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An Automated System for Analysis of Mouse Movement Activity

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In this paper an automatic system for measuring motor abilities of mice and the test results of two groups of control mice are presented. The system was designed for the benefit of experiments related to diagnostics of stroke neurological symptoms. Here we tested the validity of a system for monitoring long-term mobility of wild mice using software specifically designed for automated data gathering and analyses. The system analysed all data and filled the database with measured values characterizing the mobility of mice. Subsequent analysis revealed that there is no difference in two groups of control animals. The results indicate that this type of monitoring might constitute a reliable tool for examining neurological functions in laboratory animals.

K e y w o r d s: computed neurological examination, image analysis, image processing, motor function

1. Introduction

Stroke is a leading cause of death and disability in humans [1–4]. Management of stroke remains difficult and challenging due to highly individual disease courses and limited therapeutic options [5, 6].

The most common type of the disease, reaching up to 85% of all cases, is the focal ischemic stroke [1–6]. Currently, the only relatively highly efficient treatment measure for acute ischemic stroke is intravenous or intraarterial administration of a thrombolytic agent, recombinant tissue plasminogen activator (rtPA) [5–7]. The potential new stroke therapeutic methods are primarily tested using different experimental models of the disease, i.e. *in vitro* or in animals. In acute focal stroke, one of

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the crucial final end points confirming a therapeutic efficiency of the tested method is improvement of neurological disturbances, i.e. the symptoms caused by the focal brain injury. However, estimation of this measure in animal models is difficult due to abundance of various symptoms caused by ischemic brain injury, e.g. depending on the anatomical localisation of the ischemic lesion in the brain [5, 6].

Commonly used measurements of animal neurological symptoms can be highly subjective and tend to be of a short duration in a specific experimental environment that often adds stress to animals and confounds the data. Therefore, here is a need for more precise and reliable monitoring in extended periods under stress-free conditions.

There are many commercially available systems that can automatically monitor and analyse animals' neurological symptoms including mobility in various experiments. The most commonly used systems are based on automatic analysis of video recordings. There is a great diversity of those systems. Some of them can monitor rodent inside animal cages whereas the others operate in individually designed mazes or fields. There are systems dedicated to general tests (like Morris water maze, elevated plus maze or open field) and multi-purpose systems with ability to define individual areas and objects. Nevertheless, none of the commercially available systems were appropriate for our research. To examine general motor activity a maze with narrow corridors and suitable obstacles is necessary. Narrow and low corridors ensure that the tested animal runs through the maze in one direction thus facilitating calculation of general mobility parameters. A ramp and steps were designed to monitor the potential impairment of different movement functions, e.g. related to destruction of the motor cortex, cerebellum or the extrapyramidal system, whereas a two-way maze branch point was used to recognize symptoms such as hemianopia or neglects. The maze was also equipped with a special area for a grid to illustrate how animals manage with coordination of movements and maintaining their equilibrium. The maze should also prevent tested mice from seeing one another and ensure exactly the same conditions for each mouse. It should assure the well-being and long-lasting stay of the tested animals inside with a proper access to the fresh air, water and food, and be easily cleanable. None of commercial systems was equal to provide observation with such maze.

The required system should be able to work for at least 24 hours, distinguish three different objects (mice) and discern a lot of separate areas in the maze. It should also be fully resistant to shadows and disturbances related to a long-lasting recording (animals' excrements and dry nourishment scattered throughout the maze). For that reason we decided to create our own system, which would be the most appropriate for the comparison of mice with regard to the influence on motor abilities. Since we focused only on motor measurements, one top camcorder was fully sufficient for recording the experiments.

The aim of this work was to create an automated computer-assisted optical system with the maze and software for precise, unbiased measurement of various motor parameters, and to compare the general activity of two mice control groups in a stress-free environment.

2. Materials and Methods

2.1. System for Automatic Registration and Analysis of Animal Motor Functions

A computed system based on electronic estimation of 24-hour video monitoring of animal motor behaviour was designed and made in-house. The system for automatic registration of animal motor functions (SABN) was composed of a dry land maze made from plexiglass, a digital camcorder, and a computer with dedicated software. The camcorder was situated directly above the maze. The required picture definition was 320 x 240 pixels and all image data from the camcorder were fed to a computer by a USB interface for analysis.

