An Alternative Low-Cost Solution for Tracking Laboratory Animals

Deney Hayvanı İzlemi için Düşük Maliyetli Alternatif Bir Çözüm

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ABSTRACT

Objectives: Monitoring animal behavior under various conditions can provide important information on their neuropsychological status, including learning, memory, and cognitive activity. Several commercial tracking systems are available, but they may be too expensive for low-budget projects. This study developed an alternative solution for automated animal tracking in behavioral experiments.
Methods: The proposed system was designed to analyze a set of images sampled from a recorded video file in chronological order. The instantaneous location of the animal in each image frame was defined automatically, using a feature-extraction algorithm. Distances traveled were calculated using the coordinates of the successive instantaneous locations. The algorithm was tested using two arenas: the Morris water maze and open field test. The calculated measures were compared with those obtained manually. The internal consistency of the dataset was checked using Cronbach's alpha. The accuracy of the results was evaluated using the paired samples *t*-test and Pearson correlation, with the level of statistical significance set at $p<0.01$.
Results: A statistical comparison of the distances traveled, which were derived from the coordinates of successive locations, did not differ significantly between the manual and automatic methods ($r=0.954$ and $p=0.792$ for the Morris water maze; $r=0.996$ and $p=0.024$ for the open field test).
Conclusions: These results suggest that the algorithm is reliable and valid for estimating coordinates and may serve as a high-resolution tool for animal behavior experiments. We intend to make this software freely available to interested readers and to open feedback channels for further development.

Key Words: Behavioral animal experiment, animal tracking, behavioral measures, low cost solution, Morris water maze, open field test

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ÖZET

Amaç: Deney hayvanı davranışının özel koşullar altında izlenmesi, öğrenme, hafıza ve bilişsel aktivite de dahil olmak üzere deney hayvanlarının nöropsikolojik durumları hakkında önemli bilgiler elde edilmesine olanak sağlamaktadır. Ticari olarak mevcut olan bir çok deney hayvanı izleme sistemi, düşük bütçeli araştırma projeleri için çok pahalı olabilirler. Bu çalışma, davranış deneylerinde otomatik hayvan izlemi için alternatif bir çözüm geliştirmek amacıyla tasarlanmıştır.
Yöntem: Önerilen sistem, deney sonrası video kayıtlarından kronolojik sırada örneklenmiş bir dizi görüntüyü analiz etmektedir. Deney hayvanın anlık koordinatları, zaman bilgisi içeren ardışık görüntülerden özel bir yazılım algoritması ile otomatik olarak hesaplanmaktadır. Yazılım algoritması iki farklı arena kullanılarak test edildi: Bunlar sırasıyla Morris su tankı ve açık alan testi. Yazılım ile belirlenen ölçümler el ile elde edilenlerle karşılaştırıldı. Veri kümesinin iç tutarlılığı Cronbach'ın alfa kullanılarak kontrol edildi. Sonuçların doğruluğu, eşleştirilmiş örnekler t-testi ve Pearson korelasyonu kullanılarak değerlendirildi. İstatistiksel anlamlılık düzeyi $p<0,01$ olarak kabul edildi.
Bulgular: Deney hayvanının, ardışık görüntülerdeki koordinatlarından hesaplanan kat ettiği toplam mesafelerin, el ile belirlenen mesafelerin karşılaştırmasında istatistiksel olarak anlamlı bir farklılığa ratlanmadı (Morris su labirenti için $r = 0,954$ ve $p = 0,792$; $r = 0,996$ ve $p =$ Açık alan testi için 0,024).
Sonuç: Bu sonuçlar yazılımın, deney hayvanı koordinatlarının belirlenmesinde geçerli ve güvenilir bir algoritmaya sahip olduğunu ve davranış deneyleri için yüksek çözünürlüklü, düşük maliyetli bir alternatif olarak değerlendirilebileceğini düşündürmektedir. Bu çalışma ile, yazılımın ilgilenen araştırmacılar için serbestçe kullanılabilir hale getirmek ve geliştirilebilmesi için geri bildirim kanallarının açılması amaçlanmıştır.

