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THE MODIFIED IRWIN TEST: A COMPREHENSIVE NEUROBEHAVIORAL SCREENING TOOL FOR THE EVALUATION OF NEUROTOXICITY AND DRUG SAFETY

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ABSTRACT

Neurotoxic assessment is one of the key links in safety evaluation concerning pharmaceuticals, chemicals and Toxic environmental polutants. Different neurobehavioral screening like the Modified Irwin test has emerged as the most widely-used and reliable approach vis-à-vis functional analyses of central, peripheral nervous systems in experimental rodents. This review is a comprehensive overview of the Modified Irwin test as a basic neurobehavioral assay to detect neurotoxicity. The development of the test and the development of its methodology based on the Functional Observation Battery (FOB). It's use in a wide range of animal research including screening of the central nervous system (CNS), safety pharmacology evaluation, neurotoxicity and behavioral toxicity assessment, dose-response and time-course studies, regulatory toxicology, etc. Emphasis is placed on the ability of the Modified Irwin test to discover early functional disturbances which may be produced in the autonomic, neuromuscular, sensory and behavioral domains frequently faster than any structural neuropathological findings. Due to its noninvasiveness, adaptability, acceptance by regulatory authorities and efficiency, the Modified Irwin test continues to be a cornerstone of neurobehavioral screening tools in experimental rodent models--guiding further targeted neurotoxicological investigations, underpinning human risk assessment.

KEYWORDS: Modified Irwin test; neurotoxicity; neurobehavioral assessment; Functional Observation Battery; safety pharmacology; experimental rodents.

1. INTRODUCTION

1.1Neurobehavioral Study in Rodents

The testing and evaluation of neurotoxicity are often conducted via neurobehavioral test assays in laboratory on rodents. The Modified Irwin test is one of the most commonly used neurobehavioral test batteries employed for the initial testing of neurotoxicity. It lends an ability to observe the autonomic functions, neuromuscular activity, sensory responses and behavioural excitability in a systematic manner. Initially, Irwin Test underwent a number of changes and is now called the Functional Home cage Observations/Monitoring (FHOM) battery. The new battery consists of eight parts: posture and gait of body, general reaction and awareness, coordination of movement, involuntary movements or abnormalities, general physical condition, reflexes of the nervous system, neuromuscular responses, In the battery, evidence is also given for sensory response to visual, auditory, and tactile stimuli. This way, small changes in neurobehavioral function can be detected.[1]

There are various evaluative measures for different behavior types (Heart rate, tremble) Neurotoxicity testing generally employs functional tests. A big plus of the Modified Irwin test is that it is non-invasive, allowing for the same animal to be observed repeatedly. This makes it possible to judge the onsets and progressions of an effect. It also permits evaluations on: duration, reversibilily and long-term effects of some neurotoxic processes. Functional assays run the gamut from broad screening batteries such as the Modified Irwin test to specialized methods evaluating learning and memory. It also functions as a model guide to subsequent detailed

neurotoxicological investigation in experimental rats and mice among other laboratory animal species.[2,3]

3. Applications of the Modified Irwin Test in Animal Research

3.1 Screening of Central Nervous System (CNS) Effects

The Modified Irwin test is widely used as a primary neurobehavioral screening tool for detecting the central nervous system (CNS) effects of new chemical entities in laboratory animals, particularly rodents. With its complete observational design, the test can promptly detect functional changes in the CNS after acute or repeated test material exposure. This test screened multiple behavioral and neurological determinants, including spontaneous locomotor activity, body posture, gait, level of wakefulness and arousal, involuntary muscular twitching, trembling, anesthesia, hyperactivity and stereotyped behavior etc. Modified Irwin test offers CNS functional overview databases that make it easier to monitor a drug's potential early-stage neurotoxic or neuroactive properties.

