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Original article

Comparison of methods for the assessment of locomotor activity in rodent safety pharmacology studies

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ABSTRACT

Introduction: General neurobehavioral assays, like a modified Irwin test or a functional observational battery, are necessary for central nervous system (CNS) safety pharmacology testing near the end of the target validation (early discovery) stage of preclinical drug development. However, at earlier stages, when a greater number of test compounds must be screened for potential CNS side effects, locomotor activity assessment may be a better tool for the comparison of compounds. Methods: Spontaneous locomotor activity counts obtained from two automated test systems – an infrared beam-based activity meter (Actimeter) and the mechanical vibration-based LABORAS – were compared in rats dosed with chlorpromazine (2–8 mg/kg) or caffeine (3–24 mg/kg), p.o. A modified Irwin test was also performed to visually observe the neurobehavioral effects. Results: In all three assays, dose-dependent sedation- and excitation-related effects were observed with chlorpromazine and caffeine, respectively. The two automated activity-detection systems exhibited similar sensitivities in determining changes in locomotor activity, but with the LABORAS being more sensitive than the Actimeter in detecting caffeine-induced increases in vertical activity (rearing behavior). Discussion: Infrared beam-based activity detection systems and LABORAS provide relatively-comparable quantitative data regarding locomotor activity. Practical considerations, such as relative cost versus degree of versatility, should be considered when deciding which system to use for the screening of test compounds during the earliest stages of preclinical drug development.

1. Introduction

International Conference on Harmonisation (ICH) guidelines recommend that, prior to first-in-human studies, test compounds should be evaluated in laboratory animals to determine functional effects on three vital organ systems: the central nervous system (CNS), cardiovascular system, and respiratory system (Anon, 2001). For assessing potential CNS effects, a functional observational battery (FOB) or similar test of general neurobehavioral changes, such as a modified Irwin test, is often used. These assays, which are generally performed within the pharmaceutical industry according to Good Laboratory Practice (GLP), are important even for test compounds targeting non-CNS-related disorders (Redfern, Strang, Storey, Heys, Barnard, Lawton et al., 2005). However, there are a number of practical disadvantages to such testing including that the assays require fairly extensive training and regular intra-/inter-observer validation efforts to consistently result in accurate data, involve a relatively large amount of highly-focused time on the part of the investigator, and result in mainly qualitative data. Therefore, although general neurobehavioral testing is necessary near the end of the target validation (early discovery) stage of preclinical drug development, such testing may not be the most efficient strategy during the earlier stages of target validation when a higher number of compounds are screened and compared for potential CNS side effects. The earlier that test compounds can be prioritized or eliminated based upon their relative CNS side effect profiles, the sooner that resources can be focused on the compounds that have a greater likelihood for proceeding to clinical trials.

We have previously demonstrated that a change in spontaneous locomotor activity is an excellent preclinical indicator of CNS/neurobehavioral effects of test compounds, including effects observed in a modified Irwin test of general behavior (Lynch & Mittelstadt, 2009). That is, over a broad range of preclinical compounds tested, we found that all compounds having moderate to severe neurobehavioral effects in a modified Irwin test also exhibited statistically-significant changes in spontaneous locomotor activity. Moreover, these changes in locomotor activity always occurred at doses comparable to (i.e., within 10-fold) the lowest effective dose in the Irwin test. Therefore if, for example, a test compound was determined as having no effects on locomotor activity at doses up to 100 mg/kg, we could be fairly certain that it would have no more than mild effects in the modified Irwin test at doses up to 10 mg/kg.

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A traditional method for assessing changes in locomotor activity is via commercially-available test systems that automatically quantify interruptions of infrared beams by a rodent within a testing enclosure. Such technology has been employed for well over a quarter century (Menniti & Baum, 1981). Other than standard animal handling and injection skills, minimal training is needed by an investigator to use such automated test systems, the throughput is relatively high, and the output is non-subjective quantitative data that is well suited for comparison of a large number of test compounds. Rodent spontaneous locomotor data, which our laboratories collect very early in the drug discovery process under non-GLP conditions, is often incorporated into regulatory submissions along with GLP FOB results.

