial windows installed over the contralateral SMC. The mice were randomly assigned to receive lesion or vehicle procedures. For the MESI data, three mice were removed from the vehicle condition because their window became cloudy and their cortex was not visible. While these mice were not repeatedly imaged, they still were put under anesthesia at the same time points to correct for any

influence that repeated anesthesia may have had on behavioral results. All procedures with the mice were approved by the University of Texas Institutional Care and Use Committee, an AAALAC accredited program.

Pasta Matrix Reaching Task

Mice were both trained and tested on the PMRT. To determine their dominant forelimb, mice were placed in a plexiglass chamber and allowed to reach for Capellini pasta pieces arranged vertically outside of the chamber. Mice were able to reach and retrieve pasta through a slit in the plexiglass chamber. The dominant forelimb was determined by counting reach attempts for pasta. If a mouse made at least 20 reach attempts and 60% or greater of these were with a single forelimb then it was determined to be their dominant forelimb. After installation of the cranial window, mice then received 14 successive days of forelimb training where they were placed in the plexiglass chamber with pasta now arranged diagonal from their preferred for reaching forelimb through the slit. As such, mice were only able to successfully retrieve pasta using their dominant forelimb. Mice reached 100 times for pasta during each day of training and reach performance was calculated as no. of pieces broken/ no. of reach attempts (100). After photo-thrombotic lesions or vehicle procedures, mice were assessed with their dominant forelimb at days 3, 5, 10, and 20 at 24 hours after each imaging session.

Multi-Exposure Speckle Imaging

Cortical blood flow was assayed with MESI. Before mice received stroke or vehicle procedures, they underwent a series of 3 baseline imaging sessions, spaced at least 3 days apart, to establish basal blood flow rates. Mice then had their cortical blood flow

measured 2h, days 3, 5, 10, and 20 after lesion induction. MESI is a variation on speckle imaging which provides absolute flow rates for cortical blood flow. These images are generated by first determining the speckle contrast value (standard deviation/mean image intensity) for a 5x5 pixel cluster. Images are then acquired across 15 different exposure times and the information from each exposure time integrated to provide the final MESI frame (Kazmi et al., 2013). With MESI, images from multiple time points can be compared within and between subjects. MESI imaging was performed under isoflurane anesthesia (to maintain immobility). To correct for any influence of anesthetic plane, physiological parameters were continuously monitored (with MouseOx) and isoflurane levels adjusted such that heart rate and blood oxygenation levels varied no more than 10% across all imaging sessions.

Surgerical Procedures

Cranial Window Installations

Following determination of their preferred for reaching forelimb, mice had a cranial window installed over their contralateral region of their motor cortex. Mice were first anesthestized with 30 mg/ml ketamine and 3 mg/ml xylazine and a circular craniotomy (~3 mm in diameter) performed. Photos of the skull, window and underlying vascular patterns are taken to enable later placement of the lesion based on coordinates relative to skull landmarks. A circular glass coverslip was then placed over the opening and sealed with cyanoacrylate. Mice were then allowed three weeks of recovery before beginning any other experimental procedures and received weekly injections of carprofen.

Photothrombotic Lesions

To create a photothrombotic lesion, mice were anesthetized with 2% isoflurane and were maintained with 1% isoflurane. The mice then received Rose Bengal (.15 mg/ml i.p) and, 3 minutes following the injection, a green laser (35 mW) was focused over the forelimb area of the motor cortex through a 5X objective (.1 NA) for 15 min. The region selected as the forelimb area was estimated by images of the craniotomy taken at the time of cranial window installation. These images had both skull and cortical vascular landmarks which allowed for accurate estimation. Animals that received vehicle procedures had identical experiences but were injected with saline (solvent for Rose Bengal) instead of Rose Bengal.

Tissue Analysis

Histology

At the conclusion of the experiment, animals were overdosed with sodium pentobarbital (150 mg/kg) and transcardially perfused with 0.1 M phosphate buffer followed by 4% paraformaldehyde in the same buffer. Brains were removed and sectioned into 50 μm thick coronal sections using a vibratome. Every 300 μm, a section was mounted onto a gelatin-coated slide and later Nissl stained using toluidine blue.

