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Morphine Effects on Mouse Locomotor/Exploratory Activity: Test Dependency, Test Reliability, Uni- and Multi-Variate Analyses

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LOGGI, G., G. LAVIOLA, E. ALLEVA AND F. CHIAROTTI. *Morphine effects on mouse locomotor/exploratory activity: Test dependency, test reliability, uni- and multi-variate analyses*. PHARMACOL BIOCHEM BEHAV **38**(4) 817–822, 1991.—Drug and toxicant effects on locomotor/exploratory activity can be quite variable depending on the test and the schedule of exposure. In neurobehavioral toxicology and teratology, these interactions can affect the inferences based on the use of selected drugs as probes to assess which regulatory mechanisms are affected by one or the other treatment. The present experiments were aimed at comparing morphine effects in CD-1 mice under three conditions, namely, Varimex apparatus (VAR), toggle floor box (TOGGLE), videotape recording (VIDEO) in a home cage environment. Morphine HCl (0, 10, 33, or 100 mg/kg) was given IP 20 min before the start of a 30-min test session. The same procedure was repeated 24 h later. Results of VAR and TOGGLE tests were: dose 10 was largely ineffective; dose 33 induced depression in VAR and hyperactivity in TOGGLE; dose 100 enhanced activity in TOGGLE. There were no differences between session 1 and 2. VIDEO: Univariate analysis results showed that morphine produced a dose-dependent depression of Rearing and Grooming, and an enhancement of Crossing, again without changes due to repeated exposure. Results of Principal Component Analysis supported a response competition model of the changes observed in the mouse behavioral profile. The videorecording (VIDEO) procedure is the one providing the most accurate picture of changes in locomotor/exploratory activity and drug effects thereon, also allowing a more comprehensive statistical analysis of the relationships between various types of response changes.

Morphine Locomotor/exploratory activity Test/treatment interaction Mice Behavior Principal Component Analysis (PCA)

THE work carried out so far has often failed to provide adequate information regarding the nature of the changes in the animals' activity upon drug treatments (19). The prevailing tendency has been to use single test types for the assessment of locomotion and/or exploration levels, often disregarding possible treatment-test interactions and/or ignoring the need for multiple measurements on the same and on different response end-points (8,19). At greater magnification, it also appears that type and sensitivity of a recording device can influence the results by affecting measurements of a particular response (8, 9, 18).

In this context, it seemed important to assess the value of observational techniques which can be used to supplement, or replace, automated measurements (9, 12, 20). Besides providing concurrent multiple measurements, these techniques allow one to identify qualitative changes which otherwise escape detection (23). However, such an assessment is time-consuming and is often criticized as being "subjective," i.e., difficult to perform without introducing an experimenter bias.

Psychotropic drugs such as morphine may be classified into those which tend to increase or to decrease locomotor activity, depending on the particular dosage selected. In fact, high doses of morphine produce immobility in different rodent species (3). By contrast, when administered to particular inbred strains of mice, morphine evokes a dose-dependent locomotor hyperactivity (7). Such a hyperactivity syndrome is characterized by a stereotypic "running fit" (1,17), which usually occurs around the perimeter of the cage.

The present work was aimed at comparing in outbred mice the

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effects of morphine in two tests using automated measurements, that is, a Varimex apparatus (VARIMEX) and a toggle-floor box (TOGGLE), and in a test allowing both quantitative and qualitative observational assessments (videotape recording, VIDEO). Such a design was to diversify both the test situation (a box identical to the home cage in the case of VARIMEX and VIDEO; a totally unfamiliar environment in the case of TOGGLE) and the familiarity of the animal with any given situation (all mice were retested 24 h later in the same apparatus). The systematic investigation of treatment-test interactions performed in the present work, using a wide range of morphine doses (0, 10, 33, and 100 mg/kg), was aimed at assessing whether or not the observational approach can provide valuable information which is missed by the other approaches. In order to extract and to represent behavioral patterns in both undrugged and morphine-treated mice, Principal Component Analysis was used for VIDEO data.

