Automated Detection of Aberrant Behaviour of Mice on the Rotarod: Use of EthoVision® XT

M. Mahieu[1], R. Willems[1], L. Hoekstra[2], L. Ver Donck[1]

[1]*Dept Neuroscience, Janssen Research and Development, a Division of Janssen Pharmaceutica NV, Beerse, Belgium.*
mmahieu@its.jnj.com

[2]*Noldus Information Technology, Wageningen, The Netherlands*

Introduction

Impaired motor coordination in man due to medication can have an important impact on private life and job performance. Antipsychotic drugs in particular are known to induce extrapyramidal motoric symptoms, causing disturbed coordination of body movements. The rotarod test is widely used to evaluate drug effects on motor coordination in rodents. The principle of this test is that rats or mice are first trained to walk on a rod rotating at a certain speed. Once the animals have learned this, the effect of a test-compound on their motor performance is evaluated. Animals experiencing impaired motor coordination are unable to cope with the rotating rod and will drop off when the rotation speed exceeds their motor coordination capacity (see Figure 1). The more disturbed the animals are, the sooner they fall off the rod. However, mice show a particular coping behaviour when experiencing motor coordination problems on the rotarod: to prevent dropping off, mice can grip themselves to the rod and turn around without falling off (see Figure 2).

This behaviour then results in late (or no) falling off the rotarod, which would incorrectly indicate that the compound tested, did not disturb motor coordination. The number of times mice turn around on the rotarod therefore represents a secondary measure of disturbed motor coordination. This turnaround behaviour can be determined by visual observation, but this is challenging when 5 mice are tested simultaneously on the rotarod.

The objective of this study therefore is to automate the determination of this turnaround behaviour using video recording of the experiment in combination with image analysis software.

Methods

Male NMRI-mice (body weight 22-24 g, Charles River) were habituated to the environment for 1 week. They were first trained to walk on the rotarod (Med Associates) at constant rotation speeds in 5 min long sessions at

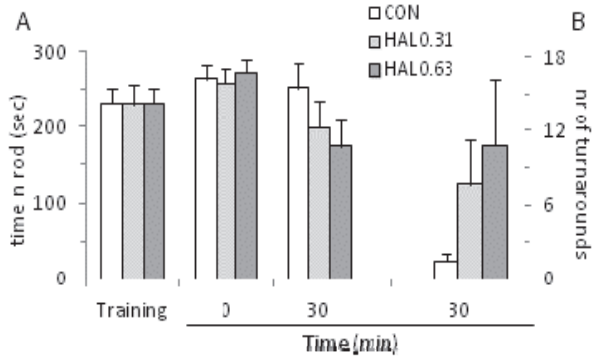

Figure 1. A: Effect of haloperidol (HAL, 0.31 or 0.63 mg/kg) on motor performance of mice on the rotarod (time spent on the rotarod); Time (min) is time after drug administration. B: number of times a mouse turns around on the rotarod. While HAL has only a very small effect on time on rod (A), the mice show a strong increase in number of turnarounds.

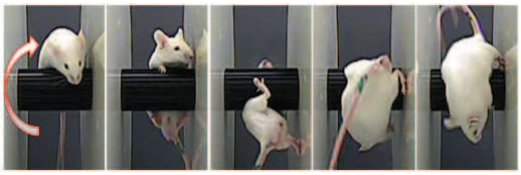

Figure 2. Time sequence (left to right) of a mouse showing coping behaviour on the rotarod by gripping itself to the rod and making a turnaround (front view). The arrow indicates the direction of rotation of the rod.

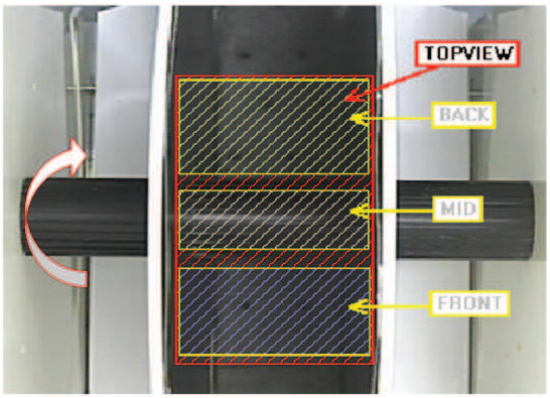

Figure 3. Top view image of one lane of the rotarod. Overlays show the area of interest (red) and the 3 distinct individual zones (yellow) covering the rod (mid) and the front and back zones as determined in EthoVison® XT. The width of the red area was set to the width of one lane of the rotarod and the length was set to 3.7 times the diameter of the rod. The arrow indicates turning direction of the rod.

16, 20 and 24 rotations per minute (rpm) at 30 min intervals; mice were placed back on the rod each time they fell off, until the 5 min session was completed. Then a session was performed with the rotarod rotating at an incremental speed starting at 4 rpm and accelerating over a 5 min period up to 40 rpm. The next day, a dose of the test-compound was administered, and mice were tested at 30 min intervals in 5 min sessions at accelerating speed. The time at which the mice fell off the rotarod was recorded, and the number of turnarounds was counted by visual observation.

