# The new experimental model for behavioral investigations in animal studies

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**Abstract.** In this paper a video file based approach to evaluate position and locomotion in animal behavior experiments is described. For this purpose original software Animal Tracker for transforming a video data to a log file which is suitable for further computational analyzes, was developed. To perform analyzes from the log file, an additional software PostProc), which enables assessment of locomotion, velocity or place preferences, was created. For video recording software called DScaler was used. This is an open source software and freely available for download. The method that we describe in this paper is based on simple video equipment and supported by three software mentioned above. This method enables performing of a wide diversity of experimental designs without limitations in time duration, color and light conditions, shape and size of experimental area and/or investigated objects. As an example, results obtained from experiments with rats in an Open-field test are included. One group of animals was treated with benzodiazepine (2 mg·kg<sup>-1</sup>, single dose, subcutaneously). This easy to use system can be implemented in most laboratories without any special training and used by investigators in the field of animal behavior research.

Key words: Animal behavior - Locomotion - Displacement - Computer evaluation

# Introduction

The behavioral investigation, such as locomotors activity, memory, anxiety, learning, aggression etc. has been used in various experimental designs. One of the most frequently used experimental design in this kind of investigation, defined as an Open-field activity test, represents a valid measure of marked changes in "anxiety-like" behaviors in drugtreated and genetically manipulated animals and can also be used for general assessment of animal basal locomotors activity and exploration. The first attempts in measurement of animal behavior occurred early in the previous century (Hall 1934). As the essential goal of any experiment is to obtain valid and exact results, it is obvious that in behavioral experiments this depends on the tools capable of sensitive tracking, recording and analyzing of the events. These tools came a long way from the first Open-field score tests (Giulian and Silverman 1975), in which only a plain box with a visible grid pattern, stopwatch and sharp eye of investigator were included in the setting.

Early semi-automated systems for detecting animal's movement were photocell-based. They automatically detected any breaking of the (visible) IR beam grid (Ericson et al. 1991). In the combination with a computer program, this method can be very accurate (Koob et al. 2006), but still it has some aspects of disadvantages such as: relatively expensive, complicate to set and use, possible influence of IR beams on animal, impossibility of sequential and retrospective analyzes, detection of movements on previously established grid, etc.

Newer methods for video analyses of animal movement appeared with the development of video and digital equipment (Godden and Graham 1983; Schwarting et al. 1993). Most of these systems are dependent on the specific hardware. Some low-cost web-cam based methods had been described, too (Lind et al. 2005; Togasaki et al. 2005). There is, however, some limitations even in the most sophisticated systems. For example: i) it depends on the specific light and arena color conditions; ii) limited experimental time duration; iii) sequential, retrospective analyses are not possible; iv) movements are detected on previously established grid, etc.

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In this work we suggest a low-cost and easy to use combination of software and hardware for recording, tracking and analyzing of animal's movement and position. The system is based on movement tracking and it is independent of the light environment, color of the animal or the arena, animal species or shape and of the background color. The software practically can analyze any video file as long as camera can record it. This method can be used for tracking movement in absolute dark conditions due to its being equipped with IR camera, which can be useful in observing circadian rhythm.

In this study we recorded movements within 24 h, but in fact there is no limitation in time because video data can be transferred to an external memory or other computers in the intranet connection. In addition, all recorded data from video file can be analyzed within any amount of space and time sequence retrospectively and since the system is based on movement, tracking from video file it is not necessary to have previously defined grid. When data from the video file are analyzed, virtual grid can be defined by software to evaluate position of the animal within any amount of space or chosen time sequence.

In order to validate accuracy of the system we were watching video file and compared it with the data obtained from analyzing programs. This process of validation was performed randomly, for random time sequences of experiment, in all examples given in this work.

### Materials and Methods

Video format of the experiment is provided using the following video equipment:

- Standard or IR camera connected to a standard PC computer with 1 GHz CPU and 256 MB of RAM memory.
- PCI TV tuner card with Philips bt878 chipset for capturing video input.
- Windows XP operating system.
- Software called DScaler for video recording (that is open source software and it is freely available for downloading at the address: http://deinterlace.sourceforge.net/).
   DScaler software records AVI file with XVID encoding, usually with half PAL resolution.

