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The usage of video analysis system for detection of immobility in the tail suspension test in mice

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Abstract

The Tail Suspension Test (TST) is a commonly used screening method for antidepressants properties of drugs in mice. To date, immobility in the TST was scored live, by an observer, or automatically, using devices in which mouse movements were detected by a strain gauge. In this study we tested whether the EthoVision video analysis system can be used reliably and accurately for automatic recording and scoring of duration of immobility in the TST. First, the duration of immobility in two mouse lines was assessed. Different mobility thresholds of the video analysis system were applied and the results compared with the duration of immobility scored manually. Next, the selected immobility threshold was applied to determine the dose-response curves for the drug venlafaxine. Finally, scores from the video analysis system were compared with scores generated by an electromechanical strain gauge device (Med Associates) and a human rater. It was found that the EthoVision system could reliably and accurately quantify the duration of immobility in the TST. The best setup was an immobility threshold ranging from 2 to 3 percentage change in the object area. The EthoVision system was effective in detecting the differences between the mouse lines and the dose response to venlafaxine. The results obtained using the video analysis system were similar to the scores yielded by a human rater and the strain gauge device.

Keywords: Video analysis; EthoVision; Tail suspension test; Immobility; Depression; Mice

1. Introduction

The Tail Suspension Test (TST) is a commonly used screening method for antidepressant properties of drugs in mice. The method is based on the observation that if a mouse is placed in a stressful situation, such as suspension by the tail, from which it cannot escape, the mouse develops an immobile posture after initial escape-oriented movements (Steru et al., 1985). The duration of immobility has been inferred as an index of "behavioral despair", where longer durations of immobility imply a greater degree of behavioral despair. An ethological perspective construes the TST as a measure of coping or adaptation, reflecting an individual's strategic response when facing a problem of survival without solution (Thierry et al., 1984). It has been shown that the duration of immobility in the TST decreases in response to many antidepressants that are used clinically, including tricyclics, selective serotonin reuptake inhibitors, monoamine oxidase inhibitors, atypical antidepressants, and electroconvulsive therapy (Steru et al., 1985, 1987; Perrault et al., 1992; Teste et al., 1990, 1993). The TST has also been used to demonstrate depression-like effects of bacterial endotoxin and interleukin-1 in mice (Dunn and Swiergiel, 2005). What is more, it has been applied to study genetic manipulations that are relevant to depression and antidepressant action (Vaugeois et al., 1996; Liu and Gershenfeld, 2001; El Yacoubi et al., 2003; Cryan et al., 2005; Crowley et al., 2006; Swiergiel and Dunn, 2006).

Since 1985 when Steru et al. introduced the TST, immobility of the subject has been quantified either manually by a trained observer during direct observation (subjective scoring) or

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automatically using devices which utilize a strain gauge to detect the movements of the subject (objective scoring) (Cryan et al., 2005). Several of these automated electromechanical strain gauge devices have been designed, the first of which was the Itematic TST from Item-Labo, which was validated by Steru et al. (1987). Now, several companies (Med Associates Inc., Georgia, VT, USA; Hamilton-Kinder, Poway, CA, USA; Neuroscience, Tokyo, Japan) offer similar systems (Crowley et al., 2004). There is also at least one specialized video analysis system (TailSuspScan, Clever Sys. Inc.) designed to analyze the behavior of mice in the TST. However, to the best of our knowledge there is no published report of the usage of a video analysis system for the detection of immobility of mice in the TST. No such paper could be found in the PubMed electronic database of biomedical literature, and no study using a video analysis system was listed in a recently published extensive review of pharmacological and genetic studies exploiting the tail suspension test (Cryan et al., 2005). To date, video analysis systems have been only occasionally applied to analyze behavior in the forced swim test (FST), which is another simple test for depression-like behavior based on immobility (passive floating) response. In this test, the inescapable aversive stimulus is provided by placing a mouse in a cylinder filled with water, from which there is no escape. The first to utilize an image analysis system (GIPS, Image House, Copenhagen) to detect immobility in the FST were Sanchez and Meier (1997). More recently, Crowley et al. (2004) successfully used The SMART II Video Tracking System from San Diego Instruments to automatically record and score the duration of immobility in the FST. There is also one report in which EthoVision version 1.90 was used to analyze behavior in the FST (Hedou et al., 2001), but in this study the video analysis system was not used to detect and score immobility directly. Hedou et al. (2001) employed EthoVision's tracking feature, which records the path of animal movement for further analysis of spatiotemporal measures, to measure the total distance covered by a rat in a cylinder during the test. The research group found that the total distance traveled was negatively correlated with the duration of immobility when scored manually, and they concluded that to assess any antidepressant activity in the FST the distance covered by an animal could be used instead of immobility.