Importantly, the Modified Irwin test distinguishes between agents that cause CNS stimulation, depression, situation neuroleptic or anxiety-like effects-and thus clusters pharmacochemical profiles with other classes of drugs. The degree of both overt and subtle behavioural alterations makes it particularly useful in the initial stages of drug discovery and lead optimization, when rapid screening combined with an index for potential neurotoxicity is essential to draw one's attention away from unwanted CNS liabilities and towards preferred compounds.[4–6]

3.2 Safety Pharmacology Evaluation (ICH S7A Core Battery)

In safety pharmacology, the Modified Irwin test provides a way to keep track of five functional endpoints. They are: level of consciousness (LOC); motor coordination; reflex integrity; and autonomic signs. Following a standard protocol using trained technologists under supervision by pharmacological and toxicological scientists, they can record each experimental animal s (or in the case of many animals indivisible groups) neurological status according to fixed criteria on instrument charts.[7] Modified Irwin test is also able to track dose-dependent and time-related effects on the CNS.[8]

3.3 Neurotoxicity and Behavioral Toxicity Assessment

Modified Irwin test is widely adopted for neurotoxicity and behavioral toxicity studies, that detecting the disturbances in nervous system function caused by environmental chemicals pesticides, solvent and all manner of industrially formulated material.[9]

This study systematically evaluated the important end point of neuromuscular tone, motor coordination and sensory reactivity as well as changes in nerve reflexes; all these are kinds of neurotoxic injury indicators. When these parameters change it is usually an indication that either the central or peripheral nervous system (or both) is disordered; Therefore even before people show outward clinical signs there may be much of neurotoxicity which cannot be identified by conventional methods.[10]

Pathological or neuropathological assessments have not been able to sufficiently reflect all aspects of the nervous system's response. Such assessments focus exclusively on anatomical and cellular damage. In contrast, the Modified Irwin test is able to reveal the early functional effects of neural injury that might otherwise remain unnoticed and yet have far greater significance for patient populations' real-world neurobehavioral outcomes.[11]

3.4 Dose–Response and Time-Course Evaluation

The test's ability to be used in the same experimental animal repeatedly permits serial observations which give a detailed description of the relationship between the dose and it's effect on the nervous system and brain behavior. This function is particularly useful in studies on neurotoxicity, where knowing how far adaptations can go and where they revert to the original state becomes essential.[12] Changes monitored systematically at various time points using the Modified Irwin test with assessments made at onset, peak impact, duration of effect and then recovery. Such longitudinal studies provide opportunities for distinguishing between transient pharmacological effects and those involving sustained or progressive neurotoxic damage. This ability to correlate behavioral alterations with dose level makes it possible to define No Observed Adverse Effect Levels (NOAELs) and Lowest Observed Adverse Effect Levels (LOAELs).[13,14]

Table 1: Applications of the Modified Irwin Test in Animal Research.

Application	Parameters Assessed	Purpose	Relevance	References
Screening of CNS effects	Spontaneous activity, posture, gait, arousal, alertness, tremors, convulsions, sedation, excitation, stereotypy	Primary screening of new chemical entities for central nervous system activity	Early identification of stimulant, depressant, neuroleptic, or anxiolytic-like effects during drug discovery and lead optimization	Irwin (1968); Gad (2007); Moser (1997)
Safety Pharmacology	Consciousness, motor coordination, reflexes,	Detection of undesirable CNS pharmacodynamic	Regulatory-relevant assay supporting risk assessment	ICH S7A (2001); Henck

(ICH S7A Core Battery)	autonomic signs, behavioral abnormalities	effects prior to first-in-human studies	and safety margins	(2019); Redfern et al. (2005)
Neurotoxicity and Behavioral Toxicity Assessment	Neuromuscular tone, coordination, reflex integrity, sensory responses	Identification of functional neurotoxic effects of chemicals and environmental agents	Detects functional disturbances that may precede structural neuropathology	Tilson & Mitchell (1992); Crofton et al. (1992); OECD (2004)
Dose–Response and Time-Course Evaluation	Behavioral and neurological changes observed at multiple time points	Characterization of onset, intensity, duration, and reversibility of CNS effects	Supports NOAEL/LOAEL determination and differentiation of transient vs. persistent effects	Irwin (1968); Moser (1991); Gad (2007)

