Supplementary Information

Brain-Machine-Interface in Chronic Stroke: Long-Term Follow-up

Supplementary methods

Exercises for home training

For home training, patients were required to perform 2 sessions of 30 to 45 minutes of exercises per day. Examples of exercises required for patients to perform at home includes:

a) bend your fingers – relax so that the fist opens (20 repetitions)

b) use a toothpaste tube (2 to 3 times a day):

- push the toothpaste tube into the paralyzed hand with the help of the non-paretic hand;

- hold it;

- unscrew the cap (with the help of the non-paretic hand);

- apply toothpaste to the toothbrush (i.e., press the toothpaste with the paretic hand, while holding the toothbrush with the healthy hand);

- screw the cap (with the help of the non-paretic hand);

- release the toothpaste tube by relaxing the paretic hand muscles (if possible), or take the toothpaste tube out of the paralyzed hand with the help of the non-paretic hand;

Supplementary results

Primary behavioral outcome measure: analyses of hand and arm scores (motor part) from the modified upper limb Fugl-Meyer Motor Assessment (cFMA)

In the C+ group 9/16 patients and in the Sham group 7/12 improved their hand FMA scores in the Post2 session as compared to baseline values, while 4/16 patients in the C+ group and 8/12 patients in the Sham group improved hand FMA scores as compared to Post1 session. In the C+ group 13/16 patients and in the Sham group 7/12 patients improved their arm FMA scores in the Post2 session as compared to baseline values, while 4/16 patients in the C+ group and 7/12 patients in the Sham group improved their arm FMA scores between Post1 and Post2 sessions.

Paired samples *t* tests were performed to evaluate changes in arm or hand FMA scores between Pre and Post2, and between Post1 and Post2 sessions. We found that while C+ group presented a significant increase in arm FMA scores from Pre (7,91±1,28) to Post2 (9,56±1,42; p=0.039) sessions, no significant difference was found on hand FMA scores from Pre to Post2 session in C+ (handPre: 3,25±0,61; handPost2: 3,87±0,74; p=0.093). Moreover, C+ group did not show significant changes on hand FMA scores between Post2 and Post1 (4,06±0,73; p=0.67) or on arm FMA scores between Post2 and Post1 (10,5±1,34; p=0.22). No significant changes were found on Sham group hand or arm FMA scores from Pre to Post2 sessions (hand FMA scores Pre: 3,32±0,88; hand FMA scores Post2: 4,17±0,99; p=0.25; arm FMA scores Pre: 9,96±2,19; arm FMA scores Post2: 10,58±1,95; p=0.23) or from Post1 to Post2 sessions (hand FMA scores Post1: 3,71±0,98; p=0.9; arm FMA scores Post1: 9,93±2,12; p=0.54).

Secondary outcome measures: GAS, MAL and Ashworth scores

We performed Wilcoxon Signed Ranks test (data were not normally distributed) to evaluate changes on MAL, GAS and Ashworth scores between sessions. Both C+ and Sham groups significantly increased GAS (C+: z=-3.37, p=0.001; Sham: z=-2.844, p=0.004) and MAL scores (C+: z=-2.041, p=0.041; Sham: z=-2.845, p=0.004) from Pre to Post2 sessions. Between Post1 and Post2 sessions we found no significant difference in C+ or Sham groups on GAS (C+: z=-1, p=0.32; Sham: z=-0,63; p=0.53) or MAL scores (C+: z=-1,071; p=0.28; Sham: z=-1,846; p=0.065).

Correlations between fMRI laterality index and motor outcome measures

To assess the influence of changes in laterality index (LI) with changes in motor outcomes, we correlated Delta of LI scores (after – before) with delta of distinct motor assessment scores between Post2 and Pre, and between Post2 and Post1. We found no significant correlations between Delta of LI and Delta of any motor assessment scores (see Supplementary Table 3). Values were Bonferroni corrected for 11 comparisons (significant p value = 0.005).

