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A novel open field activity detector to determine spatial and temporal movement of laboratory animals after injury and disease

Andrew O. Koob^a, John Cirillo^b, Charles F. Babbs^c

Abstract

Among the wide range of tests for laboratory animal behavior after neurological injury or disease, each has its benefits and drawbacks. The varied behavior that an animal exhibits makes it difficult to decide which test to use. However, a fundamental instinct for the laboratory animal is to explore when placed in a new environment. A way to test exploratory behavior is in the open field. Here, we introduce a simple activity box without the use of video equipment to determine the exploratory movement of a rat after traumatic brain injury. The activity box is an open field, and the rat explores its surroundings when placed inside. Four infrared beams are placed in both the *X* and *Y*-axes inside the box. Using a novel system to determine which beam the rat breaks, one can describe where the rat is in space and time while in the activity box. Other models can show the number of beams broken, but here we analyze the results additionally to determine the area explored, the total distance traveled by the rat, and the percent of time exploring.

Key words: Brain injury; Neurological disease; Neurodegeneration; Head injury; Behavior; Laboratory animal; Open field; Exploratory behavior

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1. Introduction

One of the most common behavioral models to study neurological injury and disease remains the open field score (Giulian and Silverman, 1975). Using this simple and effective test, we present a method to construct a novel open field system to study different aspects of exploratory behavior. Various complicated learning and memory tests such as the arm maze (Olton and Samuelson, 1976) and Morris water maze (Morris et al., 1982) were originally developed to test behavior. However, if motor ability or other behavior is impaired in addition to memory, function cannot be adequately described using learning and memory tests (Devan et al., 1996; Vicens et al., 2003).

The open field score was originally a simple test in which an animal was placed in an enclosed area sectioned into a grid. The experimenter then counted how many lines the animal crossed in a 1/2 h (Giulian and Silverman, 1975). The idea was that animals exhibit natural exploratory behavior when placed in a new environment and may cross a number of lines in the allotted time. The open field score measures an animal's mobility and exploratory behavior (Whishaw et al., 1999) and animals that are impaired may not be able to explore either due to motor or cognitive defects (O'Connor et al., 2003; Vink et al., 2003). To use the open field test, animals do not require training, and should be unfamiliar with the open field before entering it to encourage exploratory behavior.

Activity boxes were developed based on this simple model and have been used to study neurological disease by counting infrared beam breaks by an animal in the open field (Marino et al., 2003). Previously, automated systems using techniques such as video tracking and television monitoring were unable to take measurements such as percent of time exploring (Dutrieux et al., 1978; Schwarting et al., 1993; Tomkins and O'Donovan, 1981). Using infrared beams and computer software, a rat's movement in space can also be determined without interference from an experimental observer. To show the capability of this technique, we used a known method of injury that causes large decrements in the open field: the impact acceleration brain injury model (Marmarou et al., 1994). The most dramatic differences between the behavior of injured and uninjured animals with the impact acceleration injury model occur in the open field score (O'Connor et al., 2003; Vink et al., 2003). Here, we describe a novel computational technique to study movement in space and time to measure the distance traveled, area covered, and percent of time moving in the open field in a rat model of traumatic brain injury.

2. Materials and methods

2.1. *Surgical procedures*

Two rats weighing between 400 and 415 g received identical surgical procedures. First, they were anesthetized with 4% isoflurane in 99% oxygen. They were intubated endotracheally and ventilated with 1.5–2.5% isoflurane in 99% oxygen on a Harvard Apparatus Small Animal Volume Controlled Ventilator, Model 683. Body temperature was maintained with a Harvard Apparatus Homeothermic Blanket Control Unit (catalog no. BS4 50-7053-R). A midline scalp incision was performed, and the periosteum was removed to expose the skull. A metal disk, 10 mm in diameter, 3 mm wide, was firmly attached with dental acrylic and Loctite QuickTite super glue gel to the skull between the lambda and bregma sutures (Koob et al., 2005). For one rat, a 450 g brass weight was dropped from a height of 2 m onto the disc to induce a severe injury in the impact acceleration injury model (Foda and Marmarou, 1994; Marmarou et al., 1994). The second rat was a sham animal (uninjured) and did not receive impact from the weight. The impact acceleration brain injury device (Marmarou et al., 1994) causes traumatic axonal injury to numerous areas within the brain, including axonal injury to the corpus callosum, coronal radiations and brain stem, as well as subarachnoid hemorrhage in severe cases in the periventricular space and hindbrain (Foda and Marmarou, 1994). After injury or sham, the metal disc was removed and the skin was sutured. The animals received a 0.075 mg/kg intramuscular injection of buprenex and were weaned off the ventilator within an hour.