METHOD

Subjects

CD-1 male mice (25–28 g) purchased from Charles River Italia (I-22050 Calco, Italy) were housed in an air-conditioned room (temperature 21 ± 1°C, relative humidity 60 ± 10%) with lights on from 9:30 p.m. to 9:30 a.m. Pellet food (Enriched standard diet purchased from Piccioni, 25100 Brescia, Italy) and water were available ad lib. Groups of four individuals were formed and housed in $33 \times 13 \times 13$ cm Plexiglas boxes with sawdust as bedding and a metal top. One mouse from each box was randomly assigned to one of four treatment conditions, including saline (NaCl 0.9%) and three doses of morphine hydrochloride (Carlo Erba, I-25100 Milano): MORPH10 (10 mg/kg), MORPH33 (33 mg/kg), or MORPH100 (100 mg/kg). Following IP injection, the animals were immediately returned to their respective home boxes and tested 20 min later in a session lasting 30 minutes (the same session duration was used for the three test types). This procedure (weighing, injection, and testing) was repeated 24 h later. All tests were carried out between 4 and 6 p.m. The experimental designs were always counterbalanced in order to equate the representation of various groups at different test times, while repeated testing of the same animal took place at about the same time of the day. The designs were also counterbalanced for assignment of animals to different apparatuses (one of the four identical units available). The boxes and the toggle-floor cages (see later) were thoroughly cleaned by cotton pools wetted with 96% ethanol, and each recording session started after an ethanol evaporation time of approximately 15 min.

Varimex Apparatus (VIDEO)

Mice were individually introduced in a clean box of the same type as the home cage. The box was placed on a Varimex Activity Meter apparatus (Columbus Instruments, OH) set at a standard level. Only the horizontal sensor systems were used.

Toggle-Floor Box Apparatus (TOGGLE)

The apparatus consisted of a $39 \times 9 \times 17$ cm Plexiglas toggle floor box. The floor was made of metal rods (diameter 1.5 mm) set at a distance of 7 mm from each other. The floor tilted when the animal crossed the midline and was connected via a Reed relay to an Accorn computer and to recording units slightly modified from those described by Bovet and colleagues (2).

Videotape Recording Apparatus (VIDEO)

Single animals were introduced in a clean Plexiglas box (of the same type of the home cage) and videotaped using a Sony VO-5630 apparatus equipped with CH-1400CE videocameras for red lights. Each animal was recorded for 15 consecutive s every min for a total recording time of 7.5 min per session.

Behavioral Observations and Categories

Recordings were scored using a hand-held keyboard connected to an Esterline Angus, by a trained observer blind to the assignment of animals to different groups, according to a score list similar to that described by Norton (16): *Crossing*: The floor of the apparatus was subdivided by black transversal lines at a distance of 8 cm from each other. Passing the line with both forepaws was the response criterion; *Rearing*: Body inclined vertically, forequarters raised; *Grooming*: Mouth or paws on body; *Smelling*: Nose against wall or ceiling and not moving; *Scratching*: Hind foot raised against body; *Washing face*: Forepaws on head; *Bar holding*: Fore- and hindpaws on the bars of the cage metal top, body suspended.

Behaviors were recorded as single bouts, unless they were separated from a successive bout by an interval of at least 3 s.

Statistical Analysis

Separate analyses of variance (ANOVAs) considering four drug dosages as between-subject factor, two sessions and six 5-min blocks as within-subject factors were carried out for each of the three types of test, using the Greenhouse-Geisser correction when appropriate. Sessions and 5-min blocks were useful to assess between- and within-session habituation. Post hoc comparisons within logical sets of means were performed using the Tukey's hsd test.

In addition, in order to take into account correlations between the different behavioral categories in the VIDEO data, Principal Component Analysis (PCA) was performed on the total number of behavioral bouts recorded during the two test sessions. PCA is a factorial method allowing reduction in the number of variables under study by constructing new characters (factors) which are linear combinations of the original variables. Moreover, PCA does not make any assumption about an underlying probabilistic model or an a priori data structure. For these reasons this technique is exploratory rather than analytical and it suggests rather than confirms hypotheses on the structure of the phenomena under study (13).

