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Is Experimental Catalepsy Properly Measured?

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FERRÉ, S., T. GUIX, G. PRAT, F. JANE AND M. CASAS. *Is experimental catalepsy properly measured?* PHARMACOL BIOCHEM BEHAV **35**(4) 753–757, 1990.—Following logarithmic transformation (ln) of total duration of haloperidol-induced catalepsy in the rat, measured by means of the bar test, a normalization of the results is achieved. With the help of this transformation we have been able to study what are probably the most important variables involved in the measuring of experimental catalepsy and to establish some criteria for a better use of such measures: 1) repeated measures of catalepsy have to be taken in order to avoid the stress-induced inhibition of catalepsy caused by the new experimental situation, 2) the dose of neuroleptic used has to be sufficiently low to permit the measurement of total or real duration of catalepsy between two determinations, and 3) the dose of neuroleptic has to be sufficiently high to prevent the development of a learned "pseudocatalepsy."

Catalepsy Haloperidol Repeated measures "Pseudocatalepsy" Adrenalectomy Apomorphine

EXPERIMENTAL catalepsy and particularly the bar test in the rat, is a frequently used procedure to study the pharmacological effect of dopaminergic antagonists (opioids and cholinergic agonists also cause experimental catalepsy) [for a recent review see (12)]. Most of the reported studies that use this methodology record repeated measures for each animal. Sometimes repeated measures are taken, according to some authors, to check the beginning of the neuroleptic pharmacological action (5). In other cases they are performed to test the effect of a drug on neuroleptic-induced catalepsy (e.g., a putative dopaminergic agonist) (4,8). Also, repeated measures are used to study phenomena such as tolerance (3, 6, 10) or time-dependent sensitization (1). Stanley and Glick (13) pointed out that the duration of catalepsy significantly increased when repeated measures were recorded and that, as a consequence, some of the already reported results dealing with experimental catalepsy had to be reviewed. Later, Costall *et al.* (5), answering Stanley and Glick's paper, did not find any difference between repeated and nonrepeated measures of catalepsy. However, another study supporting the results of Stanley and Glick recently has been published (7), claiming that repeated measures can modify the degree of neuroleptic-induced catalepsy. In any case, due to the existence of contradictory results, it is very probable that experimental catalepsy has not yet been properly measured. In addition to the problem affecting repeated measures, there are two other clear examples of the above statement: 1) following repeated treatment with dopaminergic antagonists, some authors have described the development of tolerance (decrease of the duration of catalepsy) (3, 6, 10), while others have found time-dependent sensitization (increase of the duration of catalepsy) (1), 2) treatment with apomorphine (a dopaminergic agonist) sometimes inhibits sometimes potentiates neuroleptic-induced catalepsy (8).

As all authors use different criteria for recording catalepsy, this could be one of the most important causes of confusing results. Authors do not usually measure the total duration of catalepsy, only allowing the animal to remain on the bar up to different maximum preestablished times and they use, in addition, different rating scales. Furthermore, their scorings do not show a normal distribution and therefore are not open to the versatility and potency of parametric tests. In this work we describe a new way of analyzing repeated measures of catalepsy, which has allowed us to study what are probably the most important variables involved in the measuring of experimental catalepsy and to establish some criteria for a better use of such measures.

METHOD

Animals

In all experiments male Sprague-Dawley rats weighing 350–

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450 g were used. They were housed under 12-hr dark-light cycle, with a controlled temperature (21°C) and with free access to food and water.