Anahtar Sözcükler: Davranışsal hayvan deneyi, hayvan izlemi, davranışsal ölçümler, düşük maliyetli çözüm, Morris su tankı, açık alan testi

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INTRODUCTION

The systematic tracking of an object is used in a broad range of applications, from academia to industry. Tracking individual animal movements in the field or monitoring the position of elderly people living alone uses similar technology. Experiments based on monitoring laboratory animals have long been used as standardized tests in medicine because similar animals respond to similar stimuli in comparable ways (1). To achieve different aims, studies use different laboratory animals and various arena designs, including labyrinths (mazes); open, closed, and elevated fields; and water tanks (2).

By monitoring the behavior of an animal in a specific situation (*e.g.*, prize/penalty responses, avoidance, escape, or anxiety), important information on learning, memory, and cognitive activities as well as the function of the hippocampus, striatum, and amygdala can be gathered (3–5).

A behavioral experiment is evaluated by analyzing the actual behavioral patterns of an animal in comparison with a control group. Predefined measures used for such comparisons include the reaction time/strength/latency to certain stimuli, ratio of true to false responses, number of repetitions needed to achieve predefined performance criteria, recall time from memory, preferred locations in the arena, movement speed, distance travelled, and time to reach a target (2). Another important preparatory aspect of such experiments is to choose the best method that enables reliable, accurate, fast, easy, and economical data analysis.

Typically, data are collected off-line from video files recorded during experiments. This process is performed manually or automatically using computer-based image-analysis tools.

In the manual method, an observer makes note of predefined behavioral measures and extracts the Cartesian coordinates of the animal's instantaneous location manually (6,7). The main advantages of the conventional manual method are that it is economical and can identify irregular behavioral patterns that are impossible to extract using automated methods. However, manually extracted data have problems with reliability and repeatability due to inter- and intra-observer variability (8). Therefore, the level of observational errors should be considered when interpreting measurements made manually (9). Although repeated measurements will increase the validity, they are laborious and time consuming (7).

Computer-based automated animal-tracking systems permit highly accurate, consistent data extraction quickly. There are several commercial packages for this (Table 1). Each system focuses on the common and special demands of users, although the relatively high initial cost of these systems may be too expensive for promising low-budget projects. Free software and toolboxes are available; however, each has its own advantages, limitations, and dependencies (19–21). Therefore, we developed our own system to enable a large degree of end-user control and flexibility independent of any software that allows an alternative solution for problems that we may face in our laboratory. This study describes the main features of our stand-alone video-tracking system for behavioral animal experiments and discusses its reliability and validity.

Table 1. List of commercial software

HVS Image Video Tracking and Analysis Software	HVS Image Software Ltd	http://www.hvsimage.com/
ANY-MAZE Video Tracking Software	San Diego Instruments	http://www.sandiegoinstruments.com/
MazeScan Water Maze Software	CleverSys Inc	http://cleversysinc.com/
EthoVision® XT Video Tracking Software	Noldus Information Technology	http://www.noldus.com/
Actual-Track Video Tracking Software for Viedomix System	Columbus Instruments	http://www.colinst.com/
Smart 3.0 Advanced Video Tracking Software	Coulbourn Instruments	http://www.coulbourn.com/

METHODS

Any laboratory animal-tracking system is comprised of three subsystems. The arena where the laboratory animal moves and the video recording system (webcam) are two of these subsystems and are standard, easy-to-obtain tools. The third is the automated video-tracking and analysis software that tracks the laboratory animal on video files recorded during the experiment. The tool presented in this report was developed and tested in two different arenas: the Morris water maze (MWM) and open field test (OFT) (10,11). The video files used for validation were recorded with a standard webcam during experiments performed in the arenas mentioned above.

The program was written in Borland Delphi ver. 6.0 programming language for Microsoft Windows 98 (Microsoft Corp., Redmond, WA) or higher operating systems.

The program was designed to analyze a set of image frames already captured in temporal order from a video file. This batch capturing process can be done using readily available shareware and was not included in our main tool. The Cartesian coordinates of the animal's instantaneous location in successive images are extracted by an image-processing algorithm. Previously defined experimental measures are calculated using these data and displayed on the screen. Finally, the results are saved permanently in a spreadsheet file for further analysis. The performance of the tool was tested for experiments performed in two different arenas (10,11). The water maze arena had circular geometry with a diameter of 120 cm and open field arena had square geometry with the dimension of 60x60 cm. The operational screen design and variables were organized in a single-page user-friendly graphical interface. Figures 1 and 2 show screenshots of the video-tracking process in the MWM and OFT, respectively. Possible future upgrades to the structure of the software are being considered.

