Clinical Neurologic Indices of Toxicity in Animals

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The fundamental structures and functions of the nervous systems of animals and humans are conserved in many ways across species. These similarities provide a basis for developing common neurologic examinations for a number of species of animals and also provide a basis for developing risk assessments across species for neurologic end points. The neurologic examination requires no expensive equipment and can be conducted in the field or wherever impaired animals are identified. The proper conduct of neurologic examinations in animals assumes that the examiner has a fundamental understanding of the normal structure and function of the nervous system as well as knowledge about the spontaneous disease background of the species being studied. — Environ Health Perspect 104(Suppl 1):323–330 (1996)

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Introduction

Consideration of structure–activity relationships or the results of preliminary toxicity studies may suggest the need for more in-depth analysis of nervous system structure or function. This second tier of testing typically involves a broad-based assessment of many neurologic end points to establish whether the concerns raised by the initial assessment indicate a significant concern about neurotoxicity. A broad-based assessment typically involves observational methods designed to detect behavioral changes in experimental animals, but similar methods can be used to study neurotoxicity in domestic animals or wildlife.

This paper describes clinical methods that can be used to examine animals for evidence of neurologic deficits. The methods described are adaptable for use in many different species and are similar to those used in human neurologic evaluations. The equipment necessary to perform neurologic examinations in animals is minimal and much of it can be improvised in the field if necessary. Necessary equipment includes an examination form, a pen or pencil, a penlight, a clicker or other device to make sounds, a reflex examination hammer for larger animals, a wooden or metal blunt probe, and a pin or pointed probe.

The nervous systems of various animal species, including man, have many similarities. The basic architectural units of the nervous system, neurons with axons and dendrites, and glial cells and myelin sheaths that support and invest the neurons and their process, are essentially the same types of structures across vertebrate species. The modes of transmission of neural impulses and the physiologic bases for potentiation and inhibition of neural activity are essentially the same across species. The organization of neurons in nuclei and layers, while similar across species, is, however, also a defining difference between species. As structure and physiology are conserved across species, so are basic functions, but here again the complexity of the function that various species perform is a defining element of the species.

The similarities and differences in function are important when investigators try to interpret neurotoxicologic observations in animals. Where structure and function are similar across species, the interpretation of neural deficits can be fairly straightforward. Where structure and function are different, a sound understanding of the differences is important if the significance of neural deficits is to be understood.

Diseases that afflict areas of the nervous system which are anatomically and functionally conserved across species frequently produce similar clinical syndromes across species. Because there are a finite number of ways that the nervous system can react to a toxic insult, clinical syndromes caused by different agents can be similar. The phylogenetic conservatism built into nervous system structure and function and the limited variety of disease states provide a basis for making risk assessment extrapolations across species.

Investigators of neurotoxic diseases in animals should have an understanding of the variety of spontaneous neurologic diseases that occur in animals for two reasons. First, spontaneous neurologic disease in animals is relatively common and can act as a confounding factor in the interpretation of neurotoxicology investigations. Second, zoonotic neurotropic diseases such as rabies occur at a relatively serious frequency in some populations of wild and domestic animals. Thus, individuals investigating neurotoxic diseases in animals must be aware of how to properly restrain and handle animals potentially infected with neurotropic viruses. Unless an investigator is working with laboratory-reared animals of known health status, he or she should assume that the animal could be carrying a neurotropic virus and handle the animal accordingly. Individuals planning to study neurotoxic diseases in wild or domestic animals should discuss preexposure immunization for rabies with their personal physicians.

Investigations of neurotoxic diseases in animals should follow a standardized plan of action. Following a protocol or planned examination will reduce the likelihood that the investigator will find himself or herself without a tool necessary to complete the examination and will increase the likelihood that an opportunity to make a significant observation will not be missed. The accurate recording of all observations at the time of the examination will allow the investigator to compare clinical progress in the same animal over time, facilitate the comparison of clinical syndromes in different animals, and ensure that a complete data set exists for future interpretive...
case summaries and consultations with co-investigators. An improperly recorded investigation becomes nothing more than an anecdote over time. A format for creating records of neurologic data in animal studies has been published (1).

**Neurologic Examination**

The neurologic examination has three parts that correspond to similar activities in human clinical medicine. These include a history, a physical neurologic examination of the animal, and any follow-up laboratory studies that may be indicated by either the history or the examination. The methods described below are based on methods that have been described in the veterinary neurology literature (2–5), the neurotoxicology testing literature (1,6–9), and the author’s experience in this field. It is not realistic to assume that this paper or any other can serve as a substitute for studying the textbooks available on veterinary neurology if the reader intends to become proficient in diagnosing neurologic conditions in animals.

