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Quantitation of Rodent Catalepsy by a Computer-Imaging Technique

B. R. MARTIN,[1] W. R. PRESCOTT AND M. ZHU

Department of Pharmacology and Toxicology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298

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MARTIN, B. R., W. R. PRESCOTT AND M. ZHU. *Quantitation of rodent catalepsy by a computer-imaging technique.* PHARMACOL BIOCHEM BEHAV 43(2) 381-386, 1992.—Catalepsy is usually defined as a behavioral state in which an animal maintains an unnatural posture for an extended period of time. While numerous laboratory models have been developed for assessing catalepsy, a common problem encountered with most procedures is the difficulty in quantitating immobility. Measurement of catalepsy is still frequently subjective in nature. To eliminate this subjectivity, a computer-based technique was developed for quantitating catalepsy in mice and rats as measured on the elevated ring. The system consisted of a videocamera that was focused on either three mice or two rats. Their behavior was recorded during a 5-min session on videotape that was subsequently transmitted to a Macintosh II microcomputer via a Scion Image-Capture 2 board. A modification of the NIH *Image 1.17* public domain program allowed the image of the rat to be transformed to a purely black or white image by assigning pixel values of either 0 or 256. The subsequent captured image was preprocessed in an identical manner and each pixel was subtracted from its corresponding pixel in the previous frame. Thus, changes in animal posture between the two frames can be quantitated. One subtraction cycle (acquisition, bilevel processing, and subtraction) was repeated at an average rate of approximately one per second. To quantitate immobility by image analysis, each frame was subtracted from the previous frame during a 5-min session. The resulting data were sorted according to the magnitude of movement (number of changed pixels) and plotted vs. time. A biphasic plot resulted, showing a clear demarcation between mobility and immobility that could also easily be distinguished from background noise inherent in the video imaging system. Quantitation of the period of immobility in this fashion was found to be in close agreement with that obtained by human observation over a wide range of immobility-inducing drugs.

Catalepsy Quantitation Cannabinoids Methodology

A problem frequently encountered in determining the behavioral effects of drugs is that the observation is not readily automated or objectively quantifiable. Computer-imaging techniques provide a new approach to the acquisition and analysis of some animal behavioral data that is otherwise not readily available. These techniques have been applied in a variety of ways that include the study of turning behavior (2), measurement and control of head position in cephalometry (5), and detection of surface movements on single smooth muscle cells (26).

Catalepsy is a behavioral syndrome qualitatively distinct but not readily quantifiable. The cataleptic state of immobility can be characterized as an animal maintaining an abnormal or unnatural posture for extended period of time. Considerable attention has focused on the measurement of catalepsy as a result of many diverse drug classes eliciting this syndrome. For example, cannabinoids produce catalepsy in many laboratory animals including dogs, rabbits, guinea pigs, mice, rats, and gerbils (13,15). Catalepsy has been especially well characterized in rodents treated with opiates and neuroleptics. It has also proven to be a useful tool in characterization of the mechanism of drug action. With regard to both opiates and neuroleptics, catalepsy has been shown to be a pharmacologically distinguishable behavioral effect mediated by distinct neural pathways and mechanisms (1,4,6,10,12,14). Although opiate- and neuroleptic-induced catalepsy are distinguishable in many respects, their responses to specific antagonists most directly demonstrate the involvement of distinctly different mechanisms. The catalepsy caused by morphine is blocked by the classical opiate antagonist naloxone, while that caused by neuroleptics is not (14). Further, the catalepsy of haloperidol is decreased by anticholinergics, while that of morphine is not (7,10). Although apomorphine, a D_1-D_2 dopaminergic agonist, decreases the catalepsy caused by both prototypic drugs, it appears to be much more effective in reducing the opiate rather than the neuroleptic catalepsy (10).

The tests used to measure catalepsy vary primarily in the topography of this "unnatural" position. They include the

[1] Requests for reprints should be addressed to Dr. Billy R. Martin, Ph.D., Department of Pharmacology & Toxicology, P.O. Box 613, MCV Station, Medical College of Virginia, Richmond, VA 23298.