Catalepsy Test

To measure catalepsy all animals were taken to a soundproofed temperature-controlled (21°C) experimental room with white noise (80 dB). Catalepsy was measured by means of the bar test. The bar was made of wood and had a diameter of 1.2 cm and the height from the floor to the top of the bar was 10 cm, as Sanberg *et al.* (11) suggested that these parameters were the most appropriate when working with Sprague-Dawley rats, especially the diameter of the bar. The forepaws of the animals were gently placed over the bar and the animal timed from then until both forepaws touched the floor or until reaching the maximum time allowed in our experiment (29 min). A determination of catalepsy was performed every 30 min. Between each determination, animals were introduced into individual homecages. In our experimental room, catalepsy can be determined, simultaneously, to eight animals (there were equal bars separated by white wooden walls to prevent any animal contact). The order in which the animals were placed on the bars was always the same, being initially randomized, and the researcher who measured the duration of catalepsy did not know which treatment (haloperidol, saline, apomorphine) any of the animals had received, which had also been randomly administered (blind experiment). The number of determinations performed depended on the experiment, but the maximum were 24 which correspond to a period of time equal to the usual light period of the animal (12 hours).

Adrenalectomy

A group of animals (n = 8) underwent bilateral adrenalectomy using pentobarbital anesthesia (60 mg/kg) 24 hours before the measurement of catalepsy.

Drugs

Haloperidol (Syntex Latino, Spain) was diluted with saline and was given IP (5 ml/kg). Apomorphine (Sigma Chemicals, USA) was dissolved in saline and administered SC (1 ml/kg) immediately following its preparation, avoiding its exposure to the light. Saline was administered IP (5 ml/kg) or SC (1 ml/kg). The doses of apomorphine (1, 2 and 4 mg/kg) were within the range reported by other authors (8).

Statistics

The software SPSS ' was used for statistical studies. The procedures and tests used are indicated in each case.

RESULTS

Logarithmic Transformation (ln)

Kolgomorov-Smirnov test of normality and Barlett test of homoscedasticity demonstrate that, following logarithmic transformation (ln), the normality and homoscedasticity of all the periods from different groups of animals which are statistically analyzed cannot be discarded. Consequently, parametric statistical tests are performed (being indicated in each case).

Catalepsy Induced by Different Doses of Haloperidol

Following the administration of 0.25, 0.5, 1.0 and 2.0 mg/kg haloperidol IP to different groups of rats 30 min before the first determination of catalepsy, similar curves are obtained, all of them presenting an initial rise (periods 1 to 5) followed by a plateau (periods 6 to 10) (Fig. 1). During this plateau the group treated with 2.0 mg/kg haloperidol attained the maximum preestablished duration of catalepsy in 60% of cases, whilst the groups treated with 1.0, 0.5 and 0.25 mg/kg haloperidol attained such duration in 25%, 5% and 0% respectively. Hence, the group treated with 2.0 mg/kg haloperidol is not included in any statistical comparison, as their results considerably deviate from the measure of total catalepsy. A repeated measures analysis of variance (with polynomial contrasts) demonstrated that groups treated with 1.0, 0.5 and 0.25 mg/kg haloperidol present a significant linear increase of catalepsy during the first five periods ($p<0.01$) and that a dose dependency exists (group effect: $p<0.001$ with no significant interaction between group effect and period effect). The same statistical analysis (with different contrasts) in the plateau (periods 6 to 10) also demonstrated the existence of a dose dependency (group effect: $p<0.001$ with no significant interaction between group effect and period effect) and an absence of significative differences among periods. The periods following the plateau (periods 11 to 24) show a descent in the groups treated with 0.5 and 1.0 mg/kg haloperidol, whilst the group treated with 0.25 mg/kg haloperidol maintain catalepsy determinations similar to the plateau, the results of the last periods overlapping with the group treated with 0.5 mg/kg haloperidol.

