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An analysis of the CatWalk XT and a composite score to assess neurofunctional deficits after photothrombosis in mice

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ABSTRACT

The purpose of this study was to evaluate CatWalk's capability for assessing the functional outcome after photothrombotic stroke affecting the motor cortex of mice. Mice were tested up to 21 days after photothrombosis or sham surgery using CatWalk, and a composite score assessing functional deficits (neuroscore).

The neuroscore demonstrated deficits of the contralateral forelimb for more than two weeks after stroke. There were no asymmetric or coordinative dysfunctions of limbs detected by CatWalk. However, CatWalk data revealed impairment of locomotion speed and its depending parameters for one-week after stroke in strong correlation to the neuroscore.

Data suggest that the composite neuroscore allows to more sensitively and precisely specify and quantify photothrombosis-induced hemisyndromes than CatWalk.

1. Introduction

Methods to assess behavioural and functional deficits after experimental stroke are a prerequisite for meaningful preclinical stroke research that may translate to future therapeutic options [1–4]. Photothrombotic stroke is widely used to study the cellular and molecular mechanism underlying cerebral ischemia inflicting neurodegeneration, neuroprotection, and neuroregeneration [5–7]. In our hands, photothrombosis is a pure cortical stroke, minimally invasive, and the lesions are highly reproducible in size and location [7–9].

A bunch of standardised methods have been suggested to assess neurological (dys)function after experimental stroke, especially to evaluate gait disturbances in a longitudinal fashion [10–12]. A widespread tool for automated analysis of rodent's gait is CatWalk [13]. Since its first release in 2006, CatWalk has been used to quantify neurofunction in many experimental setups, e.g., spinal cord injury, Parkinson's disease, peripheral nerve damage, and stroke [14–17]. CatWalk XT is a registered trade mark of Noldus Information Technology, Wageningen, Netherlands.

For CatWalk gait analysis, the animal freely traverses a glass plate towards a goal box, while a high-speed camera captures its illuminated footprints. CatWalk software analyses use the data to investigate the animal's gait and generate a large number of finely graded dynamic and static gait parameters, promising to detect even subtle and nuanced changes [14,18,19].

The automatic analyses provide independence of an observer's subjective judgement, thereby increasing reliability. Nevertheless, several limitations apply including differences in the animal's compliance to traverse, deficits in the automatic classification of the footprints, locomotion speed that impacts on the analysis of limb kinematics and interrelated speed-depending parameters, all of which may result in errors [13,18–20].

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Table 1
Comparison of non-parametric Neuroscore and quantitative Catwalk parameters.

Neuroscore	Score and description
Flexion of the forelimb in suspension	0 - forelimb does not move and lies close to the trunk 1 - forelimb lies mostly close to the trunk and rarely moves 2 - forelimb mostly moves up and down and rarely lies close 3 - forelimb is mostly stretched out to the ground and rarely moves up and down 4 - regularly moveable forelimb, stretched to the ground permanently
Paw placement on a table's edge	0 - paw is immobile and hangs down, no movement 1 - paw is mostly hanging, little movements forward and backward 2 - paw is mostly hanging, moving horizontally and vertically downside the table surface 3 - paw is hanging, moves horizontally and vertically up to the table surface level 4 - paw rarely hangs down, moveable to all directions, occasional placement on the table surface 5 - normal placement, paw is immediately taken up to the table surface
Whisker-reflex	0 - not present 2 - present
Catwalk	Quantitative measures
Individual paw measures	Print area of left and right forelimb Print length of left and right forelimb Print width of left and right forelimb Mean intensity of left and right forelimb Stand of left and right forelimb Swing speed of left and right forelimb
Distances between the paws	Stride length of the left and of the right forelimb Base of support - distance between the left and right forepaw prints
Time-based relationships between the paws	Step cycle of the left and of the right forelimb Regularity index (regularity of footfall) Run average speed of locomotion Couplings - left to right forelimb - Couplings - left forelimb to left hind limb - Couplings - left forelimb to right hind limb -

We here evaluate the ability and reproducibility of the CatWalk gait assessment to depict motor deficits after cortical photothrombotic stroke in mice and relate it to a composite neuroscore [21]. Furthermore, multiple testing points were chosen to assess the CatWalk's and neuroscore's performance to depict motor deficits over time.