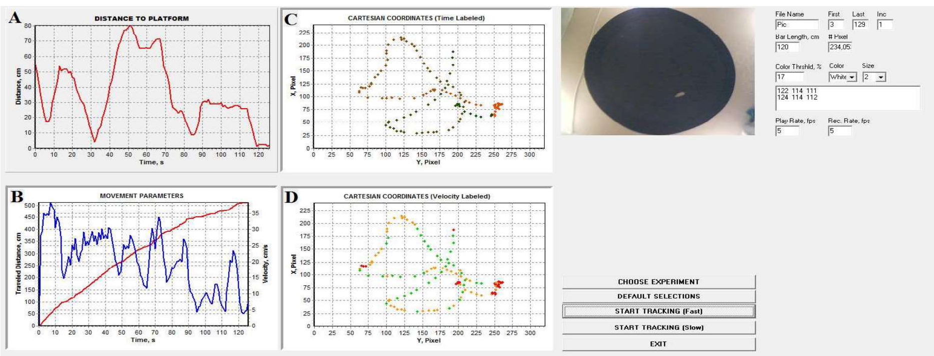

Figure 1. Screenshot of MWM test video tracking: (A) Change in the distance to hidden platform with respect to time (s) in MWM test. (B) Change in the total traveled distance (steadily increasing line) and the instantaneous movement velocity (alternating line) both with respect to time (s) were plotted on the same graphic. The primary axis denotes distance in cm and the secondary axis denotes velocity in cm/s. (C) Time is color coded in the traveled pathway plotted in x-y coordinates. (D) Instantaneous movement velocity is color coded in the traveled pathway plotted in x-y coordinates. Abbreviations; MWM: Morris water maze, OF open field

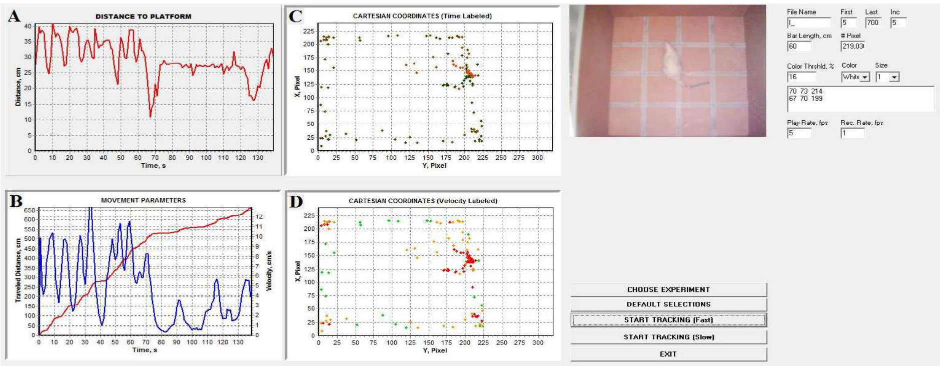

Figure 2. Screenshot of OF test video tracking: Description is the same as in Figure 1. Abbreviations; OF open field

Image Processing

To optimize the processing time and minimize errors when locating the instantaneous coordinates of the animal, the system lets the user define/adjust the region of interest, initial location, and threshold intensity of the color to be tracked. The color that is going to be scanned throughout the image may be a paint marker stuck on the head of the animal (12). This marker is then extracted using a blob-detection algorithm that labels each pixel within a blob with the same label number so that the center of the connected-component forms the coordinate of the instantaneous location. The process for automatic labeling of pixels in an image is described schematically in Figure 3.