The objective of this study was to test and validate the newly developed motion detection feature of the commercially available EthoVision video analysis program (Noldus Information Technologies, Wageningen, The Netherlands), as a method of immobility quantification in the TST. First, we assessed duration of immobility in two lines of mice that differ in depression-like behavior (Panocka et al., 2001; Juszczak et al., 2005). To determine the parameters that yield the most accurate results we used different the mobility threshold of the Noldus video analysis system and compared the obtained results with a trial where the duration of immobility was scored manually. Next, we used the selected immobility threshold to examine the dose-response curves for the antidepressant drug venlafaxine. Finally, we compared scores obtained from the video analysis system with scores from a strain gauge device (Med Associates) and manual scores from a trained observer.

2. Methods

2.1. Animals

The subjects used in this experiment belonged to the 62nd and 63rd generation of albino Swiss–Webster mice that were selectively bred in our laboratory for high (HA line) and low (LA line) magnitude of swim stress-induced analgesia (SSIA) as described earlier (Panocka et al., 1986). Briefly, mice of a parental stock were given a 3-min swim in 20 °C water and were immediately tested for pain sensitivity on a hot plate (56 °C). Pairs of males and females both displaying long or short post swim nociceptive latencies were mated to initiate the HA and LA lines, respectively. The same procedure was repeated in consecutive offspring generations. The lines are relevant to depression studies because they differ in basal performance in the FST and the TST and also the responses to desipramine in the FST and the TST (Panocka et al., 2001; Juszczak et al., 2005). The first experiment that was used to calibrate the video analysis system was performed using 12 males from the HA line (age: 5 months; weight 44.3±0.9 g) and 12 males from the LA line (age: 5 months; weight 44.4±1.8 g). The second experiment, in which the dose response to venlafaxine was tested, was performed using 48 mice from the HA line (age: 3 months; weight 39.6±0.6 g) and 46 mice from the LA line (age: 3 months; weight 38.5±0.5 g). All experimental groups were made up of 12 mice except for one which consisted of 10 mice (LA mice injected with 30 mg/kg of venlafaxine). The third experiment, where the video analysis system was compared with a strain gauge device from Med Associates, was performed on 12 mice from the HA line (age: 5 months; weight 40.3±0.7 g) and 12 mice from the LA line (age: 5 months; weight 43.3±0.5 g). All of the mice used during this experiment 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 in accordance with the guidelines and by permission of the Animal Research Ethical Committee.

2.2. Tail Suspension Test (TST)

The apparatus used in the first and second experiment was a wooden box measuring 680 (High)×365 (Wide)×280 mm (Deep), the interior of which was painted matte black to ensure a high contrast between the background and a white mouse. The apparatus was housed in a dimly lit room, and after removing the box's front wall a video camera was positioned there. A large swivel (6.5 cm in diameter and 1.5 cm long) was attached to the interior side of the box's top, and a fabric ribbon (135×17×1 mm) was attached to the bottom of the swivel. The swivel allowed for the correction of the mouse's position toward the camera so the behavior of each animal was recorded and scored in similar conditions. A mouse was suspended by its tail by placing a piece of adhesive tape 30 mm from the beginning of the tail and attaching the tape to the ribbon. After suspending the mouse its orientation was corrected by turning the swivel so that its ventral side was facing the camera. While suspended, the mouse was 120 mm away from the nearest surface and 300 mm from the camera lens. The third experiment was performed using

an automated TST system from Med Associates that was adapted to allow for video analysis of the subject during the test. The original device consisted of a box-like enclosure (32 × 33 × 33 cm) that was open on the front side. A vertical wire hanger (75 × 2 mm) with a hook at the bottom was connected to the strain gauge. In order to make conditions similar to those in the first and second experiment, we attached a fabric ribbon (135 × 17 × 1 mm) to the hook. Because the original enclosure supplied by Med Associates was not high enough to allow us to suspend a mouse from the ribbon, we removed the ceiling that the strain gauge was attached to and build a taller enclosure that was 610 (High) × 305 (Wide) × 305 mm (Deep). Finally, we lined the enclosure with matte black paper and positioned it in place of the apparatus that was used in the first video analysis experiment. The mice were suspended so that their ventral side was in front of the camera.