A more recently commercialized, automated behavior analysis system that includes a locomotor activity component is the LABORAS (Van de Weerd, Bulthuis, Bergman, Schlingmann, Tolboom, Van Loo et al., 2001). Instead of using infrared photocells, LABORAS determines activity based on weight displacement and mechanical vibrations by the rodent and translating the vibrations into electrical signals that the software then classifies into various behavioral categories. Much of the focus for use of LABORAS has been on detecting long-term, often diurnally-related behavioral changes (Quinn, Grundy, Campbell, Collier, Lawman, Stean et al., 2005; Van de Weerd et al., 2001; Wood, Goodman, van der Burg, Gazeau, Brundin, Björkqvist et al., 2008). However, a few studies have also examined acute behavioral effects such as those relevant to safety pharmacology testing (McCann, Palfreeman, Andrews, Perocheau, Inglis, Schafer et al., 2010; Quinn, Stean, Trail, Duxon, Stratton, Billinton et al., 2003).

The purpose of the current study was to compare the sensitivity of one component of the LABORAS, its ability to detect acute locomotor activity changes, to the sensitivity of a more traditional, infrared photocell-based, locomotor activity assessment system such as the Actimeter. To the best of our knowledge, there have been no published reports directly comparing data from these two automated, but dissimilar, types of test systems. Additionally, both automated systems were compared to the more labor intense, visual observation procedure of a modified Irwin test. The locomotor depressant, chlorpromazine and the stimulant, caffeine served as reference substances to perform these comparisons.

2. Methods

2.1. Animals, reference substances, and dosing

Studies were performed in accordance with French legislation concerning the protection of laboratory animals and in accordance with a valid license, issued by the French Ministry for Agriculture and Fisheries, for experiments on vertebrate animals. Male Rj:Wistar (Han) rats (190–240 g body weight at the time of testing) were purchased from Elevage Janvier (Le Genest-Saint-Isle, France) and were housed in facilities at Porsolt SAS in a temperature-regulated environment with the lights on between 07:00 and 19:00 h. Food and water were available ad libitum, except during certain portions of the modified Irwin testing and throughout the 40 min of Actimeter testing. Experimentally naïve animals were used for each test and were sacrificed (by inhalation of a mixture of 20% O_2/80% CO_2 followed by 100% CO_2) at the end of each assay (i.e., no reuse). Chlorpromazine hydrochloride was purchased from Sigma (Saint Quentin Fallavier, France), and anhydrous caffeine was purchased from Coopération Pharmaceutique Française (Melun, France). Doses of chlorpromazine tested were 2, 4, 8, and 16 mg/kg, and those for caffeine were 3, 6, 12, and 24 mg/kg (Ilbäck, Siller, & Stålhandske, 2007; Moscardo, Maurin, Dorigatti, Champeroux, & Richard, 2007). Doses were expressed as mg/kg of supplied substance, i.e., not corrected for proportion of active substance. Solutions of the reference substances were prepared fresh daily by dissolving in distilled water. The reference substances and their vehicle control (distilled water) were administered by oral (p.o.) gavage at a dosing volume of 5 ml/kg of body weight. During all experiments the observer was blinded as to the dose that each animal received, with the one exception that the identity of the vehicle control group was known during the modified Irwin test. Although blinding is essential for most neurobehavioral testing and particularly during more subjective assays like the modified Irwin test, a non-blinded vehicle control group should be available to the experimenter during modified Irwin testing, to serve as a visual comparator for "normal" animal behavior under those same testing conditions and during that particular moment in time.