4.1 A Complete neurobehavioral effect Evaluation

The Modified Irwin test thus enjoys a major advantage over other experimental strategies: It gives you a total creature function score. Animals undergoing this procedure will receive regressions in a wide range of activity parameters every 24 hours for one week, followed by lethality data during 48 hours on those subjects which survived the week of observation. Also during testing, you can plot dose effect curves for any change that may be demonstrated and if all parameters pass a statistical test. The test covers a variety of what scientists call functional domains: autonomic, neuromuscular, sensory (including visual and auditory) and behavior.[15]

4.2 Early Detection of Neurotoxicity

The early identification of neurotoxic effects that characterizes the Modified Irwin test is invaluable.[16]

4.3 Flexible and Adaptable Methodology

This test can accommodate variety in each circumstance in which insecticides are used. This test is capable of being adapted easily to cater to different experimental arrangements and needs (e. g. species (including rat, mouse and guinea pig), specific pharmacological or toxicological aims), and is consequently suitable for a variety of neurobehavioral researches.[17]

4.4 Non-Invasive and Animal-Friendly

Such human-like non-invasive observational methods involve most parameters examined in the Modified Irwin test. This lessens experimental burden on the animals, promotes ethical research behavior, and allows for repeat assessments of subjects under the same conditions.[16]

4.5 Regulatory Acceptance

This test was universally approved by international regulatory authorities to determine whether a compound can be new medicine or not, the process steps are defined as central nervous system (CNS) safety pharmacology evaluations. Its regulatory acceptance attests to the reliability and importance of this technique in evaluating neurotoxicity in nonclinical research.[17]

4.6 Efficient Screening Tool for neurotoxicity

Finally, the Modified Irwin test serves as an efficient and time-effective screening tool**,** providing a broad functional overview of CNS activity within a relatively short assessment period. This efficiency makes it especially useful during early drug discovery, lead optimization, and hazard identification stages.[17]

5. Methodology of the Functional Observation Battery Test (FOB)

The FOB combines home cage observations, open-field arena tests, reflex evaluations, neuromuscular simulation and sensory reactivity examination, autonomic function assessment, then integrates them all in one place for use as a comprehensive indicator of neural toxicity in experimental rodent models.[18-24]

5.1 **Experimental Animals**

The functional observation battery (FOB) is commonly done with rodent species, primarily rats or mice. This is because there is more than one sex for most vertebrates. Animals are typically young adults (6–10 weeks of age) and acclimated to laboratory conditions for 5–7 days before testing to maximize minimization of stress-related variation.[19]

5.2 Test Substance Administration

Depending on design, measured substances are in applied by a variety of passages: orally would be most preferable route but intra peritoneal or intravenously preferred. Dose ascertainment is carried out based on the results from acute or repeated-dose toxicity studies, with calibration of dosage level from sub toxic up to potentially toxic. An ordinary vehicle-only control group is also present for comparison.[20]

5.3 Timing of Observations

Neurobehavioral Observations are performed at defined post-dose intervals (e.g., 30-45 minutes, 1 hour, 2 hours, and 4 hours). In the repeated dose studies, assessments may be made weekly or at terminal time points to evaluate cumulative or delayed neuro-toxic effects.[21]

5.4 Home -Cage Observations

Posture, Spontaneous activity, Presence of tremors or convulsions, Vocalization, Respiration.