**The History**

An interview with the owner or caretaker of a domestic animal provides an opportunity to learn about the animal’s past (age, previous illnesses, long-standing idiosyncrasies, etc.). It also provides an opportunity for the investigator to learn about clinical signs that may occur intermittently and to question the owner at length about specific observations the owner may have made. While the owner can provide important information about the animal, the investigator should be aware that owners and caretakers frequently have preconceived opinions about an animal’s neurologic condition which color their view of the animal’s history. Interviewing or questioning the owner about the animal’s condition should continue at appropriate points during the neurologic examination.

Investigations involving wildlife should include those people who have regular contact with the animals and an opportunity to observe the animals over a period of time. These individuals may include park rangers, naturalists, or others who have opportunities to observe the animals closely and frequently.

In the laboratory setting, the investigator should have access to the study protocol. The protocol should provide important background information about the animals and their treatment history, including dose route, dosing frequency, time on test, and other planned testing. Since the neurologic examination in the laboratory setting will ordinarily be conducted blind, the investigator should not have access to information that would provide cues to identify dose groups. At some point the investigator should question the animal caretakers or other technical staff about any abnormalities they may have observed in the test animals. This is best done at the end of the neurologic examination.

**The Physical Neurologic Examination**

The following discussion presumes that a general physical examination or routine clinical observations have been completed for the animal before the neurologic examination. The results of a neurologic examination may be erroneously interpreted if the general physical status of the animal is not assessed.

In conducting a neurologic examination under experimental conditions, the investigator should be aware of technical factors that might influence the appearance of behavioral signs. For example, the route of exposure can influence clinical signs. Intraperitoneal injections of irritating substances can cause varying degrees of peritonitis and fibrosis within the abdomen. The peritoneal reaction to irritating substances may cause the abdomen to appear enlarged or pear-shaped. The behavioral responses of the animal to peritoneal injury can include abdominal tenderness and abdution of the knees, which may lead to stance and gait abnormalities. When animals are dosed orally with irritating or unpleasant tasting materials that are left on the outside of the dosing cannula, they may show an almost immediate satiation that subsides with time. Animals that have been dosed repeatedly with irritating substances may demonstrate a Pavlovian conditioned response and begin salivating before dosing or when the investigator attempts to remove the animal from its cage.

Animals that have been repeatedly handled, especially if the handling is by the same person or if the handling procedures are fairly stereotyped, will behave differently than naive animals. Animals that have been handled roughly or have been exposed to noxious substances will commonly demonstrate aspects of fear or escape behavior. To avoid such problems, the investigator should plan for time to become acquainted with the animal. Rough or inconsiderate handling is unnecessary and counterproductive. If an animal will not allow certain procedures to be performed, the investigator should try another approach to performing the test or return to the test at a later point in the examination. Any testing that requires exposure to noxious stimuli should be conducted at the end of the examination so as not to affect other aspects of the examination.

A clinical assessment should not be harmful to the animal; the use of test procedures that could result in injury to a neurologically impaired animal should be seriously discouraged. Such handling procedures are only likely to result in confounding the results of the neurologic examination.

A number of observations that have been incorporated into a neurologic examination of laboratory rats are listed in Table 1.

**Assessment of Arousal Level or State of Ableness.** The first observations of the animal that are made are those involving an assessment of the animal’s arousal level in a familiar environment. For animals that are caged, the initial observations should be made with the animal in its home cage. The animal should then be removed from its home cage or enclosure and examined in a more open environment. For toxicology studies on small animals, the animals can be released into an open field on a laboratory cart. Larger animals such as dogs, horses, or cattle can be walked on a lead. During this part of the examination, the investigator should assess whether the animal is displaying a normal level of alertness and how the animal interacts with sensory cues present in its environment. For example, if there is movement, a change in the sound level, or other sensory stimulus, observe how the animal reacts to the stimulus and how vigorous the reaction is. Does the animal lift or move its ears when there is a new sound? If there is movement, does the animal orient itself towards the movement or otherwise act if the movement is perceived?