Volume of remaining SMC

Cortical volume measurements in the sensorimotor cortex (SMC) region of both hemispheres were used as an indirect measure of lesion size. At 12.5x magnification, seven 50 μm thick Nissl stained coronal sections within the caudal forelimb area of the MC (between 2 mm anterior and 1 mm posterior to Bregma) were traced using

Neurolucida perimeter tracing software (Microbrightfield Inc). Volume was calculated using the Cavalieri method (Gunderson et al., 1988), using the product of the distance between section planes and summed cortical areas.

Quantification of Cortical Blood Flow from MESI

Images acquired through MESI provide information on absolute blood flow rate. Each imaging frame is a 400 x 600 pixel frame. This frame was deconstructed from a matrix to a vector and the 90% trimmed mean was calculated for each image. To calculate blood flow change from baseline, each image was compared with the pre-stroke baseline image that had the closest recordings from the MouseOx.

Statistical Analysis

The *in vivo* measurements of cortical blood flow were assessed across time points using repeated measures analysis of variance (ANOVA) to determine temporal specifics blood flow gain and loss (Kazmi et al., 2013). Forelimb performance was analyzed using repeated measures ANOVA as well to explore the effect of Stroke, Day, and Stroke by Day interaction. Correlational models were constructed to determine the linear relationship between cortical blood flow changes and functional motor performance. Statistics were performed using SPSS (SPSS, Inc.) and results were considered significant at $p<0.05$. Data are mean ± SEM.

Results

Reaching Performance

The mice that received a photothrombotic lesion displayed a significant reduction in forelimb performance compared to vehicle controls that was restored by 10 days after the lesion (Figure 3). In repeated measures ANOVA the interaction between change from baseline behavioral performance in mice that received photothrombotic lesions was significant when compared with those that received sham procedures ($F(4,9) = 10.962$, $p = .002$). To investigate the simple effects of the changes in baseline motor performance at days 3, 5, 10, and 20 we performed independent t-tests. These independent t-tests produced were significant for days 3 ($t(12)=-5.388$, $p<.001$) and 5 ($t(12)=-2.588$, $p=.012$). The differences disappeared by day 10 ($t(12)=-.995$, $p=.339$) and continued through day 20 ($t(12)=-.806$, $p=.436$).

Cortical Blood Flow

Mice in both groups experienced repeated imaging using MESI. Mice that received a photothrombotic lesion displayed reductions in cortical blood flow compared to vehicles for 2 hours after the stroke (Figure 4). By three days after the stroke, cortical blood flow

