SUPPLEMENTARY INFORMATION

Stage-dependent role of interhemispheric pathway for motor recovery in primates

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List of Supplementary Information:

Figures. S1 to S7

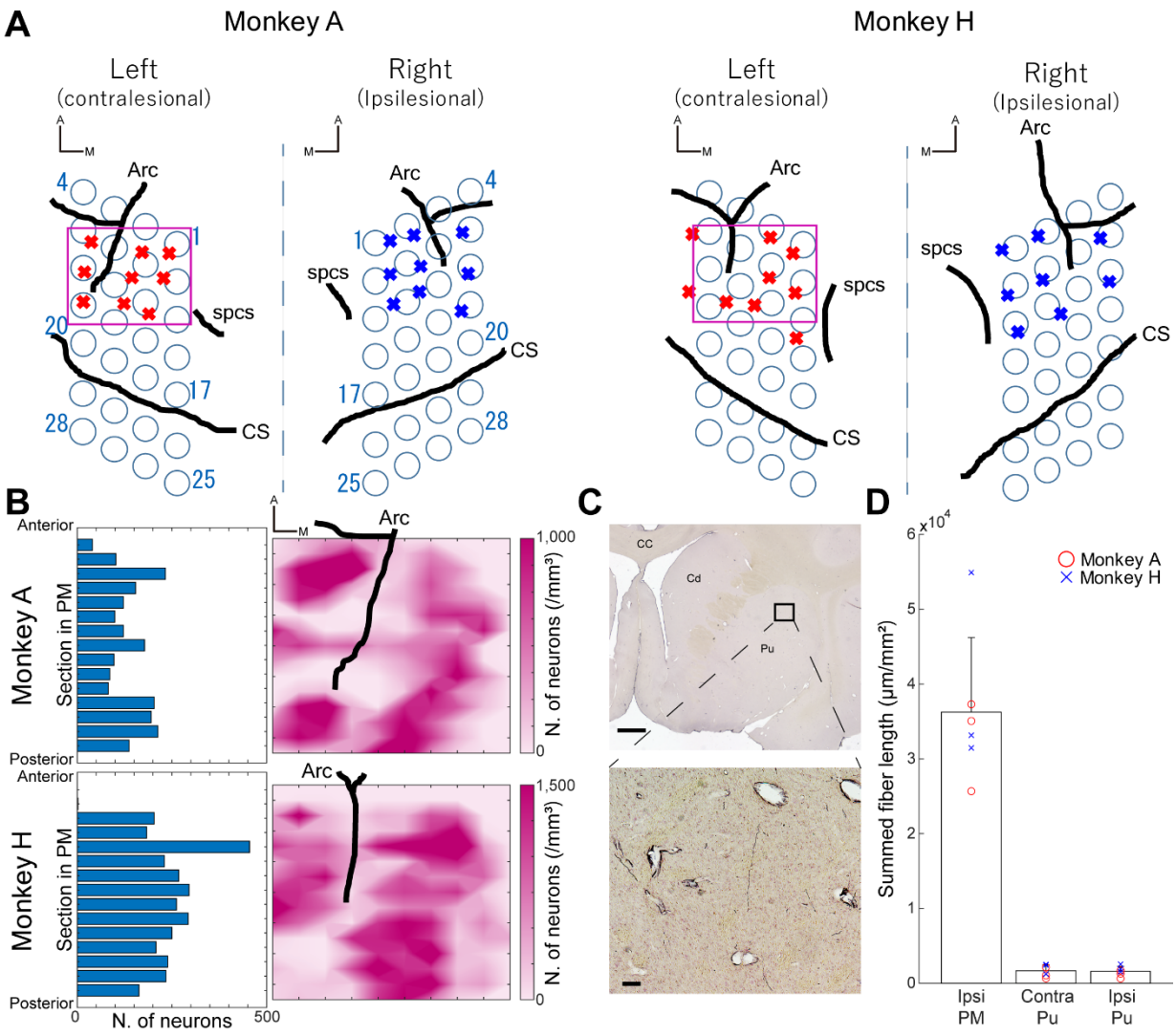
A
Monkey A
Monkey H
Left
(contralesional)
Right
(Ipsilesional)
Arc
spcs
CS
4
1
20
17
28
25
B
Anterior
Posterior
Section in PM
N. of neurons
0
500
1,000
1,500
N. of neurons (/mm³)
C
CC
Cd
Pu
D
x10⁴
Summed fiber length (µm/mm²)
Monkey A
Monkey H
Ipsi PM
Contra Pu
Ipsi Pu

Figure S1. Double viral vector injections for blockade of the unidirectional interhemispheric pathway

(**A**) Locations of vector injections and ECoG electrodes in Monkeys A and H. Red and blue crosses indicate the injection sites of AAV1-EF1α-DIO-hM4Di-mCherry and AAV2retro-CAGGS-Cre, respectively. Blue circles indicate the location of the ECoG electrodes. Magenta rectangles indicate the area we counted the labelled neurons in ***B***. (**B**) The number of the labelled neurons in each section (left) and the schematic distribution of the labelled neurons (right) in contralesional PM in Monkey A (top) and H (bottom). (**C**) Representative RFP-labelled fibers in the ipsilesional putamen of Monkey A. Rectangle in the upper figure indicates the area shown in the lower figure. Scale bar, top: 2 mm, bottom: 100 μm. Similar images were obtained from three independent brain sections. (**D**) Summed labelled fiber length in the region of ipesilesional PM and bilateral putamen in three different sections of each monkey. Data are presented as mean values +/- SD. Arc, arcuate sulcus; CS, central sulcus; spsc, superior precentral sulcus; CC, corpus callosum; Cd, caudate; Pu, putamen; A, anterior; M, medial

