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Prolonged Animal Observation by Use of Digitized Videodisplays

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SPRUIJT, B. M. AND W. H. GISPEN. *Prolonged animal observation by use of digitized videodisplays.* PHARMACOL BIOCHEM BEHAV **19**(5) 765–769, 1983.—Quantitative assessment of animal behavior requires not only much time and good training, but is always dependent upon the interpretation of the observer. Therefore we employed a commonly-used microcomputer to analyze, without intervention of a human observer, the picture obtained via videocamera, a digitizer and disk-drive. The program, written in BASIC and listed in Appendix I, generates pictures of the monitored animal in the graphics of the computer. These pictures can be analyzed immediately or stored on disk for further analysis.

Computerized animal observation   Pattern recognition   Microcomputer   Videodigitizer

IN BEHAVIORAL pharmacology one often deals with the problem of quantifying certain clearly described behaviors in different groups of animals. For the purpose of screening drugs, several typical and easily recognized behaviors are used such as akinesia, hyperactivity, hypoactivity, stereotypic gnawing, licking, biting, wet dog shake and shivering.

Often special electronic equipment adapted for particular tests has been developed to measure easily quantifiable parameters; which are derived from animal movement [5]. One disadvantage of automation by making the appropriate hardware is the limited flexibility of the equipment. Moreover, behavioral elements—like grooming—which are more defined by attitude than movement, are more difficult to measure. Often the intervention of a human observer remains a prerequisite for registrating animal behavior. This intervention not only introduces bias but is also dependent on sustained attention of the observer and might therefore interfere with the scoring consistency.

Here a system is described which can replace a time sampling method for observing two animals at the same time. In our laboratory it is used for observing the grooming behavior of two rats. Both spontaneously occurring grooming as well as peptide-induced grooming are prolonged behavioral patterns [7]. As a consequence demonstrating differences in grooming behavior requires a lot of observation time and suffers from the afore-mentioned disadvantages inherent in a human observer.

METHOD

The microcomputer used is an Apple II Plus with Applesoft BASIC and 48K RAM. Other computers compatible with Apple will do as well. The Computer is equipped with a disk-drive and a graphic printer. An essential interface is the Apple videodigitizer. This interface is connected to a standard videocamera and generates digitized pictures every 4 (or 8) seconds of the display of the camera screen. This picture will be stored in the graphics memory of the computer. The digitizer also contains microswitches for regulating contrast and brightness. The animals used for observation are housed individually in cages of Plexiglas (dimensions: 14.5×14.5×25 cm) with a black background for the required contrast with white rats [3].

Reliability is demonstrated in a small experiment in which a human observer and our proposed system were both analyzing animal behavior. Four times two rats—one experimental and one control—were observed for two hours.

The experimentals were treated with 0.3 μg/3 μl ACTH to induce the display of excessive grooming, controls received 3 μl of saline.

Software

The manual of the digitizer explains how to make a picture and display it on the screen. Different strategies for further processing the pictures can be followed: (1) immediate analysis of each picture or (2) storage on disk and application of a special program for future analysis of these pictures. Of course, storage is preferable, however, the number of pictures is restricted to the disk storage capacity available. When Apple DOS 3.3 system is used 15 pictures (8,192 bytes per picture equals 33 sectors on disk) can be stored on one disk (single sided, single density). Pattern analysis is relatively time consuming, so immediate analysis results in a reduced sampling rate, since analysis time dictates the interval between pictures analyzed.

The program listed in Appendix I is completely written in BASIC and therefore the analysis of the generated picture also requires some time.

However this can be limited by using a subroutine in assembly language for screening every point in graphics. For understanding the principles of the Apple Graphics and the way our program makes use of it, a program written in

TABLE 1

BEHAVIORAL ANALYSIS PERFORMED BY A HUMAN OBSERVER AND A COMPUTER SYSTEM

Behavior	Human Observer E–C		Computer E–C	
Grooming	139 ± 5	63 ± 5*	112 ± 18	56 ± 12*
Sitting	39 ± 5	37 ± 7	58 ± 18	53 ± 10
Sleeping	27 ± 8	119 ± 7*	24 ± 6	112 ± 8*
Rearing	71 ± 12	59 ± 8	81 ± 16	56 ± 12

The behavior of two groups of four animals was analyzed both by a human observer and the proposed computer system. **t*-test revealed significance between the two groups ($p<0.05$). C: saline-treated; E: ACTH-treated.