Interpretation of a number of other neurologic end points is affected by the assessment of level of alertness. If the central nervous system (CNS) is depressed, the activity of a number of reflexes will be altered. For example, animals intoxicated with acrylamide may develop a hypotonic gait because of peripheral nervous system (PNS) damage. Animals intoxicated with pentobarbital may also develop a hypotonic gait, but in this case the cause is not peripheral neuropathy but rather acute CNS depression. Because the mechanisms of induction of hypotonia are different in these two cases, the significance of the observation of hypotonia is also different. Hypotonia in an alert animal may be an
Table 1. A functional observational battery scoring reference sheet for laboratory rats.

| Cageside observation | Physical examination (continued) |
|-----------------------|----------------------------------|
| Body position—home cage | Nasal discharge |
| Observe the animal’s posture in its cage and while the cage is being opened | 1 None |
| 1 Normal | 2 Slight |
| 2 Flattened, limbs may be extended | 3 Moderate |
| 3 Lying on side | 4 Severe |
| 4 Sitting, head held low | Indicate if the nasal discharge is colored (e.g., porphyrin stained) |
| 5 Rearing repeatedly | |
| 6 Vertical jumping | |
| 7 Circling purposelessly | |
| 8 Pacing purposelessly | |
| 9 Whirling—lying down, wavelike movements of abdomen, alternating limb movements | |
| 10 No observation—animal in feeder | |
| Behavior while removing from cage—reactivity level | Respiratory difficulty |
| 1 Sits quietly and is easily removed or vocalizes without resisting being picked up | 1 Normal |
| 2 Runs around cage, hard to grab (with or without vocalization), or resists being removed from cage (holds on) | 2 Rales |
| 3 Freezes (with or without vocalization) or rears often, oriented toward investigator’s hand | 3 Retching |
| 4 Aggressive, tail and throat rattle (with or without vocalization), may attack | 4 Dyspneic |
| Facies—amount | 5 Gasping |
| 1 Normal | |
| 2 Slightly decreased | |
| 3 Moderately decreased | |
| 4 Severely decreased (almost none) | |
| Facies—consistency | Spontaneous vocalizations |
| 1 Normal | 1 No vocalizations |
| 2 Slightly soft | 2 1 to 5 vocalizations |
| 3 Mild diarrhea | 3 Most vocalizations occur during handling |
| 4 Severe diarrhea | 4 Vocalizes without stimulation |
| Physical examination | Body weight |
| Piloerection | Record weight to the nearest 0.1 of a gram. |
| N. None | |
| P. Present | |
| Hair coat | Neurologic examination |
| 1 Clean and groomed | Arousal level/state of alertness |
| 2 Slight (describe) | 1 Casual scratch, groom/slow spatial orientation |
| 3 Moderate (describe) | 2 I—Vigorous scratching, grooming/moderate spatial orientation or |
| 4 Severe (describe) | D—slightly reduced activity |
| Rate the severity and describe the haircoat with a comment: unkempt, alopecia; stained by feces, urine, or chemical, etc. | 3 I—Vigorous movement—slightly sharp, rapid (or darting) head or body movement, or |
| | D—moderately reduced activity |
| | 4 I—Hyperalertness (extremely vigorous movement—extremely sharp, rapid |
| | (or darting) head or body movement), or |
| | D—severely reduced activity (prostration/immobility) |
| Use ‘I’ to indicate increased arousal/alertness and ‘D’ to indicate decreased arousal/alertness (e.g., 4I or 4D). | Use ’I’ to indicate increased arousal/alertness and ’D’ to indicate decreased arousal/alertness (e.g., 4I or 4D). |
| Ataxic gait—loss of muscular coordination | Ataxic gait—loss of muscular coordination |
| 1 None | 1 None |
| 2 Slight but definite | 2 None |
| 3 Considerable without falling | 3 Incomplete extension at tarsus, no impairment of gait |
| 4 Considerable, with frequent falls, or cannot walk without falling | 3 Incomplete extension of tarsus, stifle, and hip; pelvis tipped; impaired gait, drags one limb intermittently |
| Hypotonic gait—hindlimb | 4 Drags both hindlimbs |
| 1 None | Hypotonic gait—forelimb |
| 2 Incomplete extension at tarsus, no impairment of gait | 1 None |
| 3 Incomplete extension of tarsus, stifle, and hip; pelvis tipped; impaired gait, drags one limb intermittently | 2 Incomplete extension of carpus |
| 4 Incomplete extension at tarsus, stifle, and hip; pelvis tipped; impaired gait, drags both hindlimbs | 3 Thorax held close to ground |
| Abnormal gait | 4 Thorax cannot be lifted from table |
| A, absent | |
| P, present (specify type) | |

(Continued)
Neurologic examination (continued)

Type of abnormal gait
1. Steppage—animal drags forelimbs in walking, walks on its carpus, or lifts its forelimbs unusually high to avoid dragging its toes over the ground
2. Spastic—shuffling gait with legs rigidly extended and not lifted during movement
3. Hypermetria—high-stepping gait
4. Duck walk—animal walks with adducted thighs (drawn toward median line) and laterally extended legs, causing it to assume a crouched posture
5. Scissor—forelimbs cross over in extension (in front of one another) and animal walks on balls of feet
6. Knuckling over—animal walks on its knuckles
7. Crossing over—animal crosses limbs while walking
8. Splayed walking—limbs deviated laterally