Especially designed software, named Animal Tracker, enables detection of animal's movement from video file input by estimating animal's positions in every video frame – that is 25 times in one second and produces the log file from captured video format. The estimation of position is done in every frame until the end of video file. In every frame the current time and the current position is logged to a file. The Animal Tracker program is made in C++ language. It has no graphical user interface and it is executed from command line. This means that the possibilities for setting changes are not offered in advance. Instead, a simple modification in parameter files allows changes of settings. It is a small executable file, and system requirements are not demanding.

Before placing an investigated subject into the arena, we took a short (1-5 s) recording of the background (empty arena). The initial recording is used to create the basic background picture of the open field. It is further used for determining the position of in investigated subject.

After importing this initial recording (with the duration of T1 seconds) into the memory, it is represented by matrix M1[W, H, 3, FoT1], where: W is the number of pixels at horizontal axis (camera resolution), H is the number of pixels at vertical axis (camera resolution), 3 is the matrix dimension of the RGB (red-green-blue) components required for representation of each frame, and Fo is the frame frequency per second.

Number of frames in the initial recording can be calculated as N1 = FoT1.

The initial background picture is represented by matrix *M2[W, H, 3]*. It is actually, one averaged frame, calculated as:

$$M2[i, j, k] = \frac{\sum_{l=l}^{NI} MI[i, j, k, l]}{NI}$$

where parameter *i* is in the range from 1 to *W*, parameter *j* is in the range from 1 to *H*, and parameter *k* has values of 1, 2 or 3 which represent RGB components, respectively.

After the initial recording, investigated subject was placed into the arena. This moment is considered as the start of the experiment, and following recording (with the duration of *T2* seconds) is represented by matrix *M3[W, H, 3, FoT2]*.

Number of experimental frames in the experimental recording can be calculated as N2 = FoT2.

Temporary matrix,  $T_{mp}[W, H, 3]$ , represents absolute values of the difference between initial background picture and actual experimental frame for each RGB component. Each element of those matrices is marked as 1 if the substantial difference exists or with 0 if the substantial difference does not exist. The Animal Tracker takes difference as substantial if pixel values in experimental frame are out of range defined by standard deviation in corresponding initial background picture. After that, the matrix  $T_{mp}OB[W, H]$  is created. This matrix is the result of application of logical or operation applied pixel-wise on each matrix (R, G and B matrix), and represents the union of regions of change detected on each color matrix (R, G and B matrices). The obtained binary matrix represents area at which investigated subject is detected. We consider the mean value of coordinates of all pixels marked as 1, as the referent point *Rp*, calculated from the following equation:

$$Rp = \frac{\sum_{x_{ij} \in O} x_{ij}}{card(O)}$$

where *O* is the body of all pixels marked as 1.

In further analysis, the PostProc uses the referent point for motion evaluation. Usually, processing is a few-times faster than duration of video file. Software Animal Tracker transforms and save data to a log file. The log file contains comma separated values – frame time, and estimated animal position.

PostProc software was designed and used to analyze log files obtained from Animal Tracker. The results of processing can be analyzed in the programs options. One of the options is animal movement in 2D view for entire time of experiment. These movements are shown through position dots that are taken every 0.02 s (this time is adjustable and can be reduced if needed).

In the experiments described in this paper, we used Wistar (n = 20) rats, ten male and ten female, weighing between 250 and 320 g from laboratory of experimental animals of Natural Sciences Faculty. One group of ten animals was used for control experiments, and another group of ten animals was treated with benzodiazepine, (test drug; Galenika AD, Belgrade, Serbia). Animals were held in the same environment, with water and food ad libitum and same cleaning habits, in the same room in 12 : 12 h dark/light conditions (lights on at 07:30), at the temperature of 20°C.

The arena used in this work was open field arena  $50 \times 70 \times 30$  cm, made of polyester and color shift able. Open field arena was in the same room in which animals were kept to exclude additional stress of displacement. Camera was placed 230 cm above the arena. For the test, the rat was placed in a corner of the open field and allowed to move freely.