2. Materials and methods

2.1. Study design

After a training period of five days on the CatWalk, an individual baseline score was recorded. Additionally, the baseline composite neuroscore was assessed for each animal (Table 1). For this, we analysed the function of the left and right forelimb. After successful training and baseline recordings, animals received either photothrombosis affecting the right motor cortex or sham surgery. At days (d) 1, 2, 3, 4, 7, 8, 9, 10, 11, 14, 15, 16, 17, 18, and 21, CatWalk data and the neuroscore were collected (Fig. 1). Mice of the photothrombosis group were included into the study if they had a relevant functional deficit, defined by a neuroscore maximum of 7 at day 1. Otherwise animals were excluded. In total we collected data from 7 stroke animals and 6 sham animals. At day 22, all mice were sacrificed.

2.2. Animals

Thirteen male C57BL/6 wild type mice (8 weeks old, 25 g–30 g) were purchased from Charles River Laboratories (Sulzfeld, Germany) and housed at 21 ± 1 °C room temperature, 50 ± 5% humidity, and 12/12 h light-dark cycles with free access to standard mice chow and water. The mice acclimated seven days in their housing, and the weight of each mouse was preoperatively measured and controlled at every examination day. All animal procedures comply with ARRIVE guidelines and had been approved in advance by the local governmental authorities (Landesamt für Natur-, Umwelt- und Verbraucherschutz Northrhine-Westfalia, Germany) following the guidelines of the European Union (directive 2010/63/EU).

2.3. Neuroscore

A modified version of our previously published neuroscore was used [21]. We decided to collect data for both the contra- and the ipsilateral forelimb but not the hindlimbs to focus on the photothrombosis' typical functional sequelae. To avoid additional training of gait that could possibly mask minimal deficits in Catwalk analysis, we did not perform the previously included grid-walk. The maximum score was thus modified to a maximum of 11 points in each front paw containing the forelimb flexion in suspension, placement of the front paw, and the whisker reflex (Table 1).

2.4. CatWalk

2.4.1. Training

All animals were trained for five days before photothrombosis (Fig. 1). After allowing exploration for about 15 min on the first day, mice were placed in a group of 2–3 on the CatWalk-end to easily find their home-cage, which was located behind the runway. This procedure was repeated 10 times. In the following, rodents were placed 20 cm further away from the cage each day until they were able to traverse 80 cm, including the defined runway. From day 4 on, mice were able to traverse separately the CatWalk in the defined direction without hesitation.

2.4.2. Recording criteria

CatWalk recording was performed under exclusion of daylight, and the animals always traversed from left to right. The walkway was defined as 27 cm in length and 6 cm in width. It was cleaned before and after every third run and additionally after pollution with excrements. Each mouse completed nine runs per examination day. The highspeed camera was set at a distance of 30 cm from the walkway. The camera aperture was fully opened. Using the auto detection function, the green intensity threshold was set to 0,1, the ceiling light to 17,7, and the walkway light to 17,4. Run criteria were set to 60 % speed maximum variation, 0,5 s run minimum duration, and 5 s run maximum duration. Every run had to contain two full step cycles at least. Twenty-two different gait parameters were analysed (Table 1): for the assessment

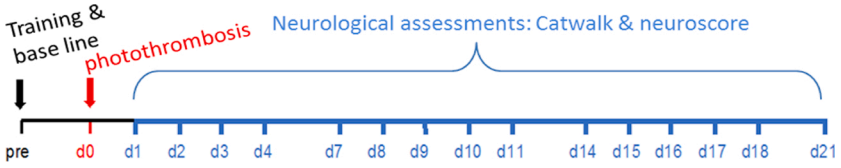

Fig. 1. Experimental design. Training with the CatWalk was performed for 5 days and baselines were recorded with the neuroscore and the CatWalk (pre). Photothrombosis was induced on day 0 and neurological function was assessed with the neuroscore and the CatWalk at multiple time points until day 21. d: day.

