



# Impairments and compensation in string-pulling after middle cerebral artery occlusion in the rat

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## ABSTRACT

Stroke is a leading cause of long-term disability in humans, and it is frequently associated with impairments in the skilled use of the arms and hands. Many human upper limb impairments and compensatory changes have been successfully modeled in rodent studies of neocortical stroke, especially those that evaluate single limb use in tasks, such as reaching for food. Humans also use their hands for bilaterally coordinated movements, dependent upon interhemispheric cortical projections, which are also compromised by unilateral stroke. This study describes middle cerebral artery occlusion (MCAO) dependent changes in the bilaterally dependent hand use behavior of string-pulling in the rat. The task involves making hand-over-hand movements to pull down a string that contains a food reward attached to its end. MCAO rats missed the string more often with both hands than Sham rats. When the string was missed on the contralateral to MCAO body side, rats continued to cycle through subcomponents of string-pulling behavior as if the string were grasped in the hand. Rats also failed to make a grasping motion with the contralateral to MCAO hand when the string was missed and instead, demonstrated an open-handed raking-like motions. Nevertheless, with repeated attempts, rats performed components of string-pulling well enough to obtain a reward on the end of the string. Thus, string-pulling behavior is sensitive to bilateral impairments but is achieved with compensatory adjustments following MCAO. These aspects of MCAO string-pulling provide a foundation for studies that investigate the efficacy of therapeutic intervention which might enhance neuroplasticity and recovery.

## 1. Introduction

Stroke is a leading cause of long-term disability most commonly affecting the upper limbs acutely in 80% of patients and chronically in 40% of patients [1]. Disruptions in bimanual coordination and fine motor skills of the hands are reported by patients following stroke, to the extent that tasks involving the use of both hands are avoided [2,3]. The most common strokes are ischemic and result in an occlusion of the middle cerebral artery (MCA). The MCA supplies blood to cortical and subcortical brain regions, including primary motor and somatosensory cortical areas responsible for the use of the face, trunk, and upper limbs [4]. In rodents the MCA occlusion (MCAO) is used to model human upper limb dysfunction, recovery, and therapeutics [5–9]. The ability to

characterize rodent performance following MCAO depends on several factors, including lesion extent/location and the type of task used to assess fine motor control [10,11]. While a variety of techniques currently exist to assess voluntary fine motor control in rodent models of stroke, tasks that can similarly assess the use of both hands would extend the utility of rodent stroke models [12]. One such task is string-pulling.

Rodents spontaneously engage in bimanually coordinated string-pulling behavior that involves hand-over-hand movements to pull in a string to retrieve a food reward attached to its end [13,14]. String-pulling behavior provides an assessment of the arms in reaching and pulling, of the hands in grasping and releasing, and in the coordination by which the two hands advance the string [15,16]. The task is described as a prototool task, because the rat must appreciate that the

*Abbreviation:* MCAO, middle cerebral artery occlusion.

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string is an instrument for obtaining a food reward, and as such, the task is sensitive to learning the appreciation of the relation between the string and the reward and motivation to acquire the reward. A strength of the task is that an animal performs many reaching movements in a single test session and in doing so, stands upright exposing the movements of the nose in following the string and the hands and arms in pulling. These behavioral features increase the ease of end point and kinematic documentation of behavior. The objective of the present study was to determine whether the string-pulling task would be sensitive to MCAO, which in the rat largely damages the lateral neocortex, including somatosensory neocortex, while largely sparing primary and secondary motor cortices. A further objective of the study was to determine if string-pulling behavior is sensitive at detecting changes in movement organization with resulting recovery and compensation after MCAO.

For the study, rat string-pulling behavior was investigated after MCAO stroke. Measures were made of the Advance, Grasp, Pull, Push, Release, and Lift arm components of multiple upward reaches to grasp the string and downward withdraws to advance it. The measures also reflect the sensory ability of the rat to locate and track the string with its nose and vibrissa as pulling takes place and any use of the mouth to supplement hand use. The end point measures of success in the string-pulling task were supplemented with many kinematic measures to reveal the topographic organization of movements used in advancing the string.

**2. Methods**

**2.1. Subjects**

A total of 15 male Long-Evans rats (*Rattus norvegicus*) were approximately three months of age at the beginning of the study; rats that did not perform during the training sessions were categorized as non-pullers and excluded from the study (n = 3). The remaining rats were randomly divided into Sham (n = 6) and MCAO (n = 6) groups. Vivarium temperatures (20–21 °C) and light (12-h light-dark cycle) conditions were consistent throughout testing. Rats were food deprived for two nights prior to beginning string-pulling behavior and provided water ad libitum. All experimental protocols (see experimental timeline Fig. 1) were approved by Southern Illinois University Carbondale Institutional Animal Care and Use Committee.

**2.2. Surgery**

The middle cerebral artery occlusion surgery was performed using methods described previously [17]. Briefly, rats were anesthetized with isoflurane (5% in oxygen) and then placed in a stereotaxic device with an integrated anesthesia port. Rats were maintained on isoflurane (1–2.5% in oxygen) for the duration of the procedure. The left skull was exposed, and a craniotomy was made to allow access to the MCA close to where it exited the rhinal fissure. The MCA was then permanently ligated with a 10–0 suture and transected. Each animal then underwent a permanent occlusion of the common carotid artery on the same side as

the MCAO and a temporary occlusion (15 min) of the contralateral common carotid artery. Permanent and temporary common carotid occlusion are necessary to produce strokes in our model, due to the robust communication in the LE Hooded brain vasculature [18–20]. Rats’ left hemispheres were chosen to receive permanent MCAO. Patients with strokes often present with unilateral permanent cerebral ischemia rather than reperfusion providing further basis for the model used in the current study. After the procedure, rats were removed from isoflurane anesthesia and allowed to recover before they were returned to their home cages.

**2.3. Apparatus**

The string-pulling apparatus was a transparent, rectangular box (19 cm × 19 cm). The apparatus sat on a table in a room with many cues. The rat remained in the testing apparatus for the entire string-pulling session within each day. In between each rat, the testing apparatus was thoroughly cleaned and prepared for the next rat.

**2.4. Procedures**

Rats were habituated to 0.5 m strings with unsalted cashews tied to the end draped in their home cage for two nights. Then, rats were trained on string-pulling across four consecutive days. String-pulling training sessions consisted of each rat pulling five 1.0 m strings within a 20-minute testing period. The following day, baseline performance was measured across four trials within a 20-minute testing session prior to surgeries. Rats were tested under the same conditions in string-pulling behavior on days 3, 7, and 14 following MCAO. As soon as the rat finished all four trials the test ended. All rats pulled in four trials within the 20 min allotted for each test.

**2.5. Behavioral analysis**

Behavioral analyses were conducted using the four string-pulling trials from all rats for each test. At least five reach and withdraw movements were evaluated from each trial per rat for each test (i.e., 20 reaches and 20 withdraws per rat each test). All reaches and withdraws when the rat was facing forward with their feet planted and forward, facing the camera were included in the behavioral analysis. String-pulling behavior where rats faced one side or the other and were not directly forward were excluded from analysis.

**2.5.1. General performance measures**

Approach and pull times were used as general measures of performance in the string-pulling task. The amount of time it took rats to approach the string once placed in the apparatus, and the amount of time it took rats to pull in the string to reach the food item at the end were used as measures of motivation to engage in and complete the string-pulling task respectively.

**2.5.2. Broad hand movement analysis**

String-pulling behavior is composed of phases of upward reaches away from the body to grasp the string and downward withdraws toward the body to pull in the string. String-pulling behavior is dependent on the ability of the hands to grasp the string to pull it in. The number of contacts (closing of digits around the string) was evaluated across testing. Misses that occurred when the rat failed to contact the string during the transition from the reach to withdraw phase was also measured across testing.

Rats use their mouth to contact the string. Thus, ratios were calculated to evaluate mouth contacts relative to left- and right-hand contacts. Values generated closer to one mean that the mouth contacted the string more than the hand, while values calculated near zero represent more hand than mouth contacts. The intermediate values represent an equal number of contacts elicited by the mouth and hand.

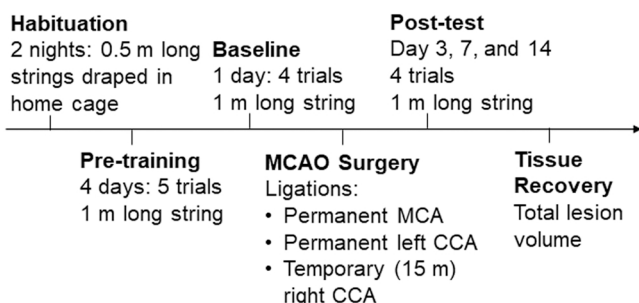


Fig. 1. A timeline shows each stage of the experiment with general details.

### 2.5.3. Sequential movement analysis

A string-pulling cycle may be further decomposed into five different movement components: Advance, Grasp, Pull, Push, and Lift. Rodents typically distribute movements evenly across all five components. Operationalized movement categories defined as Advance, Grasp, Pull, Push, or Lift were visually identified as previously described [13]. The proportion of each movement component out of the sum of the five movement components was assessed for each subcomponent during contacts (i.e.,  $\text{Contact Advances} / \sum(\text{Contact Advances} + \text{Grasps} + \text{Pulls} + \text{Pushes} + \text{Lifts})$ ) and misses (i.e.,  $\text{Miss Advances} / \sum(\text{Miss Advances} + \text{Grasps} + \text{Pulls} + \text{Pushes} + \text{Lifts})$ ) with the string separately.

### 2.5.4. Motion capture analysis

String-pulling behavior was captured at 30 frames per second with a high-definition video camera (Canon Vixia HF21) for offline analysis. This frame rate has been shown to be sufficient to capture rodents fine motor movements during string-pulling behavior [13–16,21,22]. An open-source movement tracking software, Tracker ([www.physlets.org/tracker/](http://www.physlets.org/tracker/)), was used to manually digitize the left and right hands and the nose frame-by-frame during bouts of string-pulling behavior. The environment in the string-pulling videos was scaled to real world space using the Tracker program to generate accurate XY data.