Test group of animals (n = 10) was treated with benzodiazepine (2 mg·kg<sup>-1</sup> subcutaneously) 10 min before placing in the arena. We gave this drug in order to induce locomotion impairment, just as an example of experimental design that the system is applicable. In control group (n = 10), we used saline, given in exactly the same way as test drug. Control experiments were performed by applying saline and placing animals in the open field, where video file of their locomotion for 24 h was made. There were no interference by any external stimuli on the animal during the experiment and experiment was conducted in the same room the animal was kept in. After 12 h light was turned off automatically, so that 12 : 12 h dark/light conditions were achieved in experiment. After the control experiments, we preformed the test drug experiments with the same conditions and the same number of animals. Animals were in good health and no drug or other stimuli have been given to them in order to record ordinary behavior of the animals in new environment.

Experimental data were analyzed using Student's *t*-test, were  $p \le 0.05$  was considered as statistically significant.

All the experiments were performed according to EU (86/ 609/EEC) and Local Ethical Guidelines.

# Results

After making log files by Animal Tracker, and analyzing those files by PostProc, we obtained experimental data. In Table 1, the parameters that PostProc calculate automatically are presented. These values are given for the entire control group of ten animals.

In the Fig. 1, average speed was calculated for each hour during 24 h of the control experiment. If the average speed is higher, the experimental animal is more active, and

 
 Table 1. Parameters of animal's motion calculated automatically by PostProc

$Va (m \cdot s^{-1})$	$Vmax (m \cdot s^{-1})$	S (km)	<i>Dm</i> (h)	<i>Ds</i> (h)
$0.86\pm0.098$	$3.65\pm0.29$	$18.64\pm0.59$	$6.02\pm0.61$	$17.97 \pm 1.51$

*Va*, average speed in meters per second; *Vmax*, maximal speed in meters per second; *S*, total distance traveled by animal in kilometers; *Dm*, duration of motion in hours; *Ds*, duration of stillness in hours.



**Figure 1**. Average speed calculated for every hour during 24 h of the control experiment. Note that these results are obtained from one animal in control group. The data given in this figure is calculated automatically by PostProc.



**Figure 2**. Average speed calculated for every 5 min during first 2 h of the control experiment with the line of activity. Note that these results are obtained from one animal in control group. The data given in this figure is calculated automatically by PostProc.

*vice versa.* These results are confirmed by watching the actual video recording of the experiment.

Fig. 2 represents animal's activity through its average speed calculated for every 5 min for the first two hours of the same experiment as in the Fig. 1. This period was taken to make animal's exploratory activity clearly visible.

For further analyzes of the video file, arena was divided in a virtual grid formed by lines connecting every 5 cm of the opposite sides of the open field.

In the Fig. 3, time that one of the tested animals in the control group spent in every grid section is shown. The time is displayed in minutes with accuracy of  $\pm 30$  s. In the white sections the animal spent less than 1 min. Also, the grid sec-

tion where the animal achieved maximal speed (*Vmax*) is marked. Fig. 3 further shows that animal spent most of the time in the corners of the arena.

In the experiment described above: i) *Vmax* was 3.16  $\text{m}\cdot\text{s}^{-1}$ ; ii) *Vmax* occurred in 16<sup>th</sup> second of 107<sup>th</sup> minute of experiment (animal was moving with this speed for 0.3 s); iii) *Vmax* occurred in F4 section of the virtual grid (Fig. 3).

In the same manner as in the control experiments we made the log file by Animal Tracker of data obtained in test drug experiments, and analyzed it by PostProc. In Table 2, we present parameters that PostProc has calculated automatically for test drug experiments. These values are given for the entire test group.



**Figure 3.** The amount of time animal spent in various grid section; \* = Vmax (maximal speed is achieved in F4 section of the grid); different numbers represent different duration of time animal spent in each section in minutes (section with less than 1 min time spent are left blank); the center of the arena is marked with black frame.

 
 Table 2. Parameters of animal's motion calculated automatically by PostProc for test drug experiments

$Va (m \cdot s^{-1})$	$Vmax (m \cdot s^{-1})$	S (km)	<i>Dm</i> (h)	<i>Ds</i> (h)
$0.36\pm0.061$	$1.61\pm0.16$	$2.86\pm0.24$	$2.07\pm0.12$	$21.95 \pm 2.42$

These results show that the values of *Va*, *Vmax*, *S* and *Dm* are significantly lower, while value of *Ds* is significantly higher in test drug treated animals comparing to the values in the control experiments.