Primary behavioral outcome measure: analyses of motor scores from the modified upper limb Fugl-Meyer Assessment (cFMA) in patients with mixed or subcortical lesions

While we found a significant increase in cFMA scores only in the experimental group immediately after intervention and six months after intervention, it could be argued that there are slightly more patients with mixed lesions (i.e., affecting cortical and subcortical structures) in the control group (n=9) as compared to the experimental group (n=6), and this bias could influence changes in motor outcomes after BMI-based rehabilitation. To investigate the influence of preserved cortex on motor recovery after

BMI-based rehabilitation, we analyzed changes in cFMA scores between C+ and Sham groups after intervention in patients with either subcortical lesions only, i.e., when excluding patients with mixed lesions, or with mixed lesions only, i.e., when excluding patients with subcortical lesions.

A 2-way mixed model ANOVA with time (Pre, Post1 and Post2) as within-subjects factor (repeated measures) and group (C+ and Sham) as between-subjects factor indicated that in patients with subcortical lesion only a significant time x group interaction ($F_{1,13}=6.143$, $p=0.015$), but no significant effect of time ($F_{1,13}=1.271$, $p=0.32$) or group ($F_{1,13}=1.57$, $p=0.23$). *Post hoc t-tests* indicated a significant increase in cFMA scores in the C+ group (n=10) only from Pre (10,4±2,33) to Post1 (13,7±2,74; $p=0.002$) sessions, but no significant changes between Pre and Post2 (12,1±2,82; $p=0.17$) or between Post1 and Post2 ($p=0.24$) sessions. No significant changes in cFMA scores were found in the Sham group (n=5).

Results in mixed lesion patients indicated no significant time x group interaction ($F_{1,13}=0.258$, $p=0.77$), and no significant main effect of group ($F_{1,13}=1.312$, $p=0.28$), but a significant main effect of time ($F_{1,13}=7.524$, $p=0.003$). *Post hoc t tests* indicated that patients with mixed lesions (regardless on feedback modality) significantly increased cFMA scores from Pre (11±1.88) to Post1 (13.33±2.17; $p=0.034$) and from Pre to Post2 (13.23±1,7; $p=0.005$) sessions.

Furthermore, we separated the patients in 2 groups depending on lesion location (independent on feedback group) and performed a 2-way ANOVA (delta cFMA, Lesion) to analyze the difference in recovery based on lesion location. We found no significant difference in delta cFMA scores (delta Pre-Post1 $F_{1,28}=2.197$ $p=0.149$; and delta Pre-Post2 $F_{1,26}=0.017$ $p=0.896$) depending on lesion location.

EMG agonist/antagonist ratio evolution:

We performed first a two-way ANOVA with repeated measures, taking Session (Pre,Post1 and Post2) as repeated (dependent) variable and Group (experimental and control) as independent variable. All ratios resulted in no significant interactions or main effects.

As reminder, the movements were: 1) flexion, 2) abduction of the upper arm, 3) extension of the elbow, 4) supination, 5) wrist extension and 6) finger extension

For each movement we defined 1 or 2 agonist/antagonist muscle pair, we consider relevant during the movement. For movement 4 (pronation/supination) we did not have any electrode on the main muscle involved and therefore did not calculate any muscle pair.

Mov1

Anterior vs. posterior deltoid→p-val: 0.67, $F(49, 2) = 0.39$.

Biceps vs. triceps→p-val: 0.86, $F(49, 2) = 0.15$.

Mov2

Anterior vs. posterior deltoid→p-val: 0.73, $F(49,2) = 0.32$

Biceps vs. triceps→p-val: 0.65, $F(49,2) = 0.44$

Mov3

Triceps vs. biceps→p-val: 0.44, $F(50,2) = 0.44$

Mov5

Ext digitorum vs. flexor→p-val: 0.53, $F(50,2) = 0.65$

Ext carpi ulnaris vs. flexor→p-val: 0.52, $F(49,2) = 0.67$

Mov6

Ext digitorum vs. flexor→p-val:0.6, $F(50,2) = 0.52$

Ext carpi ulnaris vs. flexor→p-val:0.36, $F(49,2) = 1.03$

Furthermore, we correlated the delta of ratio agonist-antagonist during each movement, and the delta of Ashworth and cFMA values (Post1-Pre, Post2-Pre, Post2-Post1) and we did not find any significant correlation (See Supplementary Table 6).