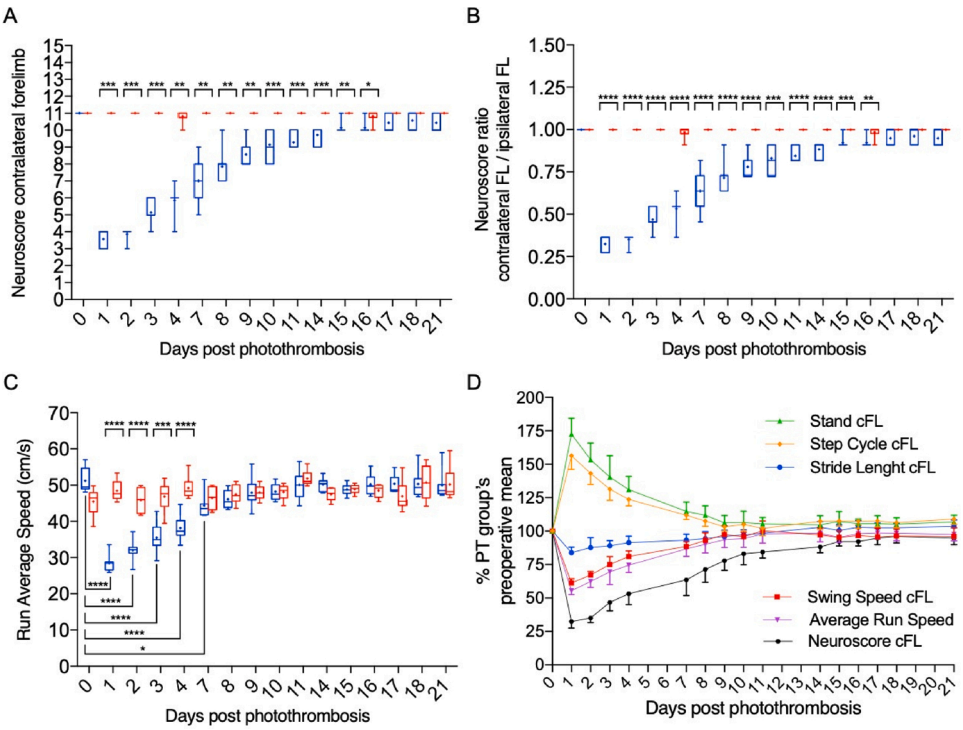

Fig. 2. Dynamics of motor recovery in neuroscore and CatWalk Neuroscore dynamics comparing the photothrombosis (blue), and sham group (red). (A) Neuroscore of the left forelimb (FL) showing the neurological contralateral deficits of the photothrombosis group. (B) Neuroscore reveals hemiparesis: Ratio of the neuroscore contralateral forelimb / neuroscore ipsilateral forelimb showing an asymmetrical deficit. C: Transient decrease of running speed on Catwalk and (D) change of velocity dependent parameters. Significance levels: *P < 0.05**P < 0.01, ***P < 0.001, ****P < 0.0001 (Sidak's multiple comparison; significant results D are not indicated for the sake of clarity).

of differences between the individual paw prints, we analysed the right and left paw's print length, print width, print area, stride length, step cycle, stand, swing speed, and mean intensity of foot print. For the assessment of paw coordination, we detected base of support, the regularity index, and the couplings -left to right front paw- (LF->RF), -left front paw to right hind paw- (LF->RH), and -left front paw to left hind paw- (LF->LH). The average run speed of each mouse was analysed. Every recorded run was automatically classified with the CatWalk XT Software 10.5 (Noldus Information Technology, Wageningen, Netherlands) and manually processed as described elsewhere [13]. Each examination day, every mouse had to complete 9 valid runs with at least two complete step cycles. From these, the arithmetic average was calculated.

2.5. Photothrombosis

Photothrombosis was performed as described previously [8,21,22]. Briefly anaesthetized mice were placed into a stereotactic frame, where a region of 3 mm x 2,5 mm of the exposed intact skull 1,5 right lateral and 1 mm posterior to bregma was illuminated with a LED cold light source (Zeiss CL6000 LED, Carl Zeiss, Oberkochen, Germany) for 15 min with an intensity of 420 lm starting 5 min after intraperitoneal injection of Rose bengal (Sigma-Aldrich, Taufkirchen, Germany). The method was carefully established beforehand to assure reproducable ischemic cortical lesions of compareable sizes. However during the study sham operation occurred without illumination to prevent any other coritcal lesioning due to individual anatomical features of each animal.