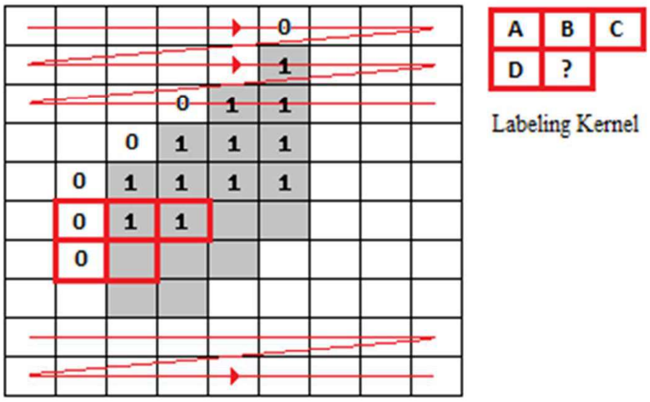

Figure 3. Automatic pixel labeling process: Labeling kernel is applied to per pixel in order to define the label at the pixel position "?" by scanning the image matrix from top left to bottom right. This process is used to check the labels of neighboring pixels (pixels A, B, C and D). If the pixels of the labeling kernel at its current position previously labeled as A=0, B=1, C=1, D=0 then the "?" has to be labeled as 1 (inspired by http://www.labbookpages.co.uk/software/imgProc/ blobDetection.html and slightly modified).

The series of Cartesian coordinates representing the locations along the path of the animal's movement are stored in an array. Since the image frames were sampled in temporal order (Figure 4), the array elements consist of time information intrinsically. The instantaneous locations forming the path that the animal followed are plotted on the graphical user interface. The distance that the animal traveled between two successive pictures (instantaneous traveled distances, ITD) was calculated by applying the Pythagorean Theorem to the respective Cartesian coordinates. The entire pathway for each arena is shown on the screen (Figs. 1C and 1D, and 2C and 2D). The time (Fig. 1C and Fig. 2C) and movement velocity (Fig. 1D and Fig. 2D) are color coded into the pathways in both plots. The screen also displays the total traveled distance (TTD), the instantaneous velocity of movement (Fig. 1B), and the distance to the hidden platform (Fig. 1A).

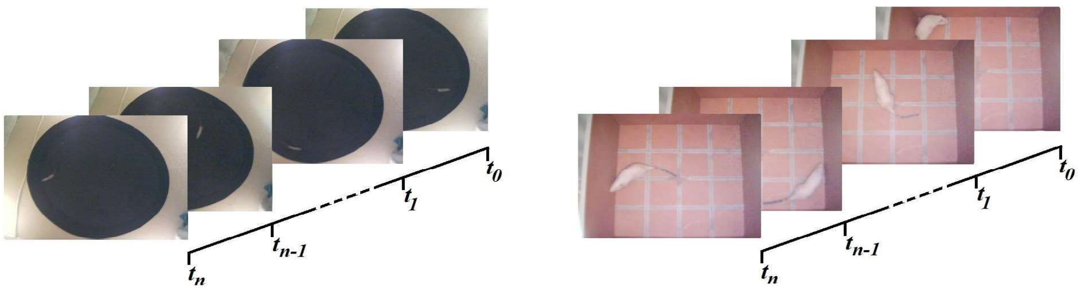

Figure 4. Demonstrative presentations of the successive image frames captured from the video files of two different experiments. Samples of MWM Test are given on the left, OF Tests samples on the right. t_1 ..t_n denotes equally spaced discrete time points. Abbreviations; MWM: Morris water maze, OF open field

Statistical Analysis

The video file from each experiment was evaluated based on the measures mentioned above both manually and using our new automatic tool. Since repeated manual data collection is accepted as a reliable, conventional method (13), the manual data were compared with the data obtained with the automatic method. Because manual data extraction by the same observer shows good intra-observer repeatability of measurements (9), we used the data for the average of three manual measurements as the reference. Reliability and validity were compared for the ITD and TTD. A statistical analysis was performed using SPSS ver. 17. The intra-observer repeatability for both data extraction methods was evaluated using the paired samples *t*-test and Pearson correlation. Cronbach's alpha was used to assess the internal consistency of the dataset. The accuracy of the predefined measures of ITD and TTD was evaluated using the paired samples *t*-test and Pearson correlation. The level of statistical significance was set to $p<0.01$.

RESULTS

A video file of a 25-s recording made during the MWM test was converted into 125 picture frames at 0.2 s inter-frame intervals. A 138-s video file of the OFT was sampled every 1 s yielding 138 data points. The Cartesian coordinates of the instantaneous locations of the animal during the entire test quantified by the manual and automatic methods are shown graphically in Figure 5A and B, respectively.