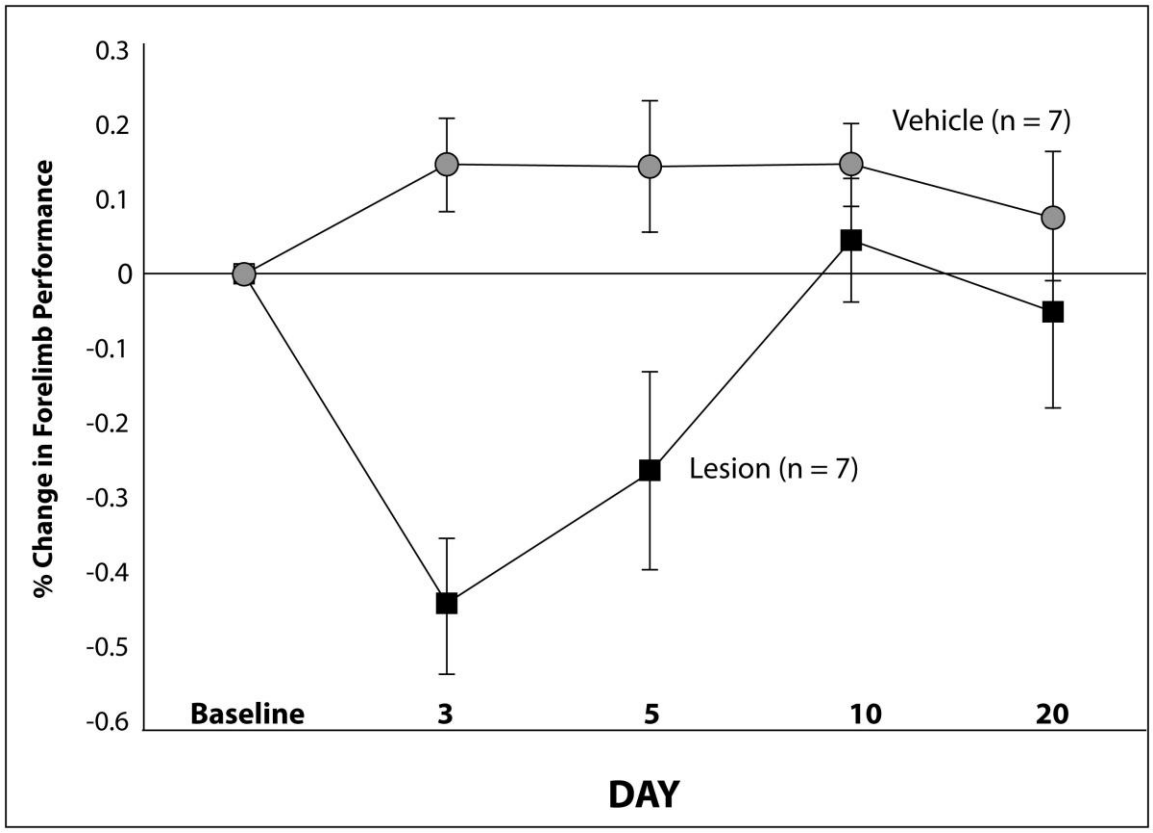


Figure 3: Forelimb reaching performance. All animals received forelimb training for 14 days before receiving a photothrombotic lesion or vehicle procedures. The baseline measurement is an average of the last three of these preoperative training days for each animal. Mice that received the lesion procedures displayed significantly decreased performance compared to sham-operated controls for days 3 and 5 after ($p<.05$). This effect was ablated by day 10 and continued to day 20 ($p>.05$). Data are displayed as mean ± SEM.

had returned to levels commensurate with baseline levels and vehicle controls. In repeated measures ANOVA there was a significant between groups and time interaction in the change from baseline cortical blood flow ($F(4,32) = 4.781$, $p = .004$). To investigate the simple effects of the changes in baseline cortical blood flow at 2 hours, and days 3, 5, 10, and 20 we performed independent t-tests. These tests revealed a

significant difference at 2 hours after the stroke ($t(9) = -2.732$, $p = .023$). All other time points were not significantly different between lesioned mice and sham-operated controls.

Lesion Size

Cortical infarcts were localized to the caudal forelimb representation of the SMC. No animals that received sham procedures displayed any evidence of cortical damage. Despite the qualitative presence of cortical damage in the lesion group, there were no significant differences in cortical volume when comparing the lesioned hemisphere to non-lesioned hemisphere ($t(6)=-1.033$, $p = .18$). In the lesion group, five of the seven mice displayed a decreased volume in the lesion hemisphere compared to non-lesion hemisphere and ranged from -3.38 mm^3 to 2.20 mm^3. Additionally, there was no significant difference in the inter-hemispheric volume difference for mice that received lesion procedures and those that received sham procedures ($t(12)=.739$, $p = .24$).

Cortical Blood Flow and Behavior Correlations

Correlations between cortical blood flow and forelimb performance were calculated for mice that received lesions. As mice that did not receive lesions were not expected to display fluctuations in cortical blood flow that reflected forelimb performance, they were not included in these calculations. To quantify the measures of interest, changes from baseline measurements were calculated for both cortical blood flow and forelimb performance. Namely, these changes for behavior were calculated as a change from baseline with respect to forelimb performance at days 3 and 20. For CBF, changes were calculated for two hours and three days with respect to heart-rate matched baselines.