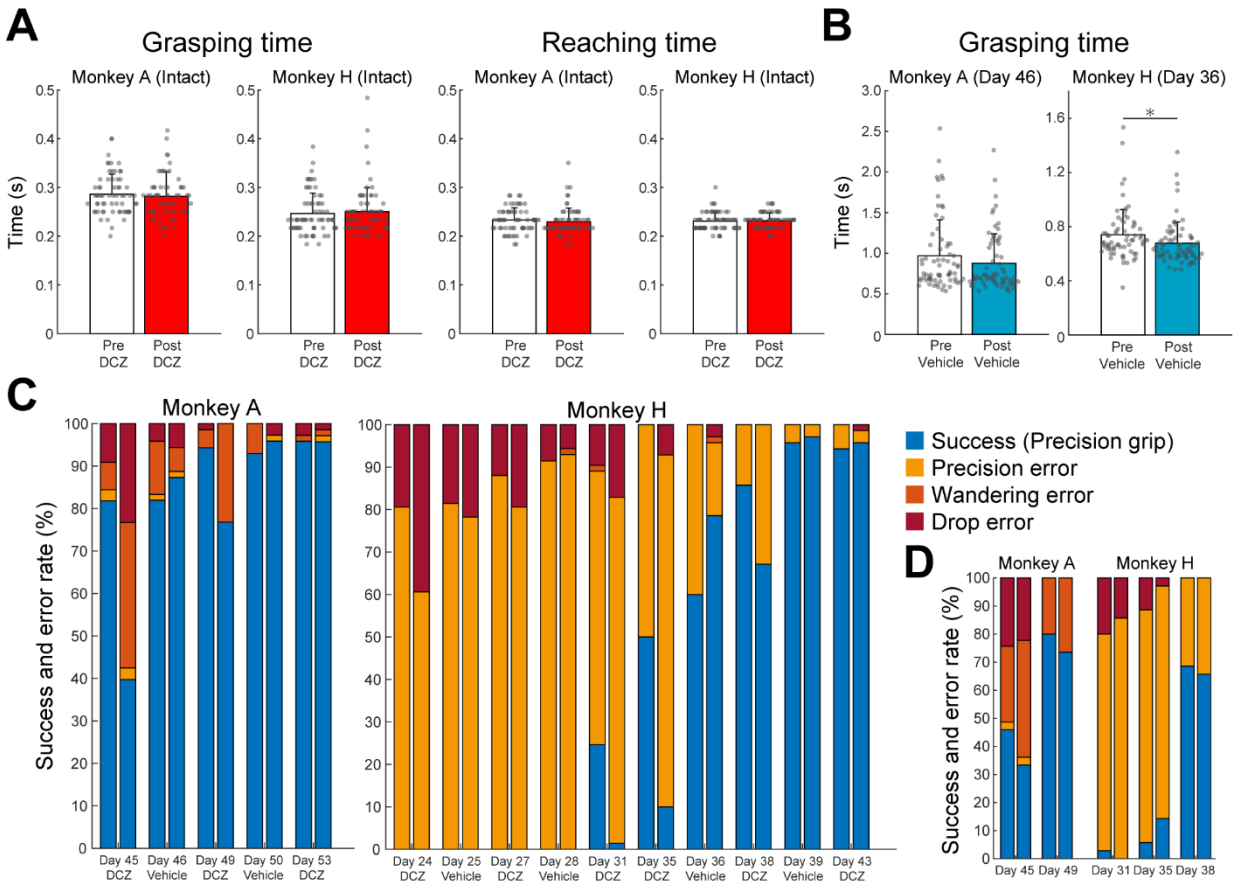
A
Grasping time
Reaching time
Monkey A (Intact)
Monkey H (Intact)
Monkey A (Intact)
Monkey H (Intact)
Time (s)
Pre DCZ
Post DCZ
B
Grasping time
Monkey A (Day 46)
Monkey H (Day 36)
Time (s)
Pre Vehicle
Post Vehicle
*
C
Monkey A
Monkey H
Success and error rate (%)
Day 45 DCZ
Day 46 Vehicle
Day 49 DCZ
Day 50 Vehicle
Day 53 DCZ
Day 24 DCZ
Day 25 Vehicle
Day 27 DCZ
Day 28 Vehicle
Day 31 DCZ
Day 35 DCZ
Day 36 Vehicle
Day 38 DCZ
Day 39 Vehicle
Day 43 DCZ
Success (Precision grip)
Precision error
Wandering error
Drop error
D
Monkey A
Monkey H
Success and error rate (%)
Day 45
Day 49
Day 31
Day 35
Day 38

Figure S2. The effects of interhemispheric blockade on the task.

