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A simple webcam-based approach for the measurement of rodent locomotion and other behavioural parameters

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Abstract

We hereby describe a simple and inexpensive approach to evaluate the position and locomotion of rodents in an arena. The system is based on webcam registering of animal behaviour with subsequent analysis on customized software. Based on black/white differentiation, it provides rapid evaluation of animal position over a period of time, and can be used in a myriad of behavioural tasks in which locomotion, velocity or place preference are variables of interest. A brief review of the results obtained so far with this system and a discussion of other possible applications in behavioural neuroscience are also included. Such a system can be easily implemented in most laboratories and can significantly reduce the time and costs involved in behavioural analysis, especially in developing countries.

Keywords: Locomotion; Displacement; Rodent; Webcam; Software

1. Introduction

Measurement of position and locomotion are central to the study of innumerable features of animal behaviour, including memory, anxiety, spatial orientation and novelty seeking. Moreover, locomotion analysis is also important to screen for neurological effects of various drugs and pharmacologically induced hyperlocomotion is one of the most widely used models to test compounds for antipsychotic activity (Kapur and Mamo, 2003; Geyer and Ellenbroek, 2003).

Analysis of animal movement has come a long way from the early days of neuroscience. For decades, little more was available to measure locomotion other than a stopwatch, an arena containing a grid pattern on its floor and a hard-labouring postgraduate student to count the number of squares crossed over a given period of time. In fact, this approach was so widely used that it still holds its ground in some recent work, particularly in the developing world (Vianna et al., 2000), due to its extremely low cost in financial terms, if not in time-efficiency.

This method was initially replaced by photocell-based systems which automatically recorded animal crossings (Ericson et al., 1991). These began to be used more than 30 years ago (Weifenbach, 1969) and gradually gained acceptance, becoming perhaps the most popular way to evaluate locomotion until recently. These systems, however, were (and still are) relatively expensive and based their estimates of locomotion on indirect discrete measures (number of crossings, as opposed to actual path measurements).

Video analysis of animal movement began to be used in the early 1980s (Godden and Graham, 1983) and started to gain wider acceptance about a decade later (Schwarting et al., 1993). However, most of these systems were and still are dependent on specific hardware (usually commercialized in a package with the software) and brought little cost improvement (Pan et al., 1996). Only recently, with the explosion of cheap "webcam" equipment, has this technology been brought to the point where it can be implemented using standard computer equipment already

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present on virtually any laboratory in the world. A few studies have been published in recent years describing webcam-based software for locomotion analysis (Lind et al., 2005; Togasaki et al., 2005); surprisingly, however, we have found little data dealing specifically with the application of such software in rodents.

We hereby propose a simple software algorithm for the tracking of rodent position by analyzing webcam-registered images. This algorithm can be easily adapted to other species and special behavioural situations through simple variations in its code. We discuss some of the myriad applications of this inexpensive methodology in the neurosciences, briefly presenting results we have obtained with it (Coitinho et al., 2002; Lourenco Da Silva et al., 2003; Dall'Igna et al., 2003, 2004, 2005; Tort et al., 2004, 2005; Dietrich et al., 2004, 2005; da Silva et al., 2005; de Oliveira et al., 2005; Kazlauckas et al., 2005) and proposing other applications to which it can be easily adapted.

2. Materials and methods

Our system consists of a program written in Pascal language through a Delphi interface, which is able to run on most Microsoft Windows® operational systems (Windows 95/98/2000/XP) and was programmed to export data to Microsoft Excel®. The software, which we have baptized as Mousetracker, was developed to analyze video files of animal experiments previously recorded through a webcam or other electronic video recording apparatus. A current version of the software can be obtained by contact with the authors at mousetracker@gmail.com for no charge. Later, improved versions may be made available commercially in the future, at prices which will still be substantially lower (around 90% cheaper) than hardware/software packages available in the market.

The program has been programmed with a simple, instinctive interface, based on a relatively small amount of buttons and functions (Fig. 1). The program occupies little hard drive space (less than 600 kB) and its hardware requirements include no more than a Pentium class or equivalent processor (we routinely use a 1.6 GHz Athlon processor with 512 MB RAM) with a webcam and proper video recording software installed. We usually set the webcam recording software to acquire videos in AVI format at 4 frames/s in 320 × 240 pixels resolution (yielding an occupation of about 500 kB of hard drive space per minute), but this is not mandatory, although the same configuration should be kept throughout a set of experiments. Microsoft Excel® software is required for exporting of the data.