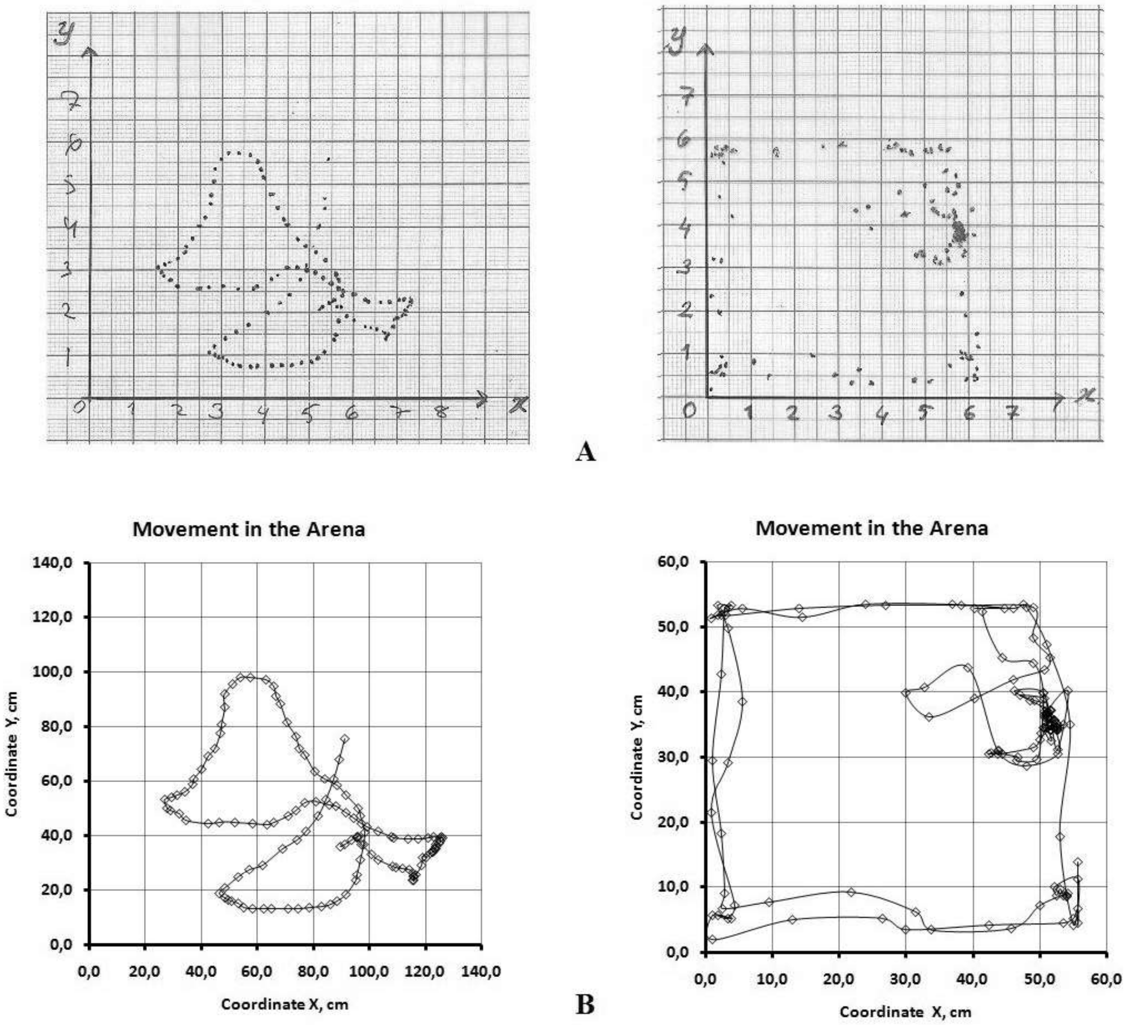

Figure 5. Instantaneous locations of the animal extracted from MWM (left) and OF (right) test video recordings plotted in the respected Cartesian coordinate system. Instantaneous locations were defined by both methods; (A) manual and (B) automatic. Data set of MWM test consist 125 points, OF test data set have 138 points. Abbreviations; MWM: Morris water maze, OF open field