**2.5.4.1. Broad nose and hand kinematic analysis.** Average moment-to-moment speed (cm/s) was examined between the left- and right-hands. Ratios were calculated to compare the speed of the nose to the left- and right-hands. Values calculated near one represent faster nose than hand movement, and values generated closer to zero mean faster hand than nose movement. The intermediate values represent equal speeds traveled by the nose and hand. Average moment-to-moment speed of the nose was also examined.

Average total distance traveled of the left- and right-hands was evaluated as ratios. Values calculated near one represent further distances traveled by the left-hand, while values generated closer to zero represent further distances traveled by the right-hand. The intermediate values represent equal distances traveled by the hands. Ratios were calculated to compare the average total distance traveled of the nose to the left- and right-hands. Values calculated near one represent further total distance traveled with the nose than the hand, and values generated closer to zero mean further total distance traveled by the hand than the nose. The intermediate values represent equal total distances traveled by the nose and hand. Average moment-to-moment speed of the nose was also examined.

**2.5.4.2. Reach and withdraw component kinematic analysis.** XY data generated by digitization via the Tracker program was segmented into reaches and withdraws based on the direction of movement (i.e., up or down). General (distance) and specific (path circuitry, concentration, heading) measures of movement organization were quantified for reaches and withdraws with the left and right hands separately across testing. Distance traveled during reaches and withdraws was evaluated for the left and right-hand for each trial. Path circuitry of reach and withdraw phases of movement was calculated by dividing the Euclidean distance by the total distance traveled. More direct paths yield values closer to one and more circuitous paths are closer to zero.

Next, circular statistics were used to evaluate the concentration of reach and withdraw endpoints and their heading directions. The variability of the directional heading of reach and withdraw phases of movement were calculated using parameter of concentration. Circular statistics were used to quantify the consistency of heading directions for sampled string-pulling trials within each testing day [23]. The start and end coordinates for both phases of movement were transformed such that the start of the path was the origin (0, 0), and the angle of the end coordinate was calculated relative to a polar coordinate system (0°: right; 90°: up; 180°: left; 270°: down). Values range from zero (variable

heading that are uniformly distributed all directions) to one (no variability in heading). These values were used to calculate average parameter of concentration for each day across testing. Typically, reach and withdraw endpoints are tightly clustered in one consistent location with little variability in heading; however, cortical damage leads to inconsistent or variable endpoints that are not tightly clustered [14,22]. This is used as a measure of movement consistency.

Reach and withdraw phases of movement are oriented in a specific direction. The directional heading of movement was evaluated by transforming the start and end coordinates of the path such that the start of the path is the origin (0,0), and the angle of the end coordinate is calculated relative to a polar coordinate system (0°: right; 90°: up; 180°: left; 270°: down).

**2.5.4.3. Nose kinematic analysis.** The nose was also tracked using the Tracker program. Then, XY data exported from Tracker was used to evaluate nose kinematics between groups. The maximum and minimum horizontal X- and vertical Y-range of movement with the nose was quantified across bouts of string-pulling behavior for each day of testing.

## 2.6. Histology

Following behavioral testing, rats were deeply anesthetized and perfused with phosphate-buffered saline, followed by 4% paraformaldehyde. Brains were stored in paraformaldehyde solution for 24 h and then moved to a 30% sucrose solution for approximately 48 h. Then, brains were sliced in to 40  $\mu\text{m}$  sections using a vibratome and stained with cresyl violet to investigate lesion volume. Lesion volumes were calculated from a series of digitized cresyl violet sections using previous described methods [24,25] and are expressed as a percentage of the total hemispheric volume. As in our previous studies, stroke lesions were limited to the cerebral cortex, and no white matter damage was observed.

## 2.7. Statistical analysis

All statistical analyses included the repeated measures of day that reflected post-surgery days 3, 7, and 14 of testing; hand, designated as left and right, was included as a variable when appropriate. Sequential movement analyses for MCAO rats included the Repeated-measure Hands. Repeated-measures ANOVAs were used to evaluate main effects and interactions on each dependent measure in the string-pulling. The Greenhouse-Geisser (G-G) correction was used in analyses where Mauchly's test indicated significant departure from the assumption of sphericity. Partial eta squared ( $\eta^2_p$ ) was used as a measure of effect size for each main effect and interaction. Linear trend and Tukey HSD tests were used for post-hoc analyses.

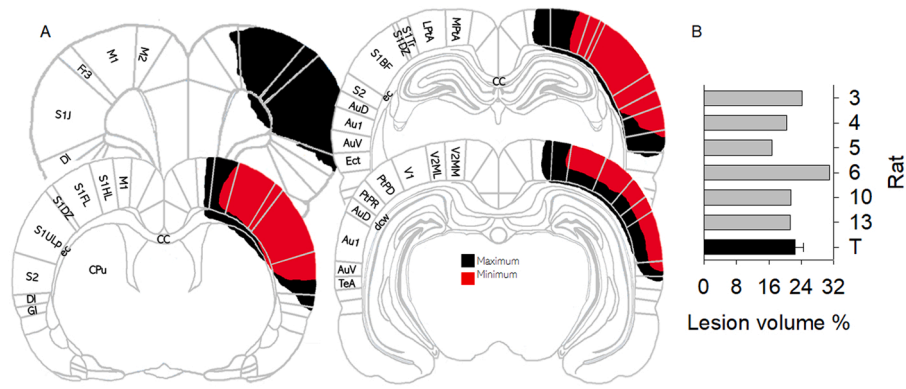
## 3. Results

### 3.1. Histology

Representative images depict minimum (red) and maximum (black) unilateral left hemisphere lesion extent following MCAO (see Fig. 2A). Total lesion volume was calculated for each rat that received a MCAO (see Fig. 2B). Extent of lesions ranged from ~16.8–30.95%. Mainly cortical regions were affected by MCAO, including primary and secondary sensorimotor areas important for skilled hand use [26]. Additional cortical brain regions damaged by MCAO encompassed the barrel cortex responsible for vibrissae movement and involved in sensorimotor processing [27].

### 3.2. String-pulling general behavioral analysis

Independent samples t-tests were used to compare performance



**Fig. 2.** Minimum (red) and maximum (black) lesion extent is shown (A) with total lesion volume as a percentage for each rat that received a MCAO (B). T represents the average total lesion volume by all rats that received a MCAO; the error bar represents the standard error of the mean.

between the Sham and MCAO group during baseline testing, prior to MCAO, on each measure. No differences were found between groups on any measures during baseline; therefore, Repeated-measures ANOVAs were used to evaluate performance between hands and groups during post-tests on days 3, 7, and 14.

Broad motivational and endpoint measures of performance were characterized to evaluate group differences in the organization of

**Table 1**  
Statistical data are shown for broad performance measures (\**p* < 0.050).

		F	df	<i>p</i>	$\eta^2p$	
Hands	Contacts					
	Day	2.572	2, 20	0.101	0.205	
	Day X Group	5.000	2, 20	0.049 *	0.333	
	Hand	6.890	1, 10	0.025 *	0.408	
	Hand X Group	0.897	1, 10	0.424	0.082	
	Day X Hand	0.886	2, 20	0.428	0.081	
	Day X Hand X Group	2.126	2, 20	0.146	0.175	
	Group	1.232	1, 10	0.293	0.110	
	Misses	Day	1.737	2, 20	0.202	0.148
	Day X Group	14.657	2, 20	0.003 *	0.594	
Hand	16.179	1, 10	0.002 *	0.618		
Hand X Group	1.421	1, 10	0.265	0.124		
Day X Hand	0.475	2, 20	0.628	0.045		
Day X Hand X Group	1.199	2, 20	0.322	0.107		
Group	55.258	1, 10	< 0.001 *	0.847		
Speed	Day	0.302	2, 20	0.743	0.029	
	Day X Group	0.384	2, 20	0.686	0.037	
	Hand	0.546	1, 10	0.477	0.052	
	Hand X Group	6.601	1, 10	0.028 *	0.398	
	Day X Hand	0.735	2, 20	0.492	0.068	
	Day X Hand X Group	2.098	2, 20	0.149	0.173	
	Group	2.998	1, 10	0.114	0.231	
	Hands, mouth, and nose	Contact ratio				
		Day	3.315	2, 20	0.057	0.249
		Day X Group	1.592	2, 20	0.228	0.137
Hand		18.122	1, 10	0.002 *	0.644	
Hand X Group		7.098	1, 10	0.024 *	0.415	
Day X Hand		4.537	2, 20	0.024 *	0.312	
Day X Hand X Group		0.213	2, 20	0.810	0.021	
Group		0.236	1, 10	0.638	0.023	
Speed ratio		Day	0.438	2, 20	0.652	0.042
Day X Group		0.346	2, 20	0.712	0.033	
Hand	1.085	1, 10	0.322	0.098		
Hand X Group	7.988	1, 10	0.018 *	0.444		
Day X Hand	1.138	2, 20	0.340	0.102		
Day X Hand X Group	0.803	2, 20	0.462	0.074		
Group	1.715	1, 10	0.220	0.146		
Total distance ratio	Day	3.569	2, 20	0.047 *	0.263	
	Day X Group	3.098	2, 20	0.067	0.237	
	Hand	1.095	1, 10	0.320	0.099	
	Hand X Group	8.150	1, 10	0.017 *	0.449	
	Day X Hand	1.311	2, 20	0.292	0.116	
	Day X Hand X Group	0.568	2, 20	0.575	0.054	
	Group	10.565	1, 10	0.009 *	0.514	