2.2. Modified Irwin test

Animals were placed in the testing room the day preceding the test. On the morning of testing, the rats were weighed and baseline rectal temperature and pupil diameter were measured approximately 2 h before the start of dosing. Animals were administered a dose of a reference substance ($n=4$ animals per dose) and then observed (continuously from 0 to 15 min post-dose, and discretely at 1/4, 1/2, 1, 2, 4 and 24 h post-dose) in simultaneous comparison with a vehicle control group. Behavioral changes, physiological and neurotoxicological signs, rectal body temperature, and pupil diameter were recorded according to a standardized observation grid derived from that of Irwin (Irwin, 1968; Porsolt, Dürmüller, Castagné, & Moser, 2007). The grid contained the following items: death, convulsions, tremor, Straub tail, altered activity, jumping, altered reactivity to touch, altered fear/startle response, altered abdominal muscle tone, aggression, fore-paw treading, head twitches, stereotypies (head movements, chewing, and sniffing), scratching, catalepsy, akinesia, abnormal gait (rolling and tip-toe), motor incoordination, loss of balance, loss of traction, loss of grasping, loss of righting reflex, loss of corneal reflex, writhing, analgesia, ptosis, exophthalmia, pupil diameter (miosis or mydriasis), piloerection, defecation/diarrhea, salivation, lacrimation, altered respiration, and rectal body temperature (hypothermia or hyperthermia).

2.3. Assessment of spontaneous locomotor activity

Animals to be tested in the Actimeters and LABORAS were placed in their testing room, on the morning of testing, approximately 2 h before the start of dosing. The rats were administered either vehicle or a dose of a reference substance ($n=10$ animals per dose) and then temporarily returned to their home cages. Prior to the start of activity testing, in order to measure their locomotor activity during both exploration and habituation phases in a novel environment, the animals were not habituated to the testing cages.

Activity testing was initiated 60 min after dosing (see below), and the resultant scores were cumulated over the 0 to 20 min assessment period (i.e., 60–80 min post-dose; exploration phase) and the 20 to 40 min assessment period (80–100 min post-dose; habituation phase). However, only data from the 0 to 20 min assessment period has been reported for chlorpromazine and from the 20 to 40 min assessment period for caffeine because those are the time periods most affected by sedative and excitatory effects, respectively. Furthermore, the reported assessment periods correspond to times when these reference substances exhibit near-maximal plasma concentrations and behavioral changes (Curry, D'Mello, & Mould, 1971; Ilbäck et al., 2007; Moscardo et al., 2007; Wang & Lau, 1998).

2.3.1. Actimeter (infrared photocell-based detection)

Before each test session, the Actimeters (Imetronic Neurosciences, Pessac, France) were validated by manually interrupting the infrared beams and verifying the correspondence between the actual number of beam breaks and the number recorded by the system. At the start of activity testing (60 min after dosing), the animals were individually placed into covered plexiglass cages (38×24×21 cm internal dimensions) contained within a darkened enclosure and connected to silent

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electronic counters (Actimeters). Each cage was equipped with four infrared photocell units, two at each end of the cage and all located 3 cm above the cage bottom, in order to assess movements within the horizontal plane. Ten additional photocell units were placed 20 cm above the cage bottom, at even intervals along the long axis of the cage, to record rearing behavior (vertical activity). The number of horizontal crossings by each animal, from one pair of (3 cm-high) photocell units to the other, was recorded by computer in 10-min intervals for 40 min. For rearing behavior, the number of individual (20 cm-high) photobeam breaks was recorded by computer in 10-min intervals for 40 min.

