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Computer assisted video analysis of swimming performance in a forced swim test: Simultaneous assessment of duration of immobility and swimming style in mice selected for high and low swim-stress induced analgesia

Grzegorz R. Juszczak [a], Paweł Lisowski [b], Adam T. Śliwa [a], Artur H. Swiergiel [a,c,*]

[a] Department of Animal Behavior, Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, 05-552 Wolka Kosowska, Poland
[b] Department of Molecular Biology, Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, 05-552 Wolka Kosowska, Poland
[c] Department of Animal Physiology, Institute of Biology, Gdansk University, Gdansk, Poland

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ABSTRACT

In behavioral pharmacology, two problems are encountered when quantifying animal behavior: 1) reproducibility of the results across laboratories, especially in the case of manual scoring of animal behavior; 2) presence of different behavioral idiosyncrasies, common in genetically different animals, that mask or mimic the effects of the experimental treatments. This study aimed to develop an automated method enabling simultaneous assessment of the duration of immobility in mice and the depth of body submersion during swimming by means of computer assisted video analysis system (EthoVision from Noldus). We tested and compared parameters of immobility based either on the speed of an object (animal) movement or based on the percentage change in the object's area between the consecutive video frames. We also examined the effects of an erosion–dilation filtering procedure on the results obtained with both parameters of immobility. Finally, we proposed an automated method enabling assessment of depth of body submersion that reflects swimming performance. It was found that both parameters of immobility were sensitive to the effect of an antidepressant, desipramine, and that they yielded similar results when applied to mice that are good swimmers. The speed parameter was, however, more sensitive and more reliable because it depended less on random noise of the video image. Also, it was established that applying the erosion–dilation filtering procedure increased the reliability of both parameters of immobility. In case of mice that were poor swimmers, the assessed duration of immobility differed depending on a chosen parameter, thus resulting in the presence or lack of differences between two lines of mice that differed in swimming performance. These results substantiate the need for assessing swimming performance when the duration of immobility in the FST is compared in lines that differ in their swimming "styles". Testing swimming performance can also be important in the studies investigating the effects of swim stress on other behavioral or physiological parameters because poor swimming abilities displayed by some lines can increase severity of swim stress, masking the between-line differences or the main treatment effects.

1. Introduction

The forced swim test (FST, Porsolt test) is both a test and a model of depression-like behavior developed for rats and mice by Porsolt [19,25]. It is based on the observation that an animal placed in an inescapable stressful situation develops an immobile posture after the initial escape-oriented movements. The duration of immobility (passive floating) has been inferred as an index of "behavioral despair", where the longer durations of immobility imply a greater degree of depression-like behavior [25]. The FST was originally designed as a screening method for antidepressants, but it is also used in studies investigating genetic mechanism underlying depression-like behaviors in rodents [8,11,23,27].

There are two serious issues encountered in behavioral psychopharmacology when quantifying animal behavior, including the FST. The first is the inaccuracy or inconsistency of subjective scoring of animal behavior and the lack of reproducibility of the results across laboratories, especially in the case of manual scoring because researchers, of necessity, apply different working definitions to the scored behaviors [3]. The second issue involves different behavioral idiosyncrasies, common in genetically different animals, that can mask or mimic the effects of experimental treatments or mutations [33]. Therefore, chances of obtaining meaningful results are maximized if behavioral deficits are identified and differentiated by

* Corresponding author. Department of Pharmacology, Toxicology and Neuroscience Louisiana State University Health Sciences Center, P.O. Box 33932, Shreveport, LA 71130-3932, USA. Tel.: +1 318 868 9923; fax: +1 318 675 7857.
E-mail address: swiergiel@yahoo.com (A.H. Swiergiel).

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combining complementary behavioral protocols [4]. These problems are especially relevant in the case of the FST. This test is almost exclusively scored manually and, despite its apparent simplicity, there is high variability in the scores of the duration of floating or swimming between individual observers [5]. To date, there have been only a few attempts to score automatically the FST behavior using video analysis [5,12,29], detection of vibrations of a cylinder with water [32], variations of water electromagnetic field [7], induction of electric currents by magnets attached to the mouse paws [31], or interruption of the photo-beam arrays [18]. None of these methods has been widely applied except for commercially available video tracking system designed by Viewpoint S.A. (Champagne au Mot d'Or, France) [6,26,28,30]. However, in the published reports that applied the Viewpoint system there was no description of parameters used to detect the immobility [6,26,28,30]. Despite making an important contribution to the development of the video analysis of depression-like behavior, the two earlier studies by Sanchez and Meier [29] and Hedou et al. [12] used parameters that had some disadvantages limiting widespread usage of the described methods (for a detailed discussion see Juszczak et al. [14]).