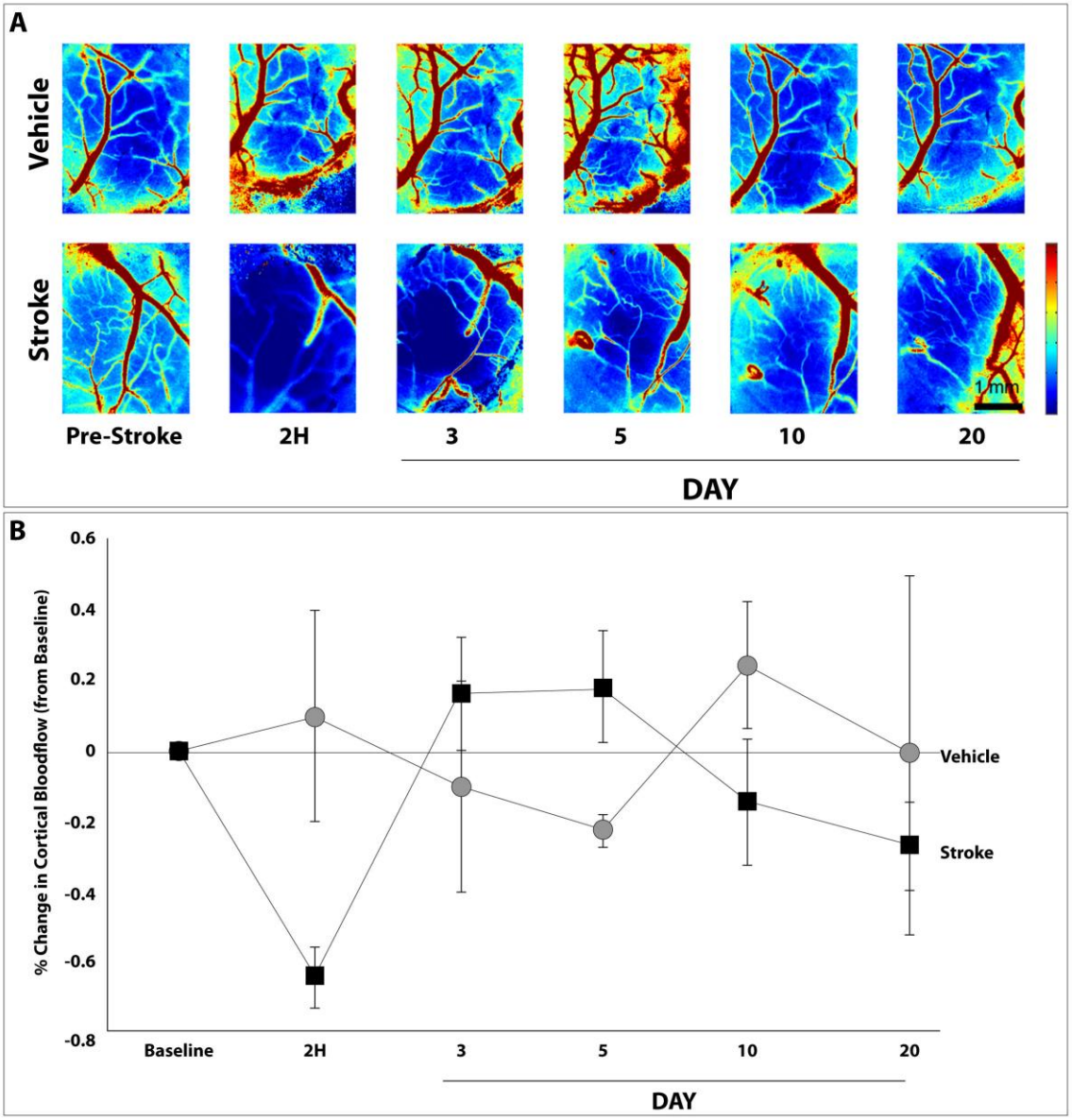


Figure 4: MESI data for lesion and sham groups. **A**: After 3 baseline imaging sessions spaced over 14 days, all mice received either photothrombotic lesions or sham procedures. All animals were then imaged 2 hours, 3 days, 5 days, 10 days, and 20 days following the lesions. **B:** Ninety % trimmed means of the MESI frames were computed and change calculated from heart-rate matched baseline frames. This data show that there was a significant reduction in cortical blood flow 2 hours after the lesion compared to vehicle controls. Furthermore, this effect was gone three days after the lesion and continued to the end point of the study.

Stroke severity (calculated as the two hour drop from baseline of CBF) displayed very strong correlations with forelimb performance at three days after stroke (r=.982, p < .001) and forelimb performance twenty days after stroke (r=.872, p=.011). CBF three days after the lesion did not display significant correlations with forelimb performance at three days after the lesion (r=.142, p = .76) or twenty days (r=-.023, p = .96).