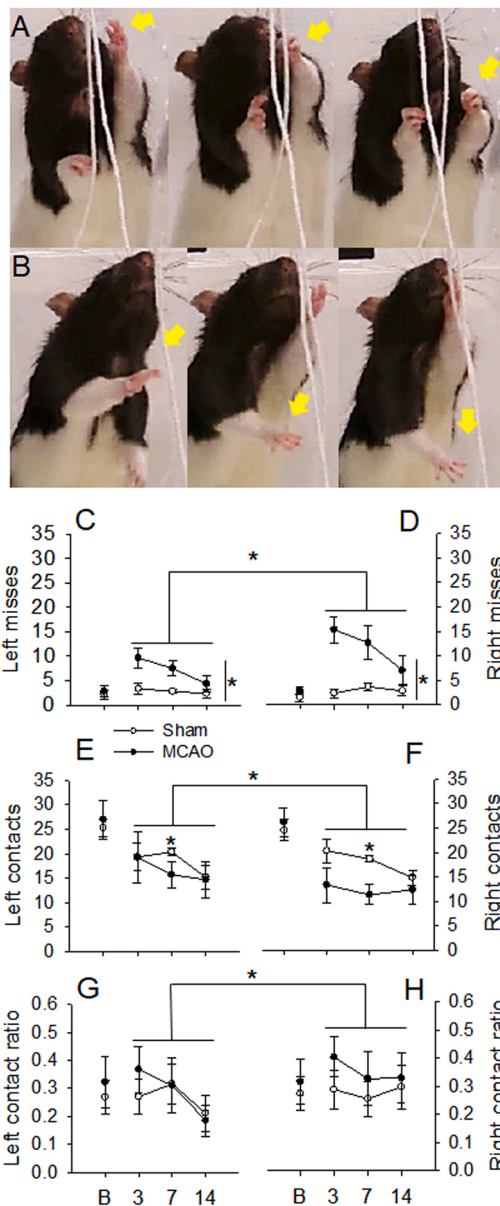
bimanual string-pulling behavior (see Table 1). Approach time and pull duration were evaluated across testing between groups as broad motivational measures of performance. The G-G correction ( $\epsilon = 0.513$ ) was used to adjust the degrees of freedom associated with the lack of sphericity in pull duration. The Repeated-measures ANOVA conducted on approach time failed to reveal a main effect of Group [ $F(1, 10) = 0.341, p = 0.572, \eta^2p = 0.033$ ] and Day [ $F(2, 20) = 2.323, p = 0.124, \eta^2p = 0.189$ ] or a Group by Day interaction [ $F(2, 20) = 0.279, p = 0.760, \eta^2p = 0.027$ ]. Further, the Repeated-measures ANOVA conducted on pull duration failed to reveal significant main effects of Group [ $F(1, 10) = 2.182, p = 0.170, \eta^2p = 0.179$ ] and Day [ $F(2, 20) = 2.140, p = 0.174, \eta^2p = 0.176$ ] or a Group by Day interaction [ $F(2, 20) = 1.810, p = 0.208, \eta^2p = 0.153$ ]. Rats took similar amounts of time to approach the string once placed in the testing apparatus as well as similar pull durations across testing.

Recall that unilateral left hemisphere MCAO results in contralateral damage to the right hand. Thus, it is expected that the right-hand may be more affected than the left-hand, and this was the case. Rats in both groups exhibited misses while attempting to contact and grasp the string to pull it in with the left- (see Fig. 3A) and right-hand (see Fig. 3B). The number of misses made with the left- and right-hands while pulling in the string were evaluated across testing between groups. A Repeated-measures ANOVA conducted on left- (see Fig. 3C) and right-hand (see Fig. 3D) misses revealed significant main effects of Group [ $F(1, 10) = 55.258, p < 0.001, \eta^2p = 0.847$ ], Day by Group interaction [ $F(2, 20) = 14.657, p = 0.003, \eta^2p = 0.594$ ], and Hand [ $F(1, 10) = 16.1709, p = 0.002, \eta^2p = 0.618$ ] yet failed to reveal other significant main effects of interactions. Following MCAO, rats exhibited an increase in misses, or failures to contact the string, with both the ipsilateral to MCAO left- and contralateral to MCAO right-hands compared to Sham rats across testing.

While misses occurred when attempting to grasp the string, all rats still engaged in grasping motions with the hands to contact and pull in the string. The number of contacts made with the string by the left- and right-hands were evaluated across testing. A Repeated-measures ANOVA conducted on left- (see Fig. 3E) and right-hand (see Fig. 3F) contacts revealed a significant Day by Group interaction [ $F(2, 20) = 5.000, p = 0.049, \eta^2p = 0.333$ ] and main effect of Hand [ $F(1, 10) = 6.890, p = 0.025, \eta^2p = 0.408$ ] with no other significant differences.

Several differences were observed for the ratios derived from mouth contacts relative to contacts with the left- (see Fig. 3G) and right-hands (see Fig. 3H). A Repeated-measures ANOVA conducted on mouth relative to each hands contacts revealed a significant Hand by Group interaction [ $F(1, 10) = 7.098, p = 0.024, \eta^2p = 0.415$ ], Day by Hand interaction [ $F(2, 20) = 4.537, p = 0.024, \eta^2p = 0.312$ ], and Hand [ $F(1, 10) = 18.122, p = 0.002, \eta^2p = 0.644$ ] with no other significant main effects or interactions. After MCAO, rats exhibited more similar amounts of contacts with their left-hand and mouth relative to Sham rats' mouth





**Fig. 3.** A frame-by-frame representative left- (A) and right-hand (B) miss is displayed; notice that the ipsilateral to MCAO left-hand and fingers grasp even when the string is missed (A), but the contralateral to MCAO right-hand exhibits extensor spasticity, or an open-raking like motion (B) across sub-components of movement (i.e., grasp, pull, push). Following MCAO, rats engaged in significantly more misses with the left (C) and right hands (D) relative to Sham rats across testing. Overall, the right-hand missed the string more than the left-hand. Sham rats engaged in more hand (E, F) contacts than MCAO rats on day 3 of testing. Overall, the left-hand contacted the string more than the right-hand. The mouth and left-hand (G) engaged in less contacts than the mouth and the right-hand (H). After MCAO, rats exhibited more similar amounts of contacts with their left-hand and mouth relative to Sham rats' mouth and left- and right-hands. Overall, this ratio decreased by hand, with the right-hand and mouth engaging in more similar amounts of contacts than the left-hand and mouth. \* $p < 0.050$ .

and left- and right-hands. Overall, this ratio decreased by hand, with the right-hand and mouth engaging in more similar amounts of contacts than the left-hand and mouth.

### 3.3. Sequential movement analyses

#### 3.3.1. Contacts

Rats sequentially organize string-pulling behavior into sub-components of movements, including an Advance to Grasp the string, a Pull and Push to move the string into the apparatus, and a release to Lift the hand and begin the next pulling cycle (see Fig. 4). A sequential analysis of the left- and right-hands when contact was made with the string revealed several differences (see Table 2).

**3.3.1.1. Lifts.** Rats engaged in a similar number of Lifts between groups, but differences were observed overall between the hands (see Fig. 4A). A Repeated-measures ANOVA conducted on Lifts revealed that the number of Lifts during reach movements significantly differed by Hand [ $F(1, 10) = 10.484, p = 0.009, \eta_p^2 = 0.512$ ] yet no other significant main effects or interactions were observed. The left-hand engaged in more Lifts than the right-hand.

**3.3.1.2. Advances.** Rats engaged in a similar number of Advances between groups, but differences were observed overall between the hands (see Fig. 4B). A Repeated-measures ANOVA conducted on Advances revealed that the number of Advances during reach movements significantly differed by Hand [ $F(1, 10) = 12.398, p = 0.006, \eta_p^2 = 0.554$ ] yet no other significant main effects or interactions were observed. The left-hand engaged in more Advances than the right-hand.

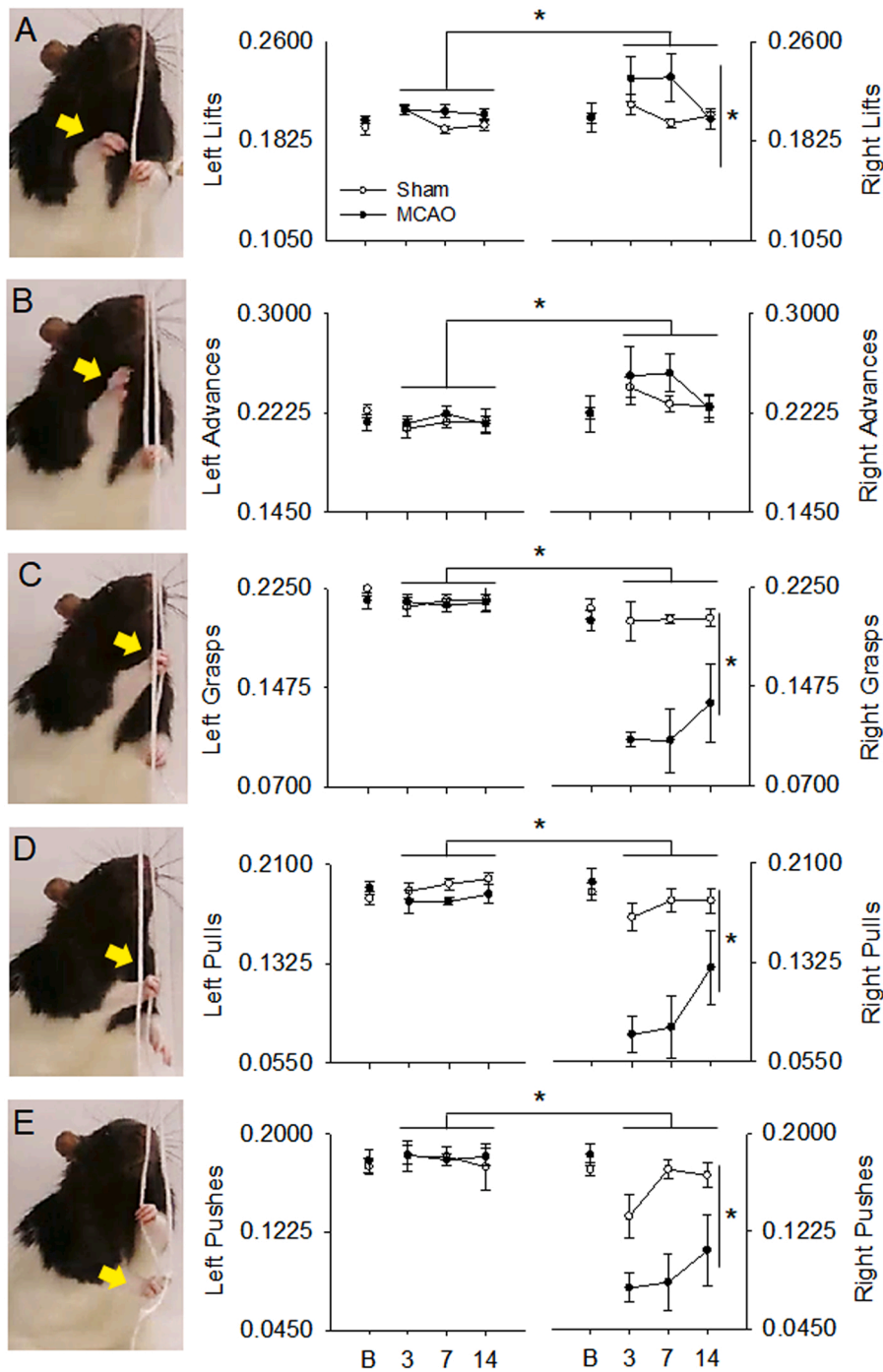
**3.3.1.3. Grasps.** Several differences were observed in the Grasp sub-component (see Fig. 4C). A Repeated-measures ANOVA conducted on Grasps revealed a significant main effect of Group [ $F(1, 10) = 18.564, p = 0.002, \eta_p^2 = 0.650$ ], Hand [ $F(1, 10) = 51.886, p < 0.001, \eta_p^2 = 0.838$ ], and Hand by Group [ $F(1, 10) = 28.479, p < 0.001, \eta_p^2 = 0.740$ ] without any other differences. Sham rats engaged in more grasps with the string overall than MCAO rats. The left-hand grasped the string more than the right-hand. Sham rats grasped the string more with the left- and right-hand relative to MCAO rats' right-hand. Further, MCAO rats grasped the string more with their left- than right-hands.

