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Long-Term Motor Deficits after Controlled Cortical Impact in Rats Can Be Detected by Fine Motor Skill Tests but Not by Automated Gait Analysis Supplementary material

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Figure legends

Figure 1: Controlled cortical impact (CCI) on the forelimb motor cortex causes severe tissue damage

The impactor tip with a diameter of 3 mm was positioned on top of the exposed forelimb area of the motor cortex contralateral to the preferred limb and hit the tissue with a target depth of 5 mm (scheme created at 3.2 mm anterior to bregma, **A**). A representative hematoxylin-eosin stained section showing the severe tissue damage six weeks after CCI (approximately 3.2 mm anterior to bregma; M1: primary motor cortex, M2: secondary motor cortex, fmi: forceps minor of the corpus callosum, aca: anterior part of the anterior commissure, **B**) further supported by quantitative analysis of the lesion volume in CCI rats (n=8) compared to control rats (n=5) with a craniotomy (*** p < 0.001, **C**).

Figure 2: CCI impairs tactile recognition of adhesive stickers underneath the preferred paw and causes reliance on the non-preferred paw during vertical exploration

In the adhesive removal test, rats designated to the CCI group were significantly faster in contacting the sticker underneath their non-preferred paw at all three timepoints, thus even before CCI (CCI n=9, control n=7; A1). The time to contact the sticker underneath the preferred paw was significantly increased two and six weeks after CCI (CCI n=10, control n=6; A2). Using the *difference score*, both animal groups again differed significantly from each other at all three timepoints (CCI n=10, control n=7; A3). CCI did not affect the

time taken to remove the stickers (non-preferred paw: CCI n=10, control n=7; preferred paw: CCI n=8, control n=6; *difference score*: CCI n=8, control n=7; **B1-3**).

After CCI, rats exhibit an increased percentage of wall contacts with their nonpreferred paw while exploring the cylinder (CCI n=9, control n=7; C1). At the same time, the percentage of wall contacts with the preferred paw was sigificantly lower compared to the control group (CCI n=10, control n=5; C2). Also, the *difference score* (here set relative to baseline values) showed a progressive asymmetry in paw use in the CCI group over time (CCI n=10, control n=7; * p < 0.05, ** p < 0.01, C3).

Figure 3: CatWalk XT analysis did not reveal any impairment in the preferred forelimb

Eight selected parameters (out of 13 parameters analyzed) describing individual paw functioning are shown (mean intensity non-preferred paw: CCI n=10, control n=7; preferred paw: CCI n=9, control n=7; *difference score*: CCI n=9, control n=7; **Fig. 3.1 A1-3**; print area non-preferred paw: CCI n=10, control n=7; preferred paw: CCI n=10, control n=7; *difference score*: CCI n=10, control n=7; **B1-3**; stride length non-preferred paw: CCI n=10, control n=7; difference score: CCI n=6; c1-3; stand non-preferred paw: CCI n=10, control n=7; preferred paw: CCI n=9, control n=7; *difference score*: CCI n=9, control n=6; **C1-3;** stand non-preferred paw: CCI n=10, control n=7; preferred paw: CCI n=10, control n=7; preferred paw: CCI n=8, control n=7; D1-3; swing mean non-preferred paw: CCI n=8, control n=7; **Fig. 3.2 E1-3**, swing speed non-preferred paw: CCI n=10, control n=7; preferred paw: CCI n=7, control n=7; preferred paw: CCI n=9, control n=7; preferred paw: CCI n=7, control n=7; preferred paw: CCI n=9, control n=7; preferred paw: CCI n=7, control n=7; preferred paw: CCI n=9, control n=7; preferred paw: CCI n=7, control n=7; preferred paw: CCI n=9, control n=7; preferred paw: CCI n=7, control n=7; preferred paw: CCI n=9, control n=7; preferred paw: CCI n=7, control n=7; preferred paw: CCI n=9, control n=7; preferred paw: CCI n=7, control n=7; preferred paw: CCI n=9, control n=7; preferred paw: CCI n=7, control n=7; preferred paw: CCI n=9, control n=7; preferred paw: CCI n=7, control n=7; preferred paw: CCI n=9, control n=7; preferred paw: CCI n=9, control n=7; preferred paw: CCI n=9, control n=7; preferred paw: CCI n=7, control n=7; preferred paw: CCI n=9, control n=7; preferred paw: CCI n=