The behavior was analyzed during a 6 min period of time, beginning with the suspension of the mouse. In the case of manual scoring immobility was defined as the time when a mouse was hanging passively without moving its paws.

2.3. Experimental procedure

Two days before the experiment the animals were transferred from the colony room to the testing room for habituation. During the first experiment the mice were tested without any treatment. During the second experiment the mice were injected intraperitoneally with venlafaxine hydrochloride (received from Dr. J. Barrett, Wyeth-Ayerst, Brinston, NJ, USA) at a dose of 7.5, 15 and 30 mg/kg in a volume of 0.1 ml/mouse, or with an equal volume of saline 30 min before test. Testing was performed between 0900 and 1300 during the light part (0700–1900 h) of the day–night cycle. The animals' behavior during the TST was videotaped using a monochromatic AV204 board video camera (AV Tech Corporation) connected to a LG video tape recorder model LV3798. The duration of immobility for each mouse was manually scored from videotape playback using The Observer 3.0 software (Noldus) by a person who was blind to any of the treatments the mice received. Each mouse was scored twice and the results for each animal were averaged. For automatic analysis of the videotapes, a PC computer with a Picolo Frame grabber was used. Digitized video images were analyzed using EthoVision 3.1 video analysis system (Noldus).

2.4. Automated analysis

2.4.1. EthoVision video analysis system

The boundaries of the arena in which the mice subjected to the TST were recognized as targets for video analysis were defined as starting from the bottom tip of the fabric ribbon and encompassing the entire body of the animal (minus any part of the tail that was attached to the fabric ribbon) during both passive hanging and active escape-oriented behaviors. Immobility was defined as periods when the percentage change in object area between video frames was below a defined mobility threshold. EthoVision assessed mobility by comparing the locations of the pixels, which are identified as belonging to the tracked animal in the current sample frame with the pixels in the previous frame. The number of relocated pixels is then expressed as a percentage of change in the object's area. In the first experiment the video clips were consecutively analyzed using the following immobility thresholds: 1, 2, 2.5, 3, and 4 percent change in object area. The second and third experiments were analyzed using the 2.5 percentage threshold. For all analyses, the following settings were chosen in EthoVision: the gray scaling method for object detection, a small image resolution, a sample rate of 12.500 video frames/second, and an averaging interval of 1 s to smooth the mobility parameter. With the latter function the average mobility for each interval was calculated by summing the mobilities of all the samples in that interval and dividing the total by the number of specified samples. The parameter of interest in the TST, which was immobility, was then determined by the EthoVision software.

2.4.2. Med Associates TST system

The total duration of immobility was measured as the time when the force of the subject's movements, converted and expressed as voltage, was below a preset threshold. An optimum threshold was determined during a preliminary study. The following settings were used during the experiment: threshold = 9, gain = 16, resolution = 10 ms. After completing the experiment the raw data were also analyzed again using a slightly higher threshold (threshold = 10), which was used previously by Crowley et al. (2004).

2.5. Statistics

Data from the first experiment and third experiments were subjected to two-way analysis of variance (ANOVA), with the method of scoring and line of mice as main effects. Data from the second experiment (dose-response to venlafaxine) were subjected to three-way ANOVA with the line of mice, treatment and method of scoring as main effects. Detailed post hoc comparisons were made with Fisher's least significant difference (LSD) test. The correlations (Pearson Correlation Coefficients, r values) were determined between the results acquired automatically using different immobility thresholds and the results obtained during manual scoring. All data analysis was performed with Statistica software, release 5.1. Values are presented as mean ± SEM.

3. Results

3.1. First experiment (system calibration)

The two-way analysis of variance indicated that there was a significant effect of the scoring method ($F(5,132) = 9.48$, $p < 0.001$). Post hoc comparisons revealed that the duration of immobility recorded automatically in the HA line by the EthoVision system using a 1% immobility threshold differed significantly from the time of immobility scored manually ($p < 0.001$). There were also significant differences in duration of immobility in the LA mouse line between scores from automated analysis using a 4% immobility threshold and scores obtained using manual recording ($p < 0.05$, Fig. 1).