Supplementary Tables

Supplementary Table 1. Correlations between frequency of home exercising and changes in motor function assessments between Post1 and Post2.

| Motor function assessments | Frequency of self-exercise at home | | | |
| | C+ | | Sham | |
Delta (Post2 - Post1)	R	p	R	p
Pearson correlations				
cFMA scores	-0.153	0.59	-0.227	0.48
EMG (isometric contraction)				
Shoulder flexion	0.081	0.78	0.084	0.82
Shoulder abduction	0.083	0.77	-0.28	0.38
Elbow extension	0.079	0.78	0.575	0.051
Arm supination	0.046	0.87	0.224	0.53
Wrist extension	-0.335	0.22	-0.464	0.13
Fingers extension	0.128	0.65	-0.448	0.15
Spearman correlations				
EMG (continuous movement)	-0.365	0.24	-0.411	0.18
GAS scores	0.144	0.61	0.29	0.36
MAL scores	0.381	0.16	0.25	0.94
Ashworth scores	-0.362	0.19	0.663	0.02

cFMA = combined Fugl-Meyer. EMG = electromyography. GAS = Goal attainment scale. MAL = Motor activity log. Significant p values <0.005 (Bonferroni corrected for 11 comparisons).

Supplementary Table 2. Correlation between Brain-Machine Interface runs and Delta scores of distinct motor assessments.

Deltas (Sessions)	Motor assessments	BMI runs C+ R (rho)	p	Sham R (rho)	p
Post1 - Pre					
Parametric	**Laterality index (LI) score**	-0.308	0.31	-0.041	0.923
	cFMA	**0.543**	**0.03**	-0.062	0.848
	EMG waveform length				
	Shoulder flexion (deltoid)	-0.234	0.38	-0.147	0.631
	Shoulder abduction (deltoid)	**-0.498**	**0.05**	-0.394	0.163
	Elbow extension (triceps)	0.113	0.68	0.012	0.969
	Arm supination (biceps)	0.395	0.13	0.066	0.838
	Wrist extension (ext. dig.)	0.054	0.84	-0.304	0.291
	Fingers extension (ext. dig.	-0.026	0.92	0.403	0.153
Non-parametric	**Ashworth scores**	-0.389	0.14	0.057	0.847
	GAS scores	0.147	0.59	0.046	0.875
	MAL scores	-0.025	0.93	0.074	0.802
	EMG (grasping movement	0.019	0.94	0.161	0.583
Post2-Pre					
Parametric	**Laterality index (LI) score**	0.012	0.97	-0.399	0.33
	cFMA	**0.761**	**0.001**	-0.555	0.06
	EMG waveform length				
	Shoulder flexion (deltoid)	-0.314	0.24	0.245	0.47
	Shoulder abduction (deltoid)	-0.084	0.76	-0.081	0.80
	Elbow extension (triceps)	0.418	0.11	0.171	0.59
	Arm supination (biceps)	-0.211	0.43	0.13	0.69
	Wrist extension (ext. dig.)	-0.026	0.92	-0.125	0.70
	Fingers extension (ext. dig.	-0.113	0.68	0.005	0.99
Non-parametric	**Ashworth scores**	0.073	0.79	0.196	0.54
	GAS scores	-0.154	0.57	-0.252	0.43
	MAL scores	-0.15	0.59	0.127	0.71
	EMG (grasping movement	0.172	0.56	-0.035	0.91

ext. dig. = extensor digitorum; cFMA = combined arm and hand Fugl-Meyer Assessment motor scores; EMG = electromyography; GAS = Goal attainment scale; MAL = Motor Activity Log. Significant values are presented in bold.