"Bhudda," "brick," and "cork" tests for rodents and perhaps even some aspects of the cannabinoid dog static-ataxia test (9,25). The bar test, typically used with rats, involves placing the animal on a flat surface with its front paws on a horizontal bar positioned 9–12 cm above the surface. The length of time the animal stays on its "haunches" with its front paws on the bar is considered a measure of catalepsy (8). In mice, catalepsy is frequently quantified by placing an animal in an unnatural position, such as on an elevated ring, and an observer determines the amount of time the animal remains immobile (19).

To eliminate the obvious subjectivity of this measurement, as well as to minimize the labor-intensive aspect of this assay, a computer-based technique was developed for automation of catalepsy in rodents as determined using the elevated ring test. A system was designed to acquire, store, and analyze the data obtained simultaneously from either two rats or three mice. This system reports catalepsy as seconds of immobility without the need for human assessment of movement.

METHOD

Animals

Male ICR mice (22–30 g) and male Sprague-Dawley rats (300 g) obtained from Dominion Laboratories (Dublin, VA) were maintained on a 14 L : 10 D cycle and received food and water ad lib. Δ^9-Tetrahydrocannabinol (THC) and Δ^8-THC were obtained from the National Institute on Drug Abuse.

Drug Preparation and Administration

The procedure of Olson et al. (18) was used to prepare micellular suspensions of Δ^9-THC and other drugs suitable for injection. Cannabinoids were dissolved (by sonication) in a 1 : 1 mixture of ethanol and emulphor (EL-620, a polyoxyethylated vegetable oil, GAF Corporation, Linden, NJ). Saline (0.9% NaCl) was added to this mixture to produce a 1 : 1 : 18 ratio of ethanol : emulphor : saline (vehicle), and the solution diluted further with vehicle to the desired concentration. Mice received intravenous injections via the tail vein and rats received intraperitoneal injections.

Apparatus and General Procedure for Determining Catalepsy

The apparatus consisted of a 5.5-cm ring attached to a stand at a height of 16 cm for mice or a 13-cm ring attached at a height of 40 cm to a ring stand for rats. A wooden black backboard (40 cm diameter) provided a contrast for animals. Each animal was placed onto a ring for 5 min. If an animal escaped from the ring by jumping or falling (due to ataxia or sedation) more than five times, then the evaluation of that animal was terminated and the immobility index was calculated based upon the total time (seconds) the animal remained on the ring. Animals that did not remain on the ring at least 2.5 min prior to five escapes failed to meet the minimum criterion of this evaluation and data were disregarded.

Quantitation by Human Observation

Experienced observers, using a stopwatch, recorded the time during a 5-min period that the animal remained in a motionless or catatonic state. During the 5-min test period, the sum of all times during which the animal remained motionless was measured to the nearest second. This value was divided by 300 s and multiplied by 100 to obtain a % immobility rating. The criterion for immobility was the absence of all voluntary movements (including snout and whisker). The only observable movements allowed during a period of "immobility" were gross body movements due to breathing.

Quantitation by Computer Imaging

A ceiling-mounted monochrome CCD camera (Panasonic, BLV-200) was focused on animals (either three mice or two rats) as depicted in Fig. 1. The 5-min experimental session was recorded on videotape and subsequently transmitted to a Macintosh II microcomputer via a Scion Image-Capture 2 board at the speed of 30 frames/s in 256 shades of gray. By a modification of the NIH *Image 1.17* public domain program, two regions of interest (ROIs) for each frame were defined for rats and three ROIs for mice so that each ROI corresponded to one animal in the picture. The ROIs consisted of greater than 56,000 individual picture elements (pixels). Despite the fact that the ROI contained a white animal on a black background, each pixel had a gray-scale value between 0 and 256. The first captured image of rats, which consisted of two ROIs, was sent to memory and transformed to a purely bilevel (black and white) image to maximize contrast. This preprocessing was accomplished by assigning pixels a value of either 0 (white) or 256 (black) based upon their gray-scale value being either below or above (respectively) a predetermined cutoff. The second captured image was preprocessed in an identical manner and each pixel was subtracted from its corresponding pixel in the previous frame. Pixels that changed between the two sequentially compared ROIs thus appear as black pixels and can be quantitated with the number of pixel changes representing the magnitude of movement.