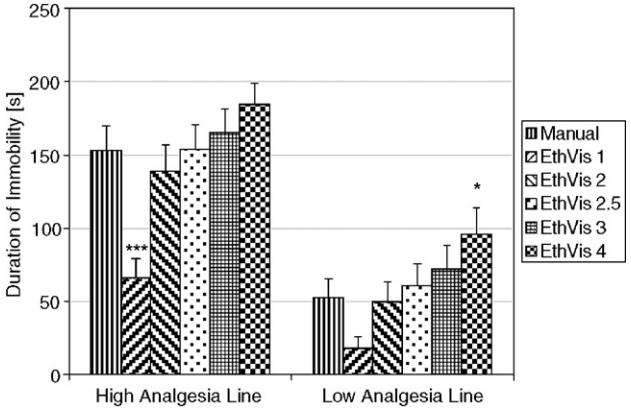

Fig. 1. Duration of immobility in TST in high (HA) and low (LA) analgesia mice. Manual — duration of immobility scored manually, EthVis 1 — automated analysis using 1% immobility threshold, EthVis 2 — automated analysis using 2% immobility threshold, EthVis 2.5 — automated analysis using 2.5% immobility threshold, EthVis 3 — automated analysis using 3% immobility threshold, EthVis 4 — automated analysis using 4% immobility threshold. *$p<0.05$, ***$p<0.001$ (compared with results for the same line during manual scoring). Values are mean±SEM.

However, regardless of the immobility threshold, all results obtained using the automated video analysis system were significantly correlated with results obtained by manual scoring. The highest correlation was achieved when we used 2.5 and 3% immobility thresholds (0.98, $p<0.001$). Correlation coefficients were smaller when the 2% (0.97, $p<0.001$), 4% (0.96, $p<0.001$) and 1% (0.91, $p<0.001$) immobility thresholds were used (Fig. 2).

Two-way analysis of variance also revealed a significant effect of the line of mice used in experiment: $F(1,132)=95$, $p<0.001$. Post hoc comparisons revealed that there were significant differences between the HA and LA line regardless of scoring method ($p<0.05$ for the EthoVision automated analysis with a 1% immobility threshold and $p<0.001$ for all other scoring methods; Fig. 1).

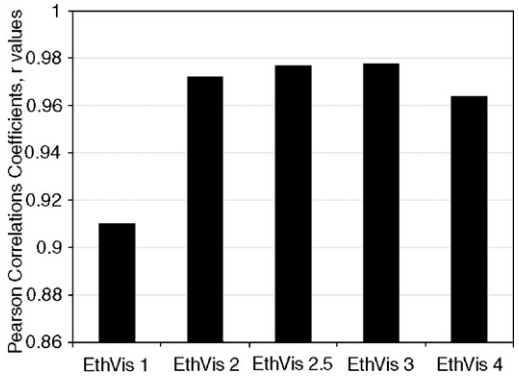

Fig. 2. Correlation between results acquired automatically using different immobility threshold and result obtained during manual scoring (Pearson Correlation Coefficients, r values). EthVis 1 — automated analysis using 1% immobility threshold, EthVis 2 — automated analysis using 2% immobility threshold, EthVis 2.5 — automated analysis using 2.5% immobility threshold, EthVis 3 — automated analysis using 3% immobility threshold, EthVis 4 — automated analysis using 4% immobility threshold.

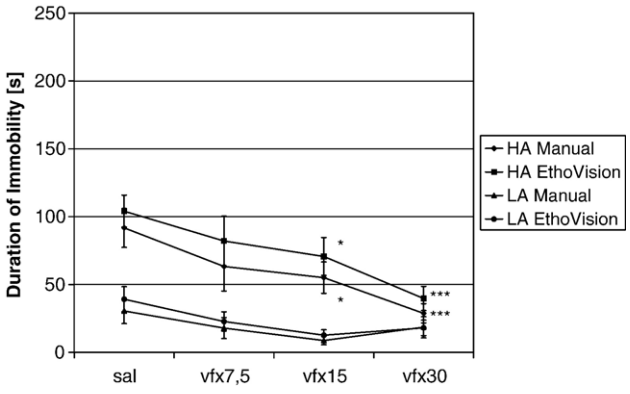

Fig. 3. Duration of immobility in TST in high (HA) and low (LA) analgesia mice. sal — mice injected with saline, vfx7,5 — mice injected with 7.5 mg/kg of venlafaxine, vfx15 — mice injected with 15 mg/kg of venlafaxine, vfx30 — mice injected with 30 mg/kg of venlafaxine, HA manual — duration of immobility in HA mice scored manually, HA EthoVision—duration of immobility in HA mice scored automatically by EthoVision system with immobility threshold set at 2.5% (see also Fig. 1), LA Manual — duration of immobility in LA mice scored manually, LA EthoVision — duration of immobility in LA mice scored automatically by EthoVision system. *$p<0.05$, ***$p<0.001$ (compared with HA mice injected with saline and scored with the same method). Values are mean± SEM.