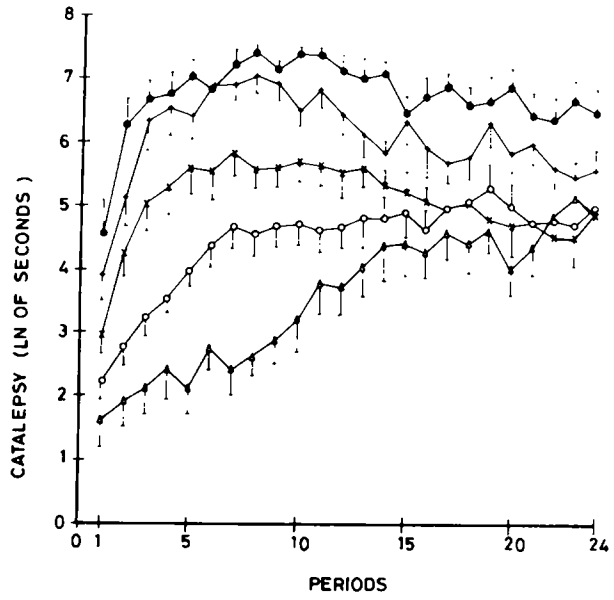

FIG. 1. Means ± SEM of catalepsy (in ln of seconds). Haloperidol or saline were administered IP 30 min before the first determination and a total of 24 tests, with an interval of 30 min between two tests, were performed. Animals were treated with haloperidol 2.0 mg/kg (●), 1.0 mg/kg (+), 0.5 mg/kg (×) or 0.25 mg/kg (○) or saline (△) (n = 8/group).

Learned Catalepsy or "Pseudocatalepsy"

The placebo group (treated with saline IP), as well as the group treated with 0.25 mg/kg haloperidol showed in the last periods a duration of catalepsy very similar to the group treated with 0.5 mg/kg haloperidol. Initially, however, the curve of the placebo group is very different from the curves of haloperidol-treated groups, being a curve characteristic of a learning process (Fig. 1).

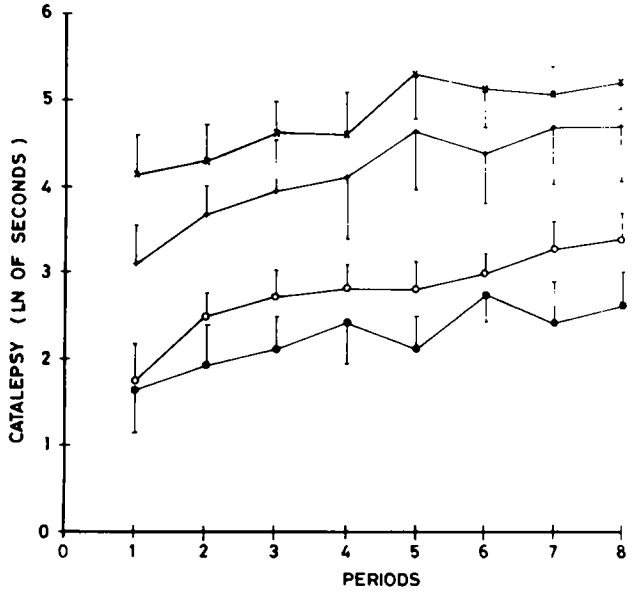

FIG. 2. Means ± SEM of catalepsy (in ln of seconds). Testing was initiated 48 hours following haloperidol IP or saline IP administration and a total of 8 tests, with an interval of 30 min between two tests, were performed. Animals were treated with haloperidol 0.5 mg/kg (○) or 0.25 mg/kg (×) or saline (+). The results of the 8 first determinations of the placebo group following saline administration are included (●) (n = 8/group).