In the Fig. 4, average speed is calculated for every hour during 24 h of test drug experiments. This figure shows that animals exploratory activity in the first 3 h of testing is significantly impaired comparing with control experiments (see Fig. 1). The significant increase in activity observed between 14<sup>th</sup> and 17<sup>th</sup> hour and later between 20<sup>th</sup> and 24<sup>th</sup> hour is still below the average speed in control experiments (see Fig. 1).

Using the parameters described above we introduced a few coefficients that can be useful for analyzing data obtained in

the experiment. Since the arena can be divided in the virtual grid, and the center and periphery of the arena can be determined, one useful tool for expressing animal's exploratory activity can be introduced. We can calculate the time spent in the center of the open field and time spent in the periphery. In this study, we considered that periphery of the arena is 10 cm from the walls, and rest of the arena was treated as center.

As an example we marked amount of time spent in the center as Tc (see Fig. 3) and time spent at the periphery as Tp and then introduced the coefficient named motion distribution (*Md*) as:

Md = Tc/Tp

Md can be calculated for the entire experiment or for any time sequence of the experiment. In the control experiments, Md value (mean  $\pm$  SE), is calculated in following manner:

 $Tc = 11,176 \pm 1283$  s

 $Tp = 75,336.6 \pm 6452$  s

 $Md = Tc/Tp = 0.148 \pm 0.0043$ 

In the Fig. 5, *Md* values through 24 h of experiment are represented, by calculating *Md* for every hour.



**Figure 4.** Average speed calculated for every hour during 24 h of the test drug experiment, with the line of activity. Note that these results are obtained from one animal in the test drug experiment. The data given in this figure is calculated automatically by PostProc.



**Figure 5.** *Md* values through 24 h of experiment, by calculating *Md* for every hour from one control experiment. Note that these results are obtained from one animal in control group. The data given in this figure is calculated automatically by PostProc.



**Figure 6.** *Md* values from one control experiments and one test drug experiment (with benzodiazepine). Note that these results are obtained from one animal in each experimental group.

Combining with the example from control test group of the experiments, we can show *Md* correlation between one of the controls and one of the test drug experiments (Fig. 6):

Control experiment	Test drug e	xperiment
Tc = 11,473 s	Tc = 1968  s	_
<i>Tp</i> = 72,114 s	Tp = 82,356	5 s
Md = Tc/Tp = 0.16	Md = Tc/Tp	p = 0.02

Significantly lower values of *Md* in test drug experiments (with benzodiazepine) indicate impairment of explorative activity.

Another parameter that we introduce is obtained by dividing duration of motion (Dm) with duration of stillness (Ds). This coefficient we named module of activity (Am), and it is calculated as:

Am = Dm/Ds

In the control experiments, Am value, mean  $\pm$  SE, is calculated in this manner:

 $Dm = 21,678 \pm 2231$  s  $Ds = 64,772 \pm 5443$  s  $Am = Dm/Ds = 0.33 \pm 0.0064$  If *Am* is higher it indicates that animal was more active in motion. We can calculate this value for any period of time, and compare these values through out the entire experiment. In this way, *Am*, as a function of time, can be obtained. This is shown in the Fig. 7, which shows *Am* for every hour for 24 h of experiment.

To see in practice how *Am* can be used we calculated and compared *Am* for one animal from control group with one from test drug experiments (Fig. 8):

Control experiment	Test drug experiment
<i>Dm</i> = 22,459.7 s	<i>Dm</i> = 6234,8 s
Ds = 70,090.9  s	<i>Ds</i> = 73222,2 s
Am = Dm/Ds = 0.3	Am = Dm/Ds = 0.08
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It is obvious that *Am* is much lower in the test drug experiments (with benzodiazepine) which indicates locomotors impairment.

