Review Article

Evaluating Protocols for Assessing Toxicological Effects of Ethnopharmacological Agents on the Central Nervous System

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Abstract

The goal in drug development is to create medications that have the greatest therapeutic effectiveness while causing the fewest negative side effects. The continuous expansion of research on the therapeutic properties of ethnopharmacological substances indicates a growing likelihood of harmful effects resulting from these agents, especially those that may impact the central nervous system (CNS). Due to the unique nature of the central nervous system (CNS) and the blood-brain barrier, which controls the passage of foreign substances into the brain, it is crucial to exercise caution and verify that ethnopharmacological agents already in use do not unintentionally and excessively affect the CNS. It is becoming increasingly necessary to analyse the potential toxicity of ethnopharmacological drugs and understand the specific pathways by which these compounds can be harmful to the central nervous system (CNS). A narrative review is needed for the methods used in biochemical testing and behavioural tests to evaluate toxicity, which can be identified by biochemical and histological abnormalities as well as behavioural abnormalities. The specific areas affected and their diverse functions determine the different Biochemical, histological and behavioural.

The authors carried out an online search of relevant articles in Embase, Google Scholar, JSTOR, PubMed, Proquest and Scopus from 1968 till date. The search focused on several areas such as ethnopharmacological toxicology, functional observational batteries, CNS toxicity, behavioural models, and historical perspectives of CNS toxicity testing. Out of the articles that met the criteria and addressed the scope, a total of 51 were used to create this article.

The neurotoxicity risk posed by both traditional and novel ethnopharmacological substances is a significant and immediate concern. The protocols used to detect these toxicities have not undergone recent evaluation. In addition, few researchers engage in the complete process of toxicological testing.

Keywords: Ethnopharmacology, functional observational battery; CNS toxicity; behavioural assessment, toxicology

Introduction

When developing new drugs, the objective is to produce medicines with the highest possible therapeutic efficacy while exhibiting the lowest possible adverse effects. The ever-expanding scope of research into the therapeutic potential of ethnopharmacological substances suggests that there would be an increasing risk of toxic consequences caused by these agents, particularly those that affect the central nervous system (CNS). However, whether they are used in traditional or conventional medical therapy, or even as components of food or accidental environmental exposure, ethnopharmacological substances can be potentially harmful in general, and they may even have a toxic predilection for the CNS. The term ethnopharmacology refers to "the interdisciplinary scientific exploration of biologically active agents traditionally employed or observed by man."[1] Ethnopharmacology is an interdisciplinary field that combines natural science research on medicinal, aromatic, and dangerous plants with sociocultural studies.[2] It is frequently linked to the creation of novel pharmaceuticals, as much of drug discovery and

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development has its roots partially or wholly in ethnopharmacology.

If the search for such compounds is done in a logical and systematic manner, it is reasonable to assume that many more beneficial therapeutic agents will be found in the plant kingdom. Research found that there were many pharmacological agents that were produced commercially from numerous ethnopharmacological sources.[3] This leads one to believe that it could be beneficial to search for these entities in a logical and systematic manner; both to maximize the therapeutic potential as well as to minimize the potential adverse events that could occur. Moreover, with great power comes great responsibility; responsibility to ensure that all biological substances isolated and developed for therapeutic use in man, must be safe.

Guidelines on safety of ethnopharmacological agents have thus been developed and modified over time to secure the safety of humanity from novel but potentially toxic pharmacological agents. The CNS is one which must be protected without fail by these guidelines due to its importance; alas this has not been so. Standard toxicity testing today does not prioritize the CNS and so many drugs, potentially toxic to the CNS have flown under the radar. In fact, the observations in toxicity testing are superficial and not as far reaching as they ought to be. Moreover, testing very high doses in order not to miss effects as is currently done, impairs relevance for human exposure situations.[4] The high-dose exposure to a single substance in a short-lived animal of a few hundred grams for a short period of time tell us about human hazards or human exposure given therapeutic use of an ethnopharmacological agent. It also definitely does not reflect human diversity (such as age, gender, weight, race, co-morbidities or genetics), special susceptibilities and exposure scenarios. Most importantly, the pattern of human exposure to toxins does not involve stat doses of single chemicals but to their mixtures in very different quantities and patterns given the myriad of toxicants we come across on a daily basis as well as the different routes and means of administration. Consequently, there is a rising need for more models which are more informative of the long or short term toxicity of ethnopharmacological agents, easier to replicate, faster to reproduce and more affordable for the average experimental scientist as well as predictive of neurotoxicity.[4]

Scope of the Study

This study therefore reviewed the importance, yet potential toxicity of ethnopharmacological agents, the anatomic physiology of the Central Nervous System (CNS) and the potential risk and mechanisms by which these agents pose a danger to the CNS in particular. It also considered historical perspectives of the present day guidelines on toxicity testing as well as made recommendations on the way forward.