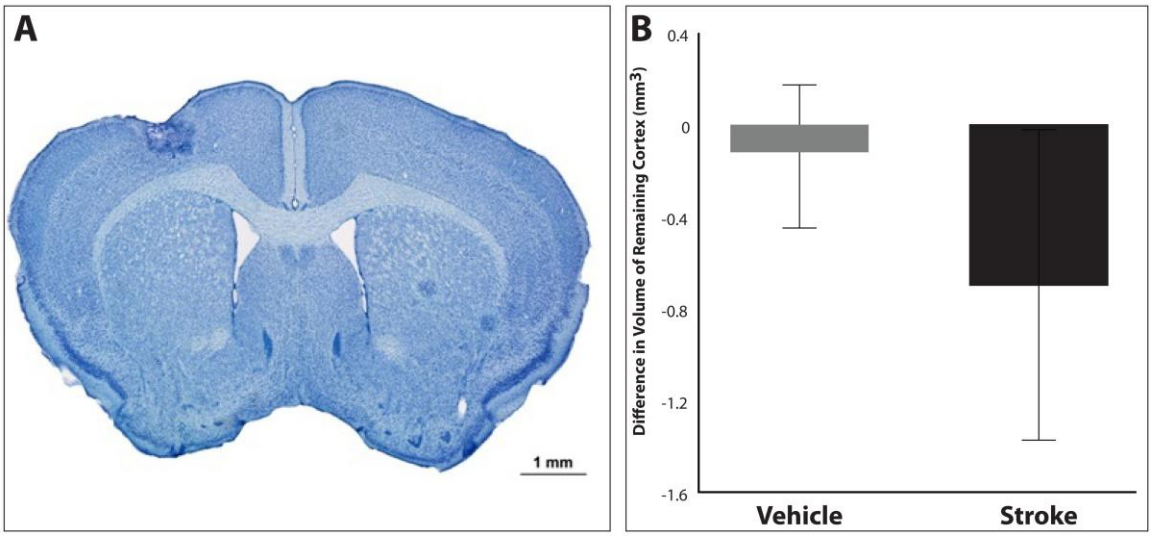


Figure 5. Representative Coronal Section of Lesion and Cortical Volume Estimation. **A**: Twenty days following the lesion mice were euthanized, perfused, and coronal sections of their brains Nissl stained. This is a representative lesion from one of the mice. **B:** Cortical areas were traced using Neurolucida software and cortical volume estimated using the Cavalieri method (∑(cortical area*Length to next section sampled)). The difference between the lesioned hemisphere and non-lesioned hemisphere was then calculated. For mice that received vehicle procedures, the side that had the cranial window installed was treated as the lesioned hemisphere. The plots here display the mean differences ± SEM.