Neurobehavioral and Neuromuscular Assessment Overview

Section	Category	Parameter Assessed	Observation
5.5 Open-Field Observations	**A. Gait and Posture**	Locomotion and body position	• Normal walking, ataxia, staggering • Body alignment (e.g., hunched or flattened posture)
	B. Reactivity and Arousal	Response to stimuli and alertness	• Response to sound or touch (tactile stimuli) • Alertness or lethargy, sedation
	C. Involuntary or Abnormal Movements	Presence of abnormal motor activity	• Tremors • Clonic or tonic convulsions • Stereotypy
5.6 manipulative and reflex assessment	**A. Handling Reactivity**	Behavior during handling	• Ease of handling • Aggression or passivity
	B. Reflex Testing	Basic neurological reflexes	• Righting reflex • Corneal reflex • Pinna reflex • Startle response
5.7 Neuromuscular Function Tests	**A. Grip Strength**	Muscle strength	• Forelimb and hindlimb grip strength measured using a grip strength meter
	B. Landing Foot Splay	Motor coordination	• Distance between hind paws following a standardized drop

Scoring Criteria of Modify Irwin test

The Modified Irwin Test scoring system typically employs an ordinal scale of 0 to 3 to assess severity of symptoms, functional impairment, and consistency of response. Each item is scored individually with total scores indicating mild, moderate or severe classification.

Scoring Criteria for Physical Factor and Gross Appearance

Physical factors and gross appearance		
1	Coat color	A= Albino, Ag=Agouti, Bl=Black
2	Body weight	Animal weight (g) before the test
3	Presence of whiskers	0 = None; 1 = A few; 2 = Most, but not a full set; 3 = A full set
4	Appearance of fur	0 = Ungroomed and disheveled; 1 = Somewhat disheveled; 2 = Well-groomed (normal)
5	Piloerection	0 = None; 1 = Most hairs standing on end
6	Patches of missing fur on face	0 = None; 1 = Some; 2 = Extensive
7	Patches of missing fur on body	0 = None; 1 = Some; 2 = Extensive
8	Wounds	0 = None; 1 = Signs of previous wounding; 2 = Slight wounds present; 3 = Moderate wounds present; 4 = Extensive wounds present

Scoring Criteria for Behavioural Observations in a Novel Environment (Open- Field Assessment)

Observation of behavior in a novel environment		
9	Transfer behavior	0 = Coma; 1 = Prolonged freeze (>10 sec.), then slight movement; 2 = Extended freeze, then moderate movement; 3 = Brief freeze (a few seconds), then active movement; 4 = Momentary freeze, then swift movement; 5 = No freeze, immediate movement; 6 = Extremely excited ("manic")
10	Body position	0 = Completely flat (on stomach); 1 = Lying on side; 2 = Lying on back; 3 = Sitting or standing; 4 = Rearing on hind legs; 5 = Repeated vertical leaping
11	Spontaneous activity	0 = None, resting; 1 = Casual scratch, groom, slow movement; 2 = Vigorous scratch, groom, moderate movement; 3 = Vigorous, rapid/dart movement; 4 = Extremely vigorous, rapid/dart movement
12	Respiration rate	0 = Gasping, irregular; 1 = Slow, shallow; 2 = Normal; 3 = Hyperventilation
13	Tremor	0 = None; 1 = Mild; 2 = Marked
14	Palpebral closure	0 = Eyes wide open; 1 = Eyes 1/2 closed; 2 = Eyes closed
15	Gait	0 = Normal; 1 = Fluid but abnormal; 2 = Limited movement only; 3 = Incapacity
16	Pelvic elevation	0 = Markedly flattened; 1 = Barely touches; 2 = Normal (3mm elevation); 3 = Elevated (more than 3 mm elevation)
17	Tail elevation	0 = Dragging; 1 = Horizontally extended; 2 = Elevated (Straub tail)

18	Urination	0 = None; 1 = Little; 2 = Moderate amount; 3 = Extensive
19	Defecation	Count the number of fecal boli emitted during the 3-min. period