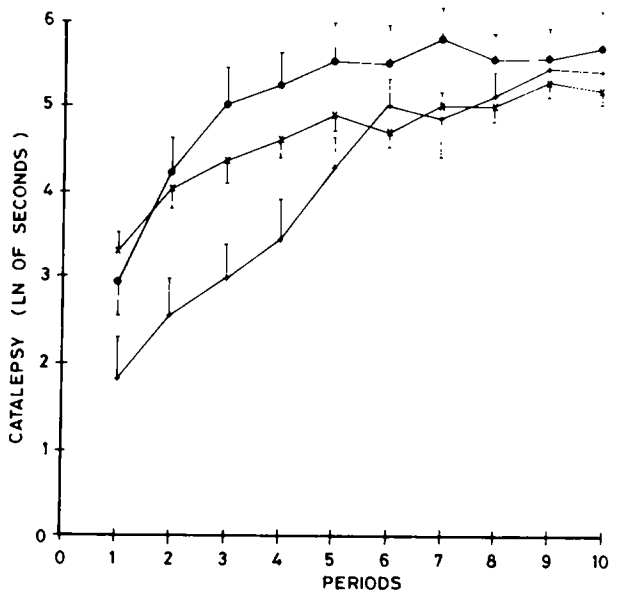

FIG. 3. Means ± SEM of catalepsy (in ln of seconds) induced by 0.5 mg/kg haloperidol IP. A total of 10 tests, with an interval of 30 min between two tests, were performed. Animals underwent the first determination 30 min (●) or 3 hours after drug administration with (×) or without (+) adrenalectomy (n = 8/group).

Forty-eight hours after haloperidol or saline administration, the animals of the groups treated with 0.25 and 0.5 mg/kg haloperidol and the placebo group were tested again during 8 periods without any treatment. These results were also compared with the first eight periods that follow the administration of saline in the placebo group. A repeated measures analysis of variance (with polynomial contrasts) demonstrated that a significant linear increase of the duration of catalepsy exists, and that the results of the placebo group and the group treated with 0.25 mg/kg haloperidol were not statistically different, being statistically greater than the results of the group treated with 0.5 mg/kg haloperidol (group effect: $p<0.001$ with no significant interaction between group effect and period effect). Furthermore, the results of the group treated with 0.5 mg/kg haloperidol were not statistically different from those of the placebo group following saline administration (Fig. 2).

Increase of the Duration of Catalepsy During the First Determinations

The catalepsy induced by 0.5 mg/kg haloperidol IP in both an adrenalectomized group and a control group was compared. Determinations began 3 hours after neuroleptic administration in both groups. A repeated measures analysis of variance (with polynomial contrast) demonstrated that during the first periods (1 to 5) a significant linear rise of the duration of catalepsy exists in both groups, although the adrenalectomized group showed significantly greater values than the control group (group effect: $p<0.001$ with no significant interaction between group effect and period effect). During the plateau (periods 6 to 10) there were no differences between the two groups, nor among periods (Fig. 3). Analyzing again the results of the group treated with the same dose of haloperidol (0.5 mg/kg) 30 min before the first determination, it can be observed that catalepsy of this group three hours after drug administration is much greater than initial catalepsy of the group in which determinations began 3 hours after drug administration (nonadrenalectomized group) (Fig. 3). A comparison between the results of the fifth period of the group treated 30 min before the first determination with those of the first period of the group treated 3 hours before the first determination demonstrated a highly significant difference (Student's t-test: $p<0.001$).

Effect of Apomorphine on Haloperidol-Induced Catalepsy

The effect of saline and 1.0, 2.0 and 4.0 mg/kg apomorphine SC in different groups of animals in which haloperidol 0.5 mg/kg IP had been administered 30 min before the first determination of catalepsy was studied. Apomorphine or saline was administered when catalepsy reached the plateau (before the sixth period) and only the differences obtained during those periods (6 to 10) were analyzed. Apomorphine shows a clear inhibitory effect on haloperidol-induced catalepsy (Fig. 4). As the curves obtained following apomorphine administration are very different from those obtained by the saline treated group, it is not possible to perform a repeated measures analysis of variance among the periods 6 to 10 to demonstrate differences among groups (being significative the interaction between the group effect and the period effect). Given that a repeated analysis of variance demonstrated an absence of significative differences among the periods 6 to 10 in the saline-treated group, it may be supposed that, in said group, each animal shows the same measure of catalepsy during such periods. Consequently the mean catalepsy that each animal presents during those five periods may be an appropriate variable to study differences among groups. A one-way analysis of variance shows the existence of significative differences in the mean duration of catalepsy of the periods 6 to 10 among the four groups: the saline-treated group and those treated with 1.0, 2.0 or 4.0 mg/kg apomorphine (Fig. 5).