The software has been initially designed to detect light-coloured animals in a dark arena, and it will be thus described, although it can be easily adapted to do the opposite (as could be of interest for experiments involving C57BL/6 mice, for example). It works through black/white differentiation, based on a grayscale threshold for animal detection. The program will then consider every pixel lighter than the threshold as being white (i.e., part of the animal) and every pixel darker than the threshold as being black (i.e., part of the arena), as shown in Fig. 2. This threshold can be modified by the user, who can visualize the "detected" areas as he varies this parameter until they include the animals, but exclude features of the background. However, we emphasize that the threshold should not be set in a level which includes only small portions of the animals as white, as this can produce fluctuations in animal detection and overestimation of movement (Fig. 2).

An ideal arena should be dark and opaque and the illumination of the room should be diffuse enough not to allow light to reflect on any point of the arena. Note that the arena we have shown in Fig. 2 is not ideal, as it contains some reflected light, which nevertheless is below the grayscale threshold level. Moreover, we stress that the arena should also be tested for the onset of light reflection when it is wet, once animal urine can be produced during experiment recordings. Still, if small white areas persist in the background, the program has a routine that allows

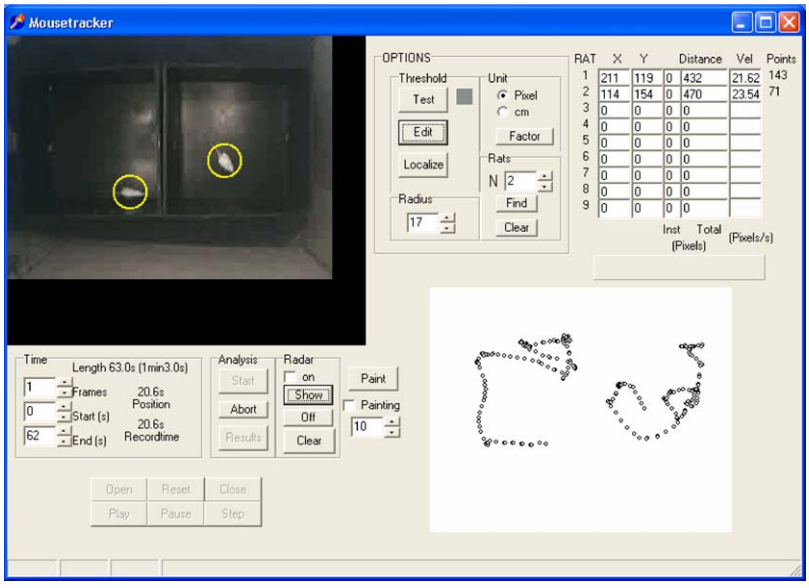

Fig. 1. User interface of the Mousetracker software. Note video display with circled animals (upper left), selected parameters and control buttons (top and lower left), locomotion counts (upper right) and path tracer (lower right).

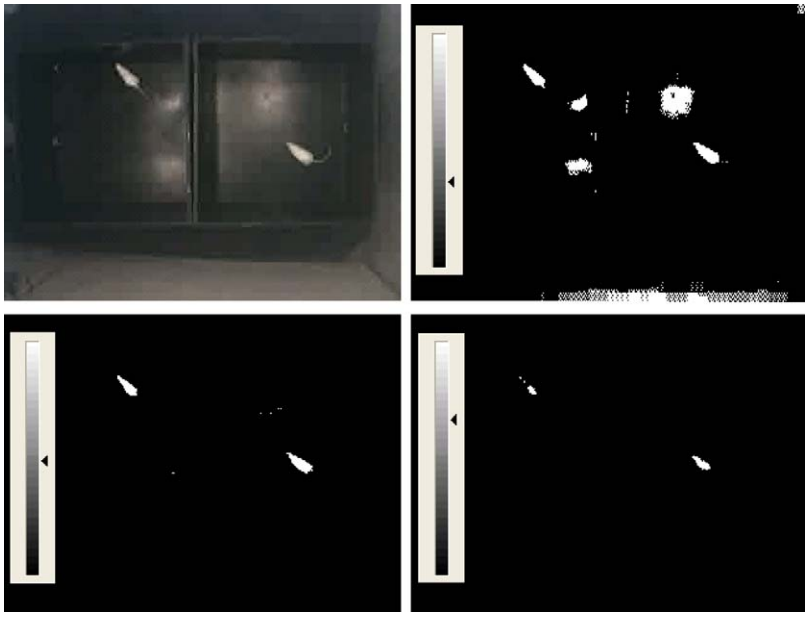

Fig. 2. Effect of the grayscale threshold on animal detection. (a) An actual frame from the video file, (b) an example of a threshold set too low, which includes lighter areas in the background, (c) the adequate threshold, with minimal background interference and (d) an example of a threshold set too high, with inadequate sampling of the animals.

them to be painted "black" with the aid of the mouse, leading them to be ignored by the system throughout the analysis period.