(**A**) Reaching and grasping times before (white bars) and after (red bars) DCZ administration before lesioning in Monkey A (Pre-DCZ: 70 trials, Post-DCZ: 70 trials) and H (Pre-DCZ: 70 trials, Post-DCZ: 70 trials). Data are presented as mean values +/- SD. There was no significant difference (two-sided Wilcoxon rank-sum test; grasping time: $P = 0.81$ [Monkey A], 0.60 [Monkey H]; reaching time: $P = 0.59$ [Monkey A], 0.70 [Monkey H]). (**B**) Grasping time before (white bars) and after (blue bars) vehicle administration 46 days after lesioning in Monkey A (Pre-vehicle: 72 trials, Post-vehicle: 71 trials), and 36 days after lesioning in Monkey H (Pre-vehicle: 70 trials, Post-vehicle: 70 trials). Data are presented as mean values +/- SD. *$P < 0.05$ (two-sided Wilcoxon rank-sum test; $P = 0.12$ [Monkey A], 3.4×10^{-3} [Monkey H]). (**C**) Rate of the success and each error type before and after DCZ or vehicle administration in the early recovery stage. (**D**) Rate of the success and each error type in the first and second half of the session after DCZ administration.

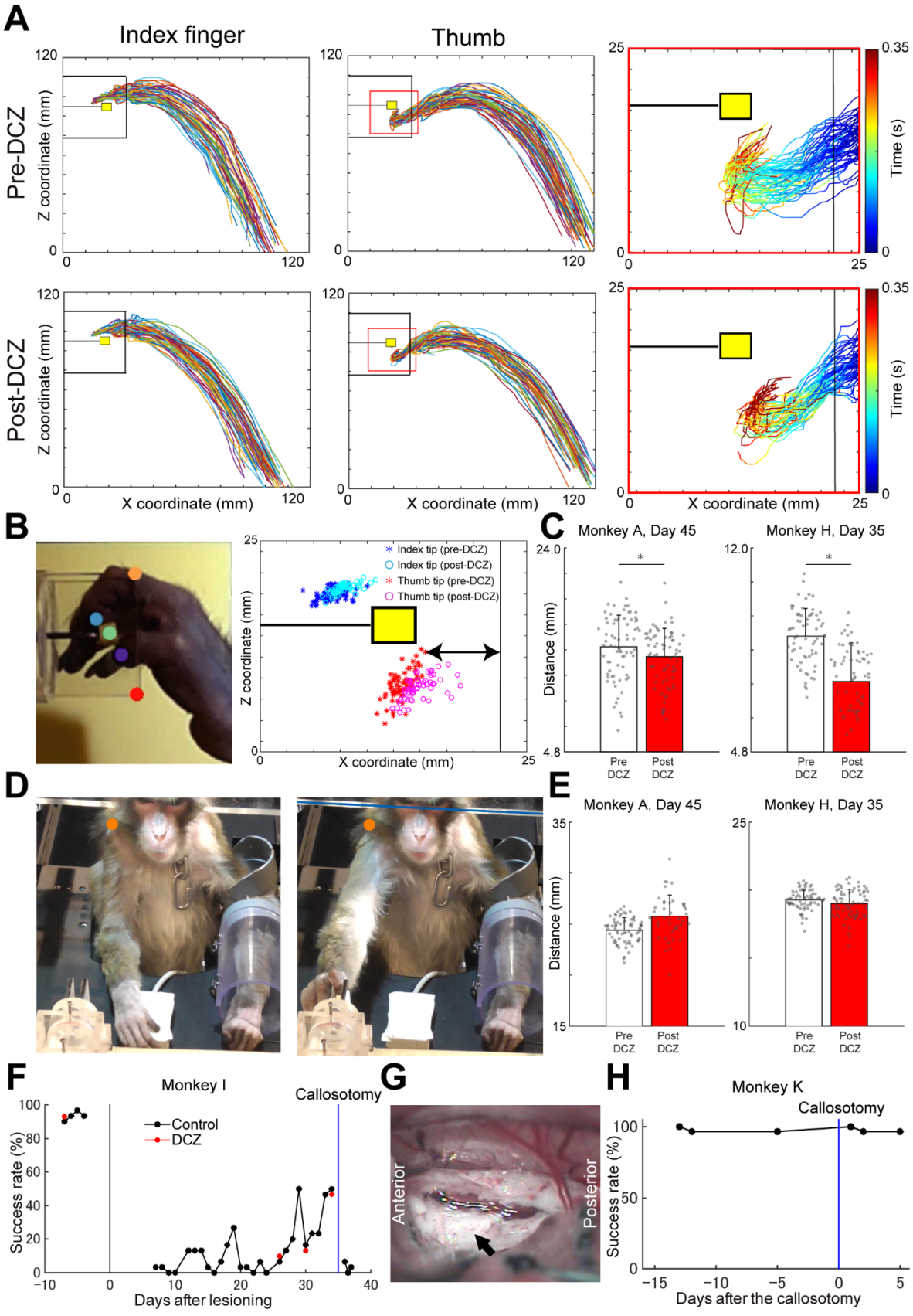
A
Index finger
Thumb
Pre-DCZ
Post-DCZ
Z coordinate (mm)
X coordinate (mm)
Time (s)
B
Index tip (pre-DCZ)
Index tip (post-DCZ)
Thumb tip (pre-DCZ)
Thumb tip (post-DCZ)
C
Monkey A, Day 45
Monkey H, Day 35
Distance (mm)
Pre DCZ
Post DCZ
D
E
F
Monkey I
Callosotomy
Control
DCZ
Success rate (%)
Days after lesioning
G
Anterior
Posterior
H
Monkey K
Days after the callosotomy