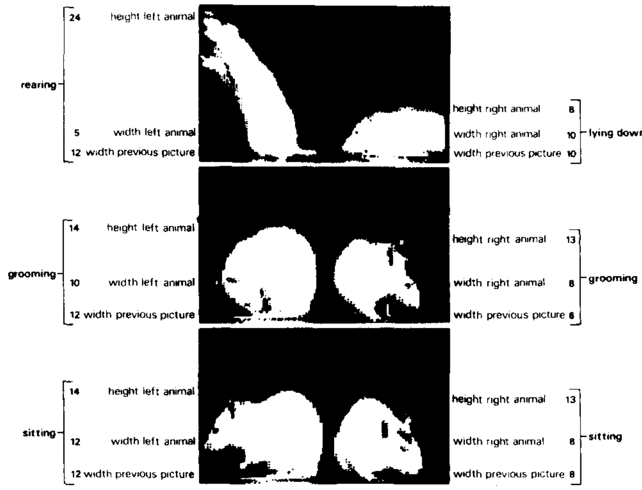

FIG. 1. Three examples of monitored behavior of two animals in two cages. The numbers represent the calculated values for height and width according to the computer. Behavior was determined for both animals as is shown. The black spot on the head of the animal is caused by the dental cement for cannula implantation.

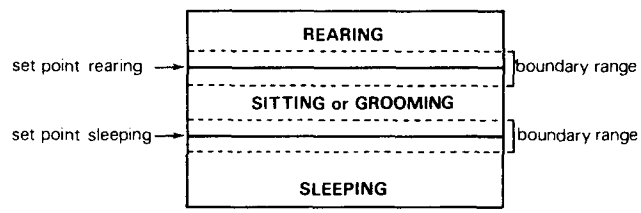

FIG. 2. The lines present the heights which determine classification of animal behavior. If the height falls within a boundary range (dotted line), the width is also taken into account (see text).

BASIC seems more appropriate and is therefore listed and used for the results in Table 1.

In analyzing the two-dimensional display two main strategies can be followed. First, one can give certain examples of different behavioral elements and let the computer compare the digitized pictures with the given pictures; the example with the closest resemblance to the monitored behavior determines the classification. A "rest" category can be used if no resemblance with any example is found. The more graphics pages the computer has the more attractive this approach is (an Apple has only two pages for its graphics (8,192 bytes each).

Secondly, one can transform the digitized pictures to easily recognizable geometrical figures deduced from the posture of the behavior in study. The program shown divides the screen into two halves each containing a picture of an animal. From both pictures the height and the width of the animal at half its height are measured. The width of the previous picture remains available as a cue for classifying behavior and is used for determining movement.

According to the results of Traber [8] and Bolles [1] most time is spent in sitting, grooming, sleeping and exploration. It is our purpose to discriminate between these four behavioral categories. Classification of animal's posture is performed according to the height, the width and the width of the fore-going posture. As can be seen in Fig. 1, which demonstrates three pictures from the screen, rearing and sleeping are rather easy to distinguish. Rearing is characterized by a large height and a small width; sleeping is marked by a small height and a large width. The postures with intermediate values for height and width, grooming and sitting, are more difficult to distinguish; both width and height can be similar in the two postures, therefore a parameter for movement is used as a discriminant. A sitting animal has the same width over the different samples, while a grooming animal is constantly moving. When the animal is not rearing or sleeping the width and the height of that scan and that of the preceding scan are used for discriminating grooming and sitting. A boundary range is defined in case the height is close to the set point of rearing or sleeping (see Fig. 2). The program uses the height as a main key; when the animal is obviously sleeping or rearing—respectively height larger or smaller than respectively set point plus or minus the range—the width is not taken into account. This range is variable and can be changed in the program (see Appendix I, variable UN). When the posture indicates grooming or sitting the width of the preceding picture is also used for discrimination. The set point must be given for every run of the program, so one can always adapt the values according to the position of the camera with respect to the cage, which is very crucial in this set up. The first part of the program provides projection of the given set points on the monitor, so you can check and, if necessary, adapt them to your requirement.

The program counts every point which makes up the picture on the screen—or every second or third point depending on the scanning rate—as either white or black.

The scanning rate of the screen is varied by altering the step size; the bigger the step size the faster and more inaccurate the scanning performed.