Once the grayscale threshold is set, the user has to identify the initial position of each animal by using the mouse. The software can analyze as many animals as desirable, providing that each animal remains in a separate part of the arena and the walls dividing them are large enough to prevent animals from approaching each other excessively. Moreover, the separate arenas should be symmetrically distributed around the camera's location to avoid distortion. In measuring locomotion, we have routinely analyzed up to eight mice on each trial.

Besides the grayscale threshold and the initial position of the animals, the user must also inform a value (in pixels) for a parameter called "radius". This variable has this name because, after localizing the animal, the program draws a circle with such a radius around the position of the animal, in a way that the user can be confident that the program is working well (see Fig. 1).

The central algorithm for the localization of the position of the animals on each frame is summarized in Fig. 3. For each animal, the system will automatically calculate the "center of white" (analogously to the calculation of a mass center) in a square area centered on the initial position of the animal and with a side length equal to twice the selected radius, as exposed in Fig. 3A and B. This "limited-radius" search for the animal was found to greatly improve processing speed as opposed to calculations performed on the whole arena. After finding the center of white (i.e., the position of the animal), the program proceeds to the next video frame. It can also be set to skip video frames on a multiple of a parameter called "frames", which has a default value of 1. In this new frame, the program will again calculate the center of white on a square of the same area, but this time centered on the position of the animal in the previous frame (Fig. 3B and C). This procedure is then repeated until the end of the analysis.

The process described above occurs very quickly (around 50–350 ms per frame, depending on the number of animals and radius value, on a 1.6 GHz processor with 512 MB RAM), in a way that the user has the impression of a continuous displacement of the circle containing the animal. Therefore, the conversion of the results is usually faster than the actual length of the video file (i.e., the conversion of a 1-min long video including two animals with a radius of 10 pixels takes about 14 s; analysis

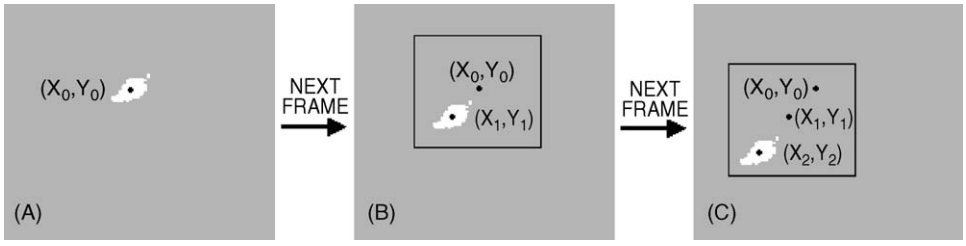

Fig. 3. Scheme of the system's central algorithm: (A) initial position (X_0, Y_0) of the animal should be informed manually with a mouse click. (B) The software then analyzes the next video frame, searching for the center of white on a square area centered on (X_0, Y_0), which corresponds to the new position of the animal (X_1, Y_1). (C) Similarly, in the next frame, the program searches for the animal's new position (X_2, Y_2) in a square centered on (X_1, Y_1), and so on. Based on the information of the position (X_t, Y_t) of the animal on each time t, it is possible to obtain the displacement, velocity, path and time spent in an area of interest, among other variables.

of eight animals at the same time will increase this to 55 s). Shorter frame intervals, higher numbers of animals and greater radius values cause the program to run more slowly. If the radius value is too small, the program can eventually lose track of an animal if its dislocation exceeds the area of the square from one analyzed frame to the next. If this happens, however, the user can easily click on the animal to allow the computer to track it again. It is also possible to pause the analysis and manually modify parameters such as radius and grayscale threshold after the program is running if needed.

By knowing the position of the animals at each point in time, the software can then compute locomotion, speed and acceleration. A button click can also access a feature which traces the animal's path on a white screen (Fig. 1). Moreover, for behavioural tasks in which animal position/place preference is a concern (e.g. object recognition and water maze probe tests), the user can select an area of interest in the arena, and the software will also obtain data on time spent within and outside of that area at the same time that it computes locomotion.