Figure S3. Blockade of the interhemispheric pathway affects recovered hand dexterity. (**A**) Trajectories of the index finger and thumb tips viewed from the left side during the task before and after DCZ administration; 35 days after lesioning in Monkey H. Each trajectory was plotted by the timing just before the monkey retrieved the food. Red rectangles in the middle figures indicate the areas shown in the right figures. The colour of trajectories in the right figures indicate the time from when the monkey's thumb touched the slit. (**B**) Position of the index finger and thumb tips when the monkey retrieved the food before and after DCZ administration; 35 days after lesioning in Monkey H. Line with arrows indicates the distance calculated and shown in ***C***. (**C**) Distance of the thumb tip from the slit entrance at the timing when the monkeys retrieved the food before and after DCZ administration; 45 days after lesioning in Monkey A and 35 days after lesioning in Monkey H. Data are presented as mean values +/- SD. *$P < 0.05$ (two-sided Wilcoxon rank-sum test, Monkey A: $n = 65$ trials [Pre-DCZ], 56 trials [Post-DCZ], $P = 0.033$; Monkey H: $n = 66$ trials [Pre-DCZ], 61 trials [Post-DCZ], $P = 1.9 \times 10^{-7}$). (**D**) Example pictures during the task 45 days after lesioning in Monkey A. Orange circles indicate the position of the ipsilesional shoulder used for the analysis shown in ***E***. (**E**) Distance of the ipsilesional shoulder during grasping from the neck plate (blue line in ***D***) before and after DCZ administration; 45 days after lesioning in Monkey A and 35 days after lesioning in Monkey H. Data are presented as mean values +/- SD. (two-sided Wilcoxon rank-sum test, Monkey A: $n = 70$ trials [Pre-DCZ], 43 trials [Post-DCZ], $P = 0.42$; Monkey H: $n = 70$ trials [Pre-DCZ], 70 trials [Post-DCZ], $P = 0.11$). (**F**) Success rate of precision grip in Monkey I with partial callosotomy between the bilateral PMs during recovery from l-CST lesioning. Red circles indicate the results after DCZ administration. The black vertical line indicates the day of l-CST lesioning and the blue line indicates the day of callosotomy. (**G**) A partially dissected corpus callosum between the premotor cortex. The black arrow indicates the corpus callosum. (**H**) Success rate of precision grip in Monkey K with partial callosotomy without l-CST lesioning. The blue line indicates the day of callosotomy.

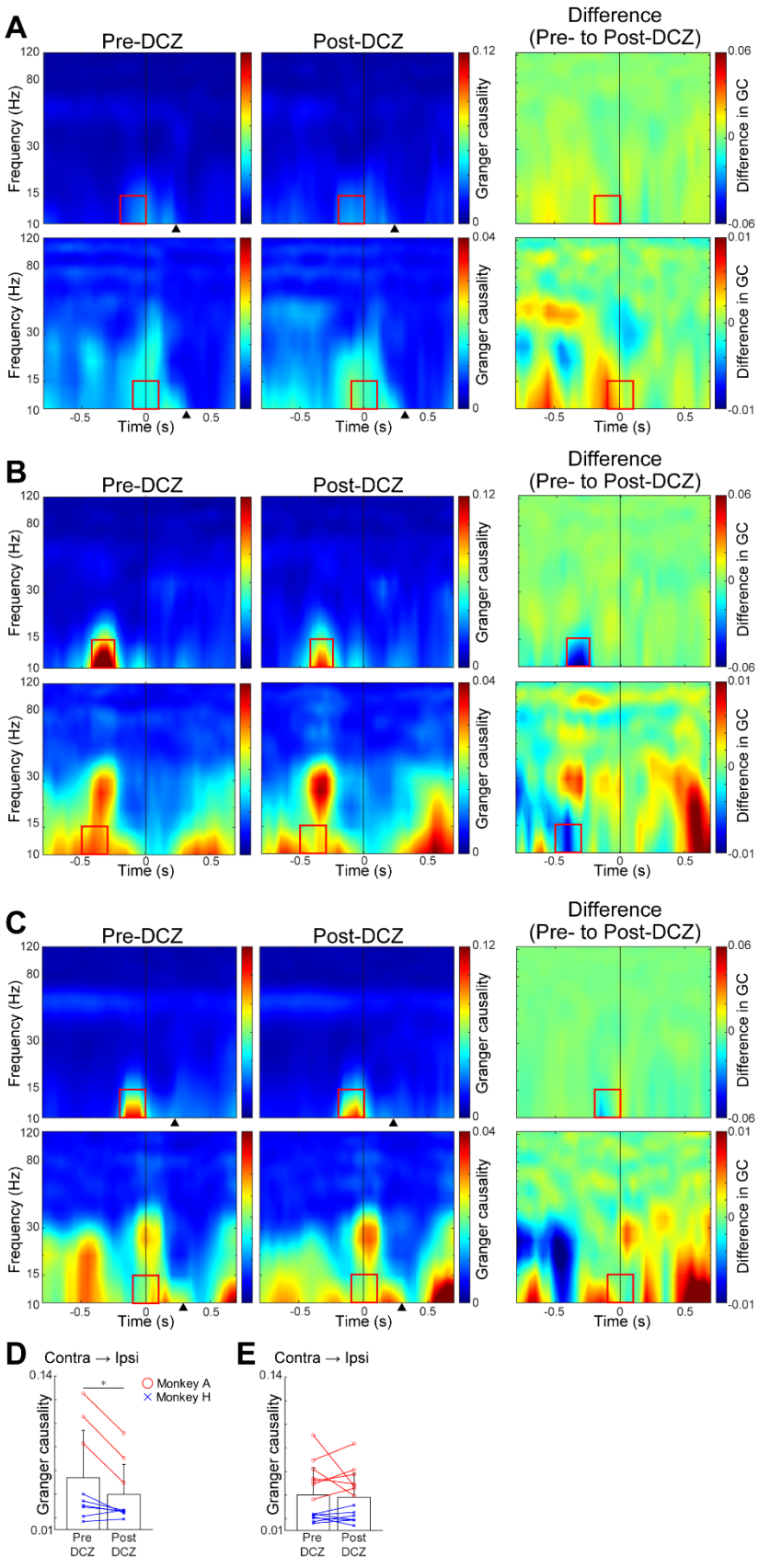
A
Pre-DCZ
Post-DCZ
Difference
(Pre- to Post-DCZ)
Frequency (Hz)
Granger causality
Difference in GC
Time (s)
B
C
D
Contra → Ipsi
Monkey A
Monkey H
Pre DCZ
Post DCZ
E
Contra → Ipsi