One subtraction cycle (acquisition, bilevel processing, and subtraction) was repeated at an average rate of approximately one per second. The precise time of each cycle (loop time) was determined by the computer as part of each analysis. The exact loop time was applied to each cycle by utilizing the internal system clock of the computer at the start and end of each experimental session and dividing by the total number of completed cycles.

RESULTS

To quantitate movements as described in the Method section, each ROI is subtracted from the previous ROI so that the number of changed pixels in the resulting subtracted image should be directly proportional to the movement of the subject. Therefore, low numbers of changed pixels indicate immobility whereas movement generates high numbers of changed pixels. To illustrate the difference between movement and immobility, an animal was treated with either haloperidol (1 mg/kg, IP) or vehicle and catalepsy was evaluated 30 min later. The number of changed pixels per frame were then plot-

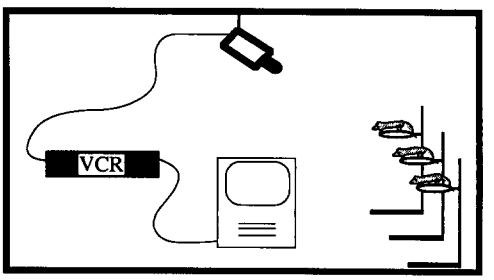

FIG. 1. Diagram of the automated catelepsy apparatus.

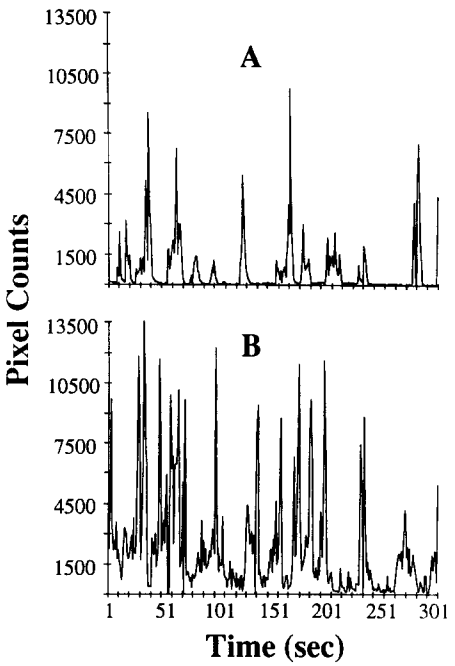

FIG. 2. Chronological representation of movement. The movement pattern as number of changed pixels/frame for a rat 30 min after an IP injection of 1 mg/kg haloperidol is presented in the upper panel (A) whereas the movement of a rat injected with vehicle is shown in the lower panel (B). The abscissa represents 1-s frames plotted in chronological order.

ted in chronological order in Fig. 2. The movement patterns of the neuroleptic-treated (Fig. 2A) and vehicle-treated (Fig. 2B) animals are easily distinguishable. Periods of low changed pixel counts (immobility) are seen to be interspersed with those containing high changed pixel counts (movement). This pattern of movement is consistent with that normally observed during a 5-min observation session.