3.2. Second experiment (dose-response to venlafaxine)

The three-way analysis of variance indicated that there was a significant effect of the line of mice: $F(1,172)=69$, $p<0.001$, and drug $F(3,172)=9.58$, $p<0.001$. The effect of scoring method was insignificant $F(1,172)=2.88$, $p>0.09$. A significant line x drug interaction was revealed: $F(3, 172)=3.38$, $p<0.05$. Venlafaxine significantly decreased duration of immobility only in HA line. Post hoc comparisons revealed that when behavior was scored manually, the duration of immobility in HA mice

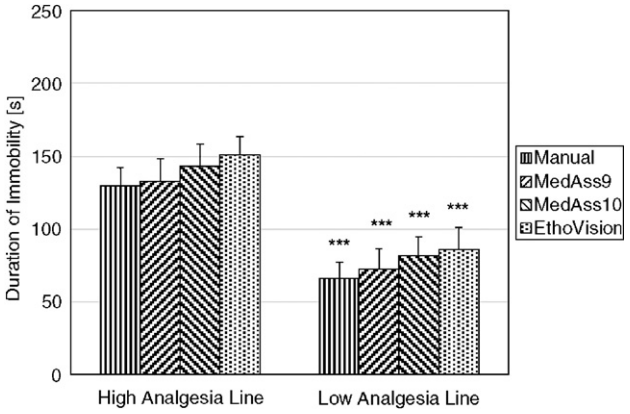

Fig. 4. Duration of immobility in TST in high (HA) and low (LA) analgesia mice. Manual — duration of immobility scored manually, MedAss9 — duration of immobility scored automatically by strain gauge device (Med Associates Inc.) with immobility threshold = 9, MedAss10 — duration of immobility scored automatically by strain gauge device (Med Associates Inc.) with immobility threshold=10, EthoVision — duration of immobility scored automatically by EthoVision video tracking system with immobility threshold set at 2.5% (see also Fig. 1). **$p<0.01$ (compared with HA mice scored with the same method). Values are mean±SEM.

injected with saline differed significantly from HA mice injected with 15 mg/kg of venlafaxine ($p<0.05$), from HA mice injected with 30 mg/kg of venlafaxine ($p<0.001$) and from all LA mice irrespective of their treatments ($p<0.001$). The same levels of significance were found for results obtained using automated analysis (Fig. 3). Data obtained using manual scoring were significantly correlated with results obtained using video analysis system (0.9, $p<0.001$).

3.3. Third experiment (comparison of strain gauge and video analysis systems)

The two-way analysis of variance indicated that there was a significant effect of the line of mice: $F(1,88)=37$, $p<0.001$. The effect of scoring method was insignificant: $F(3,88)=0.84$, $p>0.47$. Post hoc comparisons revealed that when behavior was scored manually the duration of immobility in HA mice differed significantly from the duration of immobility in LA mice ($p<0.001$). The same level of significance was found for differences in time of immobility between lines for all automated methods of scoring (Fig. 4). Data obtained during manual scoring were significantly correlated with results scored automatically by the strain gauge device (0.94, $p<0.001$ for both 9 and 10 immobility threshold) and with results scored by the EthoVision video analysis system (0.85, $p<0.001$).