Figure S4. The change of the interhemispheric connectivity by the blockade.

(**A**) Averaged time-frequencygram of GC from the ipsilesional to contralesional PMd before (left figure) and after (middle figure) DCZ administration and their difference (right) in the early recovery stage of Monkey A (top) and Monkey H (bottom). Time 0 (black line) indicates movement onset. Red rectangles indicate the time frequency ROI that was used for the calculations shown in *Figure 3H*. Black triangles at the bottom indicate the onset of grasping. (**B**) Averaged time-frequencygram of GC from the contralesional to ipsilesional PMd in the early recovery stage aligned by the grasping onset (black line) in Monkey A (top) and Monkey H (bottom). (**C**) Averaged time-frequencygram of GC from the contralesional to ipsilesional PMd in the late recovery stage of Monkey A (top) and Monkey H (bottom). (**D**) Averaged value of GC in the ROI (shown in ***B***) in the early recovery stage of both monkeys (A and H) aligned by the grasping onset. Each line plot indicates the result of each experimental day in the early recovery stage ($n = 9$ days). *$P < 0.05$ (two-sided Wilcoxon sign-rank test; $P = 0.027$). (**E**) Averaged value of GC in the ROI (shown in ***C***) in the late recovery stage of both monkeys (A and H). Error bars indicate standard deviation. (two-sided Wilcoxon sign-rank test, $n = 14$ days, $P = 0.67$)