Since software automatically calculates the time animal spent moving in the range between stillness (Vo) and average speed (Va) and amount of time moving in range between Va and Vmax, we introduced another potential new tool/option. The percentage of time that animal spent in a velocity range we called velocity distribution (Vd). The percentage of time that animal spent in a velocity range from Vo to Va we marked as  $Vd_1$ , while  $Vd_2$  represents the percentage of the time animal spent in velocity range from Va to Vmax. We can easily calculate the time animal spends moving, not only in those two ranges but in any range of velocity that we want to observe. It can be done by simply setting "speed limit" to different points. This parameter can be calculated and displayed for any portion of time and for any section of space of arena partially. These values were calculated for each hour of 24 h from one of the control experiments, and displayed in the Fig. 9.

Each column in the Fig. 9 represents one hour of the experiment and two colors of the columns represent percentage of the time that animal spent moving in  $Vd_1$  range and  $Vd_2$ 



**Figure 7.** *Am* values through 24 h of experiment, by calculating *Am* for every hour from one control experiment. Note that these results are obtained from one animal in control group. The data given in this figure is calculated automatically by PostProc.



**Figure 8.** *Am* values from one control experiment and one test drug experiment (with benzodiazepine). Note that these results are obtained from one animal in each experimental group.

range. The highest level of  $Vd_2$  was achieved in the first two hours and then again from  $18^{\text{th}}$  to  $20^{\text{th}}$  hour.

#### Discussion

Presently used systems for behavioral experiments are based on two main approaches: IR beam-based and video file-based systems.

IR beam-based systems are complicated to set and use, do not have possibility for sequentional and retrospective analyses because they detect movements on previously established grid. In addition, there is a question of possible influence of IR beam on the investigated subject (Koob et al. 2006).

The video file based systems for behavioral experiments already exist, as commercial products. These systems with graphical interface are capable to record a large number of experimental parameters (Lind et al. 2005; Togasaki et al. 2005). The experimental model described in this work is also capable to record the same parameters as other video file based systems mentioned above, but at reasonable lower cost. In addition, our model provides the possibility for calculation of two new parameters (Md and Am). In our experimental model we implemented two separate originally developed open software (Animal Tracker and PostProc), with no common graphical interface. Almost unlimited modifications of Animal Tracker and/or PostProc are possible by simple changes in parameter files. With this possibility of modification, we are able to create various experimental models in order to meet specific requirements.

Our experiments included video files of 24 h or more, but XVID compression occupies less than 4 GB of hard drive space and can fit to only one DVD, and can be reanalyzed, or shipped to different location and distributed or stored in any desirable way. Even further, the experiment can be recorded on the computers in the intranet connection which means that the duration of experimental time is almost unlimited. Video file can be encoded with any other standard encoder, and video file can be saved with any program for video recording and filmed with any kind of camera. Also, it can be taken under almost any illumination, color or other possible criteria of filming. Animal movements are detected by separating animal and background part of the video frame and thus animal can be of any size or shape, and (most importantly) of any color. Also, the background can be in any color or shape, and the illumination can go from bright colored to almost full dark conditions. As mentioned in Introduction section, we use IR camera, and any additional equipment such as LED is unnecessary. When during 24 h of experiments light conditions in the room automatically switch from light to dark, the algorithm for detection automatically adjusts background image.



**Figure 9.** Percentage of time spent in  $Vd_1$  speed range and  $Vd_2$  speed range calculated for every hour during 24 h of experiment from one of the control experiments. Note that these results are obtained from one animal in control group. The data given in this figure is calculated automatically by PostProc.

After making video file with Animal Tracker software, PostProc is taking the position dots every 0.02 s (this time is adjustable and can be reduced if needed). These dots are connected by lines following the animal movements, producing in this way the line of total movement of the animal in the experiment. Another option of this program is to create virtual grid of any size and shape dividing arena in segments. This program also calculates the number of position dots in every segment of the grid and since position dots are taken every 0.02 s, the number of dots represent the time (amount of dots multiplied by 0.02) that animal spent in specific part of the grid. If animal stays in one place without moving, the program continuously calculates at each 0.02 s the number of dots, although graphically it is represented as one dot because animal is not moving. Furthermore, certain time sequences during the experiment can be extruded and animal's location in particular time frame can be determined, where it came from to the specific section and where it left to after this. In addition, to this there is one more option called activity. This view shows exact speed in pixels/second (this can be transformed in to  $m \cdot s^{-1}$ ) on the y axes and the time on x axis, and can also be extruded for any time sequence of the experiment. This option gives values of duration of experiment (or duration of the time sequence we choose), duration of movements and Ds, and also the values of Va and Vmax. Since stillness of the animal is rarely absolute, for example animal can make rearing and grooming movements staying in same place of the arena, we involved speed threshold for movements called speed limit. This threshold provides choice if the movement of the part of body is to be assessed as moving or not. Every speed below this threshold is taken as stillness and above as movements.