Scoring Criteria for Reflexes and Reactions to Simple Stimul

Reflexes and reactions to simple stimuli		
20	Touch escape	0 = No response; 1 = Mild (escape response to firm stroke); 2 = Moderate (rapid response to light stroke); 3 = Vigorous (escape response to approach)
21	Positional passivity	0 = Struggles when restrained by tail; 1 = Struggles when restrained by neck (finger grip, not scruffed); 2 = Struggles When held supine (on back); 3 = Struggles when restrained by hind legs; 4 = Does not struggle
22	Trunk curl	0 = Absent; 1 = Present
23	Reaching reflex	0 = None; 1 = Upon nose contact; 2 = Upon vibrasse contact; 3 = Before vibrasse contact (18mm); 4 = Early vigorous extension (25mm)
24	Body tone	= Flaccid, no return of cavity to normal; 1 = Slight resistance; 2 = Extreme resistance, board like

Scoring Criteria for Physiological and Reflex Measures during Supine Restraint

Measures recorded during supine restraint		
25	Plantar surface skin color	0 = Blanched; 1 = Pink; 2 = Bright, deep red flush
26	Heart Rate	0 = Slow, bradycardia; 1 = Normal; 2 = Fast, tachycardia
27	Limb Tone	0 = No resistance; 1 = Slight resistance; 2 = Moderate resistance; 3 = Marked resistance; 4 = Extreme resistance
28	Abdominal Tone	0 = Flaccid, no return of cavity to normal; 1 = Slight resistance; 2 = Extreme resistance, board like
29	Righting Reflex	0 = No impairment; 1-10 = Number of seconds required to right Air Righting Reflex 0 = No impairment; 1-10 = Number of seconds required to right
30	Air Righting Reflex	0 = None; 1 = Slight margin of sub-maxillary area; 2 = Wet zone entire sub-maxillary area
31	Salivation	0 = Absent; 1 = Present
32	Provoked Biting	0 = Absent; 1 = Present

Scoring Criteria For Motor Coordination and Limb Strength Assessments

Motor coordination		
33	Grip strength	0 = None; 1 = Slight grip, semi-effective; 2 = Moderate grip, effective; 3 = Active grip, effective; 4 = Unusually effective
34	Inverted screen (s)	0-60 = Number of seconds before falling
35	Wire maneuver	0=Active grip with hindlegs; 1= Difficulty to grasp with handlegs; 2= Unable to grasp with handlegs; 3=Unable to lift hindlegs, falls within second; 4= Falls immediately
36	Wire Hand	0=Active grip with hindlegs; 1= Difficulty to grasp with handlegs; 2= Unable to grasp with handlegs; 3=Unable to lift hindlegs, falls within second; 4= Falls immediately
37	Pole climb	0= Climbs down within 30 sec; 1= Turns and climbd up the pole; 2= Turns but then freezes; 3= Does not move within 30 seconds or climbs but not off the pole; 4= Falls off.

DISCUSSION ON THE METHODOLOGY OF MODIFIED IRWIN TEST

A thorough, multi-phase observational framework called the Modified Irwin Test was created to assess mice' neurobehavioral reactions following the injection of a drug. It embodies the idea that several criteria should be evaluated within the same subject and proceeds in an organised manner through home cage observations, open-field assessments, manipulative testing, and neuromuscular evaluations. The mice' pre-test habituation reduces stress-related variability, enabling precise attribution of pharmaceutical effects. In order to ensure scientific rigour for regulatory submissions, the test uses GLP-compliant protocols with unblinded controls and suitable dosage routes. It is easier to distinguish between actual pharmacological effects and procedural confounders when observations are made at predetermined intervals. Reproducibility and statistical validity are encouraged by the semi-quantitative scoring method used throughout the observation phases. It differs from simply behavioural examinations in that it incorporates physiological endpoints with behavioural assessments.