The Cartesian coordinates were used to calculate the parameters of interest. Before validating these parameters, the reliability of the extracted data was evaluated by determining the repeatability and internal consistency for the ITD (Table 2). The results indicated that the three pairs of manually extracted measurements were strongly positively correlated. The weakest pairs for the MWM and OFT were "Pair 1&2" ($r=0.816$, $p=0.323$) and "Pair 1&2" ($r=0.985$, $p=0.608$), respectively. The correlations were stronger for the automatically extracted data pairs. Except for the two measurement pairs from the MWM test, which were strongly correlated ($r=0.983$, $p=0.352$), the other four measurements of both tests made with the automatic method were identical ($r=1.000$, p=N/A). The analysis revealed no significant difference between any of the measurement pairs. Cronbach's alpha (lowest alpha=0.942) also revealed good internal consistency for the repeated measurements (14).

The ITDs obtained with the manual and automatic methods were compared using the paired samples *t*-test (Table 3). The ITD differences and comparisons were given in Figure 6A and B, respectively. There were no significant differences between the manual and automatic methods ($r=0.954$, $p=0.792$ for the MWM and $r=0.996$, $p=0.024$ for the OFT).

Table 2. Reliability test results of the three measurements made by manual and automatic methods. The pairs of the three measurement combinations were compared by Pearson correlation (correlation coefficient, r) and paired samples t-test (t and p values). Cronbach's Alpha (Alpha) tested the internal consistency of repeated measurements. Abbreviations; MWM: Morris water maze, OF open field, ITD: instantaneous traveled distances, N: number of data points, SEM: standard error of mean

	Test Arena	ITD Measurement Pairs	N	Paired Differences, cm		r	t	p	Alpha
				Mean	SEM				
Manual	MWM	1&2	125	0.1193	0.0326	0.816	-0.992	0.323**	
		2&3	125	-0.0951	-0.0229	0.848	0.862	0.390**	0.942
		1&3	125	0.0242	0.0096	0.891	-0.286	0.775**	
	OF	1&2	138	0.0304	-0.0037	0.985	-0.514	0.608**	
		2&3	138	-0.0065	0.0012	0.991	0.149	0.882**	0.996
		1&3	138	0.0239	-0.0026	0.988	-0.455	0.649**	
Automatic	MWM	1&2	125	-0.0307	0.0054	0.983	0.935	0.352**	
		2&3	125	0.0307	0.0054	0.983	0.935	0.352**	0.996
		1&3	125	0.0000	0.0000	1.000	*	*	
	OF	1&2	138	0.0000	0.0000	1.000	*	*	
		2&3	138	0.0000	0.0000	1.000	*	*	1.000
		1&3	138	0.0000	0.0000	1.000	*	*	

* Value cannot be computed because SEM is zero
** Level of statistical significance is 0.01

Table 3. Validity test results of the three measurements. Mean ITDs calculated by manual and automatic methods were compared by Pearson correlation (correlation coefficient, r) and paired samples t-test (t and p values). Abrevations; MWM: Morris water maze, OF open field, ITD: instantaneous traveled distances, N: number of data points, SEM: standard error of mean

Test Arena	Mean ITD, cm		N	Paired Differences, cm		r	t	p
	Auto	Manual		Mean	SEM			
MWM	4.3732	4.2957	125	0.0145	-0.0118	0.954	0.264	0.792**
OF	3.6226	3.6081	138	-0.0770	-0.0037	0.996	-2.282	0.024**