**3.3.1.4. Pulls.** Similar differences were observed during the Pull sub-component as the Grasp (see Fig. 4D). A Repeated-measures ANOVA conducted on Pulls revealed a significant main effect of Group [ $F(1, 10) = 36.415, p < 0.001, \eta_p^2 = 0.785$ ], Hand [ $F(1, 10) = 25.092, p < 0.001, \eta_p^2 = 0.715$ ], and Hand by Group [ $F(1, 10) = 10.948, p = 0.008, \eta_p^2 = 0.523$ ] without any other differences. Sham rats engaged in more pulls with the string overall during withdraws than MCAO rats. The left-hand pulled the string more than the right-hand. Sham rats pulled the string more with the left- and right-hand relative to MCAO rats' right-hand. Further, MCAO rats pulled the string more with their left- than right-hands.

**3.3.1.5. Pushes.** Differences were also observed in the distribution of the Push subcomponent (see Fig. 4E). A Repeated-measures ANOVA conducted on Pushes revealed a significant main effect of Group [ $F(1, 10) = 9.501, p = 0.012, \eta_p^2 = 0.487$ ], Hand [ $F(1, 10) = 36.563, p < 0.001, \eta_p^2 = 0.785$ ], and Hand by Group [ $F(1, 10) = 13.960, p = 0.004, \eta_p^2 = 0.583$ ] without any other differences. Sham rats elicited more pushes with the string during withdraws overall than MCAO rats. The left-hand pushed the string more than the right-hand. Sham rats pushed the string more with the left- and right-hand relative to MCAO rats' right-hand. Further, when MCAO rats pushed the string, they did so more with their left- than right-hands.

#### 3.3.2. Misses

Next, separate movement analyses conducted when MCAO rats missed the string revealed changes in subcomponents between the left and right hands across testing. Rats in the Sham group did not engage in



**Fig. 4.** An ipsilateral to MCAO left-hand Lift (A), Advance (B), Grasp (C), Pull (D), and Push (E) during movement when contact was made with the string is displayed along with corresponding left- and right-hand graphs. MCAO resulted in greater advances and less grasps, pulls, and pushes relative to Sham rats. Overall, the right-hand exhibited greater lift and advance subcomponent ratios, and the left-hand had weaker grasp, pull, and push subcomponent ratios. \* $p < 0.050$ .

enough misses to investigate subcomponents of movement organization. Therefore, sequential analyses of movement subcomponents during misses were only conducted for rats that received a MCAO to further investigate the nature of misses with the left- and right-hands.

**3.3.2.1. Lifts.** Lifts with the left- and right-hand during misses were also evaluated across testing (see Fig. 5A). A Repeated-measures ANOVA conducted on lifts revealed a significant main effect of Hand [ $F(1, 10) = 27.008, p < 0.001, \eta_p^2 = 0.730$ ] and Day [ $F(2, 20) = 8.920, p = 0.002, \eta_p^2 = 0.471$ ] yet failed to reveal a significant Hand by Day interaction [ $F(2, 20) = 0.477, p = 0.628, \eta_p^2 = 0.046$ ]. Rats that

received a MCAO engaged in more right- than left-hand lifts across testing. Further, lifts with both hands decreased as a function of day.

**3.3.2.2. Advances.** Advances toward the string with the left- and right-hands were evaluated during misses for rats that received a MCAO across testing (see Fig. 5B). A Repeated-measures ANOVA conducted on advances revealed that MCAO rats engaged in more left- than right-hand advances across testing [ $F(1, 10) = 27.008, p < 0.001, \eta_p^2 = 0.730$ ]. Further, left- and right-hand advances increased as a function of Day [ $F(2, 20) = 8.920, p = 0.002, \eta_p^2 = 0.471$ ], but there was not a significant Hand by Day interaction [ $F(2, 20) = 0.477, p = 0.628, \eta_p^2 = 0.046$ ].

**Table 2**

Statistical data are shown for the sequential movement analysis of sub-components when contact was made with the string (\*p < 0.050).

		F	df	p	$\eta^2_p$
Lifts	Day	1.791	2, 20	0.192	0.152
	Day X Group	1.187	2, 20	0.326	0.106
	Hand	10.165	1, 10	0.010 *	0.504
	Hand X Group	2.498	1, 10	0.145	0.200
	Day X Hand	0.674	2, 20	0.521	0.063
	Day X Hand X Group	1.087	2, 20	0.356	0.098
Advances	Group	10.484	1, 10	0.009 *	0.512
	Day	0.775	2, 20	0.474	0.072
	Day X Group	0.530	2, 20	0.597	0.050
	Hand	12.398	1, 10	0.006 *	0.554
	Hand X Group	0.349	1, 10	0.568	0.034
	Day X Hand	1.680	2, 20	0.212	0.144
Grasps	Day X Hand X Group	0.253	2, 20	0.779	0.025
	Group	1.398	1, 10	0.264	0.123
	Day	0.790	2, 20	0.468	0.073
	Day X Group	0.461	2, 20	0.637	0.044
	Hand	51.886	1, 10	< 0.001 *	0.838
	Hand X Group	28.479	1, 10	< 0.001 *	0.740
Pulls	Day X Hand	0.354	2, 20	0.706	0.034
	Day X Hand X Group	0.465	2, 20	0.635	0.044
	Group	18.564	1, 10	0.002 *	0.650
	Day	2.495	2, 20	0.108	0.200
	Day X Group	0.920	2, 20	0.415	0.084
	Hand	25.092	1, 10	< 0.001 *	0.715
Pushes	Hand X Group	10.948	1, 10	0.008 *	0.523
	Day X Hand	1.540	2, 20	0.239	0.133
	Day X Hand X Group	1.439	2, 20	0.261	0.126
	Group	36.415	1, 10	< 0.001 *	0.785
	Day	0.925	2, 20	0.403	0.087
	Day X Group	0.650	2, 20	0.533	0.061
	Hand	36.563	1, 10	< 0.001 *	0.785
	Hand X Group	13.960	1, 10	0.004 *	0.583
	Day X Hand	2.454	2, 20	0.111	0.197
	Day X Hand X Group	0.412	2, 20	0.668	0.040
	Group	9.501	1, 10	0.012 *	0.487

**3.3.2.3. Grasps.** After MCAO, rats continued to engage in grasps even when the string was missed with the left- and right-hands (see Fig. 5C). However, a Repeated-measures ANOVA conducted on grasps revealed a significant main effect of Day [F (2, 20) = 4.762, p = 0.020,  $\eta^2_p$  = 0.323], with grasps when the string was missed decreasing across testing. No significant effect of Hand [F (1, 10) = 0.144, p = 0.712,  $\eta^2_p$  = 0.014] or Day by Hand interaction [F (2, 20) = 1.262, p = 0.305,  $\eta^2_p$  = 0.112] was observed.

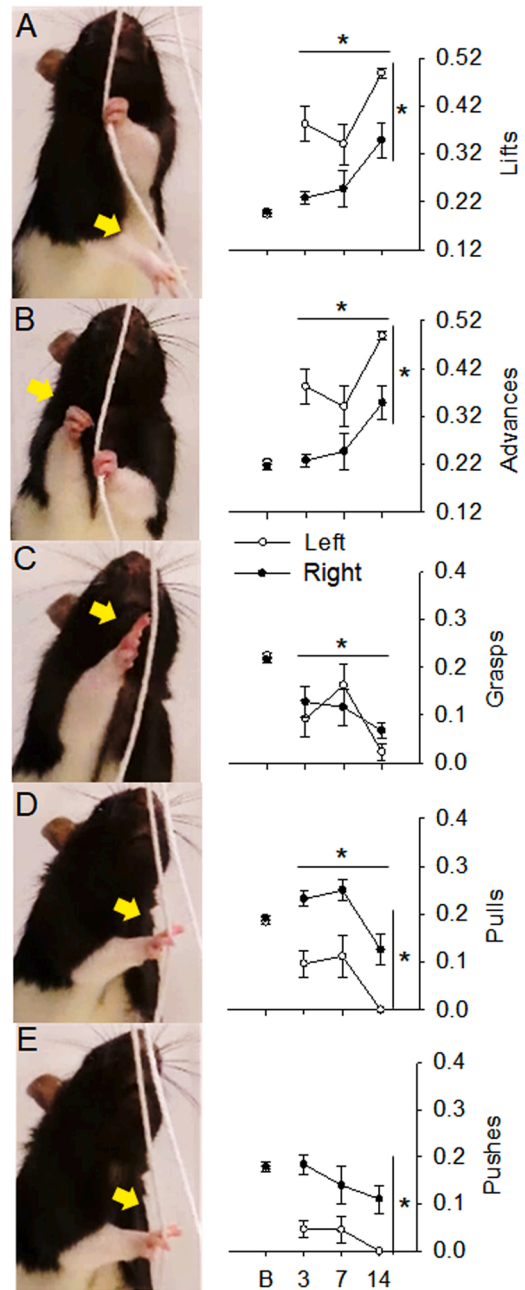
**3.3.2.4. Pulls.** When rats missed the string following MCAO they cycled through pulls with the left- and right-hand (see Fig. 5D). A Repeated-measures ANOVA conducted on pulls revealed a significant main effect of Hand [F (1, 10) = 47.496, p < 0.001,  $\eta^2_p$  = 0.826] and Day [F (2, 20) = 10.165, p < 0.001,  $\eta^2_p$  = 0.504] yet failed to reveal a significant Hand by Day interaction [F (2, 20) = 0.028, p = 0.972,  $\eta^2_p$  = 0.003]. Rats that received a MCAO engaged in more right- than left-hand pulls across testing. Overall, pulls with both hands decreased across testing.