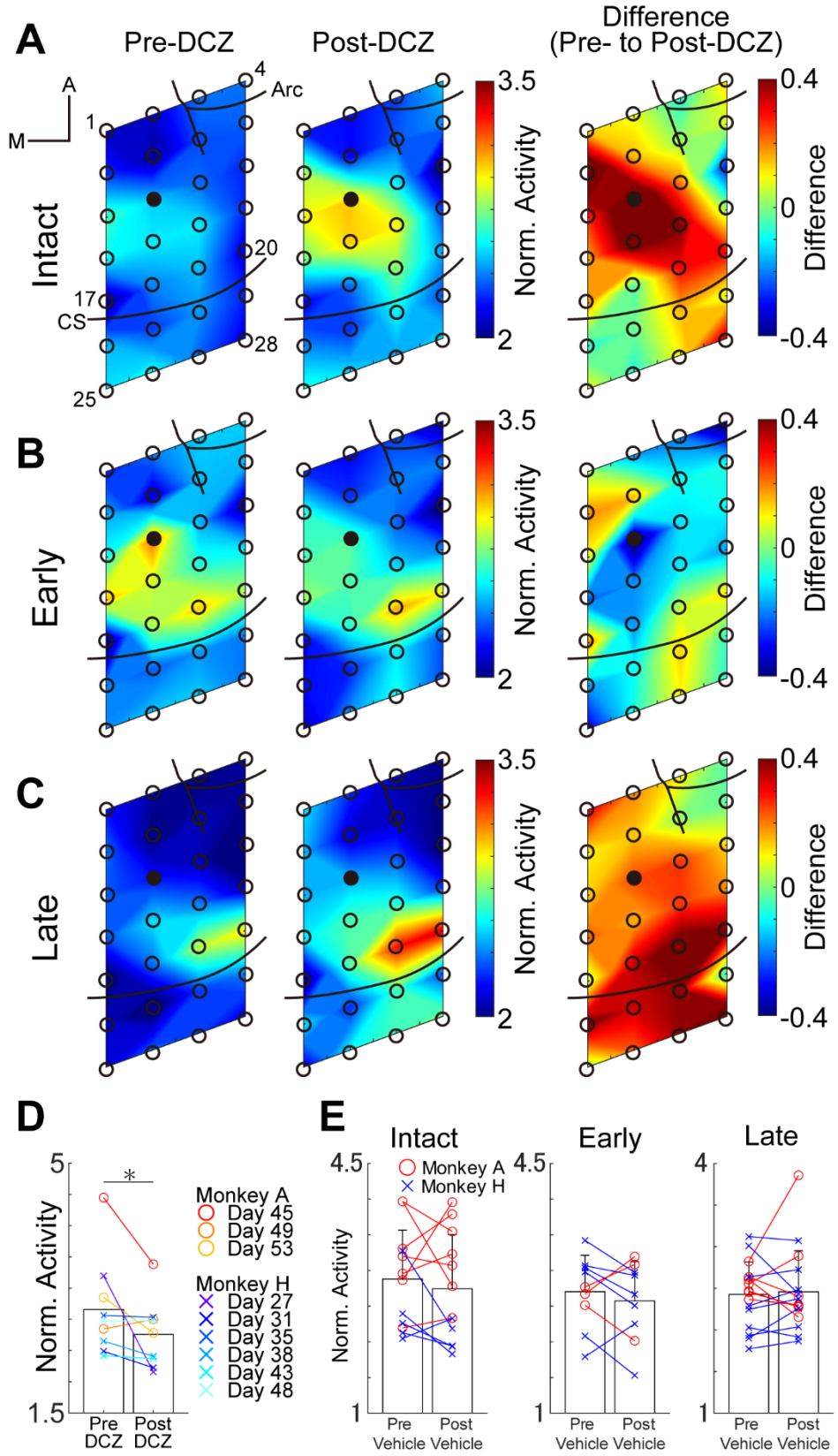
A
Pre-DCZ
Post-DCZ
Difference
(Pre- to Post-DCZ)
A
M
1
4
Arc
20
17
CS
28
25
Intact
3.5
2
Norm. Activity
0.4
0
-0.4
Difference
B
Early
C
Late
D
Norm. Activity
5
1.5
*
Pre
DCZ
Post
DCZ
Monkey A
Day 45
Day 49
Day 53
Monkey H
Day 27
Day 31
Day 35
Day 38
Day 43
Day 48
E
Intact
Early
Late
4.5
1
4
Monkey A
Monkey H
Pre
Post
Vehicle
Vehicle

Figure S5. Interhemispheric facilitation during recovery.

(**A-C**) The averaged brain activity at the ipsilesional sensorimotor cortex in the intact state (***A***), early recovery stage (***B***), and the late recovery stage (***C***) of both monkeys. Each circle indicates each electrode (Ch 1-28). Black filled circle indicates Ch 10 shown in *Fig.4*. Colour indicates the average normalized activity in the low frequency band around the movement onset before (left) and after (middle) DCZ administration and the difference (right) in activity before and after DCZ administration. A, anterior; M, medial. (**D**) Averaged value of normalized brain activity (ECoG) at the ipsilesional PMd (Ch 10) in the low frequency band around the movement onset in the early recovery stage of both monkeys (A and H), taken from Fig. 4D. Each colour indicates each experimental day after lesioning. (**E**) Averaged value of normalized brain activity (ECoG) at the ipsilesional PMd (Ch 10) before and after vehicle administration in the intact state (left), early recovery stage (middle), and late recovery stage (right) of both monkeys. There was no significant difference in activity between before and after vehicle administration in any stage. Error bars indicate standard deviation. (two-sided Wilcoxon sign-rank test; intact, $n = 12$ days, $P = 0.52$; early, $n = 9$ days, $P = 0.43$; late, $n = 16$ days, $P = 0.92$.)