2.1.1. The Maze

The dimensions of the maze were: 36 cm in length, 53 cm in width and 6 cm in height, with the width of the corridors not exceeding 4.3 cm. The maze was completely enclosed with ventilation holes drilled in to the lid. As shown in Fig. 1, each zone of the maze (of which there were 12) was numbered accordingly to track the location of the mouse and the time spent in each correctly.

Three identical separate mazes were placed side by side to enable three mice to be tested simultaneously. Each maze was separated from its adjacent one by

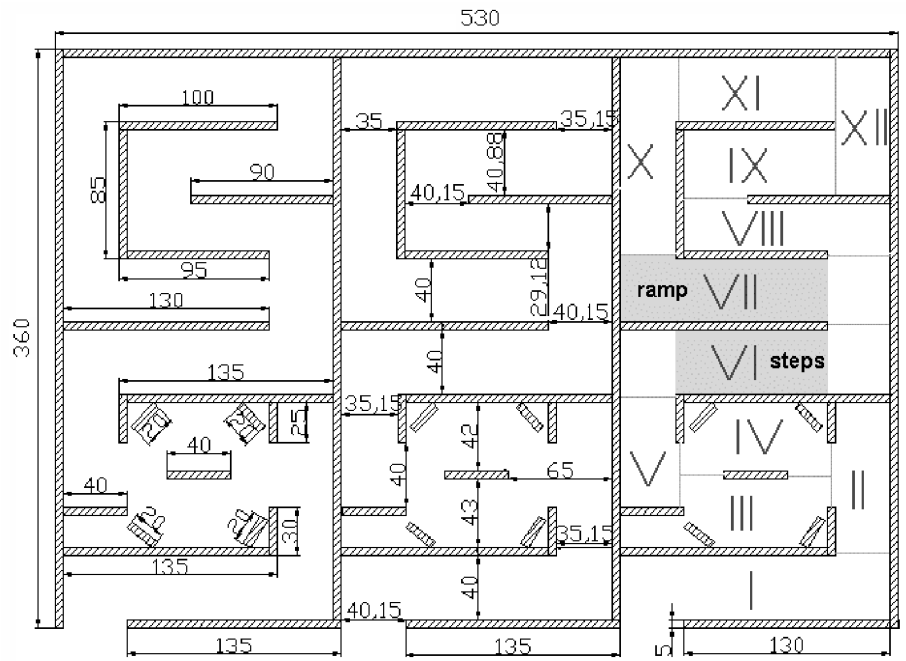

Fig. 1. The labyrinth schematic view with dimensions [in mm]

opaque white walls to prevent tested mice from seeing one another. Nevertheless, we cannot exclude that the tested mice could hear or contact each other by olfactory senses. In addition the floor of the labyrinth was made white to enable a better contrast between a mouse and the equipment. Each maze also contained objects such as steps of the length 9.5 cm (zone VI), a ramp – 13 cm (zone VII), and a corridor branch (zones III and IV) enforcing test animals to make a decision which route to take. Food (zone IX) and water (zone I) containers were placed in different parts of the labyrinth to encourage the animals to overcome the obstacles and run around its corridors.

2.1.2. Software

The software consisted of the following elements: a program for recording experiment results, directly connected to the camcorder and recorded compressed frames; a program for analysing the collected data; and a database with an interface. Figure 2 shows the general schema of the software.

C++ was used for writing the programs and MySQL for generating the database. A threshold value, image definition, speed of frame capturing and other camcorder options could be set by a user. The field of analysis was set by marking two opposite corners, necessary for calculating the mesh of the corridors (Fig. 3(a)). The light rectangle in the Fig. 3(a) marks the area where corners of the maze should be situated out whereas the dark lines determine the calculated mesh. Recording could begin only after the proper manual calibration (the user have to match the real mesh of the maze with the computed one). The software computes the position of each zone and obstacle and the ramp on the basis of the calibrated mesh. Proper calibration is essential for getting correct analysis results, which are in the form of a set of images including all details necessary for further calculation. After loading images to the analysing software, every image was changed into a binary picture (Fig. 3(b)). The recorded objects (animals) were localized on the basis of a colour and a size of a group