Lastly, note that locomotion and other variables can be obtained for different intervals of time within the video file's length. Therefore, after data are obtained, the user selects the time intervals (i.e., blocks of 30 s, 5 min, etc.) for the data to be exported to an Excel® spreadsheet.

3. Results and discussion

3.1. Validation

To confirm the validity of our method in measuring locomotion, we performed 5-min recordings of 22 animals in a 50 cm × 50 cm square arena with our webcam system. The locomotion data obtained for these animals with the software was compared with manually obtained analysis of the number of crossings of each animal after dividing the arena in 5 cm × 5 cm squares. Linear correlation of these two variables (automated and manual locomotion measurements) was performed (Fig. 4) and yielded a highly significant correlation coefficient (r) of 0.976 ($p < 0.001$). Moreover, we believe the minor deviations from the straight line seen in the figure are likely to represent limitations inherent to estimating locomotion by manual counting of crossings, rather than inaccuracies of the software in measuring this parameter. This strong correlation, therefore, makes one comfortable that the software does indeed provide reliable measurements of locomotion.

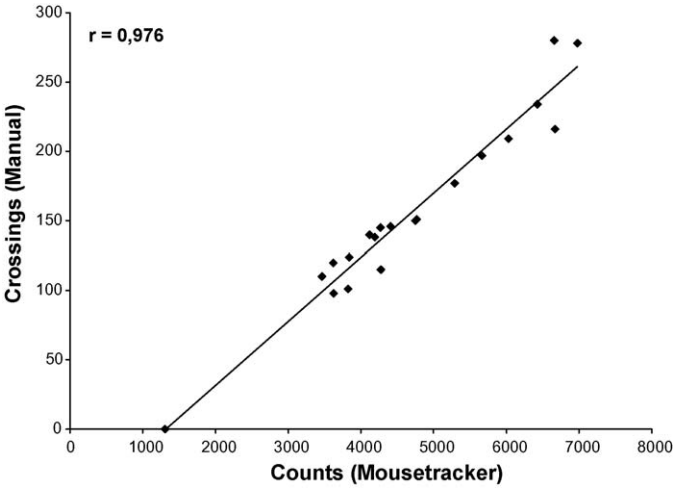

Fig. 4. Correlation between manual recording of number of crossings (y-axis) and automated registering of locomotion (number of "counts" or pixels traveled) by the Mousetracker software (x-axis). Points are very close to the straight line and statistical analysis yields a strong and significant correlation coefficient ($r = 0.976$, $p < 0.001$).

3.2. Applications

3.2.1. Evaluation of spontaneous locomotor activity

Perhaps the most obvious application for a system designed to measure locomotion is the evaluation of spontaneous locomotion, as shown in Fig. 5a. By simply exposing an animal to an open field, one can look for motor impairment caused by drugs and other interventions on animal locomotion. Although this is rather nonspecific, as increases or decreases in locomotion can occur due to a variety of causes (including some not related to motor impairment), this can be useful as a screening test. In this setting, we have used our system to show evidence of motor impairment by chronic, low dose exposure to methylmercury in drinking water (Dietrich et al., 2005).

3.2.2. Drug-induced hyperlocomotion

Models of drug-induced hyperlocomotion have been traditionally used as predictive models to screen for the antipsychotic activity of different compounds. Although the links between the pathophysiology underlying psychiatric illness such as schizophrenia and the increased locomotion induced by compounds such as amphetamine and the *N*-methyl-D-aspartate (NMDA) receptor blockers phencyclidine (PCP) and dizocilpine (MK-801) are unclear, these animal models have shown good predictive validity for the antipsychotic activity of various drugs (Ninan and Kulkarni, 1999; O'Neill and Shaw, 1999; Geyer and Ellenbroek, 2003).

The use of our software allows us to monitor animals in these models for a reasonably long period of time. Therefore, one can analyze locomotion before and after the injection of a hyperlocomotion-inducing compound and the effect of potential antipsychotic drugs in blunting this response. As can be seen in Fig. 5b and e, both amphetamine and MK-801 produce marked increases in total locomotion which gradually decrease with waning of the drugs. A similar effect, although more subtle, can be observed with caffeine (Fig. 5d), which also increases dopaminergic tonus in the central nervous system (Cauli and Morelli, 2005). Note also the "priming" response of multiple injections of amphetamine, which potentiate the response in subsequent administrations (Fig. 5c).