2.3.2. LABORAS (mechanical vibration analysis)

Before each test session, the LABORAS (Metris b.v., Hoofddorp, The Netherlands) was calibrated using the calibration procedure and reference weights supplied by Metris. At the start of activity testing (60 min after dosing), the animals were individually placed into covered polycarbonate/Makrolon type IIIh cages (37×21×24 cm internal dimensions) in the LABORAS, all of which resided in a darkened testing room. Food and water were available throughout the 40 min of testing. Data were automatically analyzed for frequency (counts) of horizontal and vertical activity as well as for the following, additional behavioral parameters: duration of horizontal and vertical activity; frequency and duration of grooming, drinking, feeding, immobility, and undefined behaviors (i.e., all behaviors that do not fit into the previous categories); and total distance traveled, average speed during locomotion, maximum speed, and duration at maximum speed.

2.4. Data analysis

No formal statistical analysis was conducted on the data from the modified Irwin testing. Data from the Actimeter and LABORAS testing were analyzed using 2-way ANOVA (with the test systems and treatments as the analysis factors) followed by 1-way ANOVAs (for each treatment) and Dunnett's two-tailed test (comparing reference substance dosing groups with vehicle control) for post hoc analysis (InVivoStat; http://invivostat.co.uk). The level of significance was set at $P<0.05$.

3. Results

3.1. Modified Irwin test

Animals dosed with chlorpromazine exhibited a number of signs (Table 1), but the predominant ones were decreased abdominal muscle tone, slightly decreased activity, and decreased fear/startle response (to an investigator's fingers snapping). These sedation-related effects were generally dose dependent, and they were noted only at the 1/2, 1, 2 and 4 h post-administration time points. For the sign of decreased activity, only the 8 and 16 mg/kg dosing groups were observed as having this effect.

Animals dosed with caffeine also exhibited a number of signs (Table 2), with stereotypies (increased head movements and sniffing behavior), slightly to moderately increased activity, increased fear/startle response, and increased respiratory rate being the predominant ones. These excitation-related effects were generally dose dependent, and they were noted only during the 15-min continuous observation period (immediately after dosing) and at the 1/4, 1/2, 1 and 2 h post-administration time points. For the sign of increased activity, all four dosing groups were observed as having this effect.

3.2. Spontaneous locomotor activity

Statistical comparisons of the effects of the two reference substances in the two test systems using two-way ANOVA determined statistically significant effects for chlorpromazine and caffeine on both horizontal and vertical activity count data ($P<0.001$ for the factor treatment for all four comparisons). One-way ANOVAs with Dunnett's two-tailed tests were then performed for each treatment, and the data for each test system is reported below.

3.2.1. Actimeter

Chlorpromazine decreased spontaneous locomotor activity in a dose-dependent manner, with statistically-significant effects on horizontal activity at 8 and 16 mg/kg and on vertical activity at 4, 8 and 16 mg/kg (Fig. 1). By comparison, caffeine produced dose-dependent increases in spontaneous locomotor activity, with statistically-significant effects on horizontal activity at 6, 12 and 24 mg/kg and on vertical activity at 12 and 24 mg/kg (Fig. 2).

3.2.2. LABORAS

Chlorpromazine decreased spontaneous locomotor activity in a dose-dependent manner, with statistically-significant effects on both horizontal and vertical activity at 8 and 16 mg/kg (Fig. 1). By comparison, caffeine produced dose-dependent increases in spontaneous locomotor activity, with statistically-significant effects on horizontal activity at 6, 12 and 24 mg/kg and on vertical activity at all four doses tested (Fig. 2). Similar to the data for frequency, horizontal and vertical activity duration were dose-dependently decreased with chlorpromazine and increased with caffeine, but the statistically-significant effects for duration oftentimes began at higher doses than those for counts (data not shown).

3.2.3. Comparison of Actimeter with LABORAS

In addition to statistically-significant effects for the factor of treatment (described above), two-way ANOVA determined a statistically significant interaction effect between caffeine treatment and the test system for horizontal activity counts ($P<0.001$). This can be observed graphically in Fig. 2 where increasing doses of caffeine caused increased horizontal activity and the Actimeter reported a smaller relative increase in counts than did LABORAS. By comparison, treatment and test system interactions were not significant for the data on the effects of caffeine on vertical activity counts nor on the effects of chlorpromazine on both horizontal and vertical activity counts ($P>0.05$ for all 3 comparisons).