2.2. *Open field activity detector*

Twelve hours after injury or sham, animals were placed in a Plexiglas activity box (100 cm × 100 cm × 20 cm) at night in a darkened room (Fig. 1). Food was placed over the center of the box and the box was thoroughly cleaned with water between experiments to encourage the rat not to engage in thigmotaxic behavior. 4.5 cm off the ground, eight infrared beams in an X-Y matrix, 20 cm apart, were counted by a Veeder-Root Series 7999 Mite Totalizer (ID# 79998D-110, Gurnee, IL) when broken by the rat. The lag time between counts was 14 ms. At 1/2 and 1 h, the number of total beam breaks was tabulated.

Fig. 1. Activity box for open field score. The dimensions of the activity box used for the open field score. The infrared beams (red lines) are an equidistant 20 cm apart. The box is 100 cm $\times$ 100 cm $\times$ 20 cm. Four beams in the X and Y direction are numbered 1–4. The beams are 4.5 cm high relative to the bottom of the box. The rat is placed in the middle at the beginning of the test and allowed to explore the area. Food is placed over the center of the box. A totalizer counter registers the number of beams the rat breaks. A computer system registers what beam is being broken to determine where the rat is in space and time.

Separate from the totalizer, custom-designed in house software recorded the state of the infrared beams at 200 ms intervals. With these data, the experimenter could determine the rat's position in space and time over the course of the experiment. The intent is to detect the animal's movement in real time. Fig. 2 shows an outline of the circuitry in the open field activity detector. The infrared beams are comprised of QED123 infrared emitters (LEDs; Fairchild Semiconductor, South Portland, ME) driven with a 150 ms pulse every 3.5 ms. The receivers at the other side of the box consist of L14G1 infrared detectors (Fairchild Semiconductor, South Portland, ME), a two-transistor amplifier and wave shaper, and a TTL inverter IC. The eight outputs are wired to the I/O pins of a BASIC Stamp Microcontroller BS2 module (Parallax Inc., Rockland, CA: U5, Fig. 3).

Whenever a beam is broken, this condition is sent to the appropriate I/O pin of the BASIC stamp. The beam break is not detected as a momentary event, but rather as a continuous event. If an animal blocks a beam for 5 min, the beam is recorded as being broken for the duration of the 5 min. The basic stamp contains custom software which reads the status of the eight infrared beams simultaneously, and sends the current conditions through a serial port connection at 200–250 ms intervals (Fig. 3).

The serial port is connected via a cable to a computer running MS-DOS or Windows. The software on the computer reads the data coming into the serial port. The *X* and *Y* coordinates from 1 to 4 in each axis at each time interval are determined based on which beam the rat is breaking. If the rat is not in front of a beam in either the *X* or *Y* direction, the coordinates are listed halfway between the beam the rat broke previously and the beam the rat breaks next. Additionally, if the rat is blocking two beams simultaneously in one axis, the software determines the animal is breaking one beam—either the beam the rat broke immediately previous to blocking two beams, or the beam the rat breaks immediately afterwards. In the case that the animal breaks a single beam immediately before and immediately after breaking two beams simultaneously, the beam the animal breaks before takes precedence.

Fig. 2. Outline of the system. The infrared beams emit a signal to a detector. The signal is converted to data that is registered by the PC. See Fig. 3 for schematic representation of the dotted box.