** Level of statistical significance is 0.01

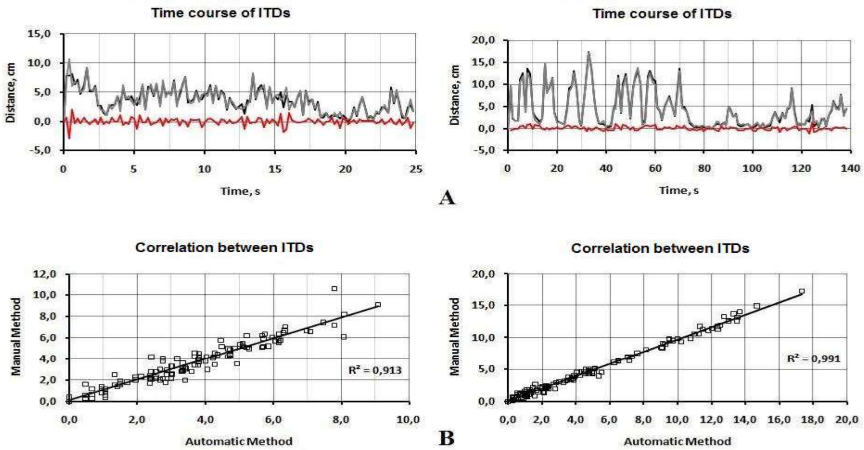

Figure 6. Correlation between manual and automatic methods. (A) Time course of ITDs calculated from manual (gray line) and automatic (black line) data for MWM (left) and OF (right). The time course of the difference between the methods was also given in the same graphic (red line) in the order to emphasize on its negligible magnitude. (B) Scatter plot presentation of manual and automatic ITDs showed strong correlation ($R^2 > 0.91$). Abbreviations; MWM: Morris water maze, OF open field, ITD: instantaneous traveled distances

TTD is calculated by summing the ITDs over the test time. Therefore, the alternating (randomized) nature of the instantaneous measuring errors (*i.e*., positive and negative values) could cancel the total error in TTD. For MWM, the TTD was 448.8 and 447.7 cm for the manual and automatic methods, respectively. This corresponds to a 0.24% total measuring error within the 25-s MWM experiment. In the OFT, the TTD was 601.9 and 592.6 cm by the manual and automatic methods, respectively, for the 138-s experiment, resulting in a total error of 1.55%.

DISCUSSION

Laboratory animal behavioral experiments are a widely used, efficacious tool in the evaluation of the neural system, learning, memory, and cognitive function; assessment of anxiety/depression status; and experimental neuropharmacology studies (15–18). Under certain circumstances, experimental animals may perform special behaviors such as grooming and rearing. Small animals may sometimes move so quickly that they are difficult to follow, even with high-technology tracking systems. Therefore, conventional monitoring by the naked eye is still important in the evaluation of behavioral experiments (12). However, there is increasing effort to analyze such experiments objectively with automated systems. These include computer-based automatic animal tracking using video recordings and image analysis algorithms (15–18).

In this paper, we introduced laboratory animal tracking software that analyzes a video file of an experiment as an alternative to costly commercial tools. The software was tested in two different arenas. In general, the validity and reliability of a measuring tool can be assessed by defining its static transfer characteristics, especially the repeatability, internal consistency, and accuracy of the measurements. Since no measurement tool is valid unless it is reliable, before evaluating the accuracy of a new tool it is important to verify the repeatability and internal consistency of the measurements first (9,14).

The accuracy of the data extracted using our tool was assessed by comparing it with the results obtained using a manual method. The subjectivity of the manual measurements was minimized by making repeated data extractions by the same observer; this showed that a desirable reliability level (9,14) was obtained with the manual measurements (worst case; r=0.816, p=0.323, alpha=0.942). A comparison of the automatic and manual measurements found a good correlation between the methods (worst case; r=0.954, p=0.792). The total measurement error when compared with the manual method ranged from 0.24 to 1.55% depending on the size of the test arena.

The correlation analysis revealed that the algorithm used for color scanning in our tool produced reliable, accurate results when compared with the manual method. Although our results were encouraging for experiments performed in two different arenas, several additional areas should be investigated. These include performing extended experiments with various laboratory animals and in different arenas, and advanced feature-extraction algorithms for fast, specific body movement analysis (*e.g*., grooming, rearing, and stretching). Future goals are to add other implementations, including real-time analysis; high-speed, high-quality video recording; and batch video capture.

In conclusion, the current version of the software presented here is a valid, accurate tracking tool for laboratory animal behavioral experiments. Considering its abilities and limitations, we believe that this tool can be used reliably in low-budget projects. We intend to share the software with interested readers for their projects that may have possible contributions in this area. The software can be obtained directly from the corresponding author (leyla3b@yahoo.com) free of charge.

Conflict of interest
No conflict of interest was declared by the authors.

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