4. Discussion

As expected (Ilbäck et al., 2007; Moscardo et al., 2007), rats dosed with chlorpromazine and caffeine exhibited sedation- and excitation-related effects, respectively. Changes were automatically detected by both the Actimeter and LABORAS test systems, and they were visually confirmed during the modified Irwin testing. In all three assays, the effects were generally dose-related, and some changes were determined at even the lowest dose of each reference substance tested.

The Actimeter and LABORAS test systems exhibited equal sensitivity for detecting changes in horizontal activity counts, as was observed during chlorpromazine and caffeine exposure. That is, whenever the Actimeter or LABORAS identified a statistically-significant effect on horizontal activity counts, the other system also identified this effect at the same dose. For vertical activity counts (rearing behavior), data were generally similar between the two test systems, although the Actimeter detected significant activity changes at one lower dose than did LABORAS when assessing chlorpromazine-induced decreased activity, while LABORAS identified significant effects at two lower doses than did the Actimeter when assessing caffeine-induced increased activity. The apparent difference in sensitivity with chlorpromazine appears more likely due to happenstance than to a true effect: these data greatly overlapped, so much so that a 2-way ANOVA found no statistically-significant difference between the test systems. In contrast, the apparent difference in sensitivity for caffeine was a more robust effect in that it was observed for two of the four doses tested. The reason for this sensitivity difference

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t1.1 **Table 1**

t1.2 Effects of chlorpromazine in a modified Irwin test.

Dose (mg/kg, p.o.)		2							4							8							16						
Observation time		0-15m	15m	30m	60m	120m	240m	24h	0-15m	15m	30m	60m	120m	240m	24h	0-15m	15m	30m	60m	120m	240m	24h	0-15m	15m	30m	60m	120m	240m	24h
Death																													
Convulsions Tremor Straub tail	Excitation																												
Increased activity: marked Increased activity: moderate Increased activity: slight	Excitation																												
Jumping Increased reactivity to touch Increased fear/startle Increased abdominal muscle tone Aggression	Excitation																												
Fore-paw treading Head twitches Stereotypies (head movements) Stereotypies (chewing) Stereotypies (sniffing) Scratching	Stereotypy																												
Catalepsy Akinesia Abnormal gait (rolling) Abnormal gait (tip-toe) Motor incoordination Loss of balance Loss of traction Loss of grasping	Motor																												
Loss of righting reflex Loss of corneal reflex	Sedation																												
Decreased activity: marked Decreased activity: moderate Decreased activity: slight	Sedation																												
Decreased reactivity to touch Decreased fear/startle Decreased abdominal muscle tone	Sedation																												
Writhing Analgesia	Pain																												
Ptosis Exophthalmia Miosis[a] Mydriasis[a] Piloerection Defecation/diarrhea Salivation Lacrimation	Autonomic																												
Increased respiration Decreased respiration Hypothermia[a] Hyperthermia[a]	Other measures																												

The shading indicates the number of rats exhibiting the signs (or the intensity for the signs with footnote "a"): ▒: 1/4 (or slight), ▒: 2/4 or 3/4 (or moderate) and ■: 4/4 (or marked).
[a]Evaluated by comparison of the mean scores obtained in chlorpromazine- and vehicle-treated animals.

t1.34

was not determined in the present study, but it may have been due, at least in part, to slight differences between the testing environments (e.g, dimensions of the test enclosures, degree of darkness, ambient noise, and presence or absence of food and water during testing, etc.) resulting in the greater increase in caffeine-induced vertical activity counts in the LABORAS.