Body position—open field
Normal
Abnormal (describe)
Flattened, limbs may be extended
Lying on side, nonresponsive
Sitting, head held low
Rearing repeatedly
Vertical jumping
Circling purposelessly
Pacing purposelessly
Withing—lying down, wavelike movements of abdomen, alternating limb movements

Stereotypy
Describe:
Head flick—head shaking or backward flip of head
Head search—repetitive turning of the head from side to side, as though searching the environment
Prancing—restless shifting from one forelimb to the other, with slight turning of the body from side to side
Circling—tendency to move in circles around and along objects, or in an open environment
Waltzing—rapid turning in circles
Side-to-side rocking—side-to-side shifting, no lifting of feet

Unusual behavior
Describe:
Hallucinatory—animal appears to be responding to objects not present, visual tracking or fear withdrawal
Compulsive biting—usually of the grid floor
Self-destructive biting—usually biting of toes, with bleeding
Compulsive licking—usually of cage wall
Upright walking—on hindlimbs only
Aimless wandering—progressive, slow, plodding movements about the environment, with no apparent purpose
Retropulsion—walks backwards
Spatial disorientation—walking or stumbling into objects

Tremor severity
1. None
2. Head tremors only or body tremors slight; does not impair locomotion
3. Body tremors moderate; impairs locomotion slightly
4. Body tremors severe; prevents locomotion
Use 'R' to indicate resting tremors (tremors that occur at rest) and 'T' to indicate intentional tremor (tremors that occur in response to stimulation, occur or increase in severity only when the animal orients to an object or attempts to move) (e.g., 2R or 2T).

Convulsion
1. None
2. Slight (describe)
3. Moderate (describe)
4. Severe (describe)
Use 'S' to indicate spontaneous convulsions and 'T' to indicate convulsions induced by handling, light, sound, or other stimuli (state stimulus) (e.g., 2S or 2T).

Neurologic examination (continued)

Type of convulsion
Chewing only
Clonic tremors of entire body (clonic—alternating stiff and relaxed, may have purposefullike movement, "running")
Tonic convolution—limbs held rigidly extended (tonic—persistent contraction and spasm of a set of voluntary muscles)
Spasmatic jumping—animal repeatedly jumps or bounces in air; "popcorn seizure"

Approach response
Approach animal head-on with blunt object held approximately 3 cm from face for 4 sec
1. Slowly approaches and sniffs at object
2. No reaction or pulls away slightly or freezes
3. Jumps or turns away to avoid object
4. Jumps at object, attacks or bites

Touch response
Touch rump with blunt object
1. Turns toward site slowly or walks forward, away from stimulus
2. No reaction or turns to opposite side of touch or freezes
3. Jerks around toward object
4. Aggressive or violent reaction (with or without vocalization)

Auditory orientation
With the rat free to move about, a clicker is used to make a snapping sound outside of the animal's visual field.
Y, orient to sound
N, does not orient to sound

Muscle tone
The musculature of the limbs is palpated between the thumb and first finger.
N, normal—muscle is firm but not hard
T, Tense—muscle is tense and may be hard
S, Soft—muscle is soft and flabby and may be atrophied

Pupillary size
N, normal
D, dilated pupil
C, constricted pupil

Pinna touch response
Use blunt object to touch hairs within the external auditory canal
1. One twitch
2. More than one twitch
3. No response
4. Head shake

Righting reflex
The animal is turned on either side and its ability to return to a standing or sternal position is observed.
1. Rights when turned to either side
2. Rights with some difficulty
3. Rights from one side but not the other
4. Attempts to right but cannot or does not right when turned to either side

Visual placing
The animal is held by its tail at a level lower than the paper tray. The animal is then slowly brought up to the level of the paper tray
1. Early vigorous extension or placing before vibrissae contact
2. Placing after slight vibrissae contact
3. Placing after marked vibrissae contact or placing after nose contact
4. Unusual behavior hampers placing or none even after nose contact

Tail pinch
Grasp tail firmly with forceps 5 cm from tip of tail
1. Turns toward site slowly or walks forward, away from stimulus
2. Freezes (with or without vocalization) or immediately turns, may bite at side or pull tail away
3. Jumps forward (with or without vocalization)
4. No response or exaggerated response, rapid turning (with or without vocalization)
indication of motor impairment, whereas hypotonia in an animal that is not alert may be an indication of the degree of CNS depression. Similarly, the absence of a flexor response in a dog exposed to pentobarbital may be used to indicate a level of surgical anesthesia. The same clinical deficit in a dog exposed to methyl n-butyl ketone at relatively low exposure levels may indicate impairment of lower motor neuron function.