Despite the fact that the role of motor performance is well recognized in behavioral tests [4], swimming deficits have not attracted much attention in experiments employing the FST. Since the parameters scored during the FST are the duration of floating and/or swimming behaviors, swimming performance is an important factor that can affect conclusions drawn from the results of the FST. It has been shown that genetically modified mice can display altered swimming patterns such as swimming in a vertical position. This behavioral pattern is associated with inability to stay immobile because of a risk of drowning [15]. Other swimming deficits range from partial impairment [2] to total inability to swim [10]. Performing the FST in such genetically and behaviorally different animals, valuable as they are to determine the genetic basis of depression, is either very difficult or impossible. The significance of the swimming patterns or swimming abilities in comparisons of genetically modified mice tested in the FST has recently been suggested by Kalueff and Tuohimaa [16] but the lack of interest in the swimming performance of rodents in the FST can be due to lack of easy and objective methods of assessing swimming abilities. Few studies that investigated swimming pattern employed subjective visual assessment of swimming or time consuming manual measurement of angle between water surface and body of the mouse [9,15].

The aim of this study was to develop an automated method enabling simultaneous assessment of the duration of immobility and the depth of body submersion during swimming by means of computer assisted video analysis system (EthoVision from Noldus).

2. Methods

2.1. Animals

The subjects belonged to the 65th generation of albino Swiss–Webster mice that have been selectively bred in our laboratory for high (HA line) and low (LA line) magnitudes of swim-stress induced analgesia (SSIA) as described earlier [21]. The lines differ in a number of physiological and behavioral parameters [1,13,14,17,22]. Animals were males, 5 months old and weighed 40.4 ± 1.0 g and 40.7 ± 0.7 g (HA and LA lines, respectively). Experimental groups counted from 10 to 11 animals. Mice were housed four to six per cage on a 12-h/12-h light/dark cycle with unlimited access to food and water. All experiments were performed by permission of the Animal Research Ethical Committee.

2.2. Forced swim test (FST)

The forced swim test was performed according to the method of Porsolt et al. [24,25]. The apparatus was a Plexiglas® box tank (30 cm high, 15 cm wide and 14.5 cm deep) filled with water. A box tank was used, instead of a more usual cylinder, because filming across a cylinder filled with water, and distorting an image, was very difficult. The tank was placed inside an enclosure (128 × 65 × 100 cm) painted inside matte black that, in turn, was housed in a dimly lit observation room. The enclosure assured a high contrast between the black background and a white mouse and prevented the Plexiglas® box from mirroring the image of the room. Water was maintained at 32 °C and at a depth of 19 cm. Warm water, instead of the usually used 20–25 °C, was used because HA mice develop hypothermia at low water temperatures. A monochromatic video camera (AV Tech Corporation) was placed between the tank (19 cm from the tank) and one of the enclosure walls. The air bubbles were brushed off the walls of the tank filled with water. Setting the correct lighting conditions is important with automated analysis of the forced swim using video analysis system. In case of problems, that is when an animal is not recognized correctly one can: a) shield the Plexiglas® box from direct source of light; b) decrease intensity of illumination; c) change settings of contrast and brightness in video analysis system; d) change settings of low and high threshold limits in video analysis system; e) set minimal object size to remove reflections. The camera was connected to a PC computer with a Picolo® Frame grabber and a video recorder. Video images were analyzed using EthoVision 3.1 video analysis system (Noldus Information Technology, Wageningen, The Netherlands).

2.3. Automated video analysis using EthoVision

2.3.1. Swimming performance

A mouse was observed and tracked within a virtually delineated frame, called “arena”. The arena started just below the surface of the water and was divided into bottom (5.5 cm deep) and top (surface) zones (3 cm deep) (Fig. 1). Initial depths of the zones were based on the animals' dimensions. Also, it was assumed that mice that swim well maintain horizontal position. To differentiate between the good and the bad swimmers, the top zone had to be set up as shallow as possible but it had to encompass mathematically defined centers of the images of the animals swimming in horizontal position. Those centers of the tracked object images, called gravity points, were continuously being determined. Depending on location of the gravity point within the arena, the position of an animal within the arena was attributed to one of the two zones by the EthoVision software. Swimming performance was defined to be the duration of time spent by the animal in the top zone. The longer the time that EthoVision system attributes to the position of the animal in the top zone, the better its swimming abilities. We also reanalyzed tracks recorded by EthoVision system (gravity point positions stored in the system memory) using different depths of the top zone, to prepare the profiles of swimming performance for the two mouse lines.

2.3.2. Immobility detection

Immobility was defined as the duration of time when the velocity of mouse movement (shifting of the gravity point) decreased below 2 cm/s (parameter developed and validated by Crowley et al. [5]). Duration of immobility, based on speed parameter, was calculated by means of the “movement detection” feature of the EthoVision system (term used in the EthoVision manual). The percentage change in an object (animal) image area was based on comparisons of the locations of pixels, belonging to the tracked animal image, between the consecutive images — the comparisons allowed calculating the percentage of overlap of the object's images between two consecutive video frames [14]. For example, an animal shifting so that there is no overlap of its two consecutive images equates to 100% change, while the animal staying still so that there is a perfect overlap of two consecutive images equates to 0% change. Duration of immobility based on area change parameter was calculated by means of the