Fig. 3. Schematic diagram of the open field activity detector circuitry. Data from infrared beams being broken are converted by the system above. Onboard regulator U2 provides 5V for the digital circuitry. The remainder of the circuit is powered by the unregulated 9V input. Q1 is a 2N3906 transistor used as a light emitting diode (LED) driver and D1–D8 are the LEDs. The LEDs are arranged in two series strings of four. Q2 is the L14G1 transistor that detects the beam from the LED. The circuit of Q2, Q3, Q4, D9, and U3 represents a detector circuit for one out of eight beams. U5 (BASIC Stamp Microcontroller BS2 module) monitors the state of the eight input pins connected to the detectors, and at about 200 ms intervals it sends the data to the serial port (J2) at a rate of 9600 baud. This information is then sent to a computer to indicate *X–Y* coordinates of the rat's position in time.

Any computer capable of reading a serial port at 9600 baud can be used to read the data. In this study, we used a Pentium class computer running under Windows 2000. The simple terminal/capture program was compiled in Quick Basic (Microsoft, Redmond, WA). The function of the detectors and emitters were validated before each experiment by blocking the beams manually and confirming the correct registration by the computer software. All computer software devised is available online at <http://web.ics.purdue.edu/~cirillo/ratterm> or on request.

2.3. Area explored

To determine the amount of area explored, the coordinates of the rat's position were placed into an Excel file and graphed. If the rat was breaking a beam in the X or Y -axis, the rat's position could be determined at a specific time. If the rat was not breaking a beam, its position could be determined based on the last beam the rat broke and the next beam the rat breaks. Therefore, the position of the rat in space and time was either determined to be in front of a beam or between two beams, giving a total of nine places in each axis where it could be determined the rat explored. The percent the rat explored out of the 81 possible places was then calculated.

2.4. Distance traveled

To determine the distance the rat traveled over the course of an hour, the coordinates of the rat's position were placed into an Excel file. Using the equation $d = \sqrt{(X_1 - X_2)^2 + (Y_1 - Y_2)^2}$, starting from the second time point of data for each coordinate in time (every 200 ms), where X_1 and Y_1 are the previous coordinates and X_2 and Y_2 are the following coordinates. The result is based on an integer of 1–4 denoted by the beams on the X and Y -axes. If the rat moves diagonally or in a line parallel to the sides, the distance is noted. Knowing that the distance between each beam is 20 cm, the resulting sum of distance traveled for all time points over the course of the hour is multiplied by 0.2 to give a number in meters.

2.5. Time exploring

Using in house computer software, looking at the coordinates, if the rat was in front of a beam and then moved away from a beam in either the X or Y -axis in a block of time (200–250 ms), then that was considered a block of time during which the rat was moving and thus exploring. Likewise, if the rat was not blocking a beam and then moved in front of a beam for a block of time, that block was considered as time exploring. The total number of blocks of time the rat moved away from a beam or moved in front of a beam was divided by the overall total number of time blocks to give a percent of time exploring.

Fig. 4. Rat open field exploration: 2D representation. 2D representation of rat open field movement over the course of an hour. The X and Y -axis points (1–4) corresponded to the beams in the box. Using a novel computer system the rat's place in space was determined based on the last beam the rat broke. (A) An uninjured animal explores much of the area over the course of an hour. (B) An injured animal only explores one corner of the box. The bottom left hand corner is the Z -axis when expanded, representing time—see Fig. 7.

3. Results

Fig. 4(A) shows a two-dimensional representation of an uninjured rat's movement in the activity box over a 1 h period from the view of looking down on the box. In Fig. 4(B), the injured animal moves considerably less and only explores one quadrant of the box. The counts of beams broken were indicated by the totalizer. The injured animal only broke 103 after a 1/2 h and 147 after an hour; while the uninjured animal broke 720 at a 1/2 h and 1350 after an hour (Fig. 5(A)).