4. Discussion

In the present study we validated a video analysis system as a method for scoring immobility in the tail suspension test. To date video analysis systems have been only occasionally applied to record the duration of immobility in the forced swim test (FST) (Sanchez and Meier, 1997; Crowley et al., 2004). Although both the TST and FST expose subjects to an inescapable stressful situation to induce a state of "behavioral despair", there are important differences that make it impossible to directly apply video analysis systems adjusted to score behavioral patterns in the FST to the recording of immobility in the TST. First, there are different repertoires of escape-oriented behaviors observed in both tests. In the TST escape efforts can be classified into three types: (1) running movements, forward or backwards; (2) body torsions with attempts to catch the suspended body; and (3) body jerks (Steru et al., 1985). In the FST, active behaviors include: (1) climbing, defined as upward-directed movements of the forepaws along the side of the swim chamber; (2) paddling, defined as rather vigorous movements of the paws while staying in the same place of the chamber; and (3) swimming, usually defined as a horizontal movement throughout the swim chamber (Cryan et al., 2002). Secondly, there are important differences in the definitions of immobility in these two tests. In the FST, immobility (or floating) is defined as a time during which an animal performs only those movements which are necessary to keep afloat (Porsolt et al., 1977). In the case of the TST there is some variability in the definitions of immobility, however usually a mouse is considered immobile when it either hangs passively and completely motionless (Butterweck et al., 2003; Li et al., 2003; Bai et al., 2001), does not move its paws (Holmes et al., 2002), or there is an absence of initiated movements (Crowley et al., 2004). To sum up, whereas in the FST small movements are allowed to consider the animal immobile, in the TST it is generally required that animal should be completely motionless. It is worth noting that some authors use somewhat odd and rather subjective definitions of immobility in the TST, such as "time when mice were judged to cease escape-motivated behaviors" (Conti et al., 2002).

Another important point is way in which the image analysis systems detect immobility. In experiments applying automated video analysis for measuring behavior in the FST two different methods of immobility detection have been previously validated. The first is based on the displacement of the object's center point of gravity. Although displacement of the center point of gravity is mainly used to measure velocity and distance covered by an animal in an arena, during the open field test for example, it has also been occasionally applied to analyze behavior in FST. First, Hedou and coworkers (2001) found that the total distance covered by a rat in a cylinder is negatively correlated with the duration of immobility scored manually. Then, Crowley and coworkers (2004) applied video tracking to detect immobility in the FST defining immobility as time when the mice velocity dropped below the specified threshold of 2.0 cm/s. The second method of automated scoring of immobility in the FST is based on the change between frames of video in the locations of the pixels of the subject being tracked. This method was used by Sanchez and Meier (1997) who defined immobility in the FST as time when the change in area between two sequential images was below 700 pixels. However, the usage of a number of pixels to define immobility is not practical because this parameter changes with differently sized animals. It would be therefore impossible to apply similar thresholds to detect immobility in mice that differ in line, age, or sex because each of these conditions can affect subject size. The solution to this problem is expressing change between frames using a set percentage of pixels instead of a set number of pixels. In our experiment, this parameter utilizing percentage change in object area between the samples, has been used to measure mobility in the EthoVision version 3.0 and has been applied to detect immobility in our experiment.

The practical difference between the first and second method is that in the center of gravity method the system will not detect any movement if there is no change in the object's center point of gravity. One could easily visualize this using an example of a bar rotating along its fixed center point of gravity. The bar would be considered immobile by the system applying a first method and strongly mobile if the system utilized the second method. Therefore, detection of immobility based on comparison of the locations of the pixels belonging to the tracked animal is better because it does not depend on the position of a subject's center point of gravity. This seems to be especially important in case of the TST because the subjects hang from a fixed point and certain activities like running movements can take place without any significant change in the position of the body, thus making it difficult to clearly distinguish these activities from immobility. It should be noted that even completely immobile objects can still produce a small change in both object area and the center of gravity between consecutive video frames because of system

noise. Also, sudden changes in lighting conditions that are not perceived by humans can affect image analysis. Secondly, there are small respiratory movements and, in the TST, pendulum-like movements of the body resulting from activity preceding a bout of immobility. Therefore, it is always necessary to preset a threshold to determine what is considered immobile. The necessity for using a threshold applies also for electromechanical systems utilizing a strain gauge that convert and express the force of a subject's movements as voltage.