In focusing our search for novel antipsychotic compounds, we have found that compounds known to possess some degree of dopamine D2-receptor blockade activity such as flunarizine (Tort et al., 2005) and cinnarizine (Dall'Igna et al., 2005) are able to blunt and/or abolish the hyperlocomotion response induced by both amphetamine and MK-801 (Fig. 5e). Measurements of

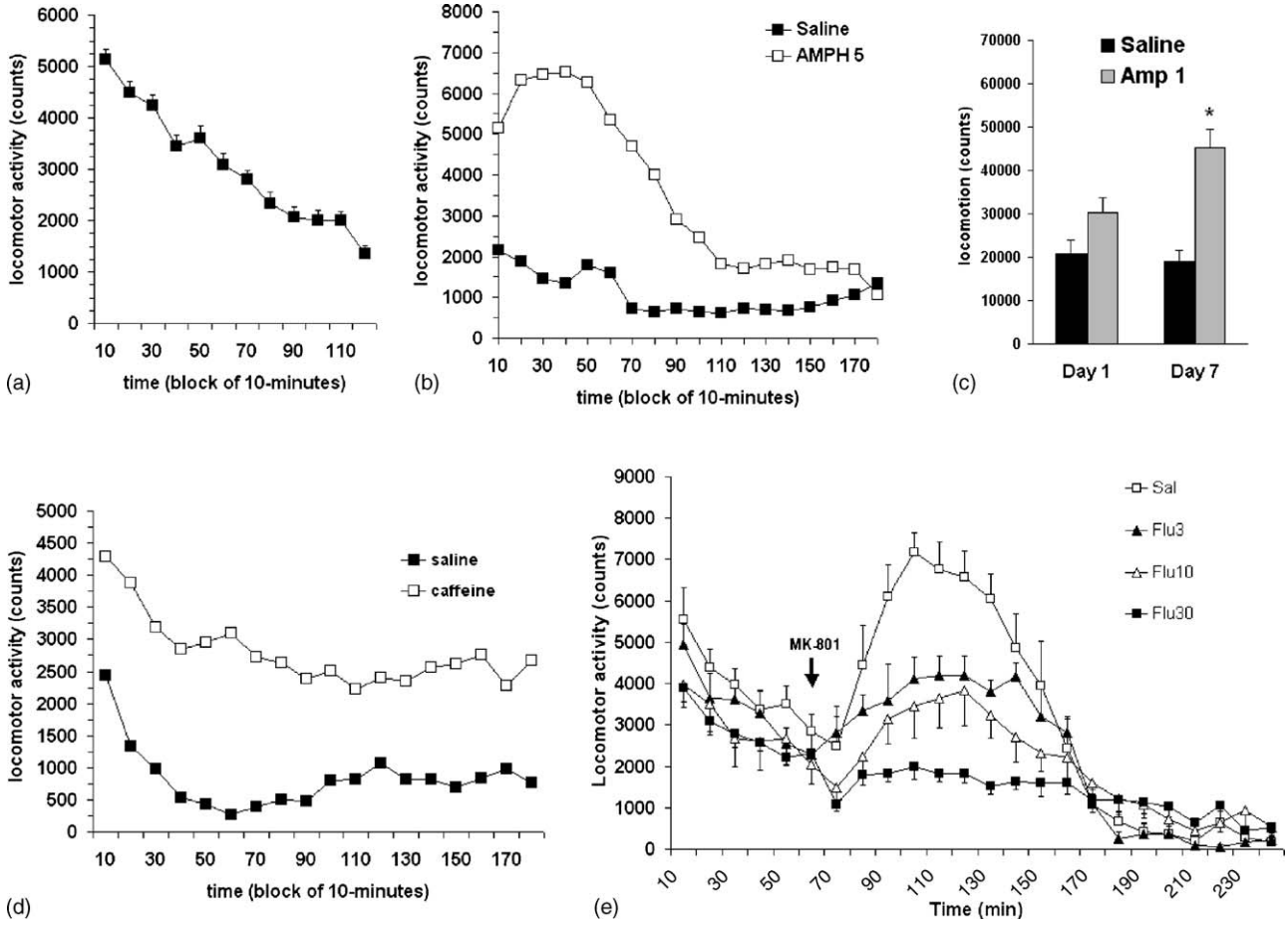

Fig. 5. Locomotion curves obtained with the system: (a) spontaneous exploration of an open field over a 2-h interval, with the *y*-axis ("counts") representing the number of pixels traveled by the animal; decreasing locomotion over time is due to habituation to the environment, (b) hyperlocomotion induced by the administration of amphetamine 5 mg/kg after the habituation period, (c) locomotion over a 2-h period after priming with amphetamine (1 mg/kg daily), evidencing greater response in the seventh administration of the drug than in the first, (d) hyperlocomotion induced by the administration caffeine 30 mg/kg after the habituation period and (e) reversal of MK-801-induced hyperlocomotion by various doses of flunarizine (Tort et al., 2005).