RESULTS

VARIMEX

The overall levels of locomotor activity showed habituation between the 1st and the 2nd session, $F(1,28) = 6.51$, $p < 0.02$ (see Fig. 1, upper graph). The effects of morphine varied as a joint function of dose and repeated measures within session, $F(15,140) = 2.75$, $p = 0.01$, while the higher order interaction between the said variables and sessions just missed statistical significance, $F(15,140) = 1.75$, $p = 0.08$. Specifically, in the first session morphine 33 when compared with saline levels, produced an overall reduction of activity in the 2nd and 3rd block (post hocs $p < 0.05$), while the other doses interfered with within-session habituation, (particularly, the high dose elevated activity in the last 5-min block $p < 0.01$). During the second session, the low and the intermediate doses produced a marked depression in the first three blocks ($p < 0.05$ or less), while the higher dose had little or no effects.

TOGGLE

Control mice clearly showed both within- and between-session habituation (see Fig. 1, intermediate graph). MORPH10 was in-

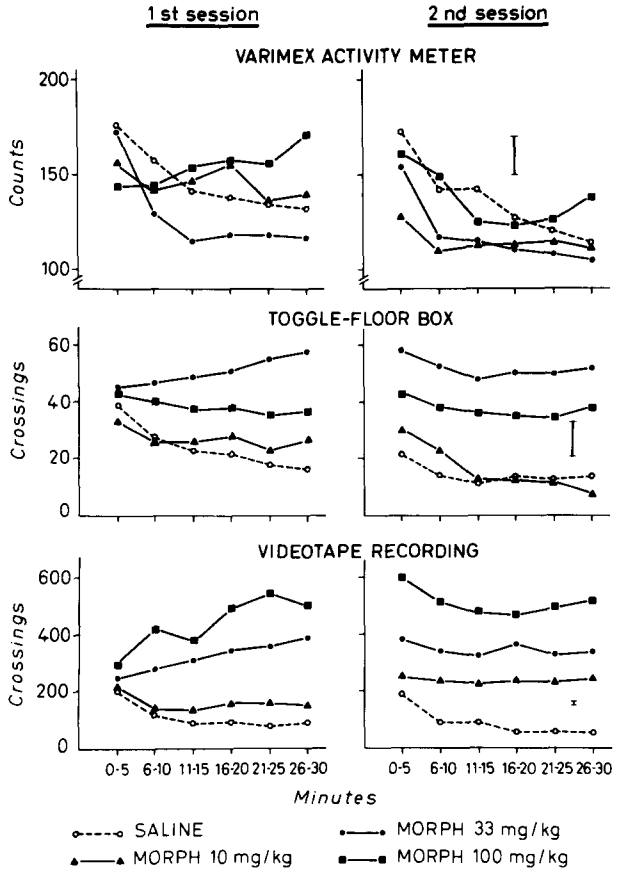

FIG. 1. Effects of IP morphine (0, 10, 33, or 100 mg/kg) upon locomotor activity in mice as determined by the use of the Varimex (upper graph), or the Toggle Floor Box (intermediate graph), or by Videotape Recording (lower graph). The same test was repeated on the same animals 24 h later (2nd session). Each point in the upper and intermediate graph represents the mean of 8 individuals, while in the lower graph it represents the sum of Crossings performed by 8 subjects during 75 s for each of the 5-min interval. The vertical bars in the figures indicate pooled S.E.M.'s derived from the appropriate error mean squares in the ANOVAs.