**3.3.2.5. Pushes.** Rats also continued to engage in pushes with the left- and right-hand during misses (see Fig. 5E). A Repeated-measures ANOVA conducted on pushes revealed a significant main effect of Hand [F (1, 10) = 41.055, p < 0.001,  $\eta^2_p$  = 0.804] yet failed to reveal a significant main effect of Day [F (2, 20) = 2.409, p = 0.116,  $\eta^2_p$  = 0.194] or Hand by Day interaction [F (2, 20) = 0.312, p = 0.735,  $\eta^2_p$  = 0.030]. Following MCAO, rats engaged in more right- than left-hand pushes across testing.

**3.4. Motion capture analysis**

**3.4.1. Overall kinematic analysis**

Average moment-to-moment speed that both hands and the nose

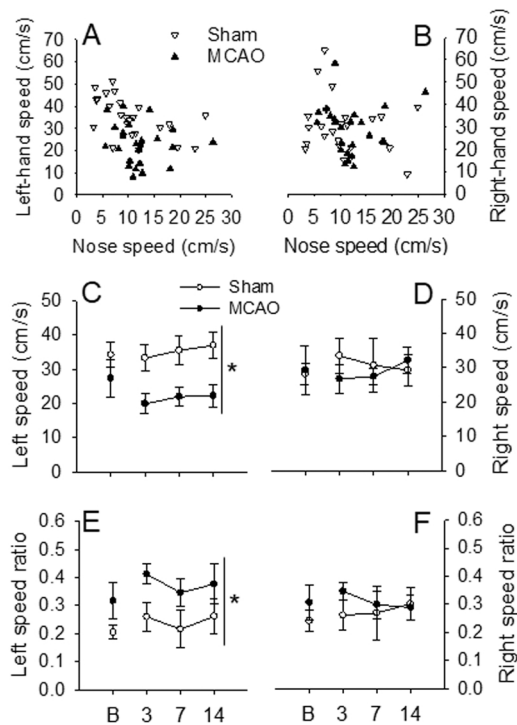


**Fig. 5.** A contralateral to MCAO right-hand Lift (A), Advance (B), Grasp (C), Pull (D), and Push (E) during movement without the string (i.e., during a miss with extensor spasticity) is displayed along with corresponding left and right-hand graphs for rats that received a MCAO. MCAO resulted in greater lifts and advances with the ipsilateral to MCAO left-hand and less pulls and pushes relative to the contralateral to MCAO right-hand. \*p < 0.050.

traveled while pulling in the string were evaluated between groups across testing (see Figure A-B). A Repeated-measures ANOVA conducted on the ratio for left- (see Fig. 6C) versus right-hand (see Fig. 6D) speed revealed a significant Hand by Group interaction [F (1, 10) = 6.601, p = 0.028,  $\eta^2_p$  = 0.398] yet there were no other significant effects or interactions. After MCAO, rats traveled slower distances with their left-hand compared to Sham rats, while no differences were observed between groups with their right-hands.

Ratios for average speed were calculated to compare speed in a standardized way between the nose and the left- (see Fig. 6E) and right-hands (see Fig. 6F) between groups across testing. A Repeated-measures ANOVA conducted on the ratio for average speed revealed a significant





**Fig. 6.** Scatterplots depict left-hand speed relative to nose speed (A) and right-hand speed compared to nose speed (B). Average speed is shown for the left- (C) and right-hands (D) across testing. After MCAO, rats traveled slower speeds with their left-hand than Sham rats. Ratios comparing nose speed to left- (E) and right-hand (F) speed are graphed across testing. MCAO resulted in the nose and left-hand traveling faster average speeds than Sham rats. \* $p < 0.050$ .

Hand by Group interaction [ $F(1, 10) = 7.988, p = 0.018, \eta^2p = 0.444$ ] without any other significant differences. Groups differed in their ratios of average speed traveled with the nose relative to the left-hand, with MCAO rats traveling more similar speeds with their nose and left-hand than Sham rats, while no differences were observed between groups with the nose relative to the right-hand.

Rats exhibited differences in total distance traveled between the left- and right-hands after MCAO (see Fig. 7A-D). A Repeated-measures ANOVA conducted on total distance ratios of the hands revealed a significant main effect of Group [ $F(1, 10) = 9.378, p = 0.012, \eta^2p = 0.137$ ] yet failed to reveal a significant effect of Day [ $F(2, 20) = 0.951, p = 0.403, \eta^2p = 0.337$ ] or Group by Day interaction [ $F(2, 20) = 1.283, p = 0.299, \eta^2p = 0.148$ ]. After MCAO, rats traveled further distances with their right-hand, while Sham rats traveled further distances with their left-hand.

Several differences were observed for the ratios derived from total distance traveled of the nose relative to the left (see Fig. 7C) and right-hands (see Fig. 7D). A Repeated-measures ANOVA revealed a significant main effect of Group [ $F(1, 10) = 10.565, p = 0.009, \eta^2p = 0.514$ ], Hand by Group interaction [ $F(1, 10) = 8.150, p = 0.017, \eta^2p = 0.449$ ], and Day [ $F(2, 20) = 3.569, p = 0.047, \eta^2p = 0.263$ ] with no other significant main effects or interactions. MCAO rats had larger ratios for total distance traveled with the nose relative to the hands than Sham rats. After MCAO, rats exhibited larger ratios with their left-hand relative to Sham rats left- and right-hands. Overall, this ratio decreased as a function of day across testing.

### 3.4.2. Nose kinematic analysis

While MCAO rats engaged in subcomponents of movement (Advance, Grasp, Pull, Push, and Lift) during bouts of string-pulling behavior, the organization of these movements were drastically altered. Importantly, following MCAO, rats engaged in whole-body

movements to initiate and perform all subcomponents of movement (see Fig. 8). Whether the string was contacted or missed with the hands, rats that received a MCAO used their entire body to advance the string. Typically, Sham rats occasionally use their mouths to contact the string to pull it in and sometimes (more rarely) use their entire body to aid in advancing the string. After MCAO, rats (see Fig. 9A) moved their noses more in the vertical Y-range relative to Sham rats (see Fig. 9B).

A repeated measures ANOVA conducted on the Y-range of nose movement revealed a significant effect of Group [ $F(1, 10) = 5.079, p = 0.048, \eta^2p = 0.338$ ] and Day [ $F(2, 20) = 5.004, p = 0.017, \eta^2p = 0.333$ ] yet failed to reveal a significant Group by Day interaction [ $F(2, 20) = 1.387, p = 0.273, \eta^2p = 0.122$ ] (see Fig. 9C). Following MCAO, rats traveled further distances in Y-range movement with the nose, and this distance decreased across testing for both groups. The minimum and maximum Y-range movement of the nose was evaluated to further investigate the nature of this difference. A Repeated-measures ANOVA conducted on the minimum Y-range of the nose revealed a significant effect of Group [ $F(1, 10) = 6.079, p = 0.033, \eta^2p = 0.378$ ] without a significant Group by Day interaction [ $F(2, 20) = 0.430, p = 0.567, \eta^2p = 0.041$ ] or effect of Day [ $F(2, 20) = 3.293, p = 0.058, \eta^2p = 0.248$ ] (see Fig. 9F). MCAO rats traveled further distances in the minimum Y-range moving all the way down to the floor at the bottom of the apparatus to advance the string, while Sham rats remained upright to pull in the string.

In contrast, no differences were observed in the maximum Y-range of the nose: Group [ $F(1, 10) = 0.004, p = 0.954, \eta^2p = 3.541e-4$ ], Day [ $F(2, 20) = 0.306, p = 0.740, \eta^2p = 0.030$ ], and Group by Day interaction [ $F(2, 20) = 0.822, p = 0.454, \eta^2p = 0.076$ ] suggesting rats reached to similar heights to manipulate the string (see Fig. 9E). Similarly, a Repeated-measures ANOVA conducted on the X-range of nose movement failed to reveal any significant differences: Group [ $F(1, 10) = 2.380, p = 0.154, \eta^2p = 0.192$ ], Day [ $F(2, 20) = 1.382, p = 0.274, \eta^2p = 0.121$ ], Group by Day [ $F(2, 20) = 0.415, p = 0.666, \eta^2p = 0.040$ ] (see Fig. 9D). Horizontal nose movement was similar between groups across testing.

Average moment-to-moment speed of the nose was also examined between groups across testing. No differences were observed by Day [ $F(2, 20) = 0.803, p = 0.462, \eta^2p = 0.074$ ], Group [ $F(1, 10) = 0.285, p = 0.605, \eta^2p = 0.028$ ], or Day by Group [ $F(2, 20) = 0.110, p = 0.896, \eta^2p = 0.011$ ]. Even though MCAO rats moved greater distances in the vertical Y-range with their nose, they traveled similar speeds relative to Sham rats.