To evaluate automated determination of turnaround behaviour, a high resolution video camera (Samsung SDC-435) was positioned 45 cm above the rod to record the session. EthoVision® XT (Noldus Information Technology, Wageningen, The Netherlands) was used to analyse the video images. A region of interest area was identified around the rod and this area was divided in 3 individual zones: the rod in the middle and the front and back area's just outside of the rod (Figure 3). The background was covered with black material such that a white mouse could be readily determined by EthoVision® XT as an object. The surface area and the center of gravity (CG) of the object were determined, as well as the occurrence of the latter in one of the zones. Also the sequential transition of the CG from middle to back to front and to middle again was determined: this sequence of events indicates a turnaround manoeuvre of the mouse. The Institutional Ethical Committee on Animal Experimentation approved the experimental protocol, in compliance with Belgian law (Royal Decree on the protection of laboratory animals dd. April 6, 2010) and the facilities are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

Results

Figure 4A shows the surface area of the object (mouse) detected in the area of interest (cf. red area in figure 3). The downward peaks represent phases during which the surface area detected suddenly decreases: this is when the mouse makes a turnaround (marked by red squares) and thus disappears for a few moments behind the rod.

However, another example in figure 4B shows that the downward peaks do not always accurately represent a turnaround manoeuvre. Surface area tracking is therefore not an appropriate measure to determine turnaround behaviour.

The zone-transition option in EthoVision® XT determines when the CG of the object detected displaces from one zone to another. When the mouse turns around on the rod, the position of its CG should change from the front to the mid zone (i.e. the mouse can only appear in the front zone and then in the mid zone when it makes a complete turnaround). This routine indeed accurately detected 12 and 7 turnarounds in figures 4 A and B

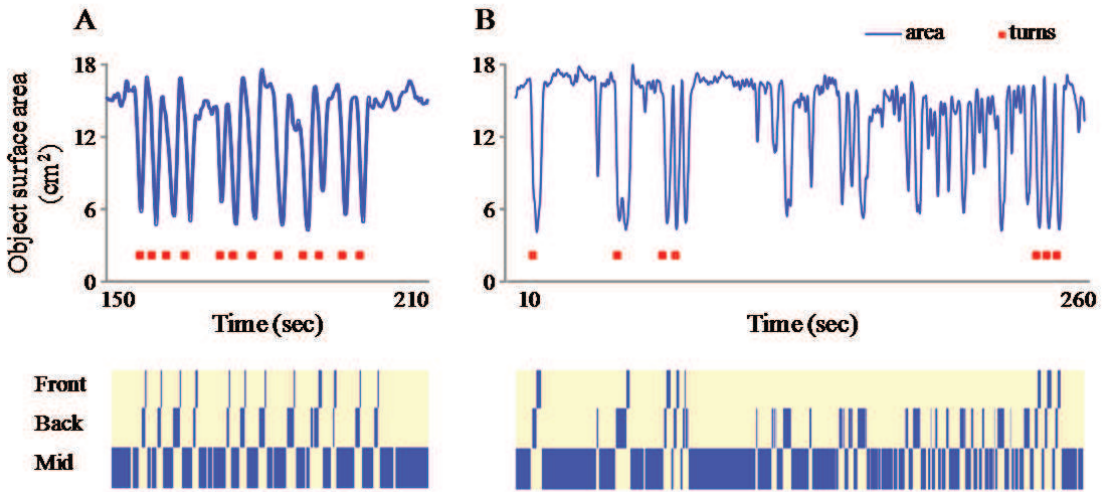

Figure 4. Top panels: examples of surface area tracking from 2 different mice treated with haloperidol; A: Sudden decreases of surface area detected by EthoVision® XT (downward peaks) correlate with visual observation of the mouse making turnarounds (red marks). B: Downward peaks recorded from this mouse do not correlate very well with turnarounds observed. Bottom panels are heat maps of occurrence of the CG of the object detected in the mid, back or front zone (blue marks). A sequential occurrence of the CG in the mid, back, front and mid zone again is consistent with visually observed turnarounds made by the mouse.

respectively. However, a limitation of this approach is that the exact timing of when these occur during the course of the experiment is not indicated.

A better option therefore is to determine the sequential transition of the CG from mid to back to front to mid again. Output from EthoVision® XT reporting the presence or absence (i.e. 1 or 0) of the CG in each of the 3 zones during a session was exported to Excel. Conditional formatting was then used to generate a heat map, colouring the field blue when the CG was detected in a zone (see bottom panels in figure 4). The horizontal axis represents the time dimension as in the graph above. A sequential appearance of the CG changing from the mid to the back to front and to the mid zone again is detected by an algorithm and reports this sequence as a turnaround. This procedure accurately detected the turnarounds as visually observed.

Conclusion

Monitoring the time that mice stay on the rotarod is not sufficient to determine impaired motor coordination in this species. EthoVision® XT can be readily used to automatically determine turnaround behaviour of mice on the rotarod. This measure is a critical parameter to assess disturbance of motor coordination in mice.