Supplementary materials for:

Characterizing spontaneous motor recovery following cortical and subcortical stroke in the rat

Authors: Sudhir Karthikeyan, M.Sc.[1,2]; Matthew Strider Jeffers, M.Sc.[1,2]; Anthony Carter, B.Sc.[1,2]; Dale Corbett, PhD[1,2,*]

Supplementary Methods

Adhesive Strip Removal Task: The adhesive strip removal task was utilized to evaluate sensorimotor impairments following stroke.[1] Two rectangular pieces of adhesive tape of equal size (20 mm × 12.5 mm) were applied with equal pressure on the plantar surface of each paw of each animal. Animals were then placed in a Plexiglas cylinder and observed by two experimenters. The time to initial contact and time to remove tape were recorded for each forepaw. Each trial continued for 2 minutes or until the animals successfully removed the tape on both paws. Animals were trained on this task daily for 5 days and their performance on day 5 was used as the measure of baseline performance. For post-stroke assessment an average of 2 trials separated by a 3 hour break was used for each time point.

Supplementary Results

Adhesive Removal Test

Two rats in the combined group failed to learn the task during the training sessions and were therefore excluded from this analysis. Following training, the remaining animals took ~5 seconds to contact and ~24 seconds to remove the tape with their dominant paw and no differences existed between groups (**Supplementary Figure 1A, C**).

Time to Contact Tape. After stroke, all groups took longer time to contact the tape with their contralateral forelimb (seconds taken: 9.4 ± 1.9 for striatal-only, 14.6 ± 5.1 for cortical-only and 38.4 ± 16.3 for the combined group); however significant post-stroke impairments could only be detected in the striatal-only group ($p<0.046$; **Supplementary Figure 1A**). These impairments were observed the first week following stroke. Repeated-measures ANOVA indicated an effect of time ($F=6.74$, $p=0.005$) but not group for post-stroke time points. There were no significant differences in the amount of improvement in the measure of time to contact between the groups (**Supplementary Figure 1B**).

Time to Remove Tape. The cortical-only and combined group took significantly longer to remove the tape with the contralateral forelimb following stroke ($p\leq0.039$; **Supplementary Figure 1C**). A significant effect of time ($F=4.24$, $p<0.005$) was observed for all post-stroke time points. Significant impairments could be detected at week 1 for the combined group and up until week 2 for the cortical-only group ($p<0.05$). There were no significant differences in the amount of improvement in the measure of time to remove between the groups (**Supplementary Figure 1D**).

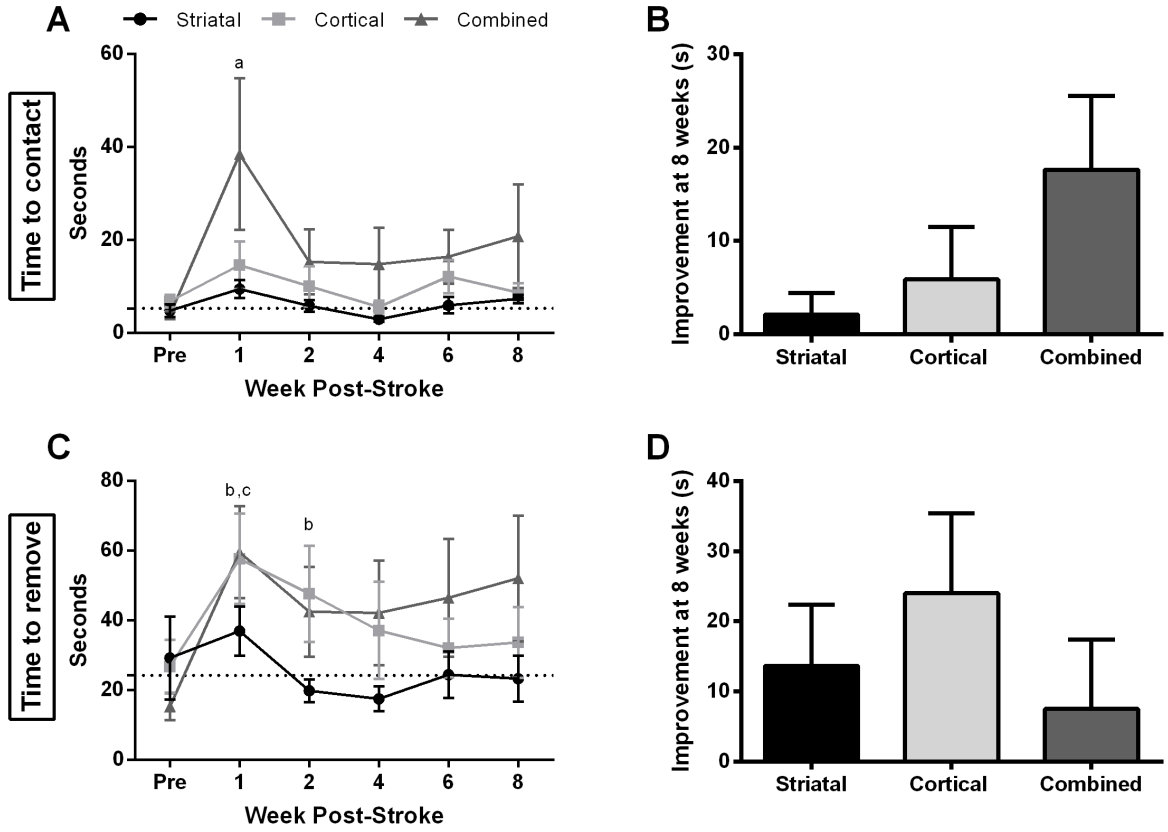
Proportion of Lesion in Forelimb versus Hindlimb Motor Cortex