To quantitate catalepsy, it is essential to accurately define that period of time whereby lack of pixel changes represent immobility. The first critical consideration concerns the complications that results from pixel changes between frames that are not due to the movement of the animal. These baseline changes arise because the high sensitivity of the image analysis system readily detects slight vibrations or movement in the apparatus. The second critical consideration involves determination of the threshold that distinguishes mobility and immobility. The third critical consideration is whether immobility must be defined by a minimum number of pixel changes (lower threshold). To address these issues, the data in Fig. 2A were sorted according to the magnitude of movement (number of changed pixels) and plotted vs. time of duration (not chronological time) in Fig. 3. The time on the abscissa was calculated by multiplying the number of frames by the computer loop time. The resulting plot has at least two distinct slopes. The flat portion of the curve represents frames with a small number of changed pixels and hence the period of catalepsy. The steeper portion of the curve increases continually to the end of the session and is considered to represent the period of movement. (For the sake of clarity, cycles with pixel counts greater than 1,000 were not plotted. They are represented as the plateau at the end of the time period.) The precise number of changed pixels that separate immobility from mobility is not easily discerned because the junction at which the respective portions of the plot meet is curvilinear. Contributing to the curvilinear portion of the line are varying degrees of mobility, as well as background noise. Therefore, it is necessary to establish the "higher threshold" of pixel changes that represent immobility. Obviously, changing this higher threshold will alter the time designated as immobility. While intuitively it does not seem necessary to impose lower limits on pixel changes for immobility, a problem frequently encountered with the ring test is animals either falling or jumping from the apparatus. Hence, during the periods when no animal is present the image analysis system would consider the animal cataleptic rather than mobile or absent. This point is further illustrated by the fact that the video recording of a dead rat generated some pixel changes, whereas the recording of the apparatus without a rat produced few if any pixel changes (data not shown). These results are most likely due to the contrast of the white animal on a black background, whereas the apparatus alone provides no contrast so that slight vibrations of the instrument offers no pixel change. Therefore, a lower threshold is considered necessary to account for animals either falling or jumping from the apparatus.

To establish the lower and higher thresholds (in terms of pixel number), the catalepsy of rats that had been treated with Δ^9-THC was determined simultaneously by human observers and by video recording for computer analysis. The data from the video recordings were analyzed using several different threshold values and the results are presented in Fig. 4. The resulting computer-generated catalepsy times were compared to those obtained from the human observers. The results in Fig. 4A demonstrate that raising the lower threshold to either 15 or 30 had relatively little effect on measurement of catalepsy whereas thresholds of either 45 or 65 results in an underestimation of the catalepsy. The results in Fig. 4B reveal that altering the higher threshold between 85 and 100 has no dramatic effect on catalepsy time. It is evident from the data in Fig. 4B that the lower catalepsy times determined by computer analysis tend to be slightly higher than those determined by human observation, whereas the higher catalepsy times determined by computer analysis are slightly lower than those de-

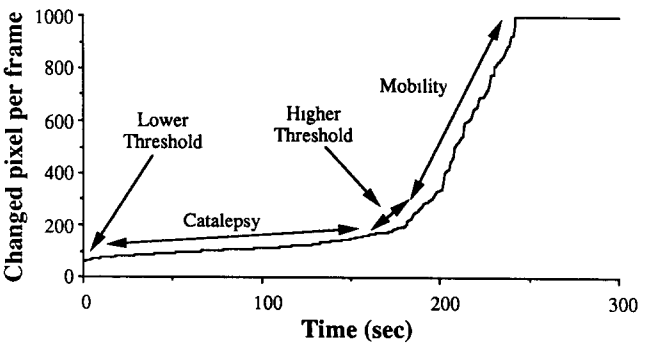

FIG. 3. Presentation of data according to number of changed pixels per frame. Data from Fig. 2A were sorted for presentation in ascending order in contrast to chronological order. The ordinate is the number of changed pixels from the preceding frame in actual time. The resulting curve is a graphic representation of the rank order of the rat's movement with the initial plateau representing the period of catalepsy during the 5-min session. The delineation between actual catalepsy and noncataleptic periods of activity is labeled "threshold." The plot is limited to 1,000 pixel counts per frame so that the presentation of the threshold could be enhanced.