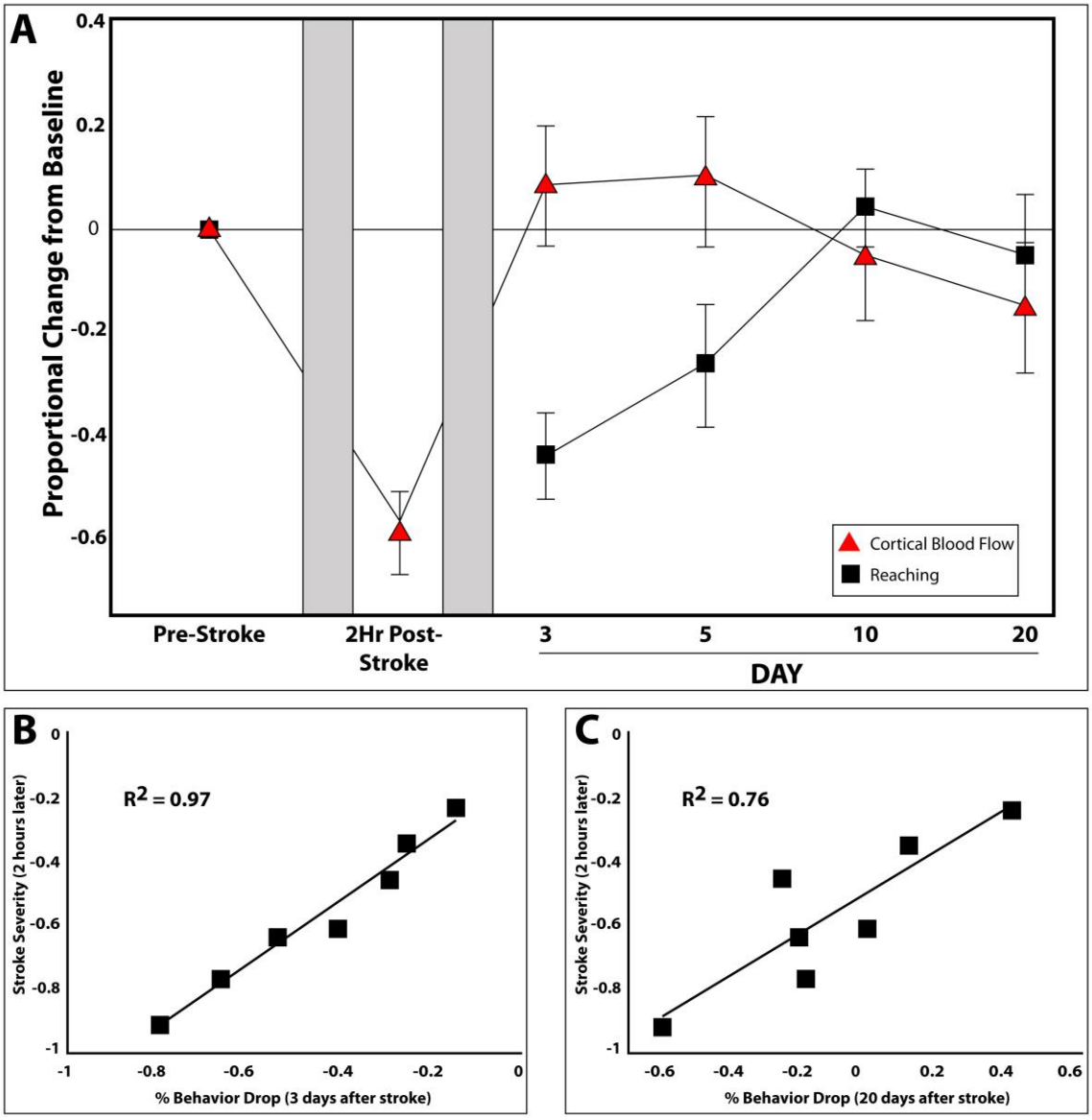


Figure 6. Comparison of cortical blood flow and forelimb performance. epresentative Coronal Section of Lesion and Cortical Volume Estimation. **A**: For animals that received a lesion, cortical blood flow was restored by three days following the stroke and forelimb performance was restored by ten days. This indicates a lag in behavioral recovery. The plots here display the mean differences ± SEM. **B**: Forelimb performance three days after the lesion displays a significant correlation with stroke severity. **C**: Stroke severity also displays a strong correlation with forelimb performance twenty days following the lesion.

Discussion

The primary goal of this project was to determine the temporal relationship between neurovascular remodeling in the SMC and functional behavioral improvements following stroke. While the presence of these events is well established, the time course of blood flow recovery had not been explored in tandem with post-ischemic motor functional changes. Recent advances in imaging techniques have allowed for the cortex of animals, especially mice, to be imaged repeatedly with microscopic resolution (Mostany, R, Portera-Cailliau, C, 2008; Yang et al., 2010; Holmaat et al., 2009; Svoboda et al., 1997; Tomita et al., 2005). This study addressed a large shortcoming by using behavioral manipulations with *in vivo* imaging to explore the relationship between motor improvements and recovery of blood flow in peri-infarct cortex. By observing the time course of cortical blood flow restoration in tandem with the recovery of skilled forelimb function following a unilateral infarct to the SMC, we aimed to establish the relationship between neuro-anatomically relevant changes in cortical blood flow with improvements in motor function.

As we were interested in combining *in vivo* imaging (Kazmi et al., 2013) with sensitive behavioral assays for forelimb motor function (Tennant, Jones, 2009), we developed a mouse model of cortical ischemia with a cranial window installed over the SMC (Holmaat et al., 2009). The mouse as a model was ideal for this study as most *in vivo* imaging techniques have been developed using mouse models (namely for their thin skulls and transgenic capabilities) (Mostany, Portera-Cailliau, 2008; Yang et al., 2010;

Holmaat et al., 2009; Svoboda et al., 1997; Tomita et al., 2005; Kazmi et al., 2013). By imaging cortical blood flow through a chronic cranial window, we using *in vivo* multi-exposure speckle imaging (MESI) (Kazmi et al., 2013) to track, follow the temporal recovery of cortical blood flow after an ischemic injury. In mice with cranial windows, a photothrombotic infarct was created in the forelimb area of SMC, which is established to result in upper extremity (forelimb) dysfunction (Farr, Whishaw, 2002; Tennant, Jones, 2009). Additionally, focused infarcts to the SMC in rodents has been shown to reliably induce upper extremity impairments (Tennant, Jones, 2009).