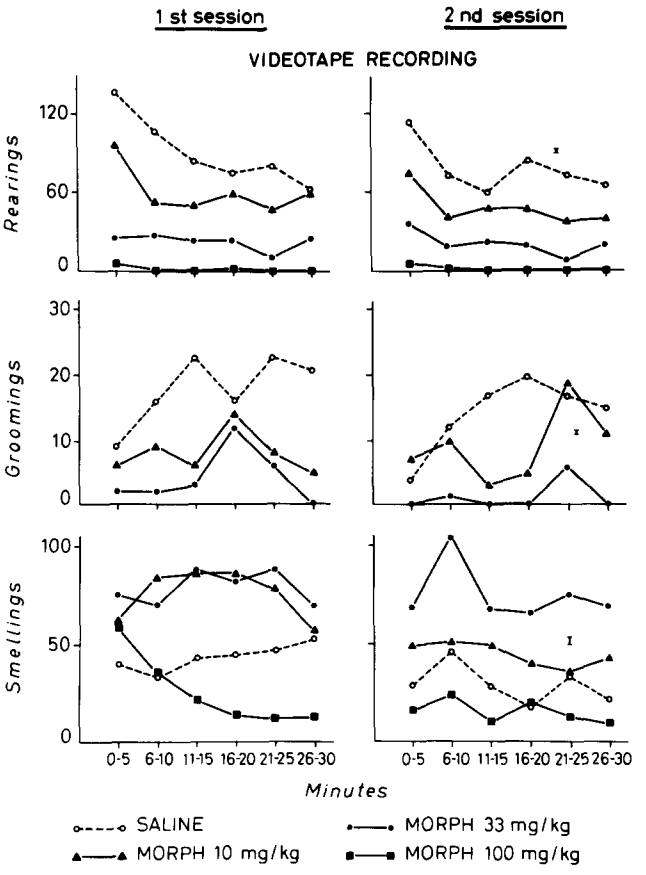

FIG. 2. Total number of behavioral bouts recorded during a 30-min session on mice injected IP with morphine (0, 10, 33, or 100 mg/kg) 20 min before testing. Data were recorded concomitantly during the same session on animals of Fig. 1 (lower graph). The vertical bars in the figures indicate pooled S.E.M.'s derived from the appropriate error mean squares in the ANOVAs.

effective, while the other two doses enhanced activity. This phenomenon was progressively more marked in the successive periods of the 1st session since the 2nd block ($p<0.05$) while it showed some attenuation in the course of the second session, drug × blocks interaction: $F(15,140)=3.05$, $p<0.01$.

VIDEO

Crossings (see Fig. 1, lower graph) were affected by morphine as a joint function of the dose, and the between- and within-session variables, $F(15,140)=2.91$, $p<0.05$. In particular, during the 1st session the low morphine dose was ineffective, while the other doses induced a hyperactivity which was more and more marked as the session progressed ($p<0.01$). On the 2nd day, morphine mice showed a dose-related hyperactivity from the start of the session ($p<0.01$). As concerns baseline levels of Rearing (see Fig. 2), they showed in both sessions a profile of within-session habituation, while morphine induced a dose-dependent decrement of Rearing, as confirmed by an interaction between drug and within-session factor, $F(15,140)=2.68$, $p=0.01$. Grooming episodes increased in the course of each session, while morphine induced a dose-related abatement of such behavior, $F(3,28)=12.96$, $p<0.001$. Further comparisons revealed a highly significant difference between controls and the MORPH33- and MORPH100-treated mice ($p<0.01$). As concerns Smelling episodes, a slight overall reduction occurred between the 1st and the 2nd session, $F(1,28)=5.44$, $p<0.05$, while morphine produced a mixed profile of effects (MORPH100 lowered, while MORPH10 and MORPH33 increased Smelling episodes), with the drug factor just missing statistical significance, $F(3,28)=2.70$, $p=0.06$.

Principal Component Analysis Applied to VIDEO Data

When applying PCA to morphine data, the percentage of variance explained by the first three factorial axes is about 87%; therefore, the structure of the phenomena was well represented considering these axes (see Table 1).

In particular (see Fig. 3), the first axis is mainly affected by crossing as opposed to self-directed behaviors (washing face, grooming, and scratching), or to other "exploratory" behaviors (rearing and bar holding). An approximate ordering of doses is evident along this axis, which explains by itself 56% of the variance. This confirms the interpretation of the first axis in PCA as a size factor of the phenomena. In particular, in our analysis, it

TABLE 1

Factorial Axes	Eigenvalues	% of Explained Variance	Cumulative %
1	3.9276	56.11	56.11
2	1.2989	18.56	74.67
3	0.8492	12.13	86.80
4	0.2970	4.24	91.04
5	0.2627	3.75	94.79
6	0.2330	3.33	98.12
7	0.1315	1.88	100.00

Principal Component Analysis: Eigenvalues and explained variance (for more details, see the Results section).

can be interpreted as a "motility" factor. Along this axis, mice injected with MORPH100 are opposed to saline-injected mice, demonstrating, therefore, the influence of the higher morphine doses on locomotion.