We assessed the relative proportion of our lesions that were in the forelimb and hindlimb motor cortex regions. We defined the forelimb motor cortex as coronal sections anterior to 0.0 mm anterior to Bregma, based on previous intracranial microstimulation studies in similar size/strain of rats.[2] Coronal sections posterior to 0.0 mm relative to Bregma were defined as hindlimb motor cortex. Using these criteria, we found that the distribution of cortical injury in the striatal group was significantly more weighted toward forelimb-associated regions than the other groups sections ($F_{2,23} = 7.728$, $p = 0.003$; **Supplementary Table 3**) using one-way ANOVA. Our cortical injections impact both the forelimb and hindlimb motor cortices; however, this is

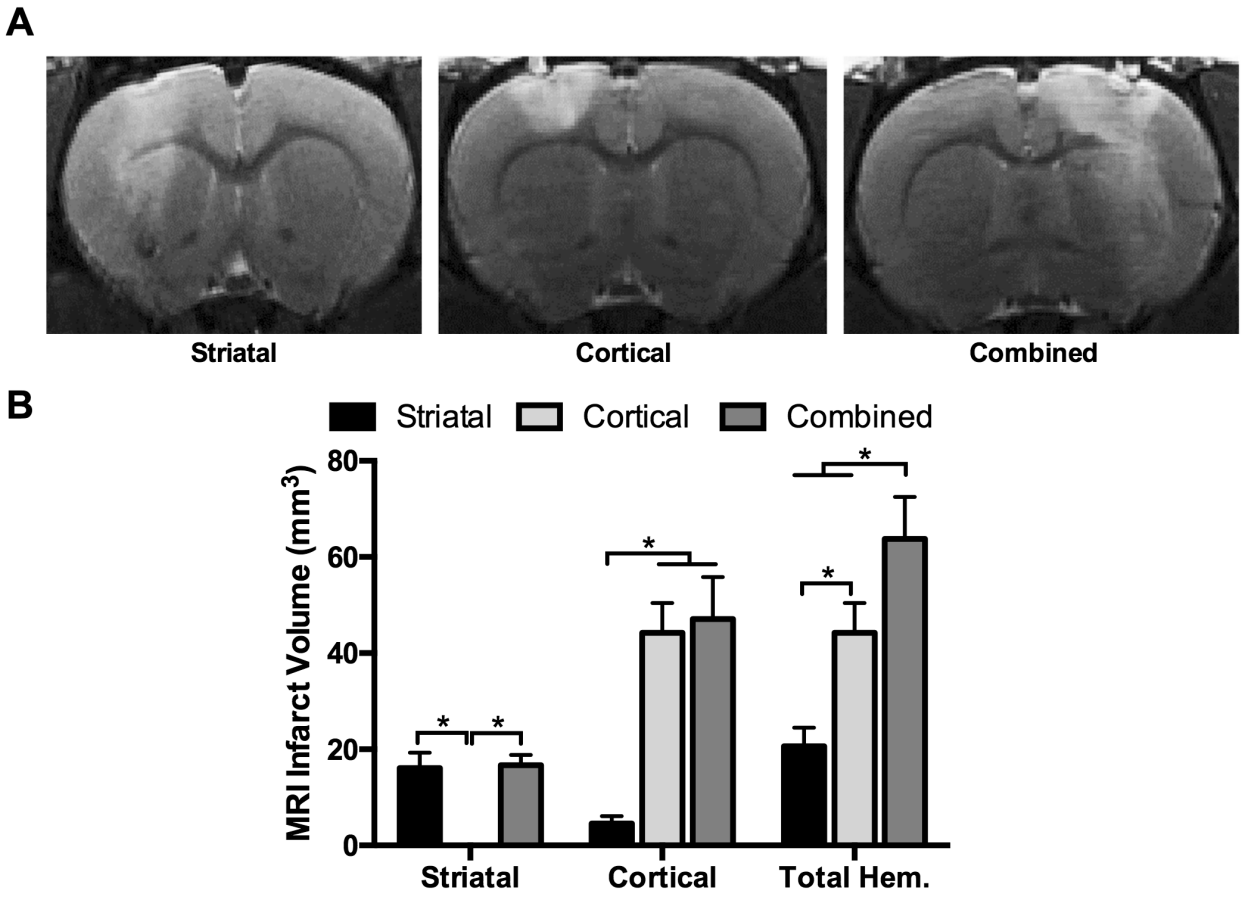
weighted more toward the forelimb regions with ~80% of the lesion in the forelimb cortex and ~20% of the lesion in the hindlimb cortex. With the striatal injection, the small amount of cortical damage from that model is even more heavily weighted towards the forelimb, with ~97% forelimb, ~3% hindlimb. Interestingly, rats in the striatal group had significant impairments in their hindlimbs on the beam walking task (Figure 5) despite many rats having no cortical damage in hindlimb-associated regions, demonstrating the importance of the striatum for hindlimb function.