2.6. Statistics

Statistical analyses were performed using Excel 2013 (Microsoft Corp.) and GraphPad Prism 6.01 (GraphPad Software). Statistical significance was set at less than the 5 % level (p-value (P) <0.05). Neuroscore and CatWalk data were tested using a 2-way mixed design ANOVA with the factors treatment (photothrombosis and sham) and time (testing days; repeated measures) followed by Sidak's multiple comparison test. For factor time all postoperative days were tested against the preoperative day using the Dunnett's multiple comparison's test. To detect differences between the contra- and ipsilateral forelimb, a ratio neuroscore assessing the contralateral forelimb / ipsilateral forelimb function was determined and analysed by a 2-way mixed design ANOVA as described above. Additionally, the neuroscore data were baseline-normalised (preoperativ mean values set as 100 %) and compared to the baseline-normalised CatWalk means with Spearman correlation analysis. Effect sizes 1d453; were calculated from the ANOVA table as follows: For each factor (treatment, timepoint or factor interaction), the factor sum of squares was divided by the total sum of squares. The resulting effect sizes were then used for an a priori calculation of sample sizes in G*power 3.1 with the following parameters: $\alpha = 0.05$, power = 0.8, number of groups = 2, number of measurements = 16, correlation among repeated measures = 0.5, nonsphericity correction $\varepsilon = 1$.

As mice are tertrapoda, their motor performance is bilaterally symmetric. When a parameter was measured at both the contralateral and the ipsilateral forelimb, a ratio of the contralateral front paw / ipsilateral front paw was determined to detect asymmetries between left and right. Thereby, locomotion speed does not influence on those ratios. To detect differences between groups and between testing days, a 2-way mixed design ANOVA was used as described above.

3. Results

3.1. Neuroscore

The neuroscore of the contralateral forelimb was significantly reduced by photothrombosis ($F(1,11) = 446.7$; $p < 0.0001$ for factor treatment). Significant differences ($p < 0.05$) compared to the sham group were found from day 1 to day 14 (Fig. 2 A). Furthermore, the neuroscore was influenced by time ($F(15,165) = 86.4$; $p < 0.0001$). Significant differences between the preoperative day and postoperative days 1–16 were found in the photothrombosis group (Fig. 2 A). Because the neuroscore of the ipsilateral forelimb was always maximum (11 for all animals at all timepoints), the ratios of contra- and ipsilateral forelimb neuroscores developed analouge to the neuroscores of the

Table 2
F-statistics of CatWalk parameters.