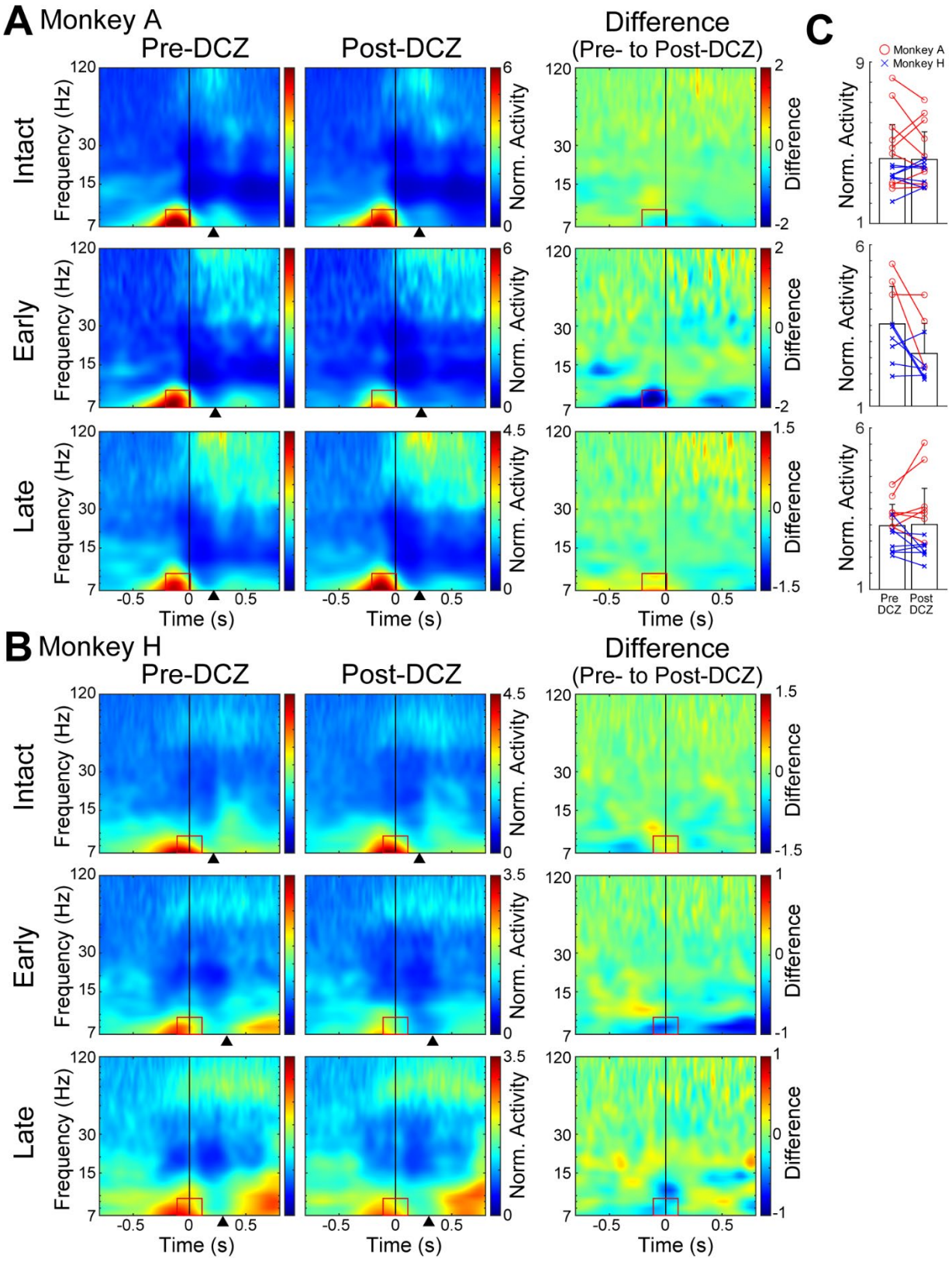
A Monkey A
Pre-DCZ
Post-DCZ
Difference
(Pre- to Post-DCZ)
Intact
Early
Late
Frequency (Hz)
Norm. Activity
Difference
Time (s)
B Monkey H
C
Monkey A
Monkey H
Pre DCZ
Post DCZ

Figure S6. The change of the activity in contralesional PMd

(**A, B**) The average normalized time-frequencygram of brain activity at the contralesional PMd (Ch 10) before and after DCZ administration in the intact state, early and late recovery stage (***A*** for Monkey A and ***B*** for Monkey H). Right figures show the difference in activity between before and after DCZ administration. Time 0 (black line) indicates movement onset. Red rectangles indicate the time frequency ROI that was used for the calculations shown in ***C***. Black triangles indicate the onset of grasping. (**C**) Averaged value of normalized brain activity (ECoG) at the contralesional PMd in the ROI in the intact state (top**,** n = 16 experiment days including 1,074 trials [Pre-DCZ] and 1,093 trials [Post-DCZ]), early recovery stage (middle**,** n = 9 days, 591 trials [Pre-DCZ] and 525 trials [Post-DCZ]), and late recovery stage (bottom**,** n = 14 days, 970 trials [Pre-DCZ] and 972 trials [Post-DCZ]) of both monkeys (A and H). Error bars indicate standard deviation. (two-sided Wilcoxon sign-rank test, P = 0.92 [top], 0.055 [middle], 0.95 [bottom]).