Convulsive Activity. Any indications of convulsive activity should be described in sufficient detail so that the investigator can determine whether the severity of the convulsive state changes over time. These observations should include information about the preictal period, if possible. For example, the investigator should note if there is a time period before the onset of the convulsive activity during which the animal’s general behavioral pattern changes so as to provide a signal that a seizure is about to occur. The events that initiate or trigger most seizures in animals are not usually known, but odors, flickering lights, a rapid change from dark to light, or other sensory stimulation can trigger seizures in animals. The manner of onset of the seizure should be noted. Does the seizure begin in a generalized manner, on only one side of the body, or first in one area of the body and then spread? Seizures can, for example, begin as vigorous chewing movements or shaking of one limb, with muscular activity spreading to include the entire body. The beginning of a seizure in a localized manner may provide information about the site of origin of a seizure. Most seizures in animals are of the tonic–clonic type in which the body is thrown into a tonic spasm followed by a period of relaxation. As the periods of spasm become shorter and the periods of relaxation longer, the seizure activity becomes a series of jerking movements (clonic phase).

The relationship between the exposure of an animal to a toxant and the occurrence of a seizure is important in determining the significance of the seizure potential of a chemical. The most common relationship seen in the laboratory environment is the occurrence of seizures due to hypoxia in moribund animals shortly before death; such seizures do not indicate that a chemical should be of concern as a convulsant. A second type of relationship involves seizures that occur in a dose-dependent manner shortly after dosing; chemicals that cause seizures in this manner are of concern, but lowering exposure levels will generally eliminate the risk associated with seizure induction. A third relationship involves chemicals that sensitize the nervous system but may not actually trigger a seizure. For example, exposure to 2,5-hexanedione is not ordinarily associated with seizure activity (the main effect of exposure is to depress the nervous system at high dose levels), but during a study with very high dose levels, a small number of animals had seizures 24 hr after dosing, when they were being examined before the next day’s dosing (JL O’Donoghue, unpublished data). A fourth type of relationship (kindling) that has been associated with electrical stimulation of the CNS and rarely with often abused drugs involves repeated exposure to subconvulsive stimuli, which eventually lead to a seizure at an exposure level that was previously not associated with seizures. When kindling is induced in laboratory animals by repetitive subconvulsive electrical stimulation, the seizures can sometimes be induced independent of the triggering stimulus, thus setting up an independent seizure cycle (10).

Assessment of Cranial Nerve Function. The position of the head and the facial features of the animal should be observed. Dysfunction of the vestibular nerve (cranial nerve VIII), vestibular nuclei, or vestibular spinal pathways may lead to abnormal positioning of the head or body and to nystagmus. Unilateral damage to the vestibular system can result in a head tilt toward the damaged side of the nervous system; with bilateral damage a head tilt will ordinarily not be present, but the animal may remain lying down and may resist attempts to get it to move. For example, when rats were given 2,4-pentanedione, some of them initially developed head tilts that disappeared within 1 to 2 days, as the vestibular pathways on the opposite side of the brain stem were damaged (11). Abnormalities in facial features may indicate dysfunction in the motor division of the facial nerve (VII) or motor innervation of the trigeminal nerve (V) to the jaw.

The eyes should be examined for prominence, papillary size (which may indicate damage to cranial nerves II or III or the sympathetic autonomic innervation to the pupil), and any positional deviation caused by damage to cranial nerves III, IV, VI, or VIII. The eyes should also be examined for nystagmus at rest and for positional nystagmus induced by moving the head quickly to either side, which can indicate damage to cranial nerve VIII. A particular group of clinical signs involving the eye, referred to as Horner’s Syndrome (enophthalmos, drooping of the eyelids, protrusion of the third eyelid [where present], and miosis), indicates that the sympathetic pathway to the eye has been interrupted. Touching the medial canthus of the eye induces a blink referred to as the palpebral reflex. The sensory portion of this reflex is served by the trigeminal nerve (cranial nerve V), while the facial nerve (cranial nerve VII) provides the motor innervation. Touching the cornea lightly induces a similar blink reflex (corneal reflex) served by the same innervation. Making a menacing movement with a finger toward the eye (without striking the eye or causing air movements to stimulate a corneal reflex) will also induce a blink reflex, but in this case the sensory input is through the optic nerve (II) while the motor response continues to come from the facial nerve (VII). Lightly touching the hairs on the inside of the ear will induce a pinna reflex that indicates normal functioning of a portion of the facial nerve.