Supplementary References

1. Bouet V, Boulouard M, Toutain J, et al. The adhesive removal test: a sensitive method to assess sensorimotor deficits in mice. *Nat Protoc*. 2009;4(10):1560-1564.
2. Touvykine B, Mansoori BK, Jean-Charles L, Deffeyes J, Quessy S, Dancause N. The effect of lesion size on the organization of the ipsilesional and contralesional motor cortex. *Neurorehabil Neural Repair*. 2016;30(3):280-292.



Supplementary Figure 1. Adhesive removal test. (A) Significant contralateral forelimb impairments were only detected in the striatal-only group ($F=6.74$, $p=0.005$). (B) There were no statistical differences between groups in the amount of improvement between post-stroke week 1 and 8 on the time to contact tape. (C) Both the cortical-only and combined group took significantly longer to remove the tape with the contralateral forelimb, whereas the striatal-only group was unaffected. Significant impairments were detected at week 1 for the combined group and up until week 2 for the cortical-only group ($F=4.24$, $p<0.005$). (D) There were no statistical differences between groups in the amount of improvement between post-stroke week 1 and 8 on the measure of time to remove tape. Values are means ± SEM. Significance markers for pre-stroke vs. post-stroke within-group differences: a = striatal-only, b = cortical-only, c = combined; $p<0.05$.



Supplementary Figure 2. Infarct volumes from magnetic resonance imaging (MRI). (A) Representative T2-weighted MRI slices showing typical stroke lesions in the three groups. (B) Quantification of infarct volumes from MRI (72 hours post-stroke). Infarct volumes estimated from early MRI were larger but correlated with volumes from cresyl violet stained tissue. The combined group had the largest total hemispheric damage. The amount of cortical damage was similar in the cortical-only and combined group. There was no difference in striatal infarct volume between the striatal-only and combined group. Values are means ± SEM. * = $p<0.05$. Total Hem. = total hemispheric infarct volume.

Supplementary Table 1. Stereotaxic injection coordinates (relative to Bregma)[a].

Group	Injection #	AP	ML	DV	Total Volume ET-1	Infarct Volume (mm^3)	ET-1 per mm^3
Striatal-only	1	+0.7	± 3.8	-7.0#	1 µl	15.1 ± 2.2	0.088 ± 0.017
Cortical-only	1	0.0	± 3.0	-1.7*	2 µl	23.1 ± 3.6	0.121 ± 0.073
	2	+2.3	± 3.0	-1.7*			
Combined	1	0.0	± 3.0	-1.7*	3 µl	49.5 ± 10.0	0.082 ± 0.049
	2	+2.3	± 3.0	-1.7*			
	3	+0.7	± 3.8	-7.0#			

[a]All injections were 1 µl in volume. Based on the infarct volumes obtained using final histological outcome (Figure 2), we calculated the concentration of ET-1 delivered in each model per unit volume of tissue damaged (µl/mm^3). Despite delivery of higher total volumes of ET-1 in different regions, there were no significant differences in ET-1 per mm^3 of damage between groups ($F_{2,23} = 1.154$, $p = 0.333$) using one-way ANOVA. ET-1 appeared to have a consistent lesion-inducing effect between groups. Values are means ± SD. Abbreviations: AP, anteroposterior; ML, mediolateral; DV, dorsoventral.* = from brain surface, # = from skull surface.