difference score: CCI n=7, control n=7; **F1-3**; step cycle non-preferred paw: CCI n=9, control n=7; preferred paw: CCI n=8, control n=7; *difference score*: CCI n=9, control n=5; **G1-3**; duty cycle non-preferred paw: CCI n=10, control n=7; preferred paw: CCI n=9, control n=7; *difference score*: CCI n=7, control n=7; **H1-3**). No significant differences between groups could be detected after CCI concerning the individual forelimbs and the *difference score*.

Figure 4: No changes in gait were measured after CCI using the CatWalk XT

All analyzed gait-related parameters, which describe coordinated movements of the four paws in concert, are shown (regularity index: CCI n=10, control n=6; **A**; base of support forelimbs: CCI n=10, control n=7; **B**; base of support hindlimbs: CCI n=10, control n=7; **C**; three-limb support: CCI n=10, control n=6; **D**; speed: CCI n=10, control n=7; **E**; cadence: CCI n=10, control n=7; **F**; couplings isilateral front – contralateral hind: CCI n=10, control n=7; **G**). None of the parameters was significantly changed after CCI when comparing CCI rats to controls.

Figure 5: Pellet eating with the preferred paw is significantly deteriorated after CCI

In the Montoya staircase test pellet eating with the non-preferred paw did not differ between CCI and control rats (CCI n=10, control n=6; A1), whereas after surgery CCI rats ate significantly fewer pellets from the staircase close to their preferred paw (CCI n=10, control n=7; A2). The pellet consumption deficit in CCI rats was also measurable when analyzing the *difference score*

comparing the number of eaten pellets between the preferred and nonpreferred paw (CCI n=10, control n=7; A3).

Three additional parameters are shown at six weeks after surgery that provide more details about deficits in pellet retrieval (**B1-3**: number of pellets eaten, **C1-3**: number of pellets remained, **D1-3**: number of pellets misplaced). After CCI, rats ate significantly fewer pellets from each individual step with their preferred limb (step 3: CCI n=10, control n=6; step 4: CCI n=8, control n=7; step 5: CCI n=6, control n=7; step 6: CCI n=7, control n=7; step 7: CCI n=10, control n=7; **B2**) while leaving more pellets untouched at their original location (step 3: CCI n=8, control n=4; step 4: CCI n=7, control n=6; step 5: CCI n=7, control n=5; step 6: CCI n=7, control n=7; step 7: CCI n=8, control n=7; **C2**). When reaching for the pellets with the preferred limb, there was an increase in the number of pellets misplaced after CCI (step 3: CCI n=7, control n=5; step 4: CCI n=8, control n=6; step 5: CCI n=7, control n=5; step 6: CCI n=4, control n=7; step 7: CCI n=8, control n=6;* p < 0.05, ** p < 0.01, *** p < 0.001, **D2**).

Figure 6: Reaching behavior with the preferred paw is severely altered after CCI

Rats showed overt abnormalities in a number of reaching behaviors related to pellet grasping and eating after CCI. Two weeks after CCI, rats had deficits in forming a "power grip", i.e. closing all digits around the pellet when achieving to grasp a pellet (CCI n=8, control n=7; **A**), followed by a problematic return of the paw towards the mouth (CCI n=8, control n=7; **B**). Pellet eating from the paw was significantly impaired both two and six weeks after CCI (CCI n=7, control n=7; ** p < 0.01, *** p < 0.001, **C**).