Additionally, the injured animal explored only 33.3% of the box while the uninjured animal explored 95.1% of the box (Fig. 5(B)). In Fig. 6(A), the movement of the uninjured rat has been expanded into a three-dimensional graph with the Z-axis indicating time in seconds. These data were plotted using an open source 3D plotting program (Williams and Kelly, 2004) to show 3D representation of rat movement. In Fig. 6(B), the three-dimensional graph shows the long periods of time the injured animal stayed in one place (indicated by vertical lines) and the amount of movement is much less.

There was also a large difference between the injured and uninjured animal in distance traveled and time spent exploring. The total distance traveled for the uninjured animal over the course of an hour was calculated to be 258.3 m; while the injured animal only traveled 15.6m (Fig. 7(A)). Additionally, the uninjured animal spent 12% of the time in the activity box exploring; while the injured animal explored 0.8% of the time (Fig. 7(B)).

Fig. 5. Infrared beam breaks and percent of area explored. (A) The injured animal broke fewer beams than the uninjured animal. Counts of beam breaks in the second 1/2 h for the injured animal were much less than in the first 1/2 h. (B) The area explored by the uninjured and injured rat were observed two-dimensionally, see Fig. 4. The data are represented as a percentage of 81 points on the box. The system was able to determine if the rat was between two beams or breaking a beam, giving a total of 9 levels in each direction. The uninjured rat covered virtually the entire area; while the injured rat covered a third of the area. Area covered does not take into consideration whether the rat revisited the same area.

Fig. 6. Rat open field movement: 3D representation. 3D representation of rat open field movement over the course of an hour, Z-axis in seconds (3600 s). The X and Y-axis (1–4) corresponded to the beams in the box. Using novel software the rat's place in space and time was determined based on the last beam the rat broke. (A) An uninjured animal explores virtually the entire space over the course of an hour. (B) An injured animal does not move (vertical lines) and prefers to sit stationary.

Fig. 7. Distance traveled and time exploring in the open field. (A) The differences between the distance traveled by the uninjured and injured animal were drastically different. The injured animal traveled 15.6 m, while the uninjured animal traveled 258.3 m. (B) If the data of the rat's position in space changed compared to the previous data, the 200 ms block of time was registered as exploration time. Exploratory behavior of the uninjured rat was much higher than that of the injured rat.

4. Discussion

In this paper, we present a defined strategy to build an open field system to study animal behavior after injury or disease. If the animal has injury, disease or neurotoxicity that effect other aspects of its behavior beside memory, a test of exploratory behavior could adequately supplement specific cognitive tests to gather a more defined picture of animal behavior.

Exploratory behavior is fundamental to the nature of the rat and open field tests can be used effectively when studying behavior of rats after treatments for neurological disease, injury, drug abuse or neurotoxicity. Additionally, hyperactivity after the use of cocaine and other psychostimulant drugs in animals results in the animal exhibiting more motility, finally resulting in conditioned response (Barr et al., 1983; Pickens and Dougherty, 1971; Tilson and Rech, 1973). This model would be an excellent way to study addictive drug behavior as a supplement to studying stereotypic anomalies. In addition to drug effects, stress and arousal can be adequately studied in the open field (Roth and Katz, 1979).

Some observers claim rats engage in typical thigmotaxic behavior in the open field by moving only along the sides of the box (Schwartz et al., 1993). Other observers have described the rat as going back to its home base in the activity box repeatedly over the course of its time in a new environment until it eventually becomes habituated (Whishaw et al., 1999). In trial experiments, we found that adding familiar food from their cage above the middle of the activity box and cleaning the box thoroughly with water between experiments discouraged the rats from exclusively exploring the sides. Additionally, activity boxes used to determine the open field score based on beam breaks do not take into account the rat staying in one place and moving a body part such as its head across only one beam. Using this model, by graphing the movement of the animal, measuring area covered and distance traveled it is possible to determine when the animal remains in one place, moving his head repeatedly across the same beam. Also, this model takes into account the fact that rats are nocturnal, and the test can be done in the dark when rats are most active.

Lastly, four beams for each axis were used in this experiment, but it is possible using the model described to add more infrared beams for a more detailed picture of the movement of the animal. Beams could also be placed higher on the sides of the open field box to study rearing behavior. By constructing this system it is possible to describe a variety of tests of laboratory animal exploration in the open field. These tests contribute to the overall behavioral picture of the laboratory animal and help us more accurately understand which treatments are effective.