In addition to small differences in sensitivity between the Actimeter and LABORAS test systems, there was a differential effect of caffeine dose on horizontal activity counts between the two systems ($P<0.001$ for the interaction of test system and treatment). That is, as increasing doses of caffeine increased horizontal activity, the Actimeter reported a smaller relative increase in counts than did LABORAS (Fig 2). This difference may be due to the fact that animals within the Actimeter must have locomotion of a sufficient distance and direction to interrupt an infrared beam before an activity count is recorded, while the LABORAS may be able to detect a smaller magnitude of locomotion and with less regard to direction. Thus, when locomotor activity was elevated by the higher doses of caffeine, LABORAS may have been better able at detecting smaller movements in addition to the relatively larger movements that the Actimeter detected. Testing of additional reference stimulants may be useful in determining whether this is a generalized, excitation-related difference between the two systems or if it is specific for caffeine.

The Actimeter and LABORAS test systems can also be compared regarding a number of practical considerations. Both systems can

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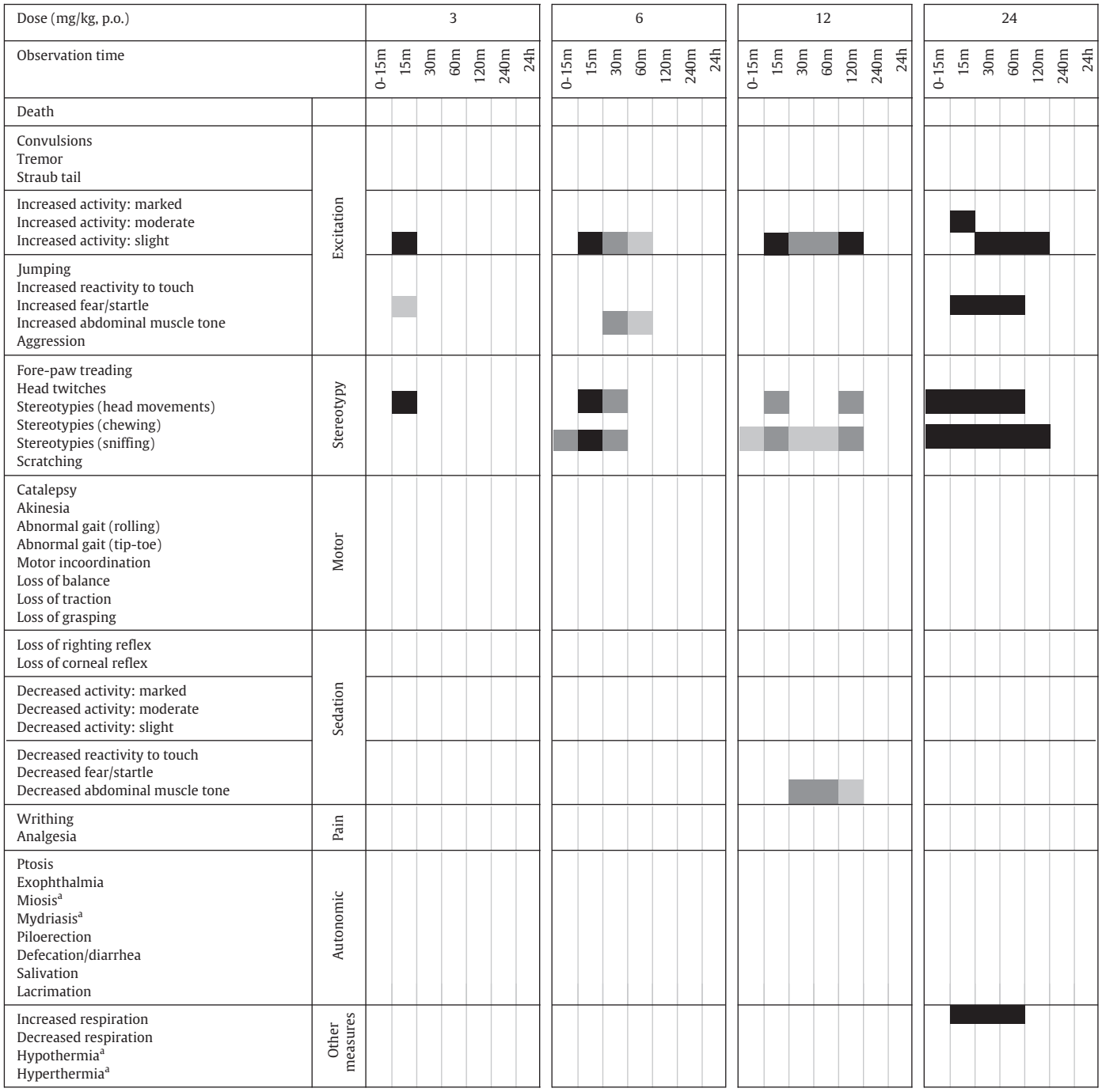
t2.1 **Table 2**
Effects of caffeine in a modified Irwin test.