Since the open-field procedure is most used for general assessment of animal basal locomotors activity and exploration, we can asses these parameters by analyzing results recorded in Fig. 1. It is shown that animal exhibits exploratory activity in the first 3 h of the control experiment while getting acquainted with the arena. After this, from 4<sup>th</sup> to 9<sup>th</sup> hour activity is significantly lower, and from 11<sup>th</sup> hour it is rapidly increasing when light switch off and rat's nocturnal activity starts. After 19<sup>th</sup> hour, activity is gradually decreasing, with the lowest average speed at 23<sup>rd</sup> hour.

Further we tried to observe this exploratory activity closer and took only first 2-h segment from this experiment. The Fig. 2 shows that the activity corresponds to a higher level of *Va*, oscillating between 1.17 m·s<sup>-1</sup> and 1.83 m·s<sup>-1</sup>. In this example the first 2 h of animal's exploratory activity is extruded, but we can extrude any time sequence from the entire experimental period. For instance, when the time period from 19<sup>th</sup> to 23<sup>rd</sup> hour is extruded (not shown) we found the activity within this period at very low level, oscillating between 0 and 0.33 m·s<sup>-1</sup>. By analyzing this sleeping period through the average speed calculated for every 5 (or less) min, we can exactly analyze the periods of absolute stillness (below the threshold of movements) and periods of the low activity. Sometimes the sleeping period can be the sole interest of investigation and in that case, the average speed (or distance) calculated for very short time durations (the limit is 0.02 s) can be analyzed. Practically this makes possible retrospective and sequential analyses for any amount of time.

To analyze animal's positioning and special component of movement we divided arena in virtual, software produced grid (see Fig. 3). This grid can be of any size or shape, because it is formatted by software as we input the certain request. The virtual grid is applicable to any kind of the arena, and most importantly, grid is determined after the experiment is done, so we can change grid format in the same experiment. So, except for just showing the special distribution of animal's motion this view enables exact and simple approach to investigate some external stimuli, such as smells, colors, electromagnetic field etc., applied to the specific section of the arena by simply assessing time that animal spent near or away from the source of those stimuli.

One of the ways to evaluate exploratory activity is to estimate the time that animal spent in the center of the field (Swiergiel 2007). If the animal spends more time in the center of the field (higher Md) we can assume that exploratory activity is higher and vice versa. Originally introduced parameters Md and Am are calculated by simply dividing of two known values. So, the question can be raised what their importance is. First of all, although these parameters are very easy to calculate they give us opportunity to observe animal's explorative potential (Md) and animal's activity potential (Am) in the function of time, observing in that way history of the whole event. For example we showed in the Fig. 7 that Md line has two peaks between 1<sup>st</sup> and 5<sup>th</sup> hour and between 19<sup>th</sup> and 21<sup>st</sup> hour indicating two phases (daily and nocturnal) of exploratory activity, but also nocturnal peak of the *Md* line can be explained as lower anxiety due to the fact that animal becomes more familiar with the arena. We also want to underline that Md can be calculated not only for center/periphery, but for any two sections of the arena that we want to. In this way we can estimate the influence of some stimuli on experimental animal (such as different smells, different colors etc.) applied to the specific sections of the arena. In similar way, Fig. 9 shows that Am line has two peaks between 1<sup>st</sup> and 3<sup>rd</sup> hour and between 20<sup>th</sup> and 22<sup>nd</sup> hour indicating two phases (daily and nocturnal) of locomotors activity. This enables us to quickly assess difference between animals in various tests.