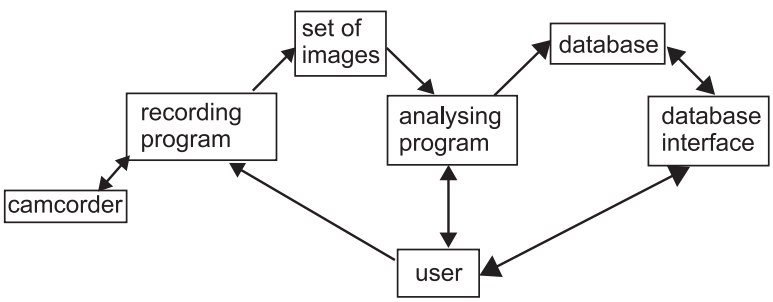

Fig. 2. A schema of the software

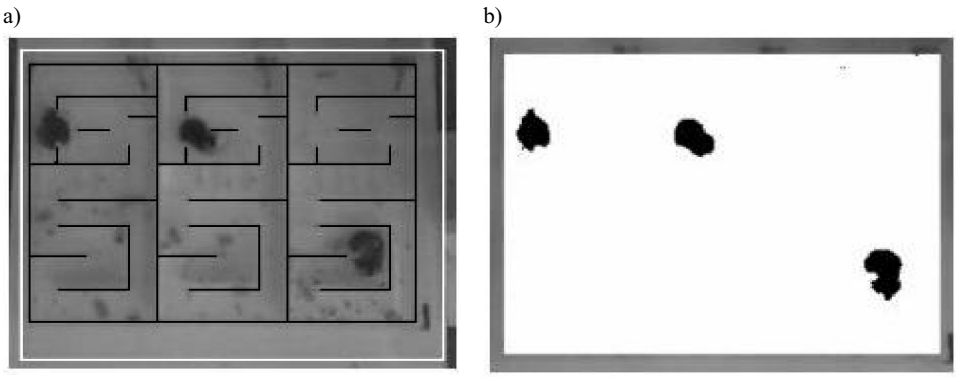

Fig. 3. Image received from camcorder (a) with marked field of analysis (light rectangle) and a net of corridors arrangement (dark lines), (b) after thresholding

of pixels. Every dark object larger than 0.5 cm in length and width was recognized as the object of interest. There was also a limitation for the maximal distance of movement during 0.5 second. If the distance was bigger than 17 cm, position of the object was recognized as incorrect. The system was fully capable of distinguishing mice from other dark objects that occur in the maze during experiments (excrements, shadows), but could function incorrectly when external disturbances appear (other objects in the field of view such as the hand of a user or improper lighting). Following calculations of the centre of gravity, the mouse's position in time was stored by the system. Values of the coordinates derived from all video frames illustrate the general draft of a rodent motion during the examination.

At the time of the last picture analysis, the system counted selected parameters of the examination:

- general mean velocity [centimetres per second (cm/s)] – quotient of the whole distance and the time of the experiment (24 hours), reflects the general velocity of rodent, including the time where a mouse was immobile.
- the mean velocity during movement [cm/s] – the quotient of the distance and time when mouse movement appeared, the movement between two following frames was noticed when the difference of distance between an actual frame and the frame 1.5 second earlier was not less than 0.6 cm (all numbers were hand-picked after numerous tests and observations). This way of comparison does not react to short in time or distance movements, it is unresponsive to resting, grooming, sniffing or stationary rotation.
- the time of immobility (the rest) [percentage of the total time of observation (%)] – the time when movement was not noticed,
- the velocity during overcoming obstacles [cm/s] – the quotient of the distance and time measured only when an object appeared in a region with obstacles (part of the zone VI – Fig. 1), regardless whether movement appeared or not.

- the velocity during overcoming the ramp [cm/s] – the quotient of the distance and time measured only when an object appeared in the region with the ramp (part of the zone VII – Fig. 1), regardless whether movement appeared or not.

A user could check those results, might write comments and sent the outcomes to the database.

These parameters are the indirect evidence of mice's physical efficiency which can illustrate the state of its cardiovascular or respiratory system.

2.2. Animals

Wild-type mice (C57BL/6J, http://jaxmice.jax.org/strain/005934.html) were obtained from the Jackson Laboratory (Bar Harbor, Maine, USA). All animals had black coat colour and were at the same age at the time of testing. All animals were kept under the same conditions of housing and lighting and were fed on standard chow *ad libitum*. Experimental procedures were approved by the Gdansk Local Ethical Committee for Animal Experimentation (Gdańsk, Poland, 2005) and were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.3. Animal Activity Monitoring

Each mouse (7 in the first group and 6 in the second) was placed in the maze and recorded continuously for 24 hours in an empty room. The camcorder frequency of sampling was set at 2 frames per second, which was fully sufficient for detecting objects moving at the speed of 9 cm/s (maximal velocity of the wild mouse) in the distance 7 cm (the minimal length of a zone in the maze). Only an object as itself was to be analysed, no details like a shape or a location of the body parts were needed, therefore low camcorder resolution (240 x 320 pixels) was sufficient for recording. Each frame was written as a jpg file. The results of the computer image analysis were output in the form of the above-mentioned five selected parameters and were additionally grouped according to the time of analysis: twenty-four hours were divided into six four-hour periods, what enabled investigation of the influence of the day cycle on the set parameters.