When the strategy of comparing the picture with standard figures is followed a good device for getting these standard figures into the computer is a graphics tablet. The postures can be drawn by hand on the tablet and can be placed in graphics automatically. (For further information see the manual of the tablet.)

RESULTS

Table 1 demonstrates how the program can distinguish saline-treated animals from ACTH-treated animals. ACTH-treated animals groomed significantly more than the saline-treated animals. This is validated by the data of the human observer. Although the drug-induced phenomenon is recognized, considerable difference between our proposed system and the human observer appears with respect to the discrimination of sitting and excessive grooming.

DISCUSSION

The results indicate some shortcomings of the proposed program. Rearing and lying down are well discriminated, however a conservative error is made when grooming behavior—performed without any movement—is scored as sitting. When the occurrence of grooming behavior is studied in prolonged behavioral session, the program works sufficiently. In our laboratory it is also used for the quantification of exploratory behavior; in this case grooming and sitting are lumped together. The advantage of this procedure lies not yet in the accuracy but in the possibility of continuous animal observation for instance in combination with continous drug administration (osmotic micropumps). Moreover the bias, which always accompanies the human interpretation of animal behavior is incorporated in a program and therefore accessible to discussion. Exchange of programs could be in the onset to more homogeneity in the way animal behavior is observed and interpreted. The equipment described has already often been used for other purposes in psychopharmacology [2, 4, 6]. When test cages are similar and compatible computers are available, minimum of investment is required to take advantage of the listed program. It is realized that the program still is far from perfect and needs modification or improvement if used in other experimental settings. Hopefully it inspires other programmers to add a required subroutine and to discuss the outcome of this type of programs with each other.