Damage to the facial nerve can lead to weakness in the muscles of facial expression resulting in drooping of the facial features (e.g., nostrils, eyes, and eyelids) or a lack of normal movement in facial features (e.g., lack of flaring of the nostrils on breathing). Damage to the motor portion of the trigeminal nerve can lead to weakness in the jaws, drooping of the lips, or difficulty eating. Damage to the sensory division of the trigeminal nerve can lead to a loss of sensation over the face and head. Weakness in the tongue or atrophy of the tongue muscles may indicate damage to cranial nerve XII; absence of a gag reflex induced by inserting a finger into the back of the mouth indicates damage to cranial nerves IX and X. When testing animals other than laboratory-raised species, protection (e.g., gloves) should be considered to prevent contact with saliva that may be contaminated with rabies virus or other neurotrophic viruses.

Testing of vision and hearing in animals can be particularly difficult because significant damage can be present with few clinical manifestations. Testing of rats can be a particular problem because light-induced retinal damage is a common problem. Menace reflexes, visual placing reflexes, and approach responses provide a rough measure of visual ability. Watching how an animal approaches and drinks from a water bowl can be very helpful in detecting the animal’s ability to use subtelr visual cues. Orientation to a finger cricket or other sharp noise provides some measure of auditory ability. A particularly important aspect of assessing visual and auditory
function in animals is how the animal responds to visual and auditory cues both in its home area and during the examination. For larger animals, walking them through a maze or observing their responses to sounds created by a whistle can help to detect visual and auditory deficits, respectively.

Olfactory ability (cranial nerve I) can be assessed by bringing an attractive odor into the vicinity of the animal, especially if the animal cannot see the source of the odor because it is hidden or because the animal is blindfolded. Most animals will orient toward and approach an odor that they find attractive or interesting. Watching how an animal finds and approaches a food bowl can also be helpful.

**Gait Abnormalities.** At some point in every investigation of the animal nervous system, it will be necessary to determine if the animal can stand and walk. If the animal cannot or will not stand, an attempt should be made to determine if the animal does not stand because of weakness or because of incoordination. If the animal being observed is a small one and is easily controlled, the investigator can easily provide the assistance necessary to place the animal in a standing position. If the animal is large, slings, ropes, and a winch may be needed to assist in raising the animal. When examining wildlife, gentle prodding may be sufficient to determine whether the animal can rise. Before attempting to move any animal, however, it is important for the observer to closely examine the animal for any evidence of spinal damage or other physical defects that might indicate that the animal should not be moved. One of the more common gait deficits reported in animal toxicology studies is ataxia. As ataxia specifically indicates the loss of muscular coordination of the gait, many of the reports that list any form of gait disturbance as ataxia may be erroneous. Common causes of ataxia are damage to the cerebellum or damage to the sensory system, which provides important information on limb position to the cerebellum and other parts of the motor system. In addition to ataxia, cerebellar damage may result in muscular weakness, dysmetria (the limbs are lifted unusually high during movement), dysmetria (the animal is unable to stop a muscular movement at the correct location and either over or under steps), or crossing-over (the limbs move across the animal’s midline while walking).

Another relatively common gait disturbance in small animals is a hypotonic gait in which the animal tends to walk without fully extending the limbs, especially the hindlimbs. If the animal has not been exposed to a CNS depressant, this gait disturbance, especially if accompanied by signs of sensory loss such as knuckling over of the toes, is suggestive of a peripheral nerve abnormality.

**Assessment of Other Somatic Sensory and Motor Functions.** The motor and sensory capabilities of an animal can be assessed with an examination of reflex activity and palpation. A number of the tested reflexes give redundant information regarding function of specific nerves, but they are used to test the same function in various ways to help reduce the likelihood that a deficit will go undetected. When the routine reflexes have been examined, the investigator may want to follow up some of the findings with additional reflexes that test other parts of the nervous system. In assessing wildlife and large animals, it may not be practical to actually pick up the animal, but observation of the animal’s movements can be used to assess function if the observer is able to dissect the animal’s activities into discrete neurologic components. For example, when checking the visual placing reflex in small animals, the investigator has the luxury of being able to pick up the animal and move it toward the edge of a table and watching to see if the animal raises its limb and places it on the table. Obviously, if the investigator is examining a horse, this cannot be done. To induce the same reflex in a horse, the investigator can place a ground pole in the path of a horse and then lead the horse toward the ground pole. If the horse can see the ground pole, it will raise its leg in anticipation of reaching the pole and stepping over the pole. Likewise a wild animal in an open area can be observed approaching an object and reacting to it by raising its leg before it strikes the object. In all three instances the animal’s ability to see an object and react in a reflex manner to its presence can be observed.