Supplementary Table 3. Spearman correlations between Delta Ashworth scores and Delta of distinct motor assessments.

| Deltas (Sessions) | Motor assessments | Delta Ashworth scores | | | |
| | | C+ | | Sham | |
		rho	p	rho	p
Post2 - Pre					
	GAS scores	-0.208	0.44	-0.203	0.53
	MAL scores	-0.28	0.29	0.633	0.036
	EMG waveform length				
	Shoulder flexion (deltoid)	-0.305	0.25	0.091	0.8
	Shoulder abduction (delto	-0.101	0.71	-0.164	0.63
	Elbow extension (triceps)	-0.172	0.53	-0.178	0.6
	Arm supination (biceps)	0.157	0.56	0.009	0.98
	Wrist extension (ext. dig.)	0.071	0.79	0.091	0.79
	Fingers extension (ext. di{	-0.101	0.71	0.055	0.87
Post2 - Post1					
	GAS scores	-0.17	0.53	0.249	0.44
	MAL scores	-0.004	0.99	-0.215	0.53
	EMG waveform length				
	Shoulder flexion (deltoid)	-0.203	0.45	-0.317	0.41
	Shoulder abduction (delto	0.088	0.75	-0.073	0.83
	Elbow extension (triceps)	-0.171	0.53	-0.077	0.82
	Arm supination (biceps)	0.31	0.24	0.438	0.21
	Wrist extension (ext. dig.)	-0.089	0.74	-0.046	0.89
	Fingers extension (ext. di{	-0.009	0.97	-0.018	0.96

ext. dig. = extensor digitorum.

Supplementary Table 4. EMG waveform length delta (after-before) values of arm and hand muscles during distinct upper limb movements

Group	EMG waveform length delta	Upper limb movements and and respective muscles					
		Shoulder flexion (deltoid)	Shoulder abduction (deltoid)	Elbow extension (triceps)	Arm supination (biceps)	Wrist extension (ext. dig.)	Fingers extension (ext. dig.)
C+	Delta 1 (Post2-Post1)	2,03±0,68	0,54±0,16	1,12±0,34	0,61±0,35	0,4±0,1	0,28±0,08
	Delta 2 (Post2-Pre)	1,98±0,69	0,67±0,18	1,33±0,36	0,56±0,32	0,44±0,14	0,25±0,99
Sham	Delta 1 (Post2-Post1)	2,51±0,78	0,67±0,3	1,34±0,3	0,09±0,08	0,81±0,33	0,45±0,19
	Delta 2 (Post2-Pre)	2,09±0,79	0,86±0,21	1,13±0,21	0,24±0,1	0,79±0,3	0,4±0,18
independent t tests							
	p value (C+ Delta 1 vs. sham De	0.66	0.69	0.65	0.27	0.36	0.35
	p value (C+ Delta 2 vs. sham De	0.92	0.5	0.64	0.4	0.31	0.42

ext. dig. = extensor digitorum. Values are given as mean ± standard error.

Supplementary Table 5. Correlation between Delta Laterality Index (LI) scores and Delta scores of distinct motor assessments.

Deltas (Sessions)	Motor assessments	Laterality Index Delta			
		C+		Sham	
		R (rho)	p	R (rho)	p
Post2 - Pre					
Parametric	**cFMA**	-0.067	0.83	0.093	0.827
	EMG waveform length				
	Shoulder flexion (deltoid)	0.101	0.74	-0.415	0.354
	Shoulder abduction (deltoi	0.131	0.67	-0.443	0.272
	Elbow extension (triceps)	0.32	0.29	0.567	0.143
	Arm supination (biceps)	-0.042	0.89	0.41	0.313
	Wrist extension (ext. dig.)	0.339	0.26	-0.275	0.51
	Fingers extension (ext. dig	0.089	0.77	-0.12	0.777
Non-parame	**Ashworth scores**	0.086	0.78	0.192	0.649
	GAS scores	0.093	0.76	0.16	0.706
	MAL scores	0.088	0.79	0.857	0.014
	EMG (grasping moveme	-0.168	0.60	-0.024	0.955
Post2 - Post1					
Parametric	**cFMA**	0.269	0.38	0.23	0.58
	EMG waveform length				
	Shoulder flexion (deltoid)	0.16	0.60	0.055	0.92
	Shoulder abduction (deltoi	0.024	0.94	-0.199	0.64
	Elbow extension (triceps)	0.464	0.11	-0.32	0.44
	Arm supination (biceps)	-0.215	0.48	-0.288	0.53
	Wrist extension (ext. dig.)	0.265	0.38	0.107	0.80
	Fingers extension (ext. dig	-0.266	0.38	0.237	0.57
Non-parame	**Ashworth scores**	0.028	0.93	-0.611	0.11
	GAS scores	-0.108	0.72	-0.378	0.36
	MAL scores	0.304	0.34	-0.198	0.67
	EMG (grasping moveme	-0.227	0.50	-0.071	0.87