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References

- Barr GA, Sharpless NS, Cooper S, Schiff SR, Paredes W, Bridger WH. Classical conditioning, decay and extinction of cocaine-induced hyperactivity and stereotypy. *Life Sci* 1983;33:1341–51.
- Devan BD, Goad EH, Petri HL. Dissociation of hippocampal and striatal contributions to spatial navigation in the water maze. *Neurobiol Learn Mem* 1996;66:305–23.
- Dutrieux G, Platel A, Deweer B. Automatic analysis of exploratory behavior in rat—an activity monitoring apparatus using a closed-circuit television. *Physiol Behav* 1978;21:721–26.
- Foda MA, Marmarou A. A new model of diffuse brain injury in rats. Part II: morphological characterization. *J Neurosurg* 1994;80:301–13.
- Giulian D, Silverman G. Solid-state animal detection system: its application to open field activity and freezing behavior. *Physiol Behav* 1975;14:109–12.
- Koob AO, Duerstock BS, Babbs CF, Sun Y, Borgens RB. Intravenous PEG inhibits the loss of cerebral cells after brain injury. *J Neurotraum* 2005;22:1092–111.
- Marino MJ, Williams Jr DL, O'Brien JA, Valenti O, McDonald TP, Clements MK, et al. Allosteric modulation of group III metabotropic glutamate receptor 4: a potential approach to Parkinson's disease treatment. *Proc Natl Acad Sci USA* 2003;100:13668–73.
- Marmarou A, Foda MA, van den Brink W, Campbell J, Kita H, Demetriadou K. A new model of diffuse brain injury in rats. Part I: pathophysiology and biomechanics. *J Neurosurg* 1994;80:291–300.
- Morris RG, Garrud P, Rawlins JN, O'Keefe J. Place navigation impaired in rats with hippocampal lesions. *Nature* 1982;297:681–83.
- O'Connor C, Heath DL, Cernak I, Nimmo AJ, Vink R. Effects of daily versus weekly testing and pre-training on the assessment of neurologic impairment following diffuse traumatic brain injury in rats. *J Neurotraum* 2003;20:985–93.
- Olton DS, Samuelson RJ. Remembrance of places passed—spatial memory in rats. *J Exp Psychol: Anim Behav Process* 1976;2:97–116.
- Pickens R, Dougherty J. Conditioning of the activity effects of drugs. In: Thompson T, Schuster C, editors. *Stimulus properties of drugs*. New York: Appleton/Century/Crofts; 1971. p. 39–50.
- Roth KA, Katz RJ. Stress, behavioral arousal, and open field activity—a reexamination of emotionality in the rat. *Neurosci Biobehav Rev* 1979;3: 247–63.

Schwartz RK, Goldenberg R, Steiner H, Fornaguera J, Huston JP. A video image analyzing system for open-field behavior in the rat focusing on behavioral asymmetries. *J Neurosci Methods* 1993;49:199–210.

Tilson HA, Rech RA. Conditioned drug effects and absence of tolerance to d-amphetamine induced motor activity. *Pharmacol Biochem Behav* 1973;1:149–53.

Tomkins PT, O'Donovan DJ. A fully automated open-field apparatus incorporating rearing detection. *Physiol Behav* 1981;26:741–46.

Vicens P, Redolat R, Carrasco MD. Spatial learning and water maze: methodology and utilities. *Psicothema* 2003;15:539–44.

Vink R, O'Connor CA, Nimmo AJ, Heath DL. Magnesium attenuates persistent functional deficits following diffuse traumatic brain injury in rats. *Neurosci Lett* 2003;336:41–44.

Whishaw IQ, Haun F, Kolb B. Analysis of behavior in laboratory rodents, modern techniques in neuroscience research. New York, NY: Springer; 1999. pp. 1243–1275.

Williams T, Kelly C. Gnuplot 4.0: an interactive plotting program. Organized by H. Bernhard-Broker, 2004.