Tactile placing in small animals is checked by moving the animal toward the edge of a surface (the animal must not be allowed to see the surface) so that the hair on the front of the digits touch the edge of the surface. If the animal is able to feel the hair movement, it will place its foot forward onto the surface. This reflex examines the integrity of the peripheral and spinothalamic sensory pathways and corticospinal and peripheral motor pathways. By touching different parts of the foot to the edge, different parts of the sensory pathway can be examined.

Proprioceptive reflexes can be examined in small animals by touching the dorsal portion of an animal’s foot to a surface such that the animal has to flip the toes up to stand on the foot. This reflex can be induced in small and large animals by passing the hand under one of the standing animal’s feet so that the dorsal part of the animal’s foot touches the surface the animal is standing on. This will cause the animal to flip its toes forward so that it can stand normally. This reflex examines the ability of the animal to sense an abnormally positioned foot and its motor ability to correct the abnormality.

In small animals, a hopping reflex can be induced in each limb by holding the animal so that its weight is on one foot and slightly pushing against the animal so that its center of gravity changes. The animal will hop to the side to regain its center of gravity. This reflex requires integration of proprioceptive, cerebellar, vestibular, corticospinal, and peripheral nervous system pathways for normal performance. A somewhat similar reflex can be induced in large animals by pushing on one side of the animal so that it is forced to move to regain its center of gravity. This reflex should not be used in large animals that have obvious motor difficulties or the animal could fall, injuring both itself and the examiner.

The extensor thrust reflex is induced in small animals by gently pushing upward on the sole of the hindpaw while the animal is held off the ground. This reflex is a segmental reflex that involves multiple spinal segments and employs peripheral sensory and motor pathways. The extensor postural thrust reflex can be induced by holding the animal under its forelimbs and lowering it toward the floor. The animal should spread its toes, extend its hindlimbs, and support itself. By shifting the animal’s center of gravity as it touches the floor, a vestibular component can be added to the reflex response.

Righting reflexes can be induced by turning the animal on its side and watching for it to turn and regain its posture. This reflex involves function of cranial nerve VIII as well as a complex array of other somotor pathways.

In animals such as dogs and cats, a patellar reflex can be induced by quickly stretching the patellar tendon and watching for extension of the lower leg. This reflex is a segmental spinal reflex and examines the integrity of the peripheral sensorimotor pathways and the associated spinal segment.

Muscle tone is assessed by gentle manipulation of the limbs and palpation of
major muscle groups. Judgments about muscle tone are generally highly subjective. Muscle strength in rodents can be measured with a grid attached to a strain gauge (1).

Perception of noxious stimuli can be assessed by induction of the flexor reflex. This reflex is induced by gently pinching the skin of the toes and assessing the strength of the limb withdrawal response. This reflex is a segmental reflex that requires intact peripheral and sensorimotor pathways and more than one spinal segment. The pinch should also induce a behavioral response from the animal, which can be used to assess whether or not the spinothalamic pathways responsible for perception of noxious stimuli are intact.

Sensation over the trunk can be assessed in small animals by touching the hairs over the back and sides of the animal with a cotton swab and watching for the animal to orient to the swab or react to it. The panniculus reflex can be induced in larger animals by gently stimulating the skin with a probe or pin and watching for the rapid contraction of the underlying muscle. The panniculus reflex is a segmental reflex that can assess function of the regional sensory and motor pathways.

**Assessment of the Autonomic Nervous System.** Bowel and bladder control are under the control of sympathetic and parasympathetic sensory and motor nerves, which originate in the sacral spinal segments. Normal voluntary control of urination and defecation involves cortical and brain stem neurons as well as the spinal reflex pathways. Chemicals that damage the spinal cord segments responsible for the reflex control of bowel and bladder function can lead to denervation of the bladder and bowel, which may result in atonia, relaxation of the anal muscles, and bladder distension. Certain chemicals have a special affinity for the autonomic nervous system and can lead to abnormalities in urination (12).

Damage to the autonomic nervous system can also lead to changes in perspiration, cardiovascular function, and digestive function.

**Abnormalities in Intracranial Fluid Pressure.** Chemicals that affect the cerebrospinal fluid pressure are relatively rare, but two potential disturbances of cerebrospinal fluid pressure exist that result in fairly obvious signs of intoxication. Some materials, e.g., hexachlorophene, increase cerebrospinal pressure by inducing intramyellic accumulation of fluid in the CNS and elsewhere (13). The increase in pressure can lead to CNS depression, edema of the optic nerve (which can be observed with an ophthalmoscope), and in dogs, head pressing. In contrast, other chemicals can damage the choroid plexus, which produces cerebrospinal fluid. The resulting clinical signs can be quite severe and widespread with apparent loss of vision and hearing, generalized CNS depression, ataxia, motor incoordination, and alteration in blood pressure (JL O'Donoghue, unpublished data).

