Data Strength Assessment of the Social-Housed Modified Irwin Test When Performed Concurrently with a CNS Tetrad Evaluation

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1 ABSTRACT

The ICH S7A guideline (2001) specifies that the effects of a test substance on motor activity, behavioral changes, coordination, sensory/motor reflex responses and body temperature should be evaluated through the use of a modified Irwin or Functional Observational Battery (FOB) to satisfy evaluations of CNS. The modified Irwin test is a qualitative CNS assessment in which the animals are evaluated in their home cage, in an arena, as well as in-hand. The test is compact and keeps manipulation to a minimum. This allows for multiple time points over the course of a day in order to detect the onset and duration of effects and decreases the risk of missing the peak effect. While the Irwin test is useful for initial observation of CNS effects, subtle changes can be difficult to quantify without statistical power. For example, as a subject acclimates to handling and exhibits increased passivity, potential depressed test substance effects may be overshadowed. These changes can confound conclusions of adversity or effect that the statistical analysis of a quantitative assessment could further elucidate.

In an effort to maximize the results of the CNS assessment, a tetrad evaluation (body temperature, measure of catalepsy, locomotor activity, and analgesic response) was performed concurrently with the Irwin Test. The tetrad evaluation maintains the time-sensitive nature of the Irwin Test while offering a quantitative component. Historically, this assessment has proven to provide accurate data with statistical support. In this study, test subjects were dosed with vehicle (saline) and positive controls (diazepam, chlorpromazine, or amphetamine). When evaluated together, the tetrad was able to identify the peak Diazepam activity at 2.5 hours versus decreased activity noted beginning at 30 minutes post-dosing in the Irwin. On the other hand, the Irwin was more sensitive to showing the positive effects (e.g. decreased grip strength, startle response, and grooming and increased pupil diameter) of the lower dose of Chlorpromazine versus the high dose. Ultimately, both aspects of the test prove to be instrumental for a holistic CNS assessment.

2 INTRODUCTION

The Irwin and tetrad assessments are currently used to assess different aspects of CNS changes following a drug's administration. Both tests exemplify strengths and weakness. The Irwin evaluates a detailed list of possible changes in a subjects' behavior while the tetrad provides a quantitative estimation in response to various situations and stimuli. Together, these tests provide a more complete view of post-dosing events in a short amount of time, allowing for multiple evaluations over the course of a few hours.

In this experiment, multiple positive controls with varying anticipated effects were used in order to establish the strength of the compilation when dealing with polar results. Also, one positive control, chlorpromazine, was dosed at multiple levels to assess the sensitivity of the combined tests when dealing with increasing responses to the same drug.

3 METHODS

- 30 (6/group) Sprague Dawley rats (7-8 weeks in age).
- All animals socially housed and acclimated to social groups for at least 3 days prior to testing.
- Animals orally dosed with vehicle (saline) or positive control (Amphetamine at 10 mg/kg, Diazepam at 50 mg/kg, and Chlorpromazine at 10 and 50 mg/kg)
- Testing performed prior to dosing and at 30, 90, 150, and 300 minutes post-dosing. Irwin testing, consisting of home cage observation followed by benchtop observation, is immediately followed by tetrad assessment. The tetrad parameters were performed in order of lowest to highest level of invasiveness (body temperature collection, catalepsy assessment, open arena observation, and then the analgesic response).

FIGURE 1
SUMMARY OF TETRAD DATA - CENTER LATENCY
Data Presented as Means ± SEM

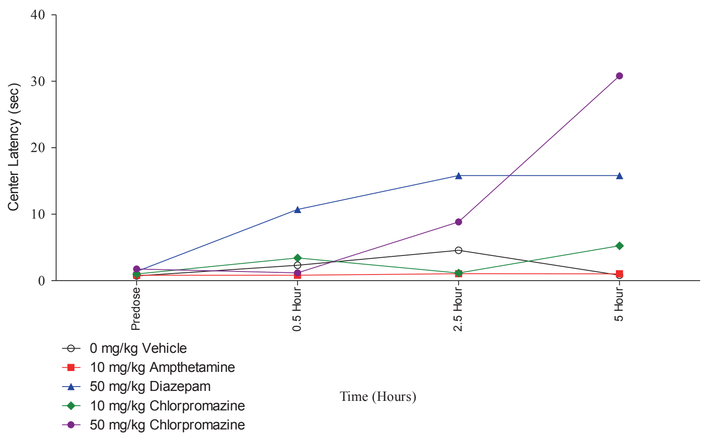
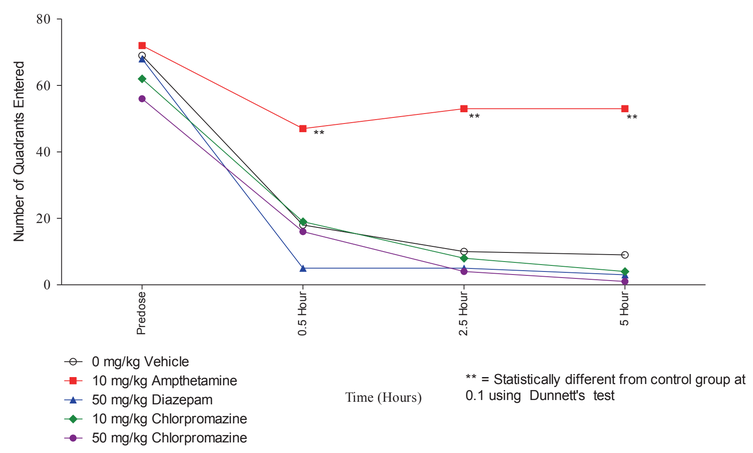
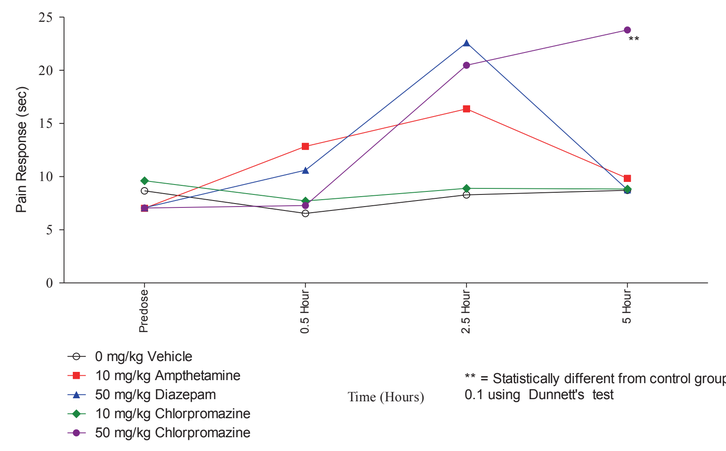