t2.2



The shading indicates the number of rats exhibiting the signs (or the intensity for the signs with footnote "a"): ▒: 1/4 (or slight), ▓: 2/4 or 3/4 (or moderate) and ■: 4/4 (or marked).
t2.4 [a]Evaluated by comparison of the mean scores obtained in caffeine- and vehicle-treated animals.

readily be used for either mice or rats. In general, the Actimeter is a less expensive system that requires slightly less calibration time, but LABORAS is more versatile in terms of the parameters that it can measure. Similar to the Actimeter (and infrared beam-based activity assessment systems in general), LABORAS produces non-subjective horizontal and vertical activity counts with relatively little effort on part of the investigator. In addition, LABORAS's data sets include horizontal and vertical activity duration as well as data regarding a number of other parameters such as grooming, drinking, feeding, distance and speed. In the current study, none of LABORAS's additional parameters were more sensitive indicators of the effects of chlorpromazine and caffeine than were horizontal and vertical activity counts (data not shown). However, other compounds have been reported as having weaker effects on activity counts than on other LABORAS measures. For example, in mice dosed with amphetamine, a statistically-significant change in activity counts was not observed until a dose of 5 mg/kg was administered, while changes in both immobility and climbing duration were observed at doses as low as 0.1 mg/kg (Quinn et al., 2006).

Data obtained from these two automated activity-detection systems can also be compared to effects visually observed during the inherently more labor-intensive modified Irwin test. In a previous study, a change

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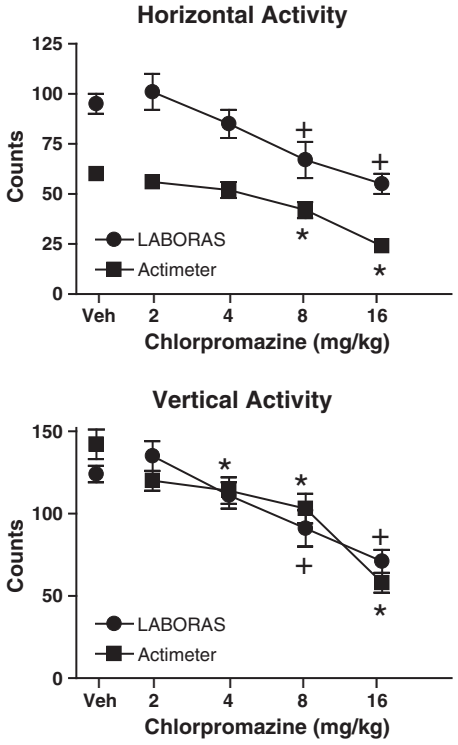

Fig. 1. Effects of chlorpromazine on horizontal and vertical activity counts in the Actimeter and LABORAS test systems. Data are from the 0 to 20 min assessment period (i.e., 60–80 min post-dose). Mean ± S.E.M.; $n = 10$ animals/group. $^{*}P<0.05$ versus the Actimeter vehicle control, and $^{+}P<0.05$ versus the LABORAS vehicle control; one-way ANOVAs and Dunnett's tests for the factor of treatment.