these effects can therefore be easily and rapidly performed by our software and can be of use in screening for drugs with potential antipsychotic activity. These results are in accordance with other evidence for antidopaminergic actions of flunarizine (Hori et al., 1998) and have warranted currently ongoing clinical studies involving this drug.

3.2.3. *Object exploration*

By focusing on animal position rather than on locomotion, other behavioural information can be obtained by the use of our system. Measures of exploratory activity, for example, are central to various models used in the evaluation of behavioural features such as anxiety (Belzung and Griebel, 2001) and memory (Rampon et al., 2000).

Exploration of a novel object or environment is an innate feature of rodent behaviour, but can be inhibited in situations in which anxiety or avoidance-related features are prominent in the animals. Our system has been innovatively used for the evaluation of object exploration as a distinguishing feature of animal temperament, which was used to differentiate mice into two groups with high and low innate exploratory activity, respectively (Fig. 6). The system was used to measure time spent in the center of an arena (in which the object to be explored was placed and "high explorer" animals tended to dwell) and in its periphery (in which "low explorers" remained, sometimes throughout the observation period). These two groups of ani-

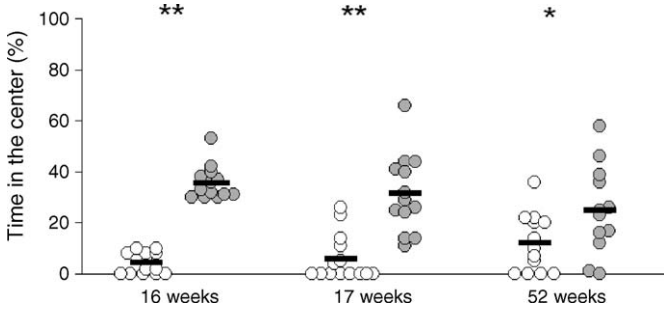

Fig. 6. Separation of high and low exploratory phenotypes among a population of CD-1 mice. The graph shows the percentage of time spent in the central portion of an arena with a central object over 5 min, as analyzed by the system. Animals were divided as high exploring (gray dots) and low exploring (white dots) animals according to their performance in the test at 16 weeks of age and differences between the two populations (dashes represent mean values for each group) remained significant when they were retested after 17 and 52 weeks of age ($^{*}p < 0.05$; $^{**}p < 0.001$) (Kazlauckas et al., 2005).

mals were later shown to behave very differently in several other behavioural tasks involving memory, anxiety and aggressiveness (Kazlauckas et al., 2005), a fact which may render the model relevant for the study of personality traits underlying mood disorders.

3.2.4. Other potential applications

The position tracking feature described above to measure object exploration can also be used in a myriad of other behavioural tasks. Object recognition memory, for example, can be measured by tracing areas of interest around two objects (only one of which has been previously explored), as mice which remember having explored an object will tend to prefer a novel object instead of the known one in a subsequent session (Rampon et al., 2000). The system can also be used to measure place preference in various anxiety tasks, such as light/dark preference and the elevated plus-maze, in which animals are given a choice between sheltered (e.g. dark chamber or closed arms) and nonsheltered environments (e.g. light chamber or open arms). Finally, although we have not yet used the system for spatial memory tasks, such as the Morris' water maze, the software is able to track the position of animal and can feasibly be used for the acquisition of both quantitative (i.e., time spent in an area) and descriptive (i.e., pathway tracing) information, provided that that adequate contrast between the animal and the pool is achieved.

4. Conclusion

In summary, we have described a simple, webcam-based software which can be run on an average personal computer and is robust enough to be useful for most behavioural tasks dealing with locomotion and/or position, as discussed above. Although the use of video tracking systems is vital to some of the tasks we have discussed, and therefore widespread among laboratories around the world, it is still done through commercially available hardware/software systems in most laboratories. By offering an alternative which can be implemented at a much lower cost, we hope to help behavioural neuroscience remain one of the few domains of knowledge in which good ideas can still be worth more than large research grants, making opportunities a bit more equal than usual for scientists all over the globe.

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