The second factorial axis is determined by the opposition between smelling and crossing: mice injected with MORPH10 and MORPH33 are clustered around the far end of the axis influenced by smelling. This can be likely explained by a dose-dependent reduction of the animal's activities aimed at cage exploring, with some residual attention at the said doses which disappears at the highest (100 mg/kg) dose (see also the Discussion section). The third axis documents an opposition between self-directed behaviors (scratching, grooming, and washing face) and "exploratory" patterns (rearing and bar holding); along this axis there is an approximate separation between saline and MORPH10 on the one side, and MORPH33 and 100 on the other.

DISCUSSION

The results reported above show similar trends of locomotor activity in undrugged animals in the different tests. In fact, a clear within-session habituation was observed in all three conditions. As concerns morphine effects, the low dose produced little or no changes, while at the intermediate dosage the drug elicited opposite effects (either "excitatory" or "inhibitory"), depending on the test. The highest dose produced a similar enhancement of locomotor activity in the various conditions, which is in agreement with previous literature data (23,24). A slight reduction of morphine effects on the 2nd session was also evident, particularly at the highest dose (100 mg/kg). This may be due either to acute tolerance or to a modification in the interactions between drug and external stimuli (see below).

Overall, the data show an unquestionable superiority of the VIDEO procedure which identified an interesting profile of changes in different directions, depending on the response endpoint. Specifically, the procedure provided 1) a detailed assessment of control activities, including (a) habituation of locomotion and rearing in both the first and the second session, (b) an opposite trend of grooming behavior, which appeared to increase when the environment became familiar, while "exploratory" activity decreased, and (c) different trends of smelling in the course of the first and second sessions; 2) a clear description of the dose-dependent locomotor activation produced by morphine, including dif-

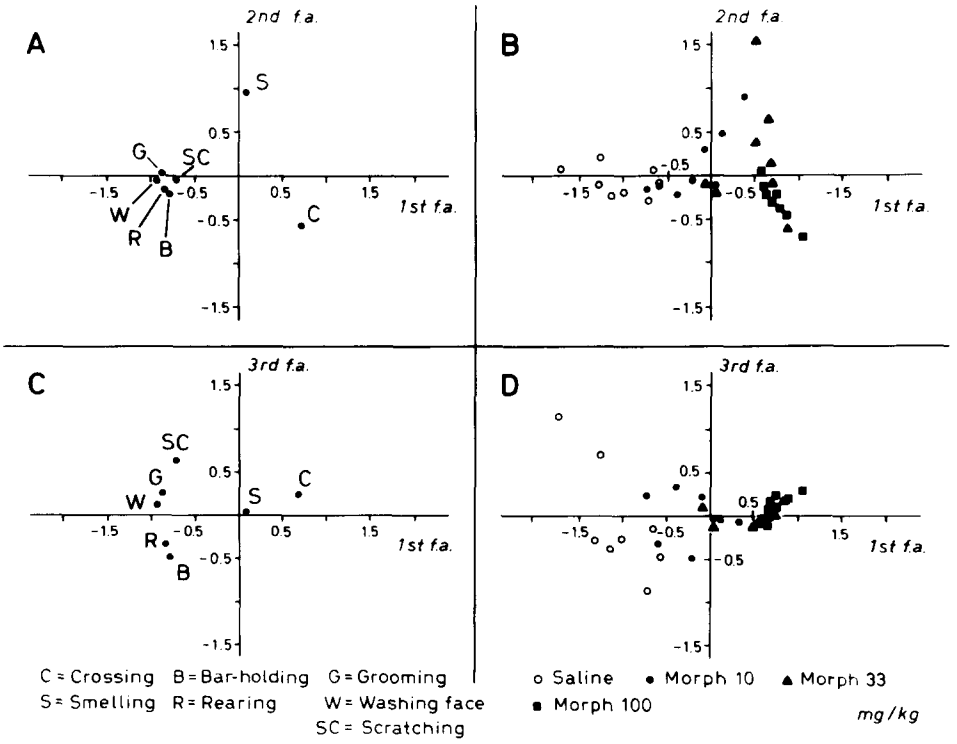

FIG. 3. Principal Component Analysis performed on the total number of behavioral bouts measured in the two sessions of test by VIDEO procedure. Factorial plans based on the 1st and the 2nd, or on the 1st and the 3rd factorial axes (f.a.). A and C quadrants represent plans of variables (behavioral categories); B and D quadrants represent plans of statistical units (individual mice). For more details, see the Results section.

ferences in habituation patterns between the first and the second session; and 3) an adequate picture of morphine changes in other responses. The latter consisted mainly of a dose-dependent depression of rearing and grooming behavior, and of a nonmonotonic change in the case of smelling (increase at the lowest and intermediate doses, and decrease at the highest dose).