ext. dig. = extensor digitorum; cFMA = combined arm and hand Fugl-Meyer Assessment motor scores; EMG = electromyography; GAS = Goal attainment scale; MAL = Motor Activity Log

Supplementary Table 6. Correlation between spasticity and impairment, and EMG agonist/antagonist ratio (ratEMG)

Group	Mov	Sess	cFMAp	cFMAR	ASHp	ASHrho
BMI	Mov1	post1.pre	0.891928	0.036952	0.784339	0.074354
BMI	Mov1	post2.pre	0.438987	0.225149	0.667721	0.126019
BMI	Mov1	post2.post´	0.809589	0.07093	0.785244	0.080185
BMI	Mov2	post1.pre	0.566039	0.155188	0.805499	0.066919
BMI	Mov2	post2.pre	0.936406	0.023514	0.818628	0.06751
BMI	Mov2	post2.post´	0.467938	0.211495	0.187473	0.374198
BMI	Mov3	post1.pre	0.073471	0.459338	0.895601	-0.03569
BMI	Mov3	post2.pre	0.502862	-0.18773	0.099706	-0.4412
BMI	Mov3	post2.post´	0.416522	0.226693	0.527073	-0.17739
BMI	Mov5	post1.pre	0.674742	0.118228	0.739326	0.093868
BMI	Mov5	post2.pre	0.923626	0.0271	0.896067	-0.03692
BMI	Mov5	post2.post´	0.859521	-0.05213	0.321431	0.286074
BMI	Mov6	post1.pre	0.034617	0.547555	0.200669	0.350201
BMI	Mov6	post2.pre	0.896309	0.036834	0.613318	0.142145
BMI	Mov6	post2.post´	0.110963	0.444864	0.121423	0.43358
Sham	Mov1	post1.pre	0.299591	-0.31187	0.345383	-0.28493
Sham	Mov1	post2.pre	0.174975	-0.46568	0.176505	-0.46423
Sham	Mov1	post2.post´	0.753589	-0.11412	0.879576	-0.05522
Sham	Mov2	post1.pre	0.471224	0.219486	0.678676	-0.12725
Sham	Mov2	post2.pre	0.369646	-0.31857	0.742938	-0.11919
Sham	Mov2	post2.post´	0.305614	0.360871	0.440015	0.276094
Sham	Mov3	post1.pre	0.333092	-0.30618	0.3497	-0.29631
Sham	Mov3	post2.pre	0.987853	-0.00522	0.514166	-0.22078
Sham	Mov3	post2.post´	0.53018	-0.22596	0.252011	-0.40005
Sham	Mov5	post1.pre	0.084198	0.496739	0.073813	0.511769
Sham	Mov5	post2.pre	0.47115	0.243202	0.392958	0.28654
Sham	Mov5	post2.post´	0.421052	0.270531	0.685875	0.13794
Sham	Mov6	post1.pre	0.979806	-0.00781	0.705441	-0.11619
Sham	Mov6	post2.pre	0.885615	0.049269	0.730942	-0.11743
Sham	Mov6	post2.post´	0.6198	0.168794	0.798403	0.087362

Column Sess indicates the delta used (Post1-Pre, Post2-Pre, Post2-Post1), cFMAp and cFMAR indicates p and R from Pearson correlation between cFMA and EMG during each movement (indicated in column MOV). ASHp and ASHrho indicates p and rho from Spearman correlation between Ashworth and ratEMG during each movement.