### 3.4.3. Reach component

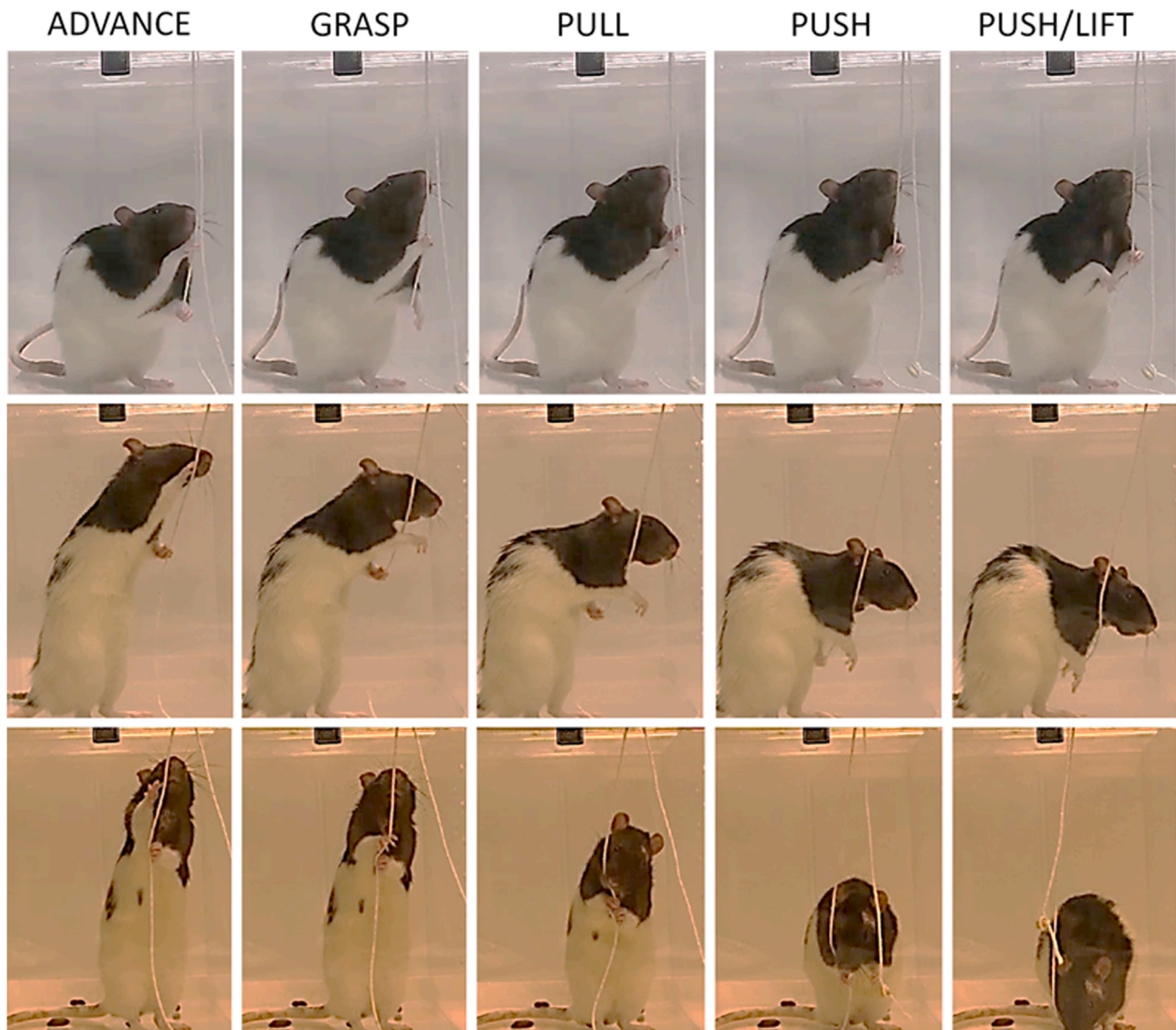
All rats engaged in left- and right-hand reaches to grasp the string with MCAO rats exhibiting selective changes in movement organization during reach paths (see Table 3). Distance traveled during reaches and the circuitry of reach paths were similar between MCAO and Sham rats. The G-G correction method was used to adjust the degrees of freedom associated with the lack of sphericity in path circuitry across Days ( $\epsilon = 0.664$ ) and for the Day by Hand interaction ( $\epsilon = 0.670$ ). A Repeated-measures ANOVA conducted on path circuitry revealed a significant effect of Hand [ $F(2, 16) = 4.045, p = 0.038, \eta^2p = 0.336$ ] yet failed to reveal a significant main effect of Group or Day by Group interaction. Overall, rats' right hands traveled more circuitous paths than their left hands. Further, the endpoints of reaches were similarly concentrated between groups.

Rats exhibited differences in heading direction of reaches that varied by hand. A Repeated-measures ANOVA conducted heading revealed a significant effect of Hand [ $F(2, 16) = 4.045, p = 0.038, \eta^2p = 0.336$ ] yet failed to reveal a significant main effect of Group or Day by Group interaction. Rats oriented their left-hands closer to  $75^\circ$  and their right-hands near  $95^\circ$ .

### 3.4.4. Withdraw component

All rats engaged in withdraws with both hands to advance the string.





**Fig. 7.** Whole body position is shown throughout the subcomponents (Advance, Grasp, Pull, Push, Lift) that make up a string-pulling cycle. The representative Sham rat (top row) depicts their typical upright stance while pulling, while MCAO rats bend their whole body to aid in advancing the string (middle and bottom row).

Distance traveled with the left and right hands during the withdraw phase of movement were evaluated on day 3, 7, and 14 between groups (see Table 4). A Repeated-measures ANOVA conducted on distance revealed a significant main effect of Day [ $F(2, 20) = 6.602, p = 0.009, \eta^2p = 0.377$ ] and a Day by Hand interaction [ $F(2, 20) = 3.532, p = 0.049, \eta^2p = 0.261$ ] yet failed to reveal other significant main effects or interactions. Distance traveled increased as a function of day. On day 14, rats' right-hands traveled further distances than their left-hand on day 3 and the right-hand on day 7.

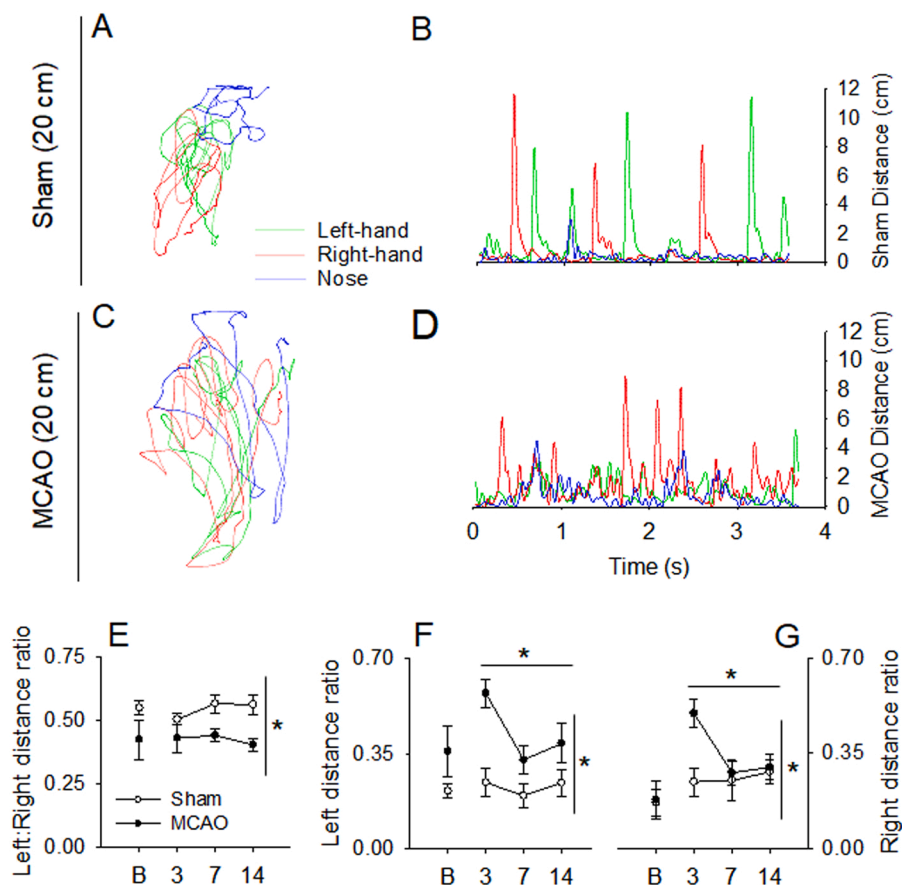
Path circuitry of the left- and right-hands during the withdraw phase of movement were measured across testing (see Fig. 10A-B). A Repeated-measures ANOVA conducted on path circuitry revealed a significant Day by Hand by Group interaction [ $F(2, 20) = 5.494, p = 0.013, \eta^2p = 0.355$ ] and Group [ $F(1, 10) = 5.573, p = 0.040, \eta^2p = 0.358$ ] yet failed to reveal other significant main effects or interactions. On day 14, MCAO rats exhibited more circuitous paths with their left- (see Fig. 10E) and right-hands (see Fig. 10F) relative to both hands of the Sham rats on day 7. On day 14, MCAO rats engaged in more circuitous paths with their right-hand relative to Sham rats left-hand on day 3 and MCAO rats right-hand on day 7. Overall, Sham rats elicited more direct withdraw hand movements than MCAO rats.

The concentration of movement with the left- and right-hand was measured on day 3, 7, and 14 for both groups (see Fig. 11A-B). A Repeated-measures ANOVA conducted on parameter of concentration revealed a significant main effect of Group [ $F(2, 20) = 6.723, p = 0.027, \eta^2p = 0.402$ ] yet failed to reveal other significant main effects or interactions. Rats in the Sham group demonstrated more concentrated endpoints with their left- (see Fig. 11E) and right-hands (see Fig. 11F).

The previous analysis revealed group differences in the parameter of concentration with the left-hand during the withdraw phase of movement. Therefore, the heading direction of the hands was not statistically evaluated during the withdraw component due to the methodology of circular statistics.