	factor treatment	factor time	time x treatment interaction
Stand	F(1,11) = 146; p = 00,029; p < 005 for photothrombosis vs sham on day 1–4	F(15,165) = 357; p < 00,001; p < 005 for day 0 vs days 1–8 in the photothrombosis group; p < 005 for day 0 vs day 11 in the sham group	F(15,165) = 320; p < 00,001
Stand ratio	F(1,11) = 1,3; p = 02,809; no posthoc testing	F(15,165) = 2,8; p = 00,006; no significant differences to day 0	F(15,165) = 1,7; p = 00,578
Swing speed	F(1,11) = 1,1; p = 03,177; p < 005 for photothrombosis vs sham on day 1, 2, 4	F(15,165) = 7,8; p < 00,001; p < 005 for day 0 vs days 1–4 in the photothrombosis group	F(15,165) = 5,7; p < 00,001
Swing speed ratio	F(1,11) = 0,4; p = 05,285; no posthoc testing	F(15,165) = 0,6; p = 08,841; no posthoc testing	F(15,165) = 1,7; p = 00,624
Step cycle	F(1,11) = 4,2; p = 00,645; p < 005 for photothrombosis vs sham on day 1–4	F(15,165) = 330; p < 00,001; p < 005 for day 0 vs days 1–7 + 21 in the photothrombosis group; p < 005 for day 0 vs day 11 in the sham group	F(15,165) = 308; p < 00,001
Step cycle ratio	F(1,11) = 0,2; p = 06,469; no posthoc testing	F(15,165) = 0,9; p = 05,476; no posthoc testing	F(15,165) = 0,8; p = 06,928
Stride length	F(1,11) = 2,3; p = 01,608; p < 005 for photothrombosis vs sham on day 1–3	F(15,165) = 213; p < 00,001; p < 005 for day 0 vs days 1–8 in the photothrombosis group; p < 005 for day 0 vs day 11 in the sham group	F(15,165) = 108; p < 00,001
Stride length ratio	F(1,11) = 1,4; p = 02,612; no posthoc testing	F(15,165) = 1,0; p = 04,979; no posthoc testing	F(15,165) = 0,2; p = 09,997
Run average speed	F(1,11) = 7,0; p = 00,231; p < 005 for photothrombosis vs sham on day 1–4	F(15,165) = 321; p < 00,001; p < 005 for day 0 vs days 1–8 in the photothrombosis group; p < 005 for day 0 vs day 11, 18, 21 in the sham group	F(15,165) = 251; p < 00,001
Print area	F(1,11) = 1,9; p = 01,927; no significant differences	F(15,165) = 1,5; p = 00,958; p < 005 for day 1, 3, 4, 8−11, 15, 21 in the photothrombosis group	F(15,165) = 1,8; p = 00,442
Print area ratio	F(1,11) = 003; p = 08,750; no posthoc testing	F(15,165) = 1,7; p = 00,548; no posthoc testing	F(15,165) = 1,2; p = 02,981
Base of support frontpaws	F(1,11) = 2193; p = 01,667; no significant differences	F15,165) = 9508; p < 00,001; p < 005 for day 0 vs days 21 in the photothrombosis group; p < 005 for day 0 vs day 9,11−21 in the sham group	F(15,165) = 1296; p = 02,095
Mean intensity	F(1,11) = 1064; p = 03,246; no posthoc testing	F(15,165) = 2,1; p = 00,094; no significant differences to day 0	F(15,165) = 1,2; p = 02,226
Mean intensity ratio	F(1,11) = 07,638; p = 04,008; no posthoc testing	F(15,165) = 159; p = 00,811; no posthoc testing	F(15,165) = 0998; p = 04,597
Print length	F(1,11) = 00,006,291; p = 09,804; no posthoc testing	F(15,165) = 1064; p = 03,935; no posthoc testing	F(15,165) = 08,015; p = 06,748
Print lenght ratio	F(1,11) = 02,399; p = 6339; no posthoc testing	F(15,165) = 09,711; p < 04,879; no posthoc testing	F(15,165) = 1571; p = 00,868
Print width	F(1,11) = 001,617; p = 09,011; no posthoc testing	F(15,165) = 2589; p = 00,016; no significant differences to day 0	F(15,165) = 04,918; p = 09,424
Print width ratio	F(1,11) = 00,235; p = 08,809; no posthoc testing	F(15,165) = 08,349; p = 06,378; no posthoc testing	F(15,165) = 09,416; p = 05,197
stride length ratio	F(1,11) = 1,4; p = 02,612; no posthoc testing	F(15,165) = 1,0; p = 04,979; no posthoc testing	F(15,165) = 0,2; p = 09,997
Couplings LF->LH	F(1,11) = 3586; p = 00,849; no posthoc testing	F(15,165) = 3243; p < 00,001; p < 005 for day 0 vs days 15 in the photothrombosis group	F(15,165) = 05,064; p = 09,347
Couplings LF->RF	F(1,11) = 008,776; p = 07,726; no posthoc testing	F(15,165) = 121; p = 02,684; no posthoc testing	F(15,165) = 1755; p = 00,452
Couplings LF->RH	F(1,11) = 06,575; p = 04,346; no posthoc testing	F(15,165) = 05,632; p = 08,994; no posthoc testing	F(15,165) = 07,367; p = 07,447
Regularity index	F(1,11) = 000,854; p = 09,280; no posthoc testing	F(15,165) = 1109; p = 03,523; no posthoc testing	F(15,165) = 04,708; p = 09,523

contralateral forelimb with identical statistical results (Fig. 2 B).