Anatomic Physiology of the Central Nervous System

Both the CNS and the peripheral nervous system (PNS) make up the subdivisions of the larger nervous system. The brain and spinal cord make up what is known as the CNS, while everything else in the nervous system is part of the PNS. The functions of the central nervous system include the reception of sensory information, processing, formation and modification of responses to that information. The CNS receives information on changes in the external or internal environment and formulates responses to these changes.[5] An account of the organization of the CNS states that the most superficial layer that encompasses the brain is known as the cerebral cortex and that it is made up of gray matter and contains billions of neurons, all of which are put to use in the execution of high-level executive activities.[6] The same authors further remark that the frontal lobe, which is found anterior to the central sulcus, is in charge of voluntary motor function, problem-solving, attention, memory, and language.[6] The motor cortex and the Broca speech area can both be found in the frontal lobe of the brain with the Broca speech area being responsible for controlling the motor activities that are necessary for producing language, whereas the motor cortex is responsible for allowing for accurate voluntary movement of our skeletal muscles.[6, 7] The somatosensory cortex is located in the region of the brain which is responsible for the processing of sensory information. Neurons in the parietal lobe take in information on various stimuli from sensory and proprioceptor cells located throughout the body, analyse the data, and use this information to construct a concept of what is being touched based on prior experiences.[7]

The caudate nucleus, putamen, and globus pallidus are the three components that make up the basal nuclei, which are also referred to as the basal ganglia. These nuclei are found deep inside the white matter of the cerebral cortex. The pallidum and striatum are both formed by these structures. The basal ganglia are the parts of the brain that control movement and coordination of the muscles and its dysregulation leads to movement/motor system disorders.[8] While many accounts have the thalamus acting as the part of the brain that acts as a relay hub,[9,10] others[11] suggest that this is a gross underestimation of the structure and function of this vital area of the brain. It is further explained that the majority of the thalamus continues to be poorly defined in terms of mapping and physiology. Thalamic relays of peripheral sensory information (visual, auditory, and somatosensory) as well as the various other types of relayed information (for example, from

cerebellum and the mamillary body) and controlling sleep and wakefulness is more researched however. [9,11]

In spite of the fact that it is one of the most inconspicuous areas of the brain, the hypothalamus plays an essential role in the regulation of homeostasis. The hypothalamus serves as a connection between the endocrine system and the CNS and regulates the body's temperature, heart rate, blood pressure, appetite, and thirst, as well as the release of a number of hormones. Furthermore, the hypothalamus is in charge of communicating with the pituitary gland in order to either release or inhibit certain hormones which include antidiuretic hormone, corticotropin-releasing hormone, gonadotropin-releasing hormone, growth hormone-releasing hormone, prolactin inhibiting hormone, thyroid releasing hormone, and oxytocin.[12]

The pons is a structure located in the brainstem. It is responsible for connecting the medulla oblongata with the thalamus.[13] It is made up of tracts that are in charge of transmitting impulses from the motor cortex to the cerebellum, as well as the medulla and the thalamus. The medulla is located near the base of the brain stem, at the point where the spinal cord enters the foramen magnum of the skull. It is in charge of autonomic functions, some of which are absolutely necessary for normal physiologic existence.[13, 14] Through the use of chemoreceptors, the medulla is responsible for monitoring respiration. These receptors have the ability to detect shifts in the chemical composition (acidity or alkalinity) of the blood which could cause compensatory alterations in respiratory rate.[14] They further state that the medulla serves as a centre for cardiovascular and vasomotor activity to control the blood pressure, the pulse, and the cardiac contractions of the body according to the requirements of the body. In the end, it is responsible for controlling reflexes such as vomiting, swallowing, coughing, and sneezing.[14]

Cerebellum: The cerebellum, also referred to as the "little brain," is the part of the brain that is in responsible for smooth, coordinated voluntary movement. The cerebellar structure is made up of the anterior, the posterior, and the flocculonodular lobes.[15] The cerebellum is home to a cerebellar circuit, which allows it to communicate with other regions of the brain via Purkinje cells and cerebellar peduncles through which it is responsible for allowing for coordinated movement in the limbs, maintaining proper posture and equilibrium.[16]

The Limbic System

The limbic system as described by some authors[17,18] comprises the piriform cortex, the hippocampus, the septal nuclei, the amygdala, the nucleus accumbens, the hypothalamus and the anterior nuclei of the thalamus. The elements of the limbic system are further stated to be responsible for controlling emotion, memory, and motivation are linked together by the fornix and the fibre tracts. In the subcortical regions are the septal nuclei, the amygdala, and the nucleus accumbens. They are responsible for pleasure, the processing of emotions, and addiction, respectively. [17]

Through a mechanism called the reticular activation system (RAS), the reticular formation, made up of neurons from the brainstem to the medulla and receiving input from the cerebral cortex, cerebellum, thalamus, hypothalamus and spinal cord, are responsible for regulating the body's overall state of consciousness. [14,19]

The Blood Brain Barrier

The arteries that vascularize the CNS possess unique features that form what is referred to as the blood–brain barrier. These blood vessels are continuous and non-fenestrated and are able to closely regulate the transit of ions, chemicals, and cells between the blood and the brain and alterations in these barrier properties are not only an important component in the pathology and progression of various neurological diseases, but they also determine, to a large extent, the rates and types of toxins that gain access to the CNS.[20] This close regulation of CNS homeostasis permits control of neuronal function and prevents neural tissue degeneration by toxins and pathogens. The adult CNS does not have a significant regenerative capacity if damaged; fully differentiated neurons are not physiologically able to replicate themselves. Since there is a continuous and steady rate of neuronal cell death in the healthy human brain throughout life and relatively low levels of neurogenesis; any increase in the natural rate of cell death caused by an accelerated access of neurotoxins into the brain would become precarious. A number of ABC energy-dependent efflux transporters (ATP-binding cassette transporters) are thus available to actively pump many of these substances out of the brain.[21] The BBB is composed of endothelial cells EC), astrocyte end-feet, pericytes (PCs), and mural cells which all biologically make up the brain's microvasculature. The EC forms a physiological barrier that is regulated by a series of anatomical, biochemical, and metabolic properties that form the capillary walls.[20,21] Additionally, these physiological barriers are regulated by interactions with various vascular, immune, and neural cells. The overall surface area of these microvessels is by far the greatest interface for blood-brain exchange for the average adult human brain.[21]

The choroid plexus epithelial cells that face the CSF create the blood-cerebrospinal fluid barrier (BCSFB) which is the second interface. There is yet another interface provided by the arachnoid epithelium, which is avascular and situated beneath the dura mata and completely encloses the CNS.[22]

Historical Perspectives

In particular, the history of ethnopharmacology had a close connection with CNS toxicity and the consumption of psychoactive substances. The use of the phrase "ethnopharmacology" was used for the first time as the title of a book on hallucinogens in 1967.[1] This notwithstanding, the concept and practice of ethnopharmacology have been around since the 16th century missionaries in Latin America. However, researchers in the 19th century such as Claude Bernard and Otto Lowi also conducted studies on the neuropharmacological effect of curare and nicotine respectively.[23,24] Since that time, however, ethnopharmacology has come a long way. In 1560, Jean Nicot de Villeman isolated the stimulant tobacco from Nicotiana tabacum. A significant amount of the data that is currently being created by ethnopharmacologists all over the world is being published in specialized publications like the Journal of Ethnopharmacology. This is because ethnopharmacological investigations are frequently motivated by the need to authenticate or maintain ancient medical practices. The natural sciences aspect of ethnopharmacology centres on understanding the biological activity of a preparation, whether this is a mixture of botanical drugs, an extract, or a single compound. Research in the field must be informed and dependent on an indepth understanding of the sociocultural rationale for the use and safety of these plant species.[25] The amount of papers published in Journal of Ethnopharmacology has more than doubled from 2003 to 2005, reflecting the significant strides made in the field.[24] However, the repercussions of this on the figurative gap between the bench and the bedside are paradoxical. Even though conventional medicine has not yet implemented the vast majority of products that claim to have a therapeutic effect in the different disease processes, there appears to be a significant increase in the knowledge, attitude, and utilization of these herbal products.[26, 27] The reasons for the lack of trust by physicians may be related to a certain lack of trust predicated upon a fear of the potential toxicity of many of these products coupled with the lack of due diligence in moving an ethnopharmacological product from theoretical to clinical practice.[24]

For the pharmaceutical and chemical (especially pesticide) industries, the testing of chemicals for nervous system effects evolved on somewhat parallel tracks. The Irwin screen, a set of observations and manipulations was frequently used in the systematic study of mice to ascertain CNS side effects of medications.[28] Its application became commonplace in the pharmaceutical sector. Although their usage for the study of behaviour itself was not widely used, cage-side observations of behaviour became a way of identifying early indicators of toxicity in early chronic toxicity studies.[29] Core battery tests, which make up the first stage of preclinical testing, focus on motor activity, behavioural modifications, coordination, sensory/motor reflex responses, and body temperature. At this stage of testing, both the FOB and Irwin methods are relevant. The core investigation results determine the necessity of follow-up research, which may involve tests of learning and memory, neurochemistry, electrophysiological assessments, and others. These principles allow the tests to be efficiently adapted when dealing with compounds for which there is a lot of existing knowledge.[28,29]

Test Routines for Determining Toxicity in General

Unraveling the inherent phytochemical properties of biological substances and determining their toxicological potential is necessary for determining whether or not these substances are safe to use. These statistics are generated from toxicological studies that are needed by national as well as international regulatory organizations; the United States Environmental Protection Agency (USEPA) and Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF) to mention a few.[29] In the first place, it is essential to ascertain the potential risk associated with any ethnopharmacological agents that possess the possibility of holding therapeutic value. In addition, because the administration and therapeutic effects of medications are typically dose-dependent, it is helpful to have a grasp of the possible dangers that each dose could pose to humans; after all, Paracelsius it was that stated "All substances are poisons; there is none which is not a poison. The appropriate dosage is what distinguishes a treatment from a poison".[30,31]

A significant majority of the guidelines for toxicological studies that are currently required for risk evaluation of plant protection products (PPPs) and other non-pharmaceuticals, were developed more than three decades ago. In the beginning, the purpose of these guidelines was to determine the dangers presented by these substances by the use of straightforward experiments on whole animals, which were very easy to carry out in the 1980s.[32,33]

Drugs, which are substances that are meant to alter physiology and biochemistry to produce pharmacological effects; almost always have adverse effects. Many ethnopharmacological agents fit this description perfectly. Therefore, just as a manufacturer of a medicine is mandated to demonstrate the desired biologic impact, any researcher attempting to demonstrate the efficacy of an ethnopharmacological substance, must also demonstrate its safety. Consequently, several techniques to evaluating toxicity of drugs and by extension ethnopharmacological substances, have developed over time.

Lorke's method

This method has two phases which are phases 1 and 2 respectively. Phase 1 requires nine animals. Three

groups of three animals each comprise the nine animals. Different test drug doses (10, 100, and 1000 mg/kg) are given to each group of animals. The animals are kept under observation for 24 hours to track their behaviour and determine the death rates. Phase 2 needs three animals, separated into three groups of one animal per group. Higher dosages of the test chemical (1600, 2900, and 5000 mg/kg) are given to the animals, who are then monitored for 24 hours for behaviour changes and mortality.[34]

In addition, Karber's procedure, which entails giving multiple groups of five animals in each varying doses of the test drug is also an alternative. Key indices in this method include the dosage differential between groups and the interval mean of the number of deaths per group.[34]

The "up-and-down" method

This is also known as the "staircase design," entails giving individual animals doses of the test drug one at a time over the course of 48 hours. The outcome of the dose that is delivered after the initial dose is administered determines the subsequent dose. It is most advocated by regulatory bodies because it limits the number of animals used in testing.[34,35]

In toxicity testing, it is common practice to ignore the relevance, occurrence, impact, and determinants of CNS toxicity. Given the many and varied neurotoxic effects that can result from the use of pharmaceutical drugs in treatment, diagnostic, and even cosmetic procedures, it is surprising to discover that this is the case as it is becoming more apparent how essential CNS affectation is to determine the overall safety and therapeutic effects of any substance, including ethnopharmacological agents.

Examinations of Biological Toxicity

The field of cosmetic usage of injectable fillers and neurotoxins is expanding at a rapid rate, and user demand for these products is rising on an annual basis. Many of these agents are of biological origin; some even of plant origin.[36] The majority of currently used anaesthetic agents with significant effects on the CNS are derived from or associated with natural products, especially plants, as evidenced by cocaine that was isolated from coca (*Erythroxylum coca*, Erythroxylaceae) and became a archetype of modern local anaesthetics; and by thymol and eugenol contained in thyme (*Thymus vulgaris*, Lamiaceae) and clove (*Syzygium aromaticum*, Myrtaceae), respectively, both of which are similar to intravenous phenolic anaesthetics.[37]

CNS Toxicity: Behavioural, Morphological and Biochemical

All brain systems consist of complex circuits or networks that span many brain regions. It is paradoxically both pedestrian and accurate to solely assign physiological function to any one or even several brain regions. Behaviour offers a sensitive way to monitor neurotoxicity. Behavioural response is a sensitive and reliable endpoint frequently used in toxicological studies in fish. In fact, behavioural effects are toxicant-specific and driven by distinct molecular mechanisms.[38] Recognition of this obvious fact as a general principle has been relatively recent, although it has been an established part of the toxic effects of certain chemicals for many years. Behavioural toxicology as a term is fairly recent. Usually, it is the physiology of the central nervous system that is monitored by behaviour, not toxicological effects. All organs are equally subject to these considerations. Function may be the easiest phenomenon to measure in the intact organism, particularly when that organism is man, for whom invasive techniques are often impractical. Hence, tests which monitor function, if they are reliable and sensitive to damage, are desirable measures of toxicity. The inherent limitations of functional tests are that many organs possess the ability to cope or even recover in the short term and can be damaged and go undetected by functional tests; yet some other organs can compensate for damage without permanent effects on function. Tolerance is the term used to describe compensatory mechanisms to effects of drugs on the CNS; in fact, tolerance and withdrawal could be two manifestations of the same compensatory mechanism, with withdrawal occurring when the counterbalancing pharmacological effect is absent.[39] Tolerance or adaptation of the central nervous system is a wide-spread phenomenon in pharmacology. Biochemical changes associated with functional tolerance have been shown to develop in many areas where feed-back mechanisms exist and which could signal the altered state to cells. As a consequence, induction of various enzymes can occur; for example, evidence has recently been presented for drug-related enzyme induction for tyrosine hydroxylase and dopamine β hydroxylase.[40] In this kind of tolerance, the behaviour may return to normal as the enzyme changes, and the enzymatic change may persist during behavioural tolerance for as long as the drug is administered. Many lesions produce temporary loss of function with recovery of behaviour in days or a few weeks.[40] A classic example is the septal lesion which produces a hyperreactive rat, but the duration of the effect is reduced by repeated handling, with recovery occurring in a few days. It has been proposed that there is a parallel return of brain catecholamines to control levels as the septal behaviour disappears.[41] Thus, tolerance or adaptation illustrate ways in which the brain can be damaged and still retain normal function.[39] Redundancy morphological reserves are particularly important in keeping function normal in the presence of morphological damage. When only a portion of a

redundant system is damaged, function is unimpaired until the reserve potential of the system is exceeded. Redundancy may exist either when there is a larger than required pool of neurons or where alternate pathways are available.[40] In either case, loss of a fraction of the neurons may not alter function. The embryology and morphology of the CNS indicate that there are pools of cells in which the individual cell is without unique properties, allowing normal function to continue in the presence of cell loss. However, there are more sensitive indicators of morphological changes in neurons than death of the cell.[40] Quite recently, paralleling the increased interest in behavioural toxicology, various investigators have turned to examination of the CNS with the light and electron microscopes. New techniques have been applied to the study of toxicants on the CNS, and some old ones, such as the Golgi stain, have been rescued from undeserved obsolescence. Thus, details of neuronal morphology are being examined and revealed in ways dramatically different from the traditional hematoxylin and eosin (H&E) stain used in histology. One area of great potential importance in the renaissance of morphology relates to the recognition of the plasticity of neuronal morphology.[42]

Behavioural tests in CNS toxicity

Early on, histopathological changes were considered the "gold standard" that defined the field of neurotoxicology, until the realization that toxicants can also alter nervous system function, for example, behaviour, in profound and varied ways emphasized the need for other types of evaluations.[29]

Tests of learning and memory, which measure a change in behaviour as a result of experience, may be considered as follow-up to positive findings perhaps in histopathological abnormalities. Cognitive evaluations in psychopharmacology and psychological research are very common, and the models, technology and expertise is expanding every day. Such paradigms include spatial or positional navigation, simple or complex conditioned responses, and operant training of positively or negatively reinforced behaviours. According to an article on assessing spatial learning and memory,[42] semantic memory, which involves remembering information on both facts and locations,[43] is an extension of the spatial encoding mechanism used by rodents for allocentric learning and memory. In spite of the fact that the hippocampus and its surrounding structures are essential for spatial learning and memory in all mammals tested to date and that in humans spatial learning and semantic and episodic memory are intertwined, memory storage and retrieval require the interaction of these structures with the prefrontal cortex and anterior cingulate cortices, as well as to a lesser extent with parietal and retrosplenial cortices.[44,45]

The procedure used informs the type of cognition that is studied; for example, learning may be evaluated with repeated training trials to measure acquisition, and memory may be repeatedly assessed across time to evaluate retention. It is important to realize that cognition cannot be directly assessed in animal models, rather inferred. Changes in motor or sensory function, or motivation, for example, may impact performance and must be taken into account in data interpretation.[29]

Specific neuropathological deficits in toxicity

The cerebellum is primarily responsible for motor integration and coordination as well as spatial learning. Cerebellar dysfunction/lesions thus present as tremor, abnormal posture, and neuromotor deficits.[46] In a comparative study, neuromuscular toxicity was detected using several behavioural tests: the FOB and tests of motor activity; studies have shown that some dopaminergic neuron dysfunction is mediated by microglia (immune cells of the brain) which could similarly mediate primary cerebellar granule neuron (CGN) death *in vitro*.[47]

Hippocampus

The hippocampus is most implicated in development and maintenance of spatial learning and memory, as well as exploratory behaviour. Cognitive tests often used to analyze hippocampal activity include spontaneous alternation, fear conditioning, Morris water maze, and radial-arm maze.[48]

Nigrostriatum

The corpus striatum area of basal ganglia is also involved in motor function, and the loss of striatal dopaminergic neuronal function produces Parkinsonism/Parkinson's disease in humans. Mouse models of nigrostriatal neuronal loss ("weaver mice") showed considerable motor dysfunction and various mechanisms resulting in injury to the nigrostriatal system produce models of Parkinsonism that can be described with various behavioral tasks such as placing, grooming or foot faults.[49]

Axon

A variety of substances produce a pattern of peripheral neuropathy in laboratory animals and humans, although the relative sensitivity of motor and sensory axons impact their specific toxicity profiles. Behavioural changes are usually observed as neuromotor (primarily) and sensory dysfunction with the hind limbs being preferentially affected in motor function tests (grip strength, limb placement); and generalized signs of ataxia, tremors, and changes in reactivity are also features of axonal affectation.[50]

Functional Observational Batteries (FOB)

The systematic evaluation of mice to determine CNS side effects of drugs typically employed a series

of observations and manipulations that became known as the Irwin screen and later modified to the functional observational battery (FOB).[29] While second-tier testing involves more complex tests that provide a more thorough description of the effects and dose-response relationships, screening, or first-tier testing, typically consists of quick or simple tests of behavior that may be used to determine whether the dose at which a chemical acts on the CNS or if at all.[51] Both levels of examinations can assess innate, or reflex, behaviours (such as locomotor activity and sensory function), which offer a comprehensive assessment of neurological affectation. However, the level of precision in these actions varies, making them potentially challenging to interpret.

In order to identify the most sensitive endpoint for a variety of toxicity tests on all organ systems though there is usually overlap since many tests localize to more than one domain which does not always predict the specific regions of the brain subserved. FOB procedures may be arranged according to domains of neurological functions.[29] Some autonomic functions, including salivation, lacrimation, and pupil size, can be measured visually, whereas others, like respiration and heart rate, may need specialized equipment or telemetry. Any number of tests can be used to assess neuromuscular function and coordination, including assessments of gait and posture, palpations of muscle tone or extensor strength, instrumental testing of grip strength in the forelimb and hindlimb, and monitoring of righting or proprioceptive responses.

Functional Observational Battery

Table 1: Functional Observational Battery[29]

Type of test	Behaviors evaluated	Specific test/endpoint
Tier 1—screening, hazard identification		
FOB, Irwin screen	Activity, excitability, sensorimotor, neuromuscular	Arousal, rearing, reactivity, gait, posture, responses, reflexes, landing foot splay, grip strength, excretions
Motor activity	Spontaneous activity	Open-field, figure-8, cage-rack chambers
Tier 2—hazard characterization		
Cognition	Learning, conditioning, memory, attention	Passive/active avoidance, water maze, T-maze, operant schedules of reinforcement
Motor	Quantitative measures: coordination, equilibrium, strength	Gait analyses, rotarod, grip strength, landing foot splay, motor activity
Sensory	Quantitative measures: stimuli-specific response	Acoustic startle response, prepulse inhibition

Table 2: Tests of CNS functions used in functional observational batteries[29]

TEST	RATIONALE
Novel object recognition test	Normal mice will spend more time exploring novel objects than familiar ones
Social recognition test	Mice with unimpaired cognitive function will spend more time sniffing novel mice than familiar mice; those with a cognitive deficit will not distinguish between familiar and novel mice (their exploration time will be similar).
Morris water maze	Tests spatial memory and long term memory by observing and recording escape latency, thigmotaxis duration, distance moved and velocity during the time spent in the MWM tank.
Barnes maze	Spatial learning and memory are assessed by measuring the time the animal spends finding the shelter box and the number of errors before finding it.
Y maze	A mouse with intact working memory, and hence intact prefrontal cortical functions, will remember the arms previously visited and show a tendency to enter a less recently visited arm.

TEST	RATIONALE
Passive avoidance	The animal learns to suppress a motor response to avoid exposure to the test area (context) associated with or predictive of the aversive event, such as a dark compartment of the passive avoidance system that is normally preferred over the brightly illuminated compartment.
Grip strength	In general, the test measurement is conducted by allowing the animal to grasp the device (e.g., grid, ring, T-bar) and then pulling it away until its grip is broken.

Conclusion

With the rapidly expanding research into ethnopharmacology, it is incumbent upon researchers to ensure novel ethnopharmacological agents are safe. Unfortunately, existing guidelines do not account for the potential harm these agents pose to the CNS. Furthermore, routine toxicity testing using the more common methods rarely account for relative duration of exposure; whereas it is established that neurotoxicity results from exposure at different rates and durations.

These toxicity testing measures could be histopathological in nature, but could also be biochemical or behavioural. Behavioural tests give a good idea as to how toxicity in different areas of the brain would present in neurotoxicity and could be more routinely applied by experimental scientists in ethnopharmacology in addition to histopathological and biochemical tests where available.

References

1. Heinrich M, Gibbons S. Ethnopharmacology in drug discovery: an analysis of its role and potential contribution. J of Phar and Pharma. 2001; 53(4): 425-32.
2. Bruhn JG, Rivier L. Ethnopharmacology–A journal, a definition and a society. J of Ethnopharm. 2019; 242: 112005.
3. Pirintsos S, Panagiotopoulos A, Bariotakis M, Daskalakis V, Lionis C, Sourvinos G, et al. From traditional ethnopharmacology to modern natural drug discovery: A methodology discussion and specific examples. Molecules. 2022; 27(13): 4060.
4. Krewski D, Andersen ME, Tyshenko MG, Krishnan K, Hartung T, Boekelheide K, Wambaugh JF, Jones D, Whelan M, Thomas R, Yauk C. Toxicity testing in the 21st century: progress in the past decade and future perspectives. Arch of Tox. 2020; 94:1-58.
5. Catala M, Kubis N. Gross anatomy and development of the peripheral nervous system. Handb of clin neur. 2013; 115: 29-41.
6. Chauhan P, Rathawa A, Jethwa K, Mehra S. The Anatomy of the Cerebral Cortex. Cere Isch. 2021: 1-6.
7. Kropf E, Syan SK, Minuzzi L, Frey BN. From anatomy to function: the role of the somatosensory cortex in emotional regulation. Braz J. of Psych. 2018; 41: 261-9.
8. Graybiel AM. The basal ganglia. Curr bio. 2000; 10(14): R509-11.
9. Ward LM. The thalamus: gateway to the mind. Wiley Interdisc Rev: Cogn Sci. 2013; 4(6): 609-22.
10. Kosif R. The thalamus: a review of its functional anatomy. Med Res Arch. 2016; 4(8).
11. Sherman SM. The thalamus is more than just a relay. Curr opin in Neurob. 2007;17(4): 417-22.
12. Meethal SV, Atwood CS. The role of hypothalamic-pituitary-gonadal hormones in the normal structure and functioning of the brain. Cell Mol Life Sci. 2005; 62(3): 257-70.
13. Odeh F, Antal M. The projections of the midbrain periaqueductal grey to the pons and medulla oblongata in rats. Eur J of Neuros. 2001; 14(8): 1275-86.
14. Sciacca S, Lynch J, Davagnanam I, Barker R. Midbrain, pons, and medulla: anatomy and syndromes. Radiog. 2019; 39(4): 1110-11125.
15. Apps R, Garwicz M. Anatomical and physiological foundations of cerebellar information processing. Nat Rev Neuros. 2005; 6(4): 297-311.
16. Ito M. Cerebellar circuitry as a neuronal machine. Prog in Neurob. 2006; 78(3-5): 272-303.
17. Roxo MR, Franceschini PR, Zubaran C, Kleber FD, Sander JW. The limbic system conception and its historical evolution. The Sci Wor J. 2011 Jan 1;11:2427-40.
18. Van der Walt E. The limbic system and the 'religious brain'. Homo Transcen. 2010; 23-39.
19. Platt B, Riedel G. The cholinergic system, EEG and sleep. Behav Bra Res. 2011; 221(2): 499-504.
20. Daneman R, Prat A. The blood–brain barrier. Col Spr Har Persp in Bio. 2015;7(1):a020412.
21. Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood–brain barrier. Neurob of Dis. 2010; 37(1): 13-25.
22. Engelhardt B, Sorokin L. The blood–brain and the blood–cerebrospinal fluid barriers: function and dysfunction. Sem in Immunopath. 2009; 31: 497-511.
23. Black J. Claude Bernard on the action of curare. Brit Med J. 1999; 319(7210): 622.
24. Gertsch J. How scientific is the science in ethnopharmacology? Historical perspectives and epistemological problems. J of Ethnopharm. 2009; 122(2): 177-183.
25. Heinrich M. Ethnopharmacology: a short history of a multidisciplinary field of research. Ethnopharm. 2015; 1-10.
26. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in

monitoring safety. Front in Pharma. 2014; 4: 66193.
27. Moke EG, Umukoro EK, Asiwe JN, Omogbiya AI, Erhirhie EO, Ben-Azu B, Anieh FU. Herbal Medicine: Education and Occupation Influences Its Practice among Residents of Port Harcourt, South-South Nigeria. Int J Pharm Phytopharm Res. 2021; 11(2): 38-44.
28. Irwin S. A rational framework for the development, evaluation, and use of psychoactive drugs. Ame J of Psych. 1968; 124(8S): 1-9.
29. Moser VC. Functional assays for neurotoxicity testing. Toxicologic pathology. 2011; 39(1): 36-45.
30. Ballabh P, Braun A, Nedergaard M. The blood–brain barrier: an overview: structure, regulation, and clinical implications. Neurob of Dis. 2004; 16(1): 1-3.
31. Lewis RW, Billington R, Debryune E, Gamer A, Lang B, Carpanini F. Recognition of adverse and nonadverse effects in toxicity studies. Tox Path. 2002; 30(1): 66-74.
32. Saghir SA. Rethinking guideline toxicity testing. Reg Tox and Pharma. 2015; 72(2): 423-8.
33. Van Norman GA. Limitations of animal studies for predicting toxicity in clinical trials: is it time to rethink our current approach?. JACC: Bas to Trans Sci. 2019; 4(7): 845-54.
34. Chinedu E, Arome D, Ameh FS. A new method for determining acute toxicity in animal models. Tox Int. 2013; 20(3): 224.
35. Parasuraman S. Toxicological screening. J of Pharma andPharmaco. 2011; 2(2): 74.
36. Abelan US, de Oliveira AC, Cacoci ÉS, Martins TE, Giacon VM, Velasco MV, et al. Potential use of essential oils in cosmetic and dermatological hair products: A review. J of Cos Dermatol. 2022; 21(4): 1407-18
37. Tsuchiya H. Anesthetic agents of plant origin: a review of phytochemicals with anesthetic activity. Molecules. 2017; 22(8): 1369.
38. Guidi C, Martínez-López E, Oliver JA, Sánchez-Vázquez FJ, Vera LM. Behavioural response to toxic elements, detoxification and organ accumulation are time-of-day-dependent in zebrafish. Chemosphere. 2023; 316: 137862.
39. Vinkers CH, Olivier B. Mechanisms Underlying Tolerance after Long-Term Benzodiazepine Use: A Future for Subtype-Selective G A B A A Receptor Modulators? Adv in Pharma and Pharmaceu Sci. 2012; 2012(1): 416864.
40. Norton S. Is behavior or morphology a more sensitive indicator of central nervous system toxicity?. Env Hea Pers. 1978; 26: 21-7.
41. Gage FH, Olton DS. L-Dopa reduces hyperreactivity induced by septal lesions in rats. Behav Bio. 1976; 17(2): 213-218.
42. Vorhees CV, Williams MT. Assessing spatial learning and memory in rodents. ILAR J. 2014; 55(2): 310-32.
43. Duan Z, Lv Z, Wang C, Chen B, An B, Zhou M. Few-shot Generation via Recalling Brain-Inspired Episodic-Semantic Memory. Adv in Neur Info Proc Syst. 2024; 36.
44. Marvel CL, Desmond JE. Functional topography of the cerebellum in verbal working memory. Neuropsych Rev. 2010; 20: 271-9.
45. Maviel T, Durkin TP, Menzaghi F, Bontempi B. Sites of neocortical reorganization critical for remote spatial memory. Sci. 2004; 305(5680): 96-99.
46. Modianos DT, Pfaff DW. Brain stem and cerebellar lesions in female rats. I. Tests of posture and movement. Bra Res. 1976; 106(1): 31-46.
47. Roqué PJ, Dao K, Costa LG. Microglia mediate diesel exhaust particle-induced cerebellar neuronal toxicity through neuroinflammatory mechanisms. Neurotox. 2016;56: 204-14.
48. Gerlai R. Behavioral tests of hippocampal function: simple paradigms complex problems. Behav Bra Res. 2001; 125(1-2): 269-77.
49. Meredith GE, Kang UJ. Behavioral models of Parkinson's disease in rodents: a new look at an old problem. Move Disors. 2006; 21(10): 1595-606.
50. Llorens J. Toxic neurofilamentous axonopathies–accumulation of neurofilaments and axonal degeneration. J of Int Med.; 273(5): 478-489.
51. Gauvin DV, Zimmermann ZJ, Dalton JA. De-risking in Tier I CNS safety assessments is the primary function of study design and technical training of laboratory staff observers. Reg Tox and Pharma. 2022; 129: 105116.