2.4. Statistical Analysis

Levene's test was used to estimate the homogeneity of variances. T-test was used to compare the parameters between two groups of animals. To analyze the impact of the day-time for each mice' population, the factorial analysis of variance (ANOVA) was performed with the two factors: a group (the first vs. the second) and the day-time (six periods of observation). The reproducibility was evaluated with statistic algorithm proposed by Bland and Altman [8].

3. Results

The T-tests for all parameters failed to reject the null hypothesis (Table 1). The ANOVA tests revealed that for the general mean velocity, the velocity during movement and the time of immobility the day-time influenced the experiment results (Table 2). The day-time of examination did not influence the differences in mobility between the two animal groups (Table 2; Fig. 4). Kind of the group had also no impact on the results (Table 2; Fig. 4). However, it is worth noting that the usual day–night cycle of the examined mice was disturbed by the permanent artificial lighting, needed for the continuous recording. The Bland-Altman test displayed a good repeatability of the measurements for all parameters except the time of immobility and the velocity at the ramp (respectively 93% and 94% of the results are located in the range of ±2 SD) (Fig. 5). All appreciable differences might be caused by different analysis options (a level of the threshold and maze calibration) and are induced by the user.

Table 1. The comparison of two groups of control subjects and results of the T-test

Characteristic	Mean 1st group	Standard deviation	Mean 2nd group	Standard deviation	T-test *p*
General mean velocity (cm/s)	1.05	0.79	0.89	0.57	0.33
Velocity during movement (cm/s)	3.71	0.96	3.44	0.78	0.21
Time of immobility (%)	79.33	13.85	79.86	12.56	0.86
Velocity at steps (cm/s)	4.20	1.75	3.78	1.06	0.24
Velocity at ramp (cm/s)	2.94	1.54	3.09	1.33	0.41

Table 2. Results of variance analysis

	General mean velocity (cm/s)		Velocity during movement (cm/s)		Time of immobility (%)		Velocity at steps (cm/s)		Velocity at ramp (cm/s)	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*P*
Day-time	**6.96**	**0.0**	**2.93**	**0.02**	**11.12**	**0.0**	1.03	0.41	0.55	0.74
Group	0.95	0.33	1.83	0.18	0.0	0.98	1.43	0.24	0.12	0.72
Day-time * group	0.66	0.66	0.25	0.94	0.65	0.66	0.27	0.92	0.45	0.81