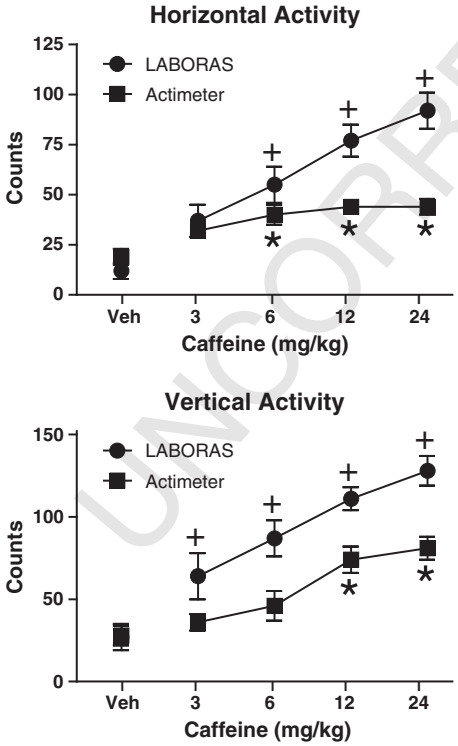

Fig. 2. Effects of caffeine on horizontal and vertical activity counts in the Actimeter and LABORAS test systems. Data are from the 20 to 40 min assessment period (i.e., 80–100 min post-dose). Mean ± S.E.M.; $n = 10$ animals/group. $^{*}P<0.05$ versus the Actimeter vehicle control, and $^{+}P<0.05$ versus the LABORAS vehicle control; one-way ANOVAs and Dunnett's tests for the factor of treatment.

in spontaneous locomotor activity (as assessed by the number of infrared beam breaks along a horizontal plane) was demonstrated to be an excellent preclinical predictor of CNS/neurobehavioral effects including those observed in a modified Irwin test, thus demonstrating the utility of locomotor activity screening during CNS safety pharmacology testing (Lynch & Mittelstadt, 2009). In the current study, the locomotor activity counts obtained from LABORAS (for both horizontal and vertical activity) nearly exactly matched (dose per dose) the activity data obtained from a modified Irwin test. In other words, whenever a statistically significant change in activity counts was observed with chlorpromazine or caffeine in LABORAS, a change in activity was also noted in the modified Irwin test. For the Actimeter system, activity counts fairly well matched the activity data obtained from the modified Irwin test, with the vertical activity measure of the Actimeter assay being slightly more sensitive than the modified Irwin test for detecting chlorpromazine-induced decreased activity, and with the modified Irwin test being more sensitive than both the horizontal and vertical activity measures for detecting caffeine-induced increased activity. Any apparent differences between the data from the modified Irwin assay and the two locomotor activity assays may be due to the subjectivity inherent to the modified Irwin test as well as to the relative specificities of the behaviors recorded during the locomotor activity testing. In addition, testing environment is known to affect locomotor activity (e.g., size, lighting conditions, familiarity, etc.), and there were a number of differences between the three approaches reported here. In particular, the modified Irwin assay involved testing under moderately bright lights as well as interaction of the experimenter with the animals.

In summary, while general neurobehavioral assays (such as the modified Irwin test employed in the current study) are necessary for CNS safety pharmacology testing near the end of the target validation stage of preclinical drug development, spontaneous locomotor activity assessment may be a better-suited assay for the screening and comparison of the larger number of compounds available during even earlier stages of development. In the present study, both the infrared beam-based Actimeter and the mechanical vibration-based LABORAS automatically and reliably detected acute changes in spontaneous locomotor activity, with comparable results between the two test systems. Practical considerations, such as relative cost versus degree of versatility, should be considered when deciding which automated system to use for the testing of target compounds.

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