DISCUSSION

Logarithmic transformation (ln) of results have allowed us to

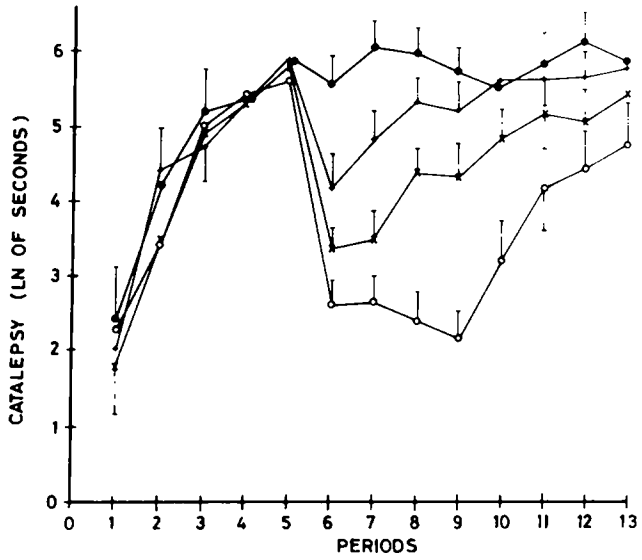

FIG. 4. Means ± SEM of catalepsy (in ln of seconds) induced by 0.5 mg/kg haloperidol IP. A total of 13 tests, with an interval of 30 min between two tests were performed. Apomorphine or saline were administered SC before the 6th determination. Animals were treated with apomorphine 1.0 mg/kg (−), 2.0 mg/kg (×) or 4.0 mg/kg (○) or saline (●) (n = 8/group).

find what we think is a suitable way of measuring neuroleptic-induced experimental catalepsy, as the total or real duration of catalepsy is recorded and, furthermore, normalization of data is achieved. Obviously, in the case of repeated measures, there is a time limit for the assessment of the duration of catalepsy which is determined by the period of time between two tests. Consequently,

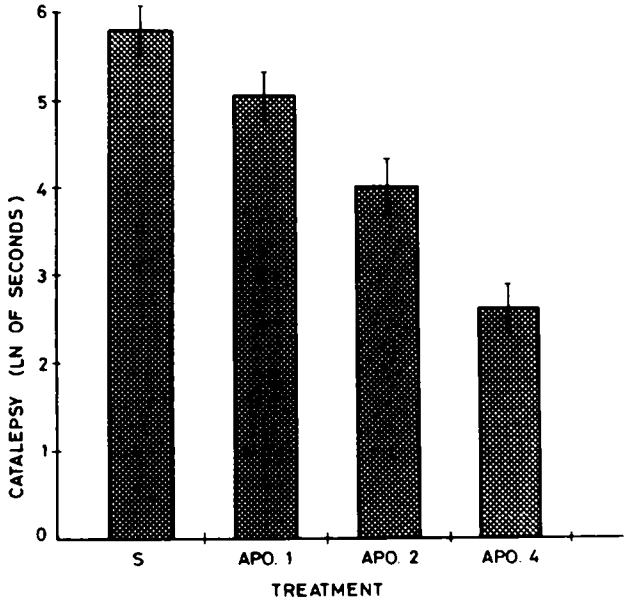

FIG. 5. Means ± SEM of 0.5 mg/kg IP haloperidol-induced catalepsy during the plateau (periods 6 to 10) of 4 different groups treated with apomorphine 1.0 mg/kg, 2.0 mg/kg or 4.0 mg/kg or saline. Apomorphine or saline were administered SC before the 6th determination (n = 8/group).

this time should not be frequently exceeded when the total duration of catalepsy is to be measured. In our experiment, for instance, we have discarded the 2.0 mg/kg haloperidol dose because the duration of catalepsy very frequently surpassed the period of time between two tests. This could explain the absence of differences from the results of the plateau between the groups treated with 1.0 and 2.0 mg/kg haloperidol, and it could also be inferred that from a given neuroleptic dose (in our experiment 1.0 mg/kg haloperidol IP), greater doses do not demonstrate a greater pharmacological action when repeated measures of catalepsy are performed.