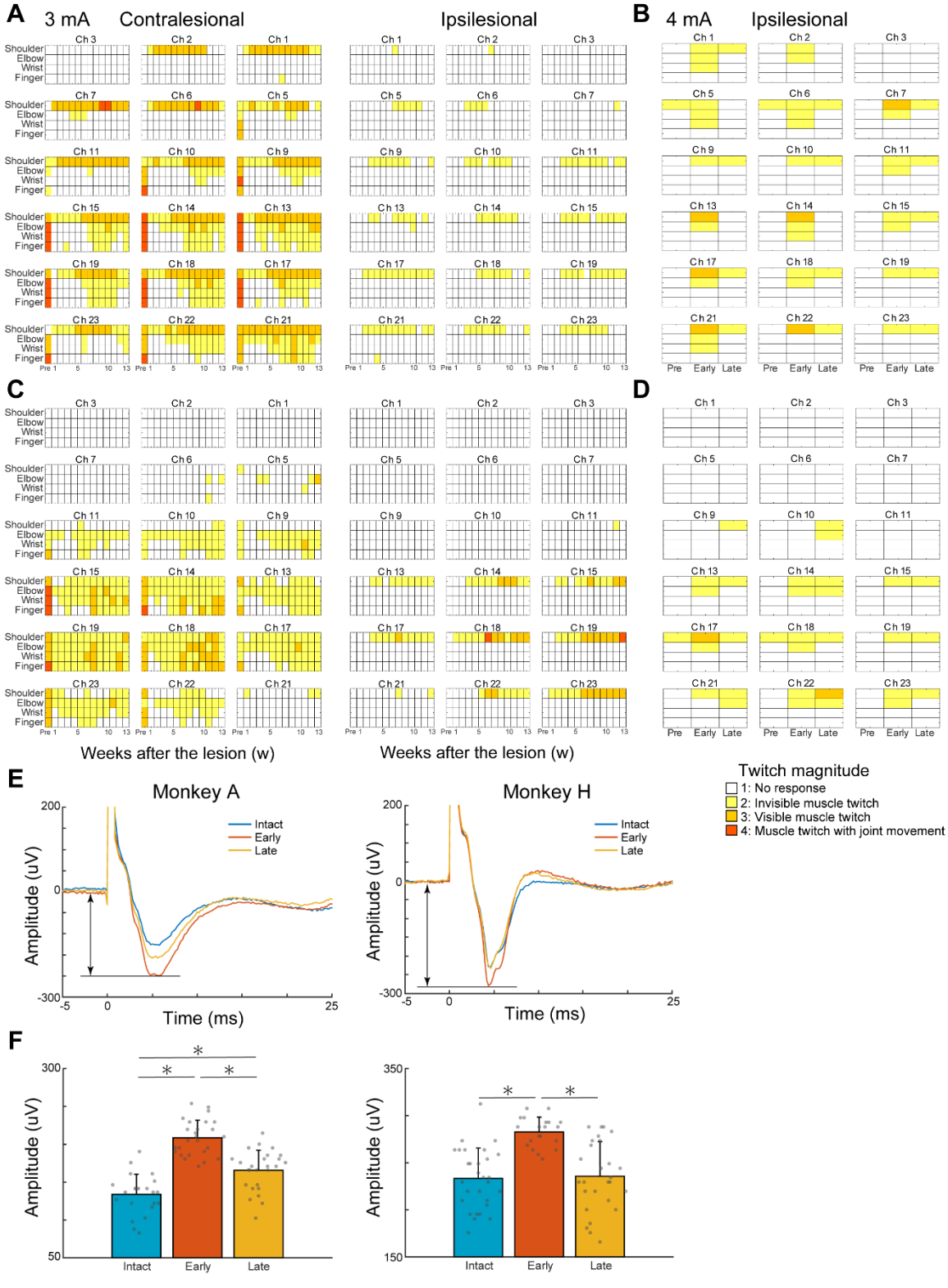
A
3 mA
Contralesional
Ipsilesional
B
4 mA
Ipsilesional
C
D
Shoulder
Elbow
Wrist
Finger
Pre
Early
Late
Weeks after the lesion (w)
Weeks after the lesion (w)
E
Monkey A
Monkey H
Intact
Early
Late
Amplitude (uV)
Time (ms)
Twitch magnitude
1: No response
2: Invisible muscle twitch
3: Visible muscle twitch
4: Muscle twitch with joint movement
F
Amplitude (uV)
Intact
Early
Late

Figure S7. Electrical stimulation demonstrating upregulation of the interhemispheric connectivity and the pathway from the ipsilesional motor cortex to the ipsilesional forelimb muscles

(**A, C**) Muscle twitches in various parts of the ipsilesional forelimb when stimulated at each channel of the ECoG electrodes on the bilateral sensorimotor cortices in Monkey A (***A***) and H (***C***). Stimulation intensity was 3 mA. Colour indicates the magnitude of muscle twitches (see inset). (**B, D**) Muscle twitches in various parts of the ipsilesional forelimb when stimulated at each channel of the ECoG electrodes on the ipsilesional cortex in Monkey A (***B***) and H (***D***). Stimulation intensity was 4 mA. (**E**) Examples of the averaged waveform of CCEPs recorded at the ipsilesional PMd (Ch 10) when stimulated at the contralesional PMd (Ch 10) in each stage (blue, intact [$n = 25$ trials, Monkey A; $n = 29$ trials, Monkey H]; orange, early [day 50, $n = 28$ trials, Monkey A; day 45, $n = 24$ trials, Monkey H]; yellow, late [day 84, $n = 30$ trials, Monkey A; day 83, $n = 27$ trials, Monkey H]). The vertical lines with arrows indicate the amplitude measured and shown in ***B***. (**F**) Averaged amplitude of CCEPs recorded at the ipsilesional PMd (Ch 10) when stimulated at the contralesional PMd (Ch 10) in each stage. Error bars indicate standard deviation. *$P < 0.05$ (one-way analysis of variance [$F = 52.04$, $P < 0.05$ for Monkey A; $F = 18.33$, $P < 0.05$ for Monkey H] followed by Bonferroni's *post-hoc* test).