3.2. CatWalk

The speed-dependent parameters stand, swing speed, step cycle, stride length, and run average speed showed significant differences between stroke- and sham animals during the first four days after photothrombosis (factor treatment; Table 2; Fig. 2 C; Fig. 3 A, C, E, G). In addition, compared to the preoperative measurement, these parameters significantly changed during the first week in the photothrombosis group (factor time; Table 2; Fig. 2 C, D; Fig. 3 A, C, E, G; blue box plots). Within 7 days after photothrombosis, forepaws had largely regained function, and by 14 days after induction of ischemia, all parameters had completely recovered to baseline (Fig. 2 C, D; Fig. 3 A, C, E, G). Quantification of function of the affected left forepaw with regard to the unaffected right forepaw, revealed no changes in stand, swing speed, step cycle, or stride length, suggesting that a hemiparesis was not detected with this system over the whole observation time (Table 2, Fig. 3 B, D, F, H). Normalised values of stand, swing speed, step cycle, and average speed strongly correlated with the neuroscore (Table 3). The most significant correlation was detected in run average speed.

Print length and print length ratio (the following applies to all ratios: contralateral paw/ipsilateral paw), print width and print width ratio, mean intensity and mean intensity ratio, regularity index, couplings LF->RF and couplings LF->RH did not show any significant difference, whether within or between groups (Table 2). Within the photothrombosis group, print area was significantly increased compared to the preoperative timepoint on day 1, 3, 4, 8−11, 15 and 21. However, significant differences to the sham group were not detected (Table 2). We propose that the low preoperative value was responsible for the significant differences rather than the influence of photothrombosis. When analysed separately with a t-test, the preoperative print area was significantly lower ($p = 0.0304$) in the photothrombosis group (0.329 ± 0.024 cm^2) compared to the sham group (0.354 ± 0.004 cm^2). Additionally, in the photothrombosis group compared to preoperative mean, couplings LF->LH was significantly decreased on day 15, also base of support on day 21 suggesting other reasons than photothrombosis. Because of the small animal numbers calculated effect sizes for neuroscore parameters were 0.37, which is between moderate and large, and suggested animal groups of 8 to expect significant results. Catwalk effect sizes were all in the small range and post hoc power analyses required animal numbers around 6–10 for velocity dependent parameters, and above 50 to reveal significant differences in left/right comparisons.