Our results demonstrate the existence of two variables which we consider of prime importance when measuring catalepsy. We have demonstrated that placebo-treated animals, when placed repeatedly with an imposed posture, "learn" to maintain, even during long-lasting periods of time, such posture. The most probable cause of said learning is the animal's avoidance of manipulation when both forepaws touch the floor, being well-known that handling is a stressor for rats (2). Consequently, the animal is forced and trained to maintain a determined posture. The lack of blind-studies could perhaps explain why other authors have not found "pseudocatalepsy" in placebo-treated rats (1,8). By means of this experimental design we have found a posteriori, that although it is sometimes easy to distinguish between neuroleptic-induced catalepsy and pseudocatalepsy, because the cataleptic animal usually keeps more still than the pseudocataleptic, they often cannot be differentiated. Hence, it was essential to establish if both behavioral responses could overlap, i.e., if the neuroleptic-induced catalepsy could have a pseudocataleptic component. To answer this question we studied the learned catalepsy of rats that 48 hours before had been repeatedly tested (24 periods) for catalepsy following neuroleptic or placebo administration. This experiment clearly showed that rats treated with low doses of haloperidol (0.25 mg/kg IP) had developed a learning process, as they presented, in the new testing, a degree of pseudocatalepsy very similar to the placebo-treated group. On the other hand, rats treated with greater doses of haloperidol (0.5 mg/kg IP) showed a pseudocatalepsy similar to the initial testing of the placebo-treated group. Consequently, we can infer the existence of a pseudocataleptic component when measuring repeatedly the catalepsy induced by low doses of neuroleptic. In accordance with this hypothesis, in the last periods of the initial testing there was an overlapping of the results from the group treated with 0.5 and 0.25 mg/kg haloperidol IP and the placebo-treated group. Pseudocatalepsy is therefore a variable that has to be considered when performing repeated measures of neuroleptic-induced catalepsy, especially when low doses are used.

The second important variable we have found arose when the initial periods of the repeated testing were analyzed. It is generally believed that the increase in the duration of neuroleptic-induced catalepsy during the first determinations has a pharmacological explanation (due to the disposition of the neuroleptic). However, by increasing the period of time between drug administration and the first test (from 30 min to 3 hours) the same response was observed during the first determinations: a linear increase during the first 5 periods followed by a plateau during the next 5 periods. In fact, an increase of the period of time between drug administration and the first test or a decrease of the neuroleptic dose produced similar changes in the cataleptic response. We think that the first determinations of catalepsy reflect an habituation of the animal to the experimental setting. Yntema and Korf (14) have demonstrated that stress inhibits haloperidol-induced catalepsy, this effect being significantly reduced by bilateral adrenalectomy, a surgical procedure that reduces the capacity of the organism to respond to stress (9). Consequently, the decrease of the stress produced by the new experimental setting could be the cause of the increased duration of catalepsy during the first determinations.

which is supported by the fact that bilateral adrenalectomy significantly increased catalepsy during said determinations.

Summarizing, we suggest the following when measuring neuroleptic-indued experimental catalepsy: 1) repeated measures have to be performed to diminish the animal stress produced by the new experimental setting, using only the results of the plateau for comparisons. 2) Logarithmic transformation (ln) of the results has to be performed in order to normalize their distribution. 3) The dose of neuroleptic used has to be sufficiently low so as to be able to measure the total or real duration of catalepsy between two determinations. 4) The dose of neuroleptic used has to be sufficiently high to avoid the development of pseudocatalepsy, which can be measured by performing a few repeated measures of pseudocatalepsy 24 hours after the last measurement of catalepsy and comparing with controls. In our study, for instance, haloperidol 0.5 mg/kg IP was found to be the most suitable dose to study the inhibition of neuroleptic-induced catalepsy caused by a dopaminergic agonist as apomorphine, as it best fulfilled the criteria mentioned above. By using these criteria we have clearly demonstrated that apomorphine produces a dose-dependent inhibition of neuroleptic-induced catalepsy in the rat.

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