At least part of these effects could be due to the sensory changes produced by morphine. In particular, the higher drug dose could result in an interference with the sensory encoding of olfactory cues (i.e., via a functional bulbectomy or a tubero-infundibular anesthesia). Neurochemical alterations in the opiate system at the level of several olfactory and brain areas have been reported to follow olfactory bulbectomy (10,14).

The results reported above also point to a greater reliability of TOGGLE than of VARIMEX data. In fact, the former provided a picture of changes in locomotor activity which replicated part of that obtained by direct observation (VIDEO), in spite of the apparatus differences: TOGGLE environment was in fact totally unfamiliar, while VIDEO (and VARIMEX) mice were scored in a box identical to the home cage, but lacking the familiar olfactory cues of the home environment. On the other hand, a mixed profile of "excitatory" and "depressive" changes was obtained by the VARIMEX test. This may be due to the fact that this test does not discriminate between motor changes in opposite directions after morphine treatment, i.e., dose-dependent enhancement of locomotion and concomitant reduction of rearing and grooming (as found in VIDEO).

As concerns these differences in activity profiles obtained by the use of different automated recording apparatuses, it has been reported that spontaneous locomotor activity is responsive to the manipulation of environmental variables (21), while the existence of separate neural mechanisms underlying different types of locomotor activity (e.g., running-wheel activity vs. stabilimeter-cage activity) has also been hypothesized by previous authors (15, 21, 22). In fact, some lesions such as those of dorsal frontal cortex and hippocampus can potentiate the effects of starvation on stabilimeter activity but not those on running-wheel activity (15). Moreover, Campbell (4) reported that running increases as a function of day of water deprivation, but the number of stabilimeter crossings actually decreases. In general, running is believed to be more sensitive to internal variables (e.g., body temperature, starvation, estrus); on the other hand, stabilimeter activity appears to be more responsive to environmental events [see, e.g., (22)]. In the case of morphine, however, running is often interpreted as being stimulation-dependent. Both morphine-induced immobility and running have been shown in rats to be under control of different environmental stimuli [see the detailed behavioral analysis of drug effects in (5,6)].

Attempts to link these previous reports with the present results can only be tentative, but certain hypotheses seem warranted. In the present study, the different activity changes produced by the same experimental manipulation (intermediate morphine dose) are apparently the consequence of the fact that different apparatuses (VARIMEX and TOGGLE), measure different responses. Perhaps, MORPH33 stimulation as previously hypothesized for starvation or water deprivation (see above), served to reduce the response threshold to environmental stimulus change. TOGGLE apparatus is known to generate a great deal of response-produced feedback which, in turn, interacting with lowered response thresholds to environmental stimuli, accounts for the enhanced level of locomotor activity.

In any event, it appears that more information can be obtained from an experiment if multiple measures of behavior are taken. In fact, drug-induced changes of locomotor activity may produce indirect effects on other responses such as exploration and/or self-directed activities. Therefore, it is necessary to test for possible direct effects of such drugs on these aspects of behavior by measuring concurrently both locomotor activity and the other response under study [see, e.g., (19)]. While investigating the covariation of the different measures, it is worth assessing the effects of a wide dose range on the different aspects of behavior, since for example an indirect effect on locomotor activity might be precluded by the finding that a particular dose affected one or the other response in the absence of an effect on locomotor activity.

In this context, the results of PCA performed on VIDEO data confirm a response competition model of the behavioral changes induced by morphine. The identification of interrelations between different response changes serves the purpose of focusing the attention on the phenomena which deserve further research aimed at clarifying the underlying processes and mechanisms; not that of defining the nature of the changes observed or the direction of causal relations. Therefore, the PCA results cannot be used to decide between a primary effect of morphine on the animal's interest in the environment and a reduction of specific behavioral responses such as smelling simply as a consequence of compulsory locomotion. However, such an approach allows one to exploit fully the available information, avoiding arbitrary selections of response end-points and abating the "noise" which can result from multiple measurements.

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