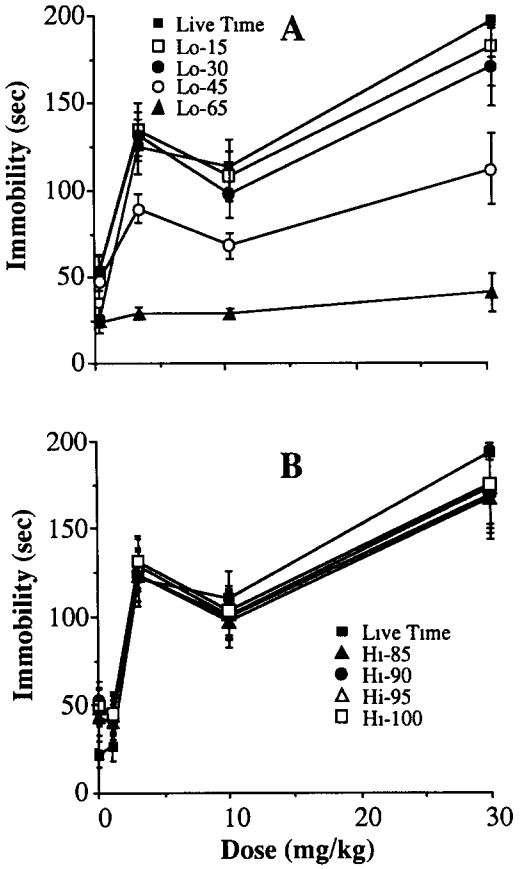

FIG. 4. Effects of alterations in thresholds on computer analysis of Δ^9-THC-induced catalepsy. Rats received an IP injection of Δ^9-THC (n = 6 rats/group) and catalepsy was simultaneously assessed by human observers and video recorded. The recordings were evaluated by the computer program by systematically altering both high and low thresholds. The results (means ± SEM) in panel A reveal the effects of altering the lower threshold while holding the higher threshold at 120. Panel B shows the effects of altering the higher threshold while keeping the lower threshold fixed at 20.

termined by human observation. For each higher threshold used in the analysis, the correlation of the computer- vs. human-determined catalepsy time alone was found to be an inadequate indication of the optimal value. As the higher threshold was systematically increased, the correlation between the human- and computer-determined catalepsy times reached a maximum value. However, further threshold increases resulted in an equally high correlation despite an obvious discrepancy between many of the human- and computer-generated times (Fig. 5). These discrepancies became evident by comparing the single mean of the human times for all rats in the data set with the corresponding value for the computer times. This comparison was made for each threshold employed in the computer analysis. The correlation was therefore weighted by dividing it by the difference between these two means (r/|diff. of means|). By this method, the optimal threshold of 158 pixels was obtained (Fig. 5).

This optimized threshold value was used for the regression analysis of computer- vs. human-determined catalepsy on an expanded data set of 206 rats. The correlation coefficient obtained was 0.94 with a slope of 0.86 (Fig. 6A). This process

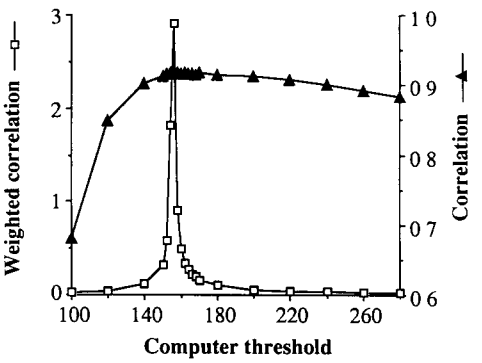

FIG. 5. Threshold optimization. The computer-generated catalepsy times of 75 rats were compared by linear regression to their times as evaluated by trained observers. The comparison was made for each computer threshold (number of changed pixels) indicated on the abscissa. The resulting correlation coefficients are plotted as the right ordinate. The left ordinate is the quotient of the correlation coefficient divided by the difference of the means of the human and computer times at each threshold.

was repeated for a data set of 70 mice. Due to the smaller size of the ROI (three per frame), the optimized threshold was determined to be 130 for this species. Linear regression analysis of the human vs. computer evaluation yielded a correlation coefficient of 0.92 and a slope of 0.91 (Fig. 6B).