In the sham group, there were significant differences between the

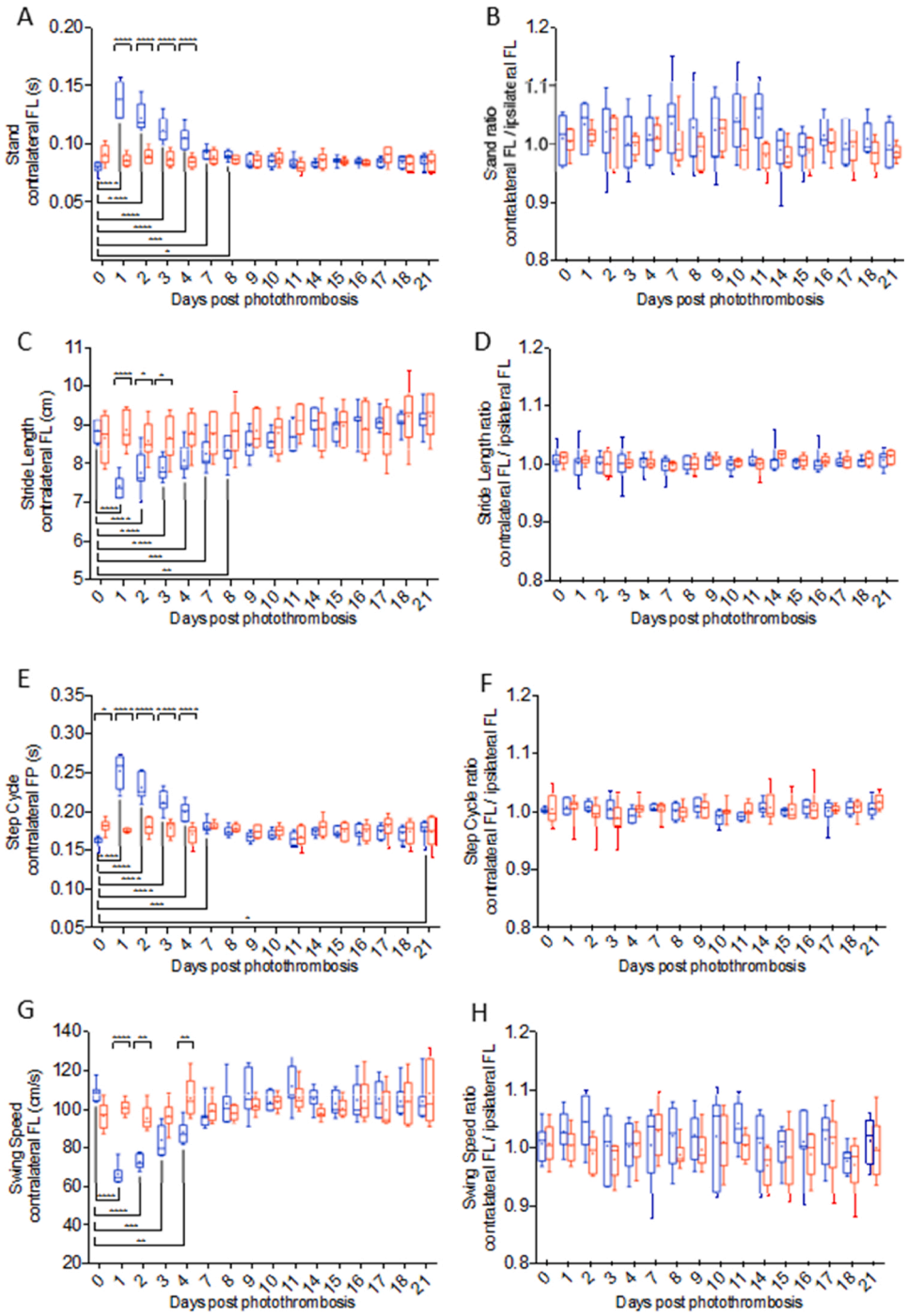

Fig. 3. Detailed gait analysis by CatWalk. Photothrombosis (blue), compared to sham group (red). A, C, E and G (left column) show parameters indicating the gait impairment and recovery within one week. Left right ratios in B, D, F and H (right column) fail to indicate unilateral deficits in contrast to the neuroscore (cf. Fig. 2 B). Significance levels: *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 (Dunnett's multiple comparison, Sidak's multiple comparison).

Table 3
Correlation of baseline-normalized contralateral forelimb (cFL) neuroscore of photothrombosis group and photothrombosis group's significantly differing baseline-normalized parameters.

	Run average speed vs. neuroscore	Stride length cFL vs. neuroscore	Stand cFL vs. neuroscore	Swing speed cFL vs. neuroscore	Step cycle cFL vs. neuroscore
Spearman's R	09,485	08,822	−0,8454	07,231	−0,6878
P value	< 00,001	< 00,001	< 00,001	00,021	00,040
95% confidence interval of R	0,8501−0,9829	0,6782−0,9600	−0,9468 - -05,914	0,3402−0,9003	−0,8861 - -02,765

preoperative measurement and day 11 with respect to stand, step cycle, stride length, and run average speed (Table 2). In addition, run average speed was also significantly different on day 18 and 21, base of support on day 9 and 11−21.

4. Discussion

For long time CatWalk has been a critically discussed issue in preclinical stroke-studies, especially with mice. To our knowledge we here present for the first time the limits of CatWalk as a tool to investigate functional deficits in mice after photothrombosis.

By comparison of the CatWalk and a composite neuroscore we found out that the neuroscore was able to specify an asymmetrical deficit of the contralateral forelimb (Fig. 2 A) until d16 after photothrombosis (Table 2, Fig. 2 B) while the CatWalk only showed symmetrical differences in speed-dependent parameters (*Stand, Swing Speed, Step Cycle and Stride Length*) present exclusively during the first week after stroke (cf. Fig. 2 C, D, Fig. 3).

The neuroscore implemented functional tests specifically address asymmetries of motor performance, especially in testing righting and placement reflexes. Given the fact the CatWalk gave "objective", rated independent results, a strong correlation with the observer dependent neuroscore results with CatWalk parameters suggested high validity and reliability of our neuroscore data (Table 3). Multiple analysing of walking parameters and individual assessment of each paw promise to reveal motor asymmetries that result in any kind of limping gait. However, CatWalk did not detect gait asymmetry, although unilateral cortical lesioning with photothrombosis was present. One possible explanation for this might be the existence of central pattern generators (CPG), i.e., neuronal networks located in the spinal cord, which regulate rhythmical movements such as gait, flying, swimming, or respiration [14,26]. In cats, rats, and humans with complete spinal cord injury, it has been shown that locomotion related movements can be generated by electrical, epidural spinal cord stimulation [27]. Studies with transgenic mice showed that left-right-integration of locomotion ultimately depends on the presence of commissural V0-interneurons [28]. Thus, data are compatible with the notion that autonomous CPG partly compensate for the contralateral front paw's deficits during gait by adjusting motor efferences. Further research into this issue is warranted.

The missing detection of hemisyndroms by the CatWalk after photothrombosis has been shown in rats before, where 48 h after ischemia a lateralized deficit was seen in the Cylinder-Test but not in CatWalk data [16]. So far only larger ischemic lesions of the brain, such as occlusion of mice's middle cerebral artery (MCAo), lead to measurable asymmetric contralateral forelimb dysfunction [12,23]. The corresponding parameters, however, were insignificant in our set-up. Other behavioural experiments after photothrombosis in mice as the Rotarod-Test [6], the Pellet-Reaching-Task [24] and the Grid-Walk-Test [25] showed motor deficits of the contralateral forelimb equivalent to our neuroscore results. In our Cat Walk data we furthermore reproduced strong correlation of speed-dependant parameters with Run Average Speed (e.g. at d0 up to d4, see Fig. 2 C, D and Fig. 3A, C, E, G) [18]. Weight changes may influence the results of gait analysis by influencing velocity and by effects of shifting body weight. However, as compared to MCAO, weight changes are minor after photothrombosis, and assessment of motor function of suspended animals makes the neuroscore robustly independent from body weight effects. A limitation of our study are relatively low n-numbers, thus further research will be needed. Hereby SRRR-guidelines (stroke recovery and rehabilitation roundtable), that had not been established yet when our data was collected, should be considered carefully [29,30]. The SRRR translational working group propose for mice with photothrombotic stroke amongst others the foot fault test, which in case of using the composite neuroscore should be included in future studies. The CatWalk, like suggested in the present study, does not play a major role in SRRR-guidelines.

In conclusion, out data suggest that the neuroscore is more suitable than the CatWalk for the evaluation of neurofunction after a photothrombotic lesion of the motor cortex in mice. It allows specifying and quantifying dysfunctional hemisyndromes for a longer period than CatWalk, suggesting higher sensitivity and specificity. Although the neuroscore is a user-dependent method, our data suggest high reliability and validity. Furthermore, the neuroscore is easier to perform and less time-consuming. If CatWalk analysis is carried out, however, we suggest that the detection of average speed is sufficient for collecting data on functional deficits.

The outcome of the present study will help to refine strategies to evaluate motor deficits after cortical lesioning in mice and may therefore in the long run reduce animal numbers necessary to evaluate therapeutic effects in preclinical CNS research.

Credit author statement

Bärmann J: Conception and design of study, acquisition of data, analysis and/or interpretation of data, Drafting the manuscript, Approval of the version of the manuscript to be published. **Walter HL:** Conception and design of study, analysis and/or interpretation of data, Drafting the manuscript, revising the manuscript critically for important intellectual content, Approval of the version of the manuscript to be published. **Pikhovych A:** Conception and design of study, acquisition of data, Approval of the version of the manuscript to be published. **Schroeter M:** Conception and design of study, acquisition of data, analysis and/or interpretation of data, Drafting the manuscript, revising the manuscript critically for important intellectual content, Approval of the version of the manuscript to be published. **Endepols H:** analysis and/or interpretation of data, Approval of the version of the manuscript to be published. **Fink GR:** revising the manuscript critically for important intellectual content, Approval of the version of the manuscript to be published. **Rueger MA:** Drafting the manuscript, revising the manuscript critically for important intellectual content, Approval of the version of the manuscript to be published

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