CONCLUSION

The Modified Irwin Test is an important neurobehavioral screening tool for the early detection of neurotoxicity and CNS drug effects in rodant (mouse) models. It employs a semi-quantitative system based on home-cage monitoring and multiple behavioral tests to provide an

extensive evaluation of the toxicity in nervous systems and can detect early functional changes before any structural damage is observed. Therefore, it became an important screening method for preclinical safety pharmacology and environmental toxicology. Its diagnostic capabilities and adherence to global regulatory standards ensure its relevance in new drug development. However, there are challenges such as subjective scoring and lack of objectivity in terms of automation. Nevertheless, the Modified Irwin Test remains relevant in neurotoxicology, contributing to risk evaluation and the creation of safer products as the field advances to more complex techniques.

REFERENCE

1. Moser VC. Functional assays for neurotoxicity testing. *Toxicol Pathol.*, 1997; 25(1): 36–45.
2. Tilson HA, Mitchell CL. Neurobehavioral techniques to assess the effects of chemicals on the nervous system. *Annu Rev Pharmacol Toxicol.*, 1992; 32: 425–450.
3. Moser VC. Functional assays for neurotoxicity testing. *Toxicol Pathol.*, 2010; 39(1): 36–45. doi:10.1177/0192623310385255.
4. Irwin S. Comprehensive observational assessment: Ia. A systematic, quantitative procedure for assessing the behavioral and physiological state of the mouse. *Psychopharmacologia.*, 1968; 13(3): 222–257.
5. Gad SC. *Drug safety evaluation.* Hoboken (NJ): Wiley-Interscience, 2007.
6. Moser VC. Functional assays for neurotoxicity testing. *Toxicol Pathol.*, 1997; 25(1): 36–45.
7. Henck JW. *J Pharmacol Toxicol Methods* 2019; 99: 106591.
8. Redfern, W. S., et al. (2005). *Cardiovascular Toxicology*, 5: 53–72.
9. Tilson, H. A., & Mitchell, C. L. (1992). *Annual Review of Pharmacology and Toxicology*, 32: 425–450.
10. Crofton, K. M., Foss, J. A., & Gray, L. E. (1992). *Fundamental and Applied Toxicology*, 18(1): 25–36.
11. OECD (2004). *Guidance Document on Neurotoxicity Testing Strategies*.
12. Irwin, S. (1968). *Annual Review of Pharmacology*, 8: 653–676
13. Moser, V. C. (1991). *Journal of the American College of Toxicology*, 10(6): 661–669.
14. Gad, S. C. (2007). *Drug Safety Evaluation*.
15. Mathiasen JR & Moser VC. *The Irwin test and functional observational battery (FOB) for assessing effects of compounds on behavior, physiology, and safety pharmacology in rodents*. Curr Protoc Pharmacol., 2018; 83(1): e43.
16. Appraisal of the state-of-the-art: modified Irwin test vs FOB in rats (Journal of Pharmacological and Toxicological Methods, 2019.
17. Irwin S. *Comprehensive observational assessment: a systematic, quantitative procedure for assessing behavioral and physiologic state in mice*. Psychopharmacologia, 1968; 13(3): 222-257.
18. Irwin, S. (1968). Comprehensive observational assessment: Ia. A systematic quantitative procedure for assessing the behavioral and physiological state of the mouse. *Psychopharmacologia*, 13: 222–257.
19. Moser, V.C. (1991). Screening approaches to neurotoxicity: The Functional Observation Battery. *Journal of the American College of Toxicology*, 10(6): 661–669.
20. OECD Test Guideline 424 (2004). Neurotoxicity Study in Rodents.
21. OECD Test Guideline 407 (2008). Repeated Dose 28-Day Oral Toxicity Study in Rodents.
22. Gad, S.C. (2007). *Drug Safety Evaluation*. Wiley-Interscience.
23. EPA (1998). Neurotoxicity Risk Assessment Guidelines.
24. Moser et al. (1995). Behavioral and neurological evaluations in rodent toxicity studies. *Neurotoxicology*, 16: 523–534.
25.