#### 4. Discussion

A unilateral MCAO produced bimanual disruptions in rat string-pulling behavior. Impaired string-pulling was characterized by persistent bilateral increases in misses with both hands and in posture changes. When the string was missed with the contralateral to MCAO hand, the rats often demonstrated an open-handed raking-like motion (i.



**Fig. 8.** Topographic plots of both hands and the nose are shown for a Sham (A) and MCAO rat (C). Corresponding moment-to-moment distance traveled is shown for each body part for a Sham (B) and MCAO rat (D). Ratios representing left-hand relative to right-hand total distance traveled shows MCAO rats traveling further distances with their right-hand relative to Sham rats across testing (E). Ratios comparing nose total distance traveled to left- (F) and right-hand (G) total distance traveled are displayed across testing. MCAO resulted in the nose and both hands traveling further total distances than Sham rats. These ratios decreased across testing. \* $p < 0.050$ .

e., extensor spasticity similar to flexor spasticity in patients after stroke) as if unable to close the hand. They also continued to cycle through subcomponents (i.e., Pulls and Pushes) of movement, as if the string had been grasped. Yet MCAO rats were still able to open their injured right-hand in preparation to grasp the string. MCAO rats also altered their posture by bending and twisting the trunk of the body and used mouth contacts with these body adjustments to pull in the string, thus using mouth and body motion as a crutch to compensate for arm/hand impairments. In contrast, Sham rats exhibited independent arm movements without whole-body assistance and made fewer mouth contacts and hand misses. Their hands made elliptical up/down excursions to grasp and pull the string, and their posture was sustained with minimal sway. In sum, the bilateral rhythmical string-pulling task revealed both arm/hand impairments and proximodistal movement compensation after MCAO stroke.

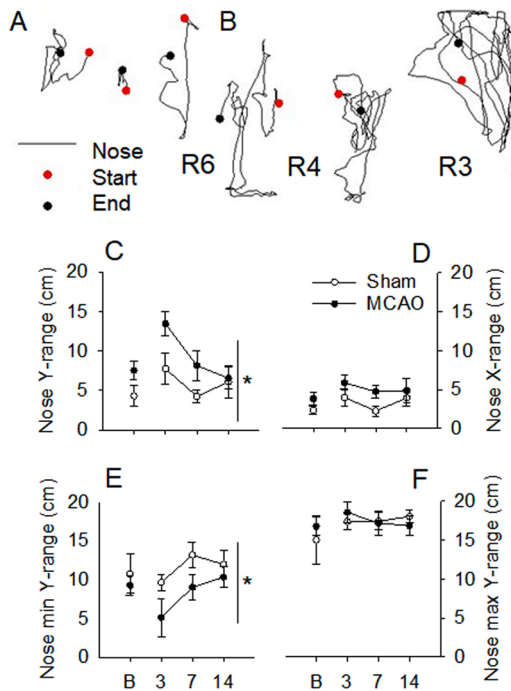
The MCA is a major artery that extends from the internal carotid artery and supplies blood to lateral portions of the parietal, frontal, and temporal lobes. The MCAO lesion model used in the current study occluded the artery just above the rhinal fissure restricting blood flow and inducing damage to the neocortex. The cortical areas irrigated by the MCA include the sensorimotor regions of the trunk, face, and limbs [4]. Patients that have experienced a stroke within the MCA often exhibit paralyses in the face, arm, or trunk of one side of the body [28]. In the present study it was found that ~16.8–30.95% of the ipsilateral somatosensory cortex was damaged by MCAO, a lesion similar to that produced in other MCAO studies (for review see [29]). The resulting cortical damage was consistent in involvement of somatosensory cortex but variable in that in two animals, portions of the motor cortices were included. Given the close relationship between the somatosensory/parietal cortex and motor cortex, it might be expected that all lesions impaired a similar sensorimotor network supporting skilled movement.

String-pulling in Sham and MCAO rats was organized differently.

When string-pulling, Sham rats typically remain upright with their torso extended to engage in multiple bilateral rhythmical Lift, Advance, Grasp, Pull, and Push movements to advance the string. Occasional mouth pulls were interspersed with hand movements. Thus, somatosensory cortex may be expected to be involved in the maintenance of posture for string-pulling, the rhythmical bilateral movements of advancing the string, and the targeted hand grasps used to purchase and release the string [14].

MCAO rats approached and pulled the string and achieved success in a similar amount of time to Sham rats, suggesting spared attentional, motivation, and compensatory motor processes required to perform the task. Nevertheless, many other aspects of string-pulling were impaired, especially in the hand/arm contralateral to the MCAO. The MCAO rats missed grasps of the string more frequently with both hands but were especially impaired with the contralateral hand that often failed to close after grasping. The stroke animals made much more use of mouth grasps to assist in advancing the string. Kinematic measures of arm movements featured further distances traveled with the injured right-hand and the nose, and changes in concentration and heading direction of movement endpoints. Lifts and advances of the arms were no longer independent but were assisted with compensatory whole-body movements. These included standing upright extended on the toes along with up and down movements of the trunk as well as twisting the whole-body to the left and right while moving downward to advance the string. Previous work has demonstrated that the compensatory use of whole-body movements to aid in fine motor control may be related to issues with movement segmentation [30].

Whereas many studies featuring single handed reaching for food by rodents have been used to investigate the consequences of stroke [8, 31–33], the string-pulling task has multiple positive features as a test for neural contributions to fine motor control. The task requires minimal preliminary training, as rats readily pull on a string placed in their cage



**Fig. 9.** Topography of the nose is plotted from a bout of string-pulling behavior for representative Sham (A) and MCAO (B) rats. Movement of the nose in the vertical Y-range (C) significantly differed between groups with rats traveling further distances after MCAO, while no changes were present in the X-range of nose movement (D). Further investigation of the min and max range of nose movement within the Y-axis revealed that after MCAO rats exhibited a significantly lower min Y-range (E) without differences in the max Y-range (F). \* $p < 0.050$ .

as part of normal investigatory behavior. The task provides many iterations of whole-body, arm, and hand movements concurrently. The behavior can be observed and documented from a frontal view, thus equating the perspective of rat and human data collection. Finally, as shown here, the task can still be performed, albeit with changes, after MCAO that compromises nearly all somatosensory cortex. In addition, successful grasps with the hands by MCAO rats were characterized by subcomponents of movement that incorporated compensatory use of the entire body. While these changes were apparent during both left- and right-hand engagement by MCAO rats, they were most apparent when the right-hand attempted to reach, grasp, and pull/push the string. This may indicate a greater limited range in movement on the impaired side. Similar behavioral deficits have been reported in rodent models of MCAO, especially in skilled reaching tasks that assesses each hand, as previously described. Thus, this study has translational value as a behavioral task that involves bilateral skilled reaching movements allowing for many ways to compensate. Separate recent studies with rodents and humans suggest that string-pulling behavior is organized similarly, in that the movements and movement sequences are similar [15,16,34]. Thus, this dynamic task affords the opportunity to investigate the effects of stroke and recovery of function in a way that is comparable in these two very different species.

Performance in the string-pulling task revealed both persistent and transient deficits following MCAO, providing evidence for potential compensation and functional recovery. Impairments that persisted at 3, 7, and 14 days after MCAO included misses with both hands, left-hand withdraw endpoint concentration, and subcomponents of movement without the string. Although enduring deficits were observed, evidence of compensation was still present. Rats that received a MCAO exhibited greater misses with both hands than Sham rats. During misses, rats engaged in more advances and lifts with the ipsilateral to MCAO hand, which suggests more attempts and effort by the nonimpaired side of the

**Table 3**

Statistical data are shown for reach kinematics (\* $p < 0.050$ ).

		F	df	p	$\eta^2p$
Distance	Day	0.877	2, 20	0.431	0.081
	Day X Group	3.294	2, 20	0.058	0.248
	Hand	3.298	1, 10	0.099	0.248
	Hand X Group	1.450	1, 10	0.256	0.127
	Day X Hand	2.740	2, 20	0.089	0.215
	Day X Hand X Group	0.672	2, 20	0.522	0.063
	Group	0.058	1, 10	0.815	0.006
	Day	1.542	1, 328	0.319	0.104
	Day X Group	0.448	1, 328	0.716	0.021
	Hand	8.625	1, 10	0.019 *	0.440
Path circuitry	Hand X Group	0.185	1, 10	0.470	0.053
	Day X Hand	0.062	1, 340	0.664	0.028
	Day X Hand X Group	1.021	1, 340	0.241	0.135
	Group	0.273	1, 10	0.582	0.031
	Day	1.236	2, 20	0.312	0.110
	Day X Group	1.988	2, 20	0.163	0.166
	Hand	0.612	1, 10	0.452	0.058
	Hand X Group	0.970	1, 10	0.348	0.088
	Day X Hand	0.480	2, 20	0.626	0.046
	Day X Hand X Group	0.092	2, 20	0.913	0.009
Concentration	Group	3.396	1, 10	0.095	0.253
	Day	1.072	2, 20	0.361	0.097
	Day X Group	2.340	2, 20	0.122	0.190
	Hand	27.537	1, 10	< 0.001 *	0.734
	Hand X Group	0.660	1, 10	0.435	0.062
	Day X Hand	0.191	2, 20	0.828	0.019
	Day X Hand X Group	1.436	2, 20	0.261	0.126
	Group	0.910	1, 10	0.363	0.083
	Day	1.072	2, 20	0.361	0.097
	Day X Group	2.340	2, 20	0.122	0.190
Heading	Hand	27.537	1, 10	< 0.001 *	0.734
	Hand X Group	0.660	1, 10	0.435	0.062
	Day X Hand	0.191	2, 20	0.828	0.019
	Day X Hand X Group	1.436	2, 20	0.261	0.126
	Group	3.396	1, 10	0.095	0.253
	Day	1.072	2, 20	0.361	0.097
	Day X Group	2.340	2, 20	0.122	0.190
	Hand	27.537	1, 10	< 0.001 *	0.734
	Hand X Group	0.660	1, 10	0.435	0.062
	Day X Hand	0.191	2, 20	0.828	0.019

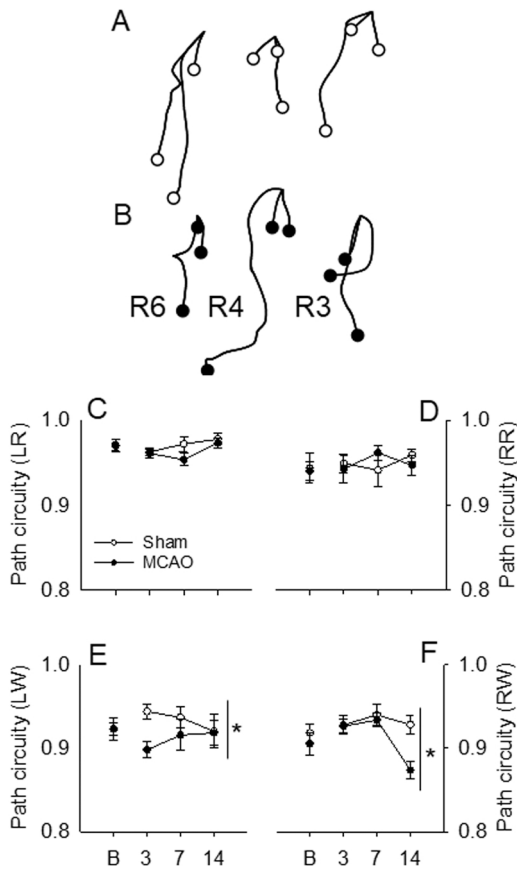
**Table 4**

Statistical data are shown for withdrawn kinematics (\* $p < 0.050$ ).