FIGURE 2
SUMMARY OF TETRAD DATA - QUADRANTS
Data Presented as Means ± SEM



** = Statistically different from control group at 0.1 using Dunnett's test

FIGURE 3
SUMMARY OF TETRAD DATA - PAIN RESPONSE
Data Presented as Means ± SEM



** = Statistically different from control group at 0.1 using Dunnett's test

Figure 4: Irwin Data: Vehicle and Positive Controls

		Vehicle Control (0 mg/kg)				Amphetamine (10 mg/kg)				Diazepam (50 mg/kg)				Chlorpromazine (10 mg/kg)				Chlorpromazine (50 mg/kg)			
Observation Time		Predose	30 min	150 min	300 min	Predose	30 min	150 min	300 min	Predose	30 min	150 min	300 min	Predose	30 min	150 min	300 min	Predose	30 min	150 miN	300 min
Lethality																					
Convulsions	Excitation																				
Tremors																					
Aggressiveness																					
Restlessness																					
Straub Tail																					
Increased motor activity																					
Increased startle response																					
Increased touch response																					
Increased alertness																					
Increased fearfulness																					
Increased pinna reflex																					
Increased corneal reflex																					
Increased body tone																					
Twitches	Stereotypy																				
Hallucinatory-like																					
Head flicking																					
Head searching																					
Compulsive sniffing/licking																					
Self-destructive biting																					
Prancing forelimbs																					
Upright walking																					
Aimless wandering																					
Circling/Waltzing																					
Retropulsion																					
Spatial disorientation																					
Catalepsy	Motor																				
Paralysis																					
Abnormal gait																					
Abnormal body carriage																					
Decreased Grip strength																					
Lethargy	Sedation																				
Loss of righting reflex																					
Passivity																					
Decreased motor activity																					
Decreased startle response																					
Decreased touch response																					
Decreased alertness																					
Decreased fearfulness																					
Decreased pinna reflex																					
Decreased corneal reflex																					
Decreased body tone																					
Decreased pain response	Pain																				
Increased Pain Response																					
Writhing																					
Vocalization																					
Exophthalmos	Autonomic																				
Piloerection																					
Salivation																					
Lacrimation																					
Ptosis																					
Increased pupil diameter																					
Decreased pupil diameter																					
Diarrhea																					
Increased urination																					
Abdominal/slower respiration	Other																				
Faster respiration																					
Cutaneous blood flow																					
Cyanosis																					
Decreased Grooming																					

The shading indicates the number of rats exhibiting the signs:
≤25% >25%-60% ≥60%

4 RESULTS AND CONCLUSION

Both the Irwin and the tetrad assessments resulted in anticipated results from both the vehicle and the positive controls. The results of the Irwin test (figure 4) accurately represented increased activity in the Amphetamine group as well as depressed activity in the Diazepam and Chlorpromazine groups, with an earlier onset and recovery for Diazepam in comparison to the Chlorpromazine. The tetrad results (figures 1-3) quantitatively supported these findings. In particular, the number of quadrants entered was able to demonstrate the increased locomotor activity of the stimulant (Amphetamine) while the center latency and analgesic response times were able to exhibit the time curves of decreased activity of the depressants (Diazepam and Chlorpromazine). The tetrad was also able to assist the Irwin in correlating the increase in changes of Chlorpromazine in the higher dose group (50 mg/kg) in comparison with the lower dose group (10 mg/kg).

The tetrad data did, as anticipated, contribute to understanding of the positive control effects further. This test allows for a more precise estimation of the peak effect and recovery of the Diazepam subjects. While the Irwin data exemplifies decreased activity from 30 minutes post-dosing to the completion of the assessments, the tetrad data (particularly center latency and analgesic response) indicates that the peak effect did not take place until 2.5 hours post dosing. Interestingly, the Diazepam pain response findings indicate that the sensitivities of the tests vary, further suggesting the use of both assessments provides a more comprehensive evaluation.

The Irwin also helped to more thoroughly examine data from the tetrad. When comparing the low and high dose of Chlorpromazine, the tetrad data shows the low dose following a similar trend as that of the vehicle for all of the analyses. However, when reviewing the Irwin assessment, it is apparent that an effect was being observed (e.g. decreased grip strength, decreased startle response, increased pupil diameter, and decreased grooming).

This experiment confirms that a CNS test that involves quantitative and qualitative analysis is an appropriate method to thoroughly assess the effects of a test compound. While this investigation confirmed the effectiveness of the dual assessment when analyzing CNS behavioral extremes (excitability vs. sedation), it is anticipated to be even more useful when differentiating subtle differences of a single compound with lesser effects.

References:

Irwin S. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiologic state of the mouse. Psychopharmacologia 1968;13(3):222-257.