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APPENDIX

```
1  HOME : VTAB 1
3  PRINT : PRINT "FOR UNDERSTANDING THE CALCULATIONS OF HEIGHT AND WIDTH READ APPLES REFERENCE MANUAL FOR DETAILS ABOUT HIGHT RESOLU
TION GRAPHICS."
4  PRINT "PROVIDE ENOUGH CONTRAST BETWEEN OBJECT AND BACKGROUND"
5  PRINT : PRINT "UNDER REM STATEMENTS THE MEANING OF USED VARIABLES IS EXPLAINED."
6  PRINT : PRINT "PLACE THE CAMERA AT THE SAME HEIGHT AS THE OBJECTS IN SUCH A WAY THAT THE OBJECTS DO'NT CROSS THE MIDLINE OF THE S
CREEN"
7  PRINT : PRINT "FOR FINISHING PRESS RESET"
8  FOR I = 1 TO 15000: NEXT I
10  HOME : VTAB 21
20 D$ =  CHR$ (4): PRINT D$"PR£2": PRINT "!*": PRINT D$"PR£0"
30  HPLOT 0,0 TO 0,159: HPLOT 140,0 TO 140,159: HPLOT 260,0 TO 260,159
40  HPLOT 0,0 TO 260,0: HPLOT 0,159 TO 260,159: FOR I = 0 TO 23:H% = I * 8: HPLOT 2,H%: NEXT I
42  FLASH : PRINT "CALIBRATING CAMERA AND DIGITIZER"
43  NORMAL : PRINT "PICTURE OKE ? (Y/N)      ": GET Y$
44  IF Y$ <  > "Y" THEN  PRINT D$"PR£0": GOTO 10
45  HOME : VTAB 21
50  INPUT "MAXIMUM WIDTH REARING";R%: HPLOT 10,0 TO 10,10:RP% = 7 * R%: HPLOT 10 + RP%,0 TO 10 + RP%,10
60  INPUT "MAXIMUM WIDTH GROOMING";G%: HPLOT 10,0 TO 10,20:GP% = 7 * G%: HPLOT 10 + GP%,0 TO 10 + GP%,20
70  INPUT "MAXIMUM HEIGHT GROOMING  ";P%: HPLOT 2,8 * (24 - P%) TO 5,8 * (24 - P%)
80  INPUT "MAXIMUM HEIGHT SLEEPING ";S%: HPLOT 6,8 * (24 - S%) TO 8,8 * (24 - S%)
90  INPUT "YOU WANT TO GIVE NEW DIMENSIONS ? (Y/N)";Y$: IF Y$ = "Y" THEN  GOTO 10
100 UN = 1
105  REM  UN=UNCERTAINTY RANGE
110  HGR :D$ =  CHR$ (4)
120 N% = 2
125  REM  N%REFERS TO STEP-SEIZE;THE BIGGER N%,THE FASTER THE PICTURE IS SCANNED AND THE LESS ACCURATELY
126  REM  R RFERS TO THE SCREEN HALF
130  PRINT D$"PR£2"
140  PRINT "!*"
150  PRINT D$"PR£0"
```

```
160 X = 0:N = 0:R = 0:
170  FOR T1 = 0 TO 2
180 TELLER = 0:T8 = 0
190  FOR T2 = T1 * 40 + 8192 TO 9088 + T1 * 40 STEP 128
200 TELLER = TELLER + 1
210  FOR T3 = 1 + R TO 20 + R STEP N%
220 X =  PEEK (T2 + T3)
230  IF X > 0 THEN N = N + 1
240  IF X > 0 AND N > 1 THEN  GOSUB 760
245  REM  N REFRES TO THE NUMBER OF POINTS WHICH MUST BE DETECTED BEFORE DECIDING THE OBJECT IS FOUND
250  IF X > 0 AND (R + N) > 21 THEN N = 0: GOTO 560
260  IF X > 0 AND N > 1 THEN N = 0: GOTO 330
270  NEXT T3
280  NEXT T2
290  NEXT T1
300  IF R = 20 THEN  GOTO 560
310  REM  DETERMINING HEIGHT LEFT ANIMAL 1000-1010
320  REM  CLASSIFYING BEHAVIOR RIGHT ANIMAL
330 RH = T2 + T3 - (8192 + (T1 * 40) + T3)
340 RH = RH / 128:AH = (2 - T1) * 8 + 8 - RH
342 HL = AH
345  REM  AH IS THE HEIGHT OF THE LEFT ANIMAL
360  PRINT "HEIGHT LEFT ANIMAL"AH
370  PRINT "WIDTH LEFT ANIMAL NOW AND BEFORE  "BL"   "LV
380  IF AH < S% - UN THEN  PRINT "LEFT ANIMAL IN A HORIZONTAL POSITION":L1 = L1 + 1: GOTO 520
390  IF AH > P% + UN THEN  PRINT "LEFT RAT REARS":R1 = R1 + 1: GOTO 520
400  IF (S% - UN) < AH AND AH < S% THEN  GOTO 420
410  GOTO 450
420  IF BL > G% THEN  PRINT "LEFT ANIMAL IN IN A HORIZONTAL POSITION":L1 = L1 + 1: GOTO 520
430  IF  ABS (LV - BL) > 0 THEN  PRINT "LEFT ANIMAL GROOMS":G1 = G1 + 1: GOTO 520
440  IF  ABS (AH - HL) > 0 THEN  PRINT "LEFT ANIMAL IS GROOMING":G1 = G1 + 1: GOTO 520
445  PRINT "LEFT ANIMAL IS SITTING":S1 = S1 + 1: GOTO 520
450  IF AH < P% - UN AND  ABS (BL - LV) > 0 THEN  PRINT "LEFT ANIMAL GROOMS":G1 = G1 + 1: GOTO 520