		F	df	p	$\eta^2p$
Distance	Day	6.062	2, 20	0.009 *	0.377
	Day X Group	1.742	2, 20	0.201	0.148
	Hand	1.583	1, 10	0.237	0.137
	Hand X Group	3.670	1, 10	0.084	0.268
	Day X Hand	3.532	2, 20	0.049 *	0.261
	Day X Hand X Group	1.994	2, 20	0.162	0.166
	Group	0.092	1, 10	0.768	0.009
	Day	2.587	2, 20	0.100	0.206
	Day X Group	0.299	2, 20	0.745	0.029
	Hand	0.006	1, 10	0.942	5.587e-4
Path circuitry	Hand X Group	0.026	1, 10	0.876	0.003
	Day X Hand	2.150	2, 20	0.143	0.177
	Day X Hand X Group	5.494	2, 20	0.013 *	0.355
	Group	5.573	1, 10	0.040 *	0.358
	Day	0.328	2, 20	0.724	0.032
	Day X Group	0.593	2, 20	0.562	0.056
	Hand	0.165	1, 10	0.693	0.016
	Hand X Group	1.426	1, 10	0.260	0.125
	Day X Hand	2.776	1, 245	0.116	0.217
	Day X Hand X Group	0.113	1, 245	0.796	0.011
Concentration	Group	6.723	1, 10	0.027 *	0.402

body to initiate engagement with the string. Further, when using the contralateral to MCAO hand, rats often engaged simultaneously in mouth contacts and pulled the string down to the left side once it was in the mouth rather than the right side. Mouth assistance speaks to the versatility of the rat and the opportunities afforded by the task for



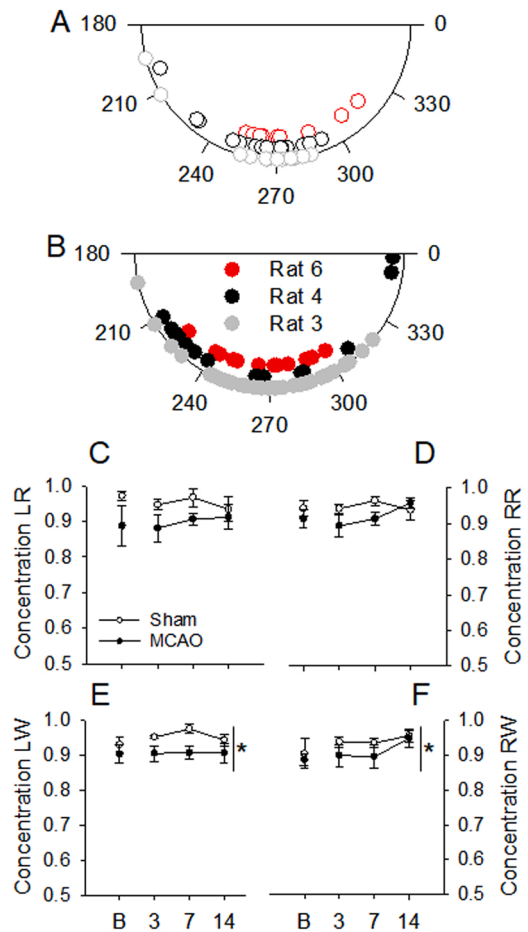


**Fig. 10.** Representative withdraw trajectories are shown for Sham (A) and MCAO (B) rats. Open circles represent path endpoints. While no differences were observed in left- (C) or right-hand (D) reach path circuitry, withdraw path circuitry differed for the left- (E) and right-hands (F) between groups. Sham rats engaged in more direct withdraw paths than MCAO rats. On day 14, MCAO rats engaged in more circuitous paths with their right-hand relative to Sham rats left-hand on day 3 and MCAO rats right-hand on day 7. Sham rats elicited more direct withdraw hand movements than MCAO rats overall.

compensation.

Compensation following brain damage is desirable of itself and can serve as an important avenue toward recovery of function. The neocortex features intratelencephalic, basal ganglia, thalamic as well as brainstem and spinal cord projections, with as many as 22 tracts from the brain to the spinal cord, thus providing many options for affecting movement. The ability of the brain to compensate following MCAO has been widely reported in the literature with varying levels of success depending on the timing and assessments used [30,35,36]. For example, compensation in digit use [37] and grasping function [38] have been previously observed in skilled reaching tasks that involve the use of one hand following MCAO. Similar compensatory strategies are observed by the hand contralateral to MCAO in unilateral skilled reaching and bilateral string-pulling tasks, such that rats exhibit an inability to close the digits but engage in digit opening motions to drag the string in against the front of the apparatus. Thus, this deficit is similar to, but less absolute than shown by Gharbawie et al. [37], in which after MCAO that includes basal ganglia damage, rats can no longer close their fingers. Perhaps the cortical impairment described here is more one of sensory than of motor inability.

Skilled reaching tasks for rodents typically require more training than does string-pulling. This may be because the string-pulling is analogous to spontaneous climbing behavior that might require hand over hand movement, or spontaneous feeding behaviors that might require alternating hand movements, e.g., pulling a grass stem that contains



**Fig. 11.** Representative topographic plots of left-hand withdraws is shown for Sham (A) and MCAO (B) rats. Filled-in circles represent the endpoints of the withdraw paths. Concentration of reach endpoints were similar (C, D), but movement with the left- (LW; C) and right-hand during withdraws (RW; D) differed by group across testing. After MCAO, rats exhibited less concentrated movement during left- and right-hand withdraws across days 3, 7, and 14 of testing. \* $p < 0.050$ .

seeds at its ends. In effect, the behavior is initially spontaneous and quickly results in the discovery that food is attached to the string. Thus acquired, the task provides many hand reaching repetitions (i.e., multiple reaches and withdraws per pulling bout) and so quickly provides ample data samples with the option to use the mouth and whole-body postural adjustments. Consequently, it may be that spontaneous recovery of function and compensation may also occur more quickly and with less training relative to skilled reaching. Because most skilled reaching tasks only assess one hand at a time, string-pulling has the advantage that the “good” hand can help the “bad” hand along. As such, it is also an ideal therapy task. It will be interesting to see whether future studies can harness the spontaneity and bimanual coordination of the hands and mouth of string-pulling behavior to enhance both compensation and recovery following brain damage.

Limitations of the current work should be considered. Performance assessments were limited to two weeks following MCAO. Previous work identified fine motor deficits using the string-pulling task acutely after a focal cortical lesion that persisted out to 70 days post-lesion [14]. Thus, future work may benefit from longitudinal assessments after MCAO to determine if similar sensitivity exists in more translational rodent models of stroke. When conducting frame-by-frame analysis, no consistent patterns emerged in the side of the face the string remained on (i.e., it moved between both sides) as broadly inspected in the current study. It is possible that 30 frames/second is not sufficient to capture



whisking in this task during string-pulling behavior with the field of view showing the entire frame of the apparatus. This study also exclusively used male rats; thus, sexual dimorphism to stroke and recovery of function and compensation [39,40] could not be documented. Further, while the use of two-dimensional analysis has been standard to-date, implementing three-dimensional analysis in future work may provide a more complete picture of the deficits and compensation abilities of rats after MCAO.

In conclusion, the string-pulling task provides a major behavioral advance in a sensitive assessment to detect changes in fine motor control following MCAO. Several of such disruptions in string-pulling behavior were observed in the rats in the present study. First, the string was missed more often with both hands after MCAO, and when the string was missed with the contralateral to MCAO hand, rats continued to cycle through the remaining subcomponents of string-pulling behavior as if the string were grasped in the hand. Second, when the string was missed, these rats also failed to make a regrasping motion with the right-hand and instead, demonstrated an open-handed raking-like motion. Third, postural changes were observed after MCAO, such that rats used their entire body as a crutch suggesting compensatory adjustments to advance the string. Lastly, changes in distance and direction that are critical to movement organization were also observed after MCAO. Despite these impairments in fine motor skills, motivation to engage in and to complete the string-pulling task to obtain a reward was preserved. This work demonstrates the importance of using detailed functional analyses of movement to detect changes in performance. Further, this study provides a foundation for future work to investigate other stroke models and to evaluate the efficacy of therapeutic interventions that have the potential to enhance neuroplasticity.

#### CRedit authorship contribution statement

All authors contributed to this study. The rodent model and data collection were performed by Muriel Hart and Joseph L. Cheatwood. Data and formal analysis were conducted by Ashley A Blackwell, Ian Q Whishaw, and Douglas G Wallace. The first draft of the manuscript was written by Ashley A Blackwell and all authors commented on previous versions of the manuscript. All authors read, reviewed, and approved the final manuscript.

#### Statements and declarations

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#### Data Availability

Data will be made available on request.

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