DISCUSSION

Catalepsy in laboratory animals has received considerable attention for several reasons. First, it is thought that there is

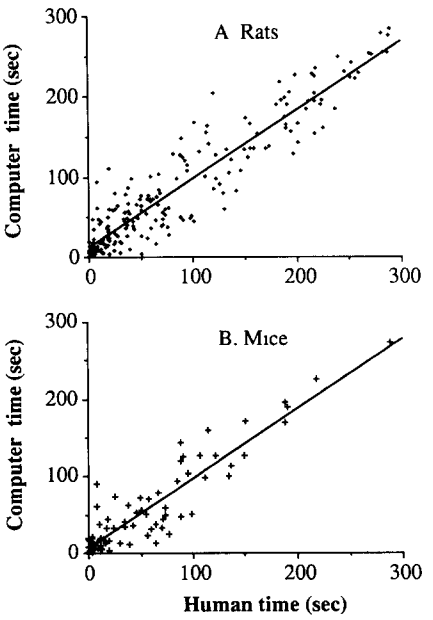

FIG. 6. Correlation of human vs. computer-generated catalepsy times for rats and mice. Animals were filmed for computer analysis and analyzed by human observers simultaneously 30 min after injection of various cataleptogens or vehicle. Computer times were generated using the optimized threshold for each species. Upper panel is for rats, n = 206, threshold 158. Lower panel is for mice, n = 70, threshold = 130.

some similarity between catalepsy and numerous pathophysiologies in the brain. On the other hand, it has been shown to be distinct from drug-induced inhibition of general locomotor activity. Second, catalepsy has become a useful behavior in studying neurochemical mechanisms as mentioned earlier. However, there are several difficulties associated with the current measurement of catalepsy, not the least of which is the use of a wide variety of measurement techniques. In addition, different laboratories use different parameters for each of the tests, which further complicates comparison of results between studies (11,24). This lack of uniformity has prompted efforts to standardize the catalepsy test, particularly the bar test (23). Another major problem associated with catalepsy arises from the fact that catalepsy has traditionally been quantitated through visual observation. To reduce the labor-intensive nature of quantitation, previous efforts have been made to automate the bar test. Moss et al. (17) developed a bar with a built-in electronic touch sensor that stops a timer with forepaw movement. More recently, Sanberg et al. (22) proposed using a commercial animal activity-monitoring system for reliably measuring catalepsy.

Although measurement of catalepsy in rats has typically relied upon the bar test, the ring test has been used for examining cannabinoid-induced catalepsy in mice (20). It should be pointed out that these two tests may not be measuring the exact same behavior. For example, grip and muscle strength are more important in the ring test while postural tonus is more important in the bar test. Another point of possible confusion might be that catalepsy in the bar tests is usually measured until the first movement of getting down from the bar, which might provide a different result than measuring total time of immobility in the ring test. Catalepsy will undoubtedly continue to be a valuable tool for studying the mechanism of action of drugs. The measurement of catalepsy has been incorporated in a battery of behavioral tests in mice that is used to evaluate cannabinoid activity (16). The automation of the elevated ring system now makes it easier to measure all behaviors in the same animal. In addition, it is important to compare catalepsy between species. In a recent report, Prescott et al. (21) adopted catalepsy in rats to compare the neuronal substrates for different behaviors produced by cannabinoids.

Of course, automated procedures must be used cautiously because there are inherent limitations in all techniques. All minor movements may not be the apparent using a video format. For example, Boren and Gallup (3) have shown that immobility is relative. Even in the absence of detectable movement by human observers, animals may still be engaged in minor movements that remain under some degree of voluntary control. On the other hand, the successful automation of catalepsy measurement eliminates an obvious source of subjectivity in the evaluation of this important drug-induced behavior. In the absence of automation, inter- and even intraobserver variability can only be addressed by increasing either the number of animals or the number of trials in an experiment. It is anticipated that this computer-image analytical system will be easily adaptable to other behaviors and other species.

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