460  IF AH < P% - UN AND  ABS (AH - HL) > 0 THEN  PRINT "LEFT ANIMAL GROOMD":G1 = G1 + 1: GOTO 520
465  IF AH < P% - UN THEN  PRINT "LEFT ANIMAL SITS":S1 = S1 + 1: GOTO 520
470  IF AH < P% AND AH > P% - UN THEN  GOTO 490
480  GOTO 510
490  IF BL% > R% AND  ABS (BL - LV) > 0 THEN  PRINT "LEFT ANIMAL GROOMS":G1 = G1 + 1: GOTO 520
500  IF BL% > R% AND  ABS (AH - HL) > 0 THEN  PRINT "LEFT ANIMAL GROOMS":G1 = G1 + 1: GOTO 520
505  IF BL% > R% THEN  PRINT "LEFT ANIMAL SITS":S1 = S1 + 1: GOTO 520
510  PRINT "LEFT RAT  REARS ":R1 = R1 + 1
520 TELLER = 0:N = 0:R = 20
530  GOTO 170
540  REM  DETERMING HEIGHT RIGHT ANIMAL 2000-2010
550  REM  CLASSIFIYNG BEHAVIOR ACCORDING TO HEIGHT AND WIDTH
560 RH = T2 + T3 - (8192 + (T1 * 40) + T3)
570 RH = RH / 128:AH = (2 - T1) * 8 + 8 - RH
572 HR = AH
575  REM  AH REFERS TO THE HEIGHT OF THE RIGHT ANIMAL
580  PRINT "HEIGHT RIGHT ANIMAL"AH
590  PRINT "WIDTH RIGHT ANIMAL NOW AND BEFOPE  "BR"  "RV
600  IF AH < S% - UN THEN  PRINT "RIGHT ANIMAL IN A HORIZONTAL POSITION":L2 = L2 + 1: CALL 62450: GOTO 130
610  IF AH > P% + UN THEN  PRINT "RIGHT ANIMAL REARS":R2 = R2 + 1: CALL 62450: GOTO 130
620  IF AH < S% AND AH > S% - UN THEN 640
630  GOTO 670
640  IF BR > G% AND  ABS (RV - BR) > 0 THEN  PRINT "RIGHT ANIMAL GROOMS":G2 = G2 + 1: CALL 62450: GOTO 130
650  IF BR > G AND  ABS (AH - HR) > 0 THEN  PRINT "RIGHT RAT GROOMS":G2 = G2 + 1: CALL 62450: GOTO 130
655  IF BR > G% THEN  PRINT "RIGHT RAT IS SITTING":S2 = S2 + 1: CALL 62450: GOTO 130
660  PRINT "RIGHT ANIMAL REARS":R2 = R2 + 1: CALL 62450: GOTO 130
670  IF AH < P% - UN AND  ABS (RV - BR) > 0 THEN  PRINT "RIGHT ANIMAL GROOMS":G2 = G2 + 1: CALL 62450: GOTO 130
680  IF AH < P% - UN AND  ABS (AH - HR) > 0 THEN  PRINT "RIGHT ANIMALS GROOMS":G2 = G2 + 1:S":                CALL 62450: G
OTO 130
685  IF AH < P% - UN THEN  PRINT "RIGHT ANIMALIS SITTING":S2 = S2 + 1: CALL 62450: GOTO 130
690  IF AH < P% AND AH > P% - UN THEN  GOTO 710
700  GOTO 730
710  IF BR > R% AND  ABS (RV - BR) > 0 THEN  PRINT "RIGHT ANIMAL GROOMS":G2 = G2 + 1: CALL 62450: GOTO 130
720  IF BR > R% AND  ABS (AH - HR) > 0 THEN  PRINT "RIGHT ANIMAL IS GROOMING":G2 = G2 + 1: CALL 62450: GOTO 130
725  IF BR > R% THEN  PRINT "RIGHT ANIMAL IS SITTING":S2 = S2;1: CALL 62450: GOTO 130
730  PRINT "RIGHT ANIMAL REARS":R2 = R2 + 1
740  PRINT D$"PR£0"
750  GOTO 130
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760  IF T1 = 0 THEN T8 = T2 + 40
770  IF T1 = 1 AND TELLER < 5 THEN T8 = 9000: GOTO 790
780 T8 = 9128
785  POKE T8,1
790 N2 = 0
800  IF R = 20 THEN LV = BR: GOTO 820
810 RV = BL
820  REM  T8 IS THE HEIGHT AT WHICH THE WIDTH IS DETERMINED
830  FOR T9 = 1 + R TO 20 + R
840 Y =  PEEK (T8 + T9)
850  IF Y > 0 THEN N2 = N2 + 1
860  IF Y <  > 0 AND N2 < 2 THEN B1 = T9
870  IF Y = 0 AND B1 <  > 0 THEN B2 = T9: GOTO 890
880  NEXT T9
890  REM  RV AND LV ARE RESPECTIVELY THE WIDTH OF RIGHT AND LEFT ANIMALS
900 BR = B2 - B1:BL = BR:B1 = 0:B2 = 0: RETURN
902  REM  STOP BY PRESSING RESET
903  REM  TYP GOTO 910 FOR FINAL RESULTS
910  PRINT "REARING LEFT ANIMAL  "R1: PRINT "REARING RIGHT ANIMAL  "R2: PRINT
911  PRINT "GROOMING LEFT ANIMAL "G1: PRINT "GROOMING RIGHT ANIMAL "G2: PRINT
912  PRINT "SITTING LEFT ANIMAL  "S1: PRINT "SITTING RIGHT ANIMAL  "S2: PRINT
913  PRINT "SLEEPING LEFT ANIMAL "L1: PRINT "SLEEPING RIGHT ANIMAL "L2
1000  END
```