Supplementary Table 2. Influence of white matter damage on stroke impairment[b].

Behavioural Task	White Matter Damage?	Pre-stroke	Week 1 Post	Week 2 Post	Week 4 Post	Week 6 Post	Week 8 Post	p-value
Staircase	No	17.6±2.1	12.3±3.4	13.6±3.6	13.5±3.2	14.0±3.3	14.5±3.3	0.146
	Yes	16.3±2.3	10.6±3.6	11.8±3.3	11.7±2.8	12.4±2.8	12.8±4.0	
Beam (forelimb)	No	93.4±7.5	85.4±12.9	83.1±15.2	88.0±10.8	88.9±9.2	88.4±9.9	0.781
	Yes	94.2±5.4	84.8±5.5	76.8±17.6	90.2±4.1	85.0±13.2	90.7±5.2	
Beam (hindlimb)	No	95.5±4.5	68.7±25.9	78.3±12.7	79.3±10.7	84.6±9.4	85.0±8.6	0.637
	Yes	97.4±1.8	69.9±21.2	83.3±10.8	79.4±14.2	86.9±13.0	85.1±16.4	
Cylinder	No	52.8±7.4	23.2±11.2	30.1±10.8	35.4±14.1	39.2±19.3	37.5±14.1	0.971
	Yes	48.8±8.7	23.6±10.8	30.7±15.4	31.2±15.4	41.2±13.3	40.9±13.1	
Tape (contact)	No	8.1±8.5	18.9±20.4	7.7±6.3	6.2±6.3	13.8±16.2	10.2±7.3	0.204
	Yes	10.4±17.7	36.3±47.1	21.7±25.2	11.6±18.3	13.5±15.3	17.7±26.3	
Tape (removal)	No	28.9±28.1	56.0±34.0	37.8±27.4	30.6±28.5	35.4±31.9	35.2±30.4	0.498
	Yes	46.7±41.5	57.8±43.4	48.7±49.8	39.9±45.5	38.7±33.3	41.7±41.5	

[b]Damage to the corpus callosum was assessed from cresyl stained sections. Approximately a third of the animals (9/26) had incidental damage to the corpus callosum that was equally distributed across the striatal, cortical and cortical + striatal stroke groups. This table shows the data for each group across time in the scale of each task (staircase = pellets retrieved; beam = % successful steps; cylinder = % impaired limb use; adhesive = seconds to contact/remove). The n of "no" = 17, n of "yes" = 9. Correlational analysis revealed no significant relationship between white matter injury and impairment on any of the behavioural tests. Values are means ± SD. P-value is the between-subjects main-effect of lesion location (no vs. yes averaged across time points) using repeated measures ANOVA. Time by group interaction was also examined but was not significant, and not shown here.

Supplementary Table 3. Proportion of lesion in forelimb vs. hindlimb motor cortex[c].

Group	7.2 to 0.0 AP (mm^3)	0.0 to -6.4 AP (mm^3)	Total (mm^3)	% Forelimb	% Hindlimb
Striatal	4.4 ± 4.2	0.2 ± 0.5	4.6 ± 4.3	97.3 ± 7.6	2.3 ± 7.6
Cortical	34.7 ± 12.1	9.5 ± 8.6	44.2 ± 18.7	81.1 ± 10.8	18.9 ± 10.8
Combined	35.7 ± 17.3	11.4 ± 10.1	47.1 ± 26.3	78.8 ± 12.2	21.2 ± 12.2
Total	25.7 ± 18.9	7.3 ± 9.0	33.0 ± 26.7	85.3 ± 13.0	14.7 ± 13.0

[c]The infarct volume in sections anterior and posterior to 0.0 mm relative to Bregma was determined from MRI images. This was used to calculate the proportion of the injury that was more likely to be in forelimb or hindlimb motor cortex (see Supplementary Results). The striatal injury had a larger proportion of what was incidental cortical injury in forelimb-associated sections than the cortical or combined groups ($F_{2,23} = 7.728$, $p = 0.003$) because the ET-1 injection needle passed through the center of the forelimb cortex en route to the striatal target

Values are means ± SD.