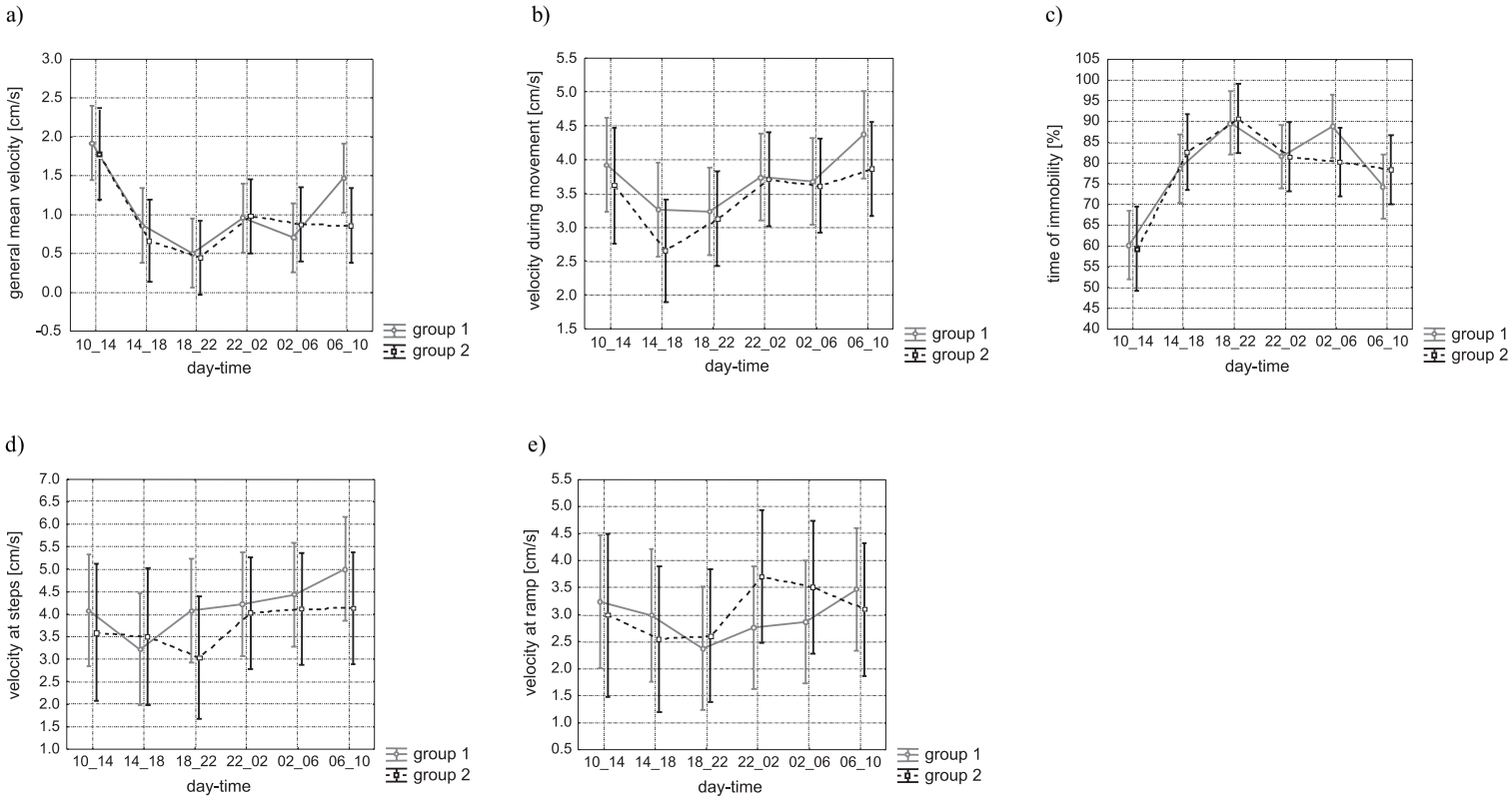

Fig. 4. Results of factorial ANOVA for: (a) general mean velocity, (b) velocity during movement, (c) time of immobility, (d) velocity at steps, (e) velocity at ramp. Vertical bars denote 0.95 confidence intervals. Horizontal axis represents ranges of time (hour) of analysed data

