Measuring pain-induced gait adaptation: the CatWalk method

A.F. Gabriel, W.M.M. Honig, M.A.E. Marcus, and E.A.J. Joosten
Pain Management and research Center, Dept.of Anesthesiology, AZM, Maastricht, The Netherlands, a.gabriel@np.unimaas.nl

The Catwalk method is an automated quantitative gait analysis that allows the objective and rapid quantification of individual paw parameters as well as parameters related to interlimb coordination. Briefly, light from a fluorescent tube is sent through a glass plate. Light rays are completely reflected internally. As soon as anything, e.g. a rat's paw, is in contact with the glass surface, light is reflected downwards. It results in a sharp image of a bright paw print. Rats are trained in order to cross the pathway without hesitation at a constant speed. The complete run is recorded via a camera placed under the glass plate. Analysis of all gait parameters is performed through the use of an appropriate software.

The computer-assisted method of locomotor analysis was previously developed to assess motor changes on spinal cord injured rodents(Koopmans et al. 2005). However, based on the correlation of Catwalk parameters with the development of mechanical allodynia as assessed with the von Frey test (Vrinten and Hamers 2003), the present study aims at demonstrating that the CatWalk method allows an automated and complete measurement of gait related changes in a model of inflammatory pain; in other words, a pain-induced gait adaptation. In order to compare the behavioral outcomes from the CatWalk to a golden standard test, the von Frey test was used to assess the development of mechanical allodynia following the CAR-injection. Both test were performed first in the acute phase (0 – 48h post injection) and in the chronic phase (1 – 4 weeks) of the carrageenan model.

Carrageenan (CAR) is an irritant that induces an inflammatory response on injection into tissues. Injected into the rat knee, it results in oedema, pain and hyperalgesia of the injected limb. CAR-induced inflammation has been described as a model of acute inflammation through the first 24 h post injection and as a model of chronic inflammation after 1 week. In this study, both acute and chronic phases were studied.

Prior to injection ($t = -1$ day), CatWalk and von Frey tests were performed in order to determine the baseline. The experimental group was injected intra-articularly, at $t = 0$, with 2 mg of λ-carrageenan (200 µl) into the knee joint of the right hind paw. The control-group was injected intra-articularly with a saline solution (200 µl) into the same joint.

In the acute phase after injection of the CAR, von Frey test of both ipsilateral and contralateral hindpaws and CatWalk analysis were performed at $t = 2.5$, 4, 24 and 48 h post-injection. The CatWalk analysis of individual paw parameters like the intensity of the paw print or the time contact with the floor showed a significant effect after CAR injection into the knee. These CatWalk parameters were highly correlated with von Frey data. Furthermore, detailed CatWalk analysis of the gait (i.e. coordinated interaction between left and right hindlimb) showed a fully coordinated locomotion following the CAR injection, implicating that the accuracy of the rat gait is not affected. From these results we concluded that the CatWalk method allows an objective and detailed detection of a pain-induced gait adaptation, correlated to the development of mechanical allodynia in the acute phase of a CAR-induced knee inflammatory pain model.

Further validation of the CatWalk technique in measuring pain related changes was obtained with the use of a standard analgesic drug, Fentanyl. Twenty four hours after the CAR-injection, rats were subcutaneously injected with Fentanyl (25µg/kg). Control rats were injected with saline solution. CatWalk and von Frey tests were performed prior to experiment, prior to Fentanyl injection and at 20, 45 and 120 minutes post-Fentanyl injection.

Von Frey results showed a significant recovery of 88% from the carrageenan-induced mechanical allodynia at 20 minutes post-Fentanyl injection. CatWalk analysis (individual paw- and coordination-related parameters) showed a significant recovery (from 50 to 82%) of the carrageenan-induced gait changes at 20 and 45 minutes post-Fentanyl injection.

If compared to the von Frey results, CatWalk analysis allows a measurement of the analgesic effect of Fentanyl in an acute inflammatory pain model which is more sensitive (less variation and longer detection of the analgesic effect), more objective (fully computerized and therefore independent of the experimenter) and more reproducible (less variability between results and thus less animals needed). Herewith, we validated the use of the CatWalk in detection of pain related gait changes. Our results strongly favor the use of the CatWalk as a new tool for preclinical assessment of analgesic drugs.

Finally, the use of the Catwalk technique in the chronic phase of the CAR-induced inflammation model was studied. Von Frey test of both ipsilateral and contralateral hindpaws and CatWalk analysis were performed at $t = 1$, 7, 14, 21 and 28 days post-injection. Von Frey results showed a significant development of mechanical allodynia 4 hours after the injection of CAR in the rat knee, and a plateau like-phase was observed up to 21 days. CatWalk parameters related to individual paw (e.g. intensity of the paw printing) showed significant changes after 24 hours, identical to those reported in the acute phase. From 7 days post-injection on, no changes could be noted in any of the CatWalk parameters. For instance, a significant reduction in the duty factor of the paw print could be measured at day post-operation 1 (DPO1) in rats from the experimental group but changes do no longer exist at DPO7. Locomotion can be considered as a daily life activity, i.e. it is a voluntarily initiated movement, repeated several times a day. It is therefore likely to explain our results in the context of habituation. Daily use of the painful limb can lead to habituation which further results in a subsequent attenuation of the behavioral gait adaptation.

In conclusion, the use of the CatWalk as a method to assess a pain-induced gait adaptation in correlation with mechanical allodynia needs to be carefully restricted to identified experimental pain models. However, when appropriated, the CatWalk technique is a powerful tool that allows an objective and sensitive measurement of the pain-induced gait adaptation.

References

1. Koopmans GC, Deumens R, Honig WM, Hamers FP, Steinbusch HW, Joosten EA. 2005. The assessment of locomotor function in spinal cord injured rats: the importance of objective analysis of coordination. *J Neurotrauma* **22(2)**:214-225.
2. Vrinten DH, Hamers FF. 2003. 'CatWalk' automated quantitative gait analysis as a novel method to assess mechanical allodynia in the rat; a comparison with von Frey testing. *Pain* **102**(1-2):203-209.