To assess forelimb function, the PMRT was used as it is established as both a reliable metric for skilled forelimb function and as a model of motor rehabilitative training, i.e., it can be used to promote improvements in skilled forelimb function after SMC infarcts (Tennant, Jones, 2009). Photothrombotic lesions were used because this method allows non-invasive infarct induction through cranial windows and minimizes the potential for other aspects of the induction procedure to disrupt vasculature (Brown et al., 2007; Brown et al., 2009). We used a cranial window installed over the SMC to allow for repeated imaging of cortical blood flow (Kazmi et al., 2013). Creating the lesions over the SMC allowed us to track motor recovery over time and determine the extent to which the blood flow changes following a stroke are temporally linked with recovery in motor function.

Early after the lesion mice displayed a significant decrease in paretic forelimb performance compared to vehicle controls. This effect was present for 5 days after the lesion and gone by 10 days after the lesion. Additionally, blood flow was reduced in mice

that received lesions and was restored to levels commensurate with baseline CBF at three days after the lesion. The behavioral data strongly correlated with the cortical blood flow data which provides support that the stroke severity (as indexed by a drop from baseline blood flow) is strongly correlated with behavioral outcomes. Blood flow recovery occurs in the peri-infarct cortex and the extent to which this area becomes re-perfused coincides with functional behavioral improvements.

Our findings here support that neuro-anatomically relevant damage is closely linked to behavioral recovery following a stroke. Further, this model can be used to explore the influence of behavioral and pharmacological manipulations on the outcome of stroke.

Methodological Pitfalls

Mice displayed plateau levels of CBF and behavioral recovery fairly early after the lesion indicating that the infarcts were not substantial enough. Additionally, while there was a trend, there was no significant difference between hemispheric volume of lesion and non-lesion hemispheres. Furthermore, in less than two weeks following the lesion mice displayed skilled forelimb performance commensurate with pre-lesion levels. More substantial infarcts will be used in future studies to capture instances of a more prolonged recovery.

Blood flow recovery occurs in the peri-infarct cortex and the extent to which this area becomes re-perfused coincides with functional behavioral improvements. However, MESI imaging only samples from the more superficial layers of the cortex, such that it is insensitive to any depth-dependent variations in blood flow recovery. All mice in this

study were perfused with *Lycopersicon Esculentum* (tomato lectin) to visualize perfused vasculature throughout the depth of remaining SMC. From histological sections, the length density of remaining capillaries will be quantified using stereological methods (virtual spherical probes; in anatomical subregions delineated based on cytoarchictecture and movement representations). To understand the depth-dependent link of vessel densities and blood flow recovery, we will separately quantify vessel densities in each layer of the cortex to alert us to the existence of depth-dependencies. We expect vessel densities and blood flow recovery to be positively linked and we will separately quantify vessel densities in each layer of the cortex to alert us to the existence of depth-dependencies.

MESI imaging was performed under isoflurane anesthesia (to maintain immobility) and blood flow rates are influenced by anesthesia level. The anesthetic plane of the animals varied greatly between imaging sessions and to control for this, physiological parameters were continuously monitored (with MouseOx) and isoflurane levels adjusted such that heart rate and blood oxygenation levels varied no more than 10% during the time for image acquisition.

Additionally, repeated anesthesia could attenuate skilled forelimb performance and recovery of CBF following an ischemic lesion. To probe for any potential confounding effects of repeated anesthesia, a subset of mice will receive imaging at only the baseline and final timepoint. If an effect of repeated anesthesia appears, additional mice that receive at fewer time points, staggered across the study to minimize anesthesia exposure. However, the data in this study suggest that this is very unlikely. Clouding of

cranial windows can prevent imaging at later time points. To minimize this complication, Carprofen (an anti-inflammatory shown to prevent clouding) was given weekly after installation of the cranial window and stopped after induction of the ischemic lesions.

Conclusions

This study provides a model of stroke which can be used to study behavioral outcomes in conjunction with *in vivo* imaging of cortical blood flow. As expected, cortical infarcts resulted in motor impairments that spontaneously improved over time. We also anticipated that the magnitude of recovery of blood flow in surviving forelimb regions is predictive of improvements in skilled forelimb function. In so doing, this model will improve the direction of experimental models of chronic stroke and inform clinical treatment approaches.

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