In the present study we found that the EthoVision system can be reliably and accurately used to assess the duration of immobility in the tail suspension test. However, it is important to choose the correct immobility threshold. We found that immobility thresholds ranging from 2 to 3 percentage change in object area yield results that do not differ significantly from scores obtained by manual scoring. These immobility thresholds are high enough to compensate for small respiratory and pendulum-like movements of the subject resulting from activity preceding a bout of immobility. The EthoVision system was effective in detecting both the dose response to venlafaxine and differences between two different lines of mice in the TST. Furthermore, results obtained using this video analysis system were similar to scores from an experienced observer and the Med Associates strain gauge device. Generally, the 2.5% immobility threshold of the EthoVision system that was used to analyze the dose-response yielded results that were slightly higher (longer immobility) than results obtained for manual scoring. In an experiment performed by Crowley et al. (2004), a strain gauge device from Med Associates also yielded scores that were slightly higher than scores generated manually. In our experiment we used two immobility threshold settings of the Med Associates TST. The lower one (threshold = 9) was selected during preliminary study in our laboratory. For comparison, we also used the higher threshold that was previously used by Crowley and coworkers (2004). Both settings of strain gauge device resulted in scores that were higher than results obtained from manual scoring. There are two possible explanations for this fact. First, automated devices can count short periods of immobility that are too short for human raters to react to, that is to press a button of a stop-watch or a key on a computer keyboard. Secondly, automated systems can also count relatively short periods of immobility that sometimes take place during body torsions. When scored manually, immobility is defined as any time when mouse is hanging passively, so the human rater does not score any periods when mice display immobility during body torsions. The next possible step in improving image analysis of behavior could be application of active shape models combined with calculation of percentage change in object area between the samples (Twining et al., 2001).

A drawback of manual scoring is that this method is tedious and can be affected by an observer's bias. Although the researchers who are scoring behavior manually are usually blind to the identity and treatments of the observed animals, it can sometimes be difficult to make scoring objective, for instance if an experiment involves different lines of mice that can easily be distinguished. Furthermore, there is some variability between the behavioral definitions of immobility in different laboratories, making it rather difficult to compare results published by different authors. In contrast, automated systems use set scoring criteria and are absolutely blind to the identity of treatment groups. Automated systems also allow for the analysis of more that one animal at the same time. However, the specialized equipment currently available to analyze behavior in the TST is expensive and therefore in many laboratories immobility in this test is still scored by direct observation. An alternative for the specialized equipment could be the usage of a new generation of video analysis systems. The systems are already widely used to distinguish the effects of antidepressants from general psychomotor stimulants by monitoring locomotor activity of rodents in a confined area like the open field, because most antidepressants do not increase activity at doses that reduce immobility in the TST or FST (Crowley et al., 2004). In fact, video analysis systems are universal tools that can be used to analyze the behavior of animals in many different tests, such as the elevated plus maze, Morris water maze, conditioned place preference, or social interaction test (Sams-Dodd, 1995; Sellings and Clarke, 2003; Salas et al., 2003). The same software and hardware can be therefore used to measure a wide range of different behaviors. Furthermore, video analysis systems that allow one to define arenas of any shape can be applied to new and more complex custom tests.

There is also another advantage of the application of video analysis systems for the analysis of behavior in the TST. It has been already recognized that when mice climb their tails, it can constitute a problem when automated strain gauges devices are used (Mayorga and Lucki, 2001; Cryan et al., 2005). Obviously, such animals must be removed from the analysis because they have learned that escape is possible (Cryan et al., 2005). When automated systems that do not allow for visual observation of the mice are used, potential climbing may influence the behavioral readout because the climbing period tends to be scored as immobility by the system (Yoshikawa et al., 2002). This is especially important because the latency for the mice to climb their tails is short (Mayorga and Lucki, 2001; our personal observations). The EthoVision system could help to overcome this problem. Before starting the experiment it is possible to define the boundaries of an arena in which objects are recognized as targets for further video analysis. When the animal disappears from the defined arena (because it climbed its tail) it is no longer recognized and tracking ends. After the experiment it can be easily inferred during inspection of results which animals climbed their tails because the sum of the duration of immobility and mobility for each animal should be equal to the duration of the entire experimental session. Secondly, it is possible to determine the minimal size of the animal in pixels below which the object is not tracked by the system. Combination of these two aforementioned methods (precisely defined arena and minimal object size) could be used to eliminate climbing from the analysis. To minimize climbing in this study we used a fabric belt because it has been suggested that attaching the mouse's tail to a fixed support can increase the tendency of a mouse to climb its tail (Yoshikawa et al., 2002; our personal observations). Taken together, the results of the present study suggest that the EthoVision software is a reliable, accurate, and rapid method for the measurement of the duration of immobility in the TST. It

allows detecting even quite small differences in behavior between the lines and small effects of antidepressants.

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