We will give an example of that: in the Fig. 10, we displayed *Md* values for the first 30 min (calculated for each minute) in one of the control experiments and in one of the test drug experiments (with benzodiazepine), which can be another way of showing the obvious exploratory impairment



Figure 10. *Md* values for first 30 min of the control experiment and of the test drug experiment. Note that these results are obtained from one animal in each experimental group. The data given in this figure is calculated automatically by PostProc.



**Figure 11.** *Am* values for first 30 min of the control experiment and of the test drug experiments. Note that these results are obtained from one animal in each experimental group. The data given in this figure is calculated automatically by PostProc.

in tests with benzodiazepine. The difference between these two lines of Md undoubtedly shows the influence of the test drug on animal's exploratory activity. Furthermore, we can choose larger time window and then we can see, for example, in which particular time the experimental drug started to induce exploratory activity impairment. In the Fig. 11, we displayed Am values for the first 30 min (calculated for each minute) of the control experiment and for the test drug experiment (with benzodiazepine), for one animal from each group (control and test drug group), which is another way of showing the obvious locomotors impairment: here we can see actual drug influence on movement and locomotors activity in the function of time. This is not only important in quick assessment of the difference between tested and control animal, but also has great significance in recording the exact time of locomotors and motion impairment, when it started, when it ended or what events in time had influence on it.

To conclude we tried to present a new easy-to use and up-to date system for capturing, analyzing and processing data obtained from animal behavior experiments together with several new potentially useful parameters/tools for assessing the data. This method can be used by investigators who do not have any particular knowledge of computer science, but who do have advance demands in their behavioral experiments.

However, our method has its limitations. The first and most serious one is that we still have to improve the system to record and assess rearing and grooming movements. This we plan to solve by advancing our Animal Tracker to record changes in the surface that represents the animal in the arena, and as this surface shrinks it will record rearing or grooming movements depending on the amount of surface decreased. It can and will be improved to record and assess number of turns, and turn directions, although these data can be extruded from log file, but still it is not done automatically.

#### References

Ericson E., Samuelsson J., Ahlenius S. (1991): Photocell measurements of rat motor activity: a contribution to sensitivity and variation in behavioral observations. J. Pharmacol. Methods **25**, 111–122

- Giulian D., Silverman G. (1975): Solid-state animal detection system: its application to open field activity and freezing behavior. Physiol. Behav. 14, 109–112
- Godden D. H., Graham D. (1983): 'Instant' analysis of movement. J. Exp. Biol. **107**, 505–508
- Hall C. S. (1934): Emotional behavior in the rat. I. Defecation and urination as measures of individual differences in emotionality. J. Comp. Psychol. **18**, 385–403
- Kooba A. O., Cirillob J., Babbsb C. F. (2006): A novel open field activity detector to determine spatial and temporal movement of laboratory animals after injury and disease. J. Neurosci. Methods 157, 330–336
- Lind N. M., Vinther M., Hemmingsen R. P., Hansen A. K. (2005): Validation of a digital video tracking system for recording pig locomotors behavior. J. Neurosci. Methods **143**, 123–132

- Schwarting R. K., Goldenberg R., Steiner H., Fornaguera J., Huston J. P. (1993): A video image analyzing system for open-field behavior in the rat focusing on behavioral asymmetries.
  J. Neurosci. Methods 49, 199–210
- Swiergiel A. H., Dunn A. J. (2007): Effects of interleukin-1 $\beta$  and lipopolysaccharide on behavior of mice in the elevated plus-maze and open field tests. Pharmacol. Biochem. Behav. **86**, 651–659
- Togasaki D. M., Hsu A., Samant M., Farzan B., DeLanney L. E., Langston J. W., De Monte D. A., Quik M. (2005): The webcam system: a simple, automated, computer-based video system for quantitative measurement of movement in nonhuman primates. J. Neurosci. Methods 145, 159–166
- Tort A. B. L., Neto W. P., Amaral O. B., Kazlauckas V., Souza D. O., Lara D. R. (2006): A simple webcam-based approach for the measurement of rodent locomotion and other behavioral parameters. J. Neurosci. Methods **57**, 91–97