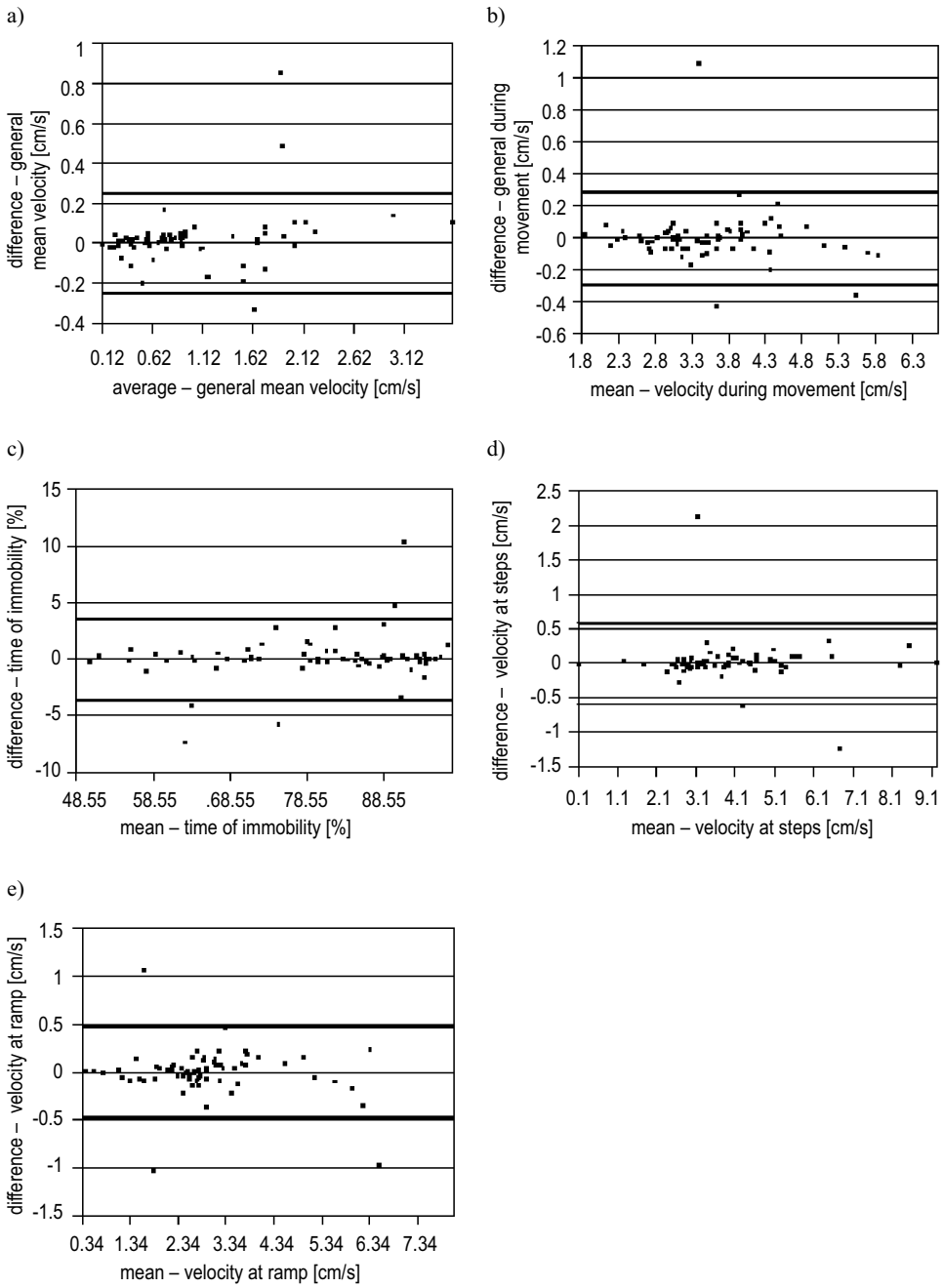

Fig. 5. Results of the Bland-Altman analysis for: (a) general mean velocity, (b) velocity during movement, (c) time of immobility, (d) velocity at steps, (e) velocity at ramp. Vertical axis presents the differences between two measurements. Horizontal axis represents mean values of the two measurements. The thick lines present variability coefficient

4. Discussion

Using the 24 hour automated monitoring system we have examined two groups of wild-type mice. The results point out that there are no differences between the tested groups. The ANOVA test demonstrates the influence of daytime on some of the parameters (the total velocity, the velocity during movement, the time of immobility), what is the effect of mice's activity day cycle. High activity between 9 am and 12 am could be caused by the stage of a new environment exploration. All results are in accordance with our assumptions. The demonstrated system did not detect any disparity between two groups of wild-type mice.

5. Conclusion

The proposed system for automatic examination of neurological motor functions has several advantages when compared with the commonly used manual neurological examination. It does not demand any researchers' engagement in the process of animal examination, which ensures unbiased testing. The animal monitoring can be performed continuously for long-term periods, ensuring robustness and high sensitivity of the results. Automated procedure and precise calibration will give the same results in each analysis of the same data, which is the common known feature of deterministic computer programs.

The maze and the software were designed to facilitate further, more precise experiments. The obstacles within the maze can be exchanged or modified and designed to test many different functions in addition to the selected for this particular study. For example, the system is capable of detecting selection of direction at corridor branch points ("corner test") or recording the tested animals giving a side view (side camcorders) in one of the corridors (back walls are painted white), that for instance might be used for grid performance testing [9]. The software is dedicated to the presented maze. To use it for a different maze a change of the code is necessary. The parameters describing motion presented in this paper make possible computing of derivative parameters, for example: the time of activity stems from the time of immobility and the past path can be calculated from the general mean velocity. A limitation of our study was the permanent lighting of the maze (no day-night cycle), necessary for a proper image recording. However, in future this could perhaps be modified with use of more sensitive video cameras or infrared sensors.

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