**Localization of Lesion Sites**

A major problem with defining nervous system lesions in animals with neurotoxicologic deficits is that there is no textbook or manual that deals in a systematic way with the diagnosis of neurotoxic syndromes in animals. Textbooks in veterinary neurology are helpful in learning to understand how to localize lesions in many neurologic disease states, but in-depth information about neurotoxic diseases in animals is limited or fragmentary.

The basic information for understanding how to localize lesions includes knowledge about where the structural correlates of certain functions are anatomically located, how the anatomical structures are physically located in relationship to each other, and how damage to an anatomical structure will manifest itself functionally.

In localizing lesions involving the cranial nerves of special sensation, the observer must look (anatomically or functionally) at every accessible part of the nerve so as to rule in or rule out the presence of damage. For example, after noting that a visual impairment exists, it would be important to know whether the cornea, lens, retina, and optic nerve were normal on ophthalmoscopic examination. The presence of normal peripheral visual pathways would indicate that the visual loss was of central origin. Evidence for loss of hearing should prompt an otoscopic examination to determine whether there is physical evidence of damage to the external ear, the tympanic membrane, and as far as possible, the middle ear. Localizing damage to the somatic sensory and motor portions of the cranial nerves requires an understanding of which cranial nerves innervate which areas of the face and head.

Localization of damage to the spinal cord can be aided by the appearance of certain signs. The sympathetic innervation to the eye originates in the hypothalamus and passes through the ventrolateral columns of the cervical spinal cord, the C8 to T1 cervical spinal roots, the sympathetic nerve trunk, the cervical ganglia, and the trigeminal nerve before finally reaching the eye. Damage to any part of this pathway can lead to unilateral Horner's syndrome. Causes of such damage include space-occupying lesions in the cervical spinal cord, anterior thorax, and neck and damage to the peripheral pathways due to trauma to the neck. Abnormalities in the function of the diaphragm may indicate damage to C5 to C7 spinal cord segments. Abnormalities in the function of the intercostal muscle may indicate damage to T2 to T8 spinal cord segments. Traumatic interruption of the spinal cord in the mid-thoracic or anterior lumbar region of some species (e.g., dogs) can result in paralysis of the hindlimbs and extension, hypertonicity, and hyperalgesia in the forelimbs. Paralysis of the hindlimbs and loss of bladder control with normal function in the forelimbs indicate an abnormality in the lumbar spinal cord.

Sensory pathways within the spinal cord are arranged differently, depending on the modality they subserve. The pathways for position sense ascend through the spinal cord without crossing in the dorsal columns until they reach the brain stem. On the other hand, the pathways involving pain and temperature sense cross within the spinal cord and ascend within the spinothalamic tracts. Thus damage to a spinal pathway on one side of the spinal column will lead to a position sense loss on the same side of the body and to pain and temperature sense loss on the opposite side of the body.

The clinical signs resulting from damage to the upper motor neurons or their pathways coming from the brain differ from those produced by damage to the lower motor neurons located in the ventral motor horns of the spinal cord. Weakness or paralysis due to upper motor neuron damage typically is associated with increased or spastic muscle tone, increased spinal reflex responses, absent or altered supraspinal reflexes, and in some species, clonus and the appearance of a crossed-extensor reflex. Weakness or paralysis due to lower motor neuron damage is typically associated with reduced muscle tone or flaccid muscles, decreased or absent spinal and supraspinal reflexes, absence of crossed-extensor reflexes, and muscle atrophy.

Chemicals that cause peripheral neuropathies in animals commonly result in early weakness in the tail muscles. In the rat, the tail is not carried as high as normal, the animal rears without use of the tail, or it rears less frequently than normal. The
animal does not extend its hindlimbs normally while walking and tends to walk with a flat-footed gait. The toes may be curled under the foot while walking (knuckling over). As the neuropathy progresses the forelimbs may be affected as well. Segmental reflexes will give weak responses and some of them (e.g., patella reflex) may disappear. The muscles, particularly those of the hindlimb, will be hypotonic and may be atrophied.

The effort expended in localizing a lesion in the nervous system is worthwhile because it requires the investigator to go about collecting data in an organized manner. It also encourages the development of a differential diagnosis, which may lead to the identification of additional tests that should be conducted to more specifically identify the anatomical basis for the observed neurotoxicity.

Other Tests
Following the physical portion of the neurologic examination, additional tests should be considered to follow up on leads discovered during the examination. The additional work could include hematology, clinical chemistry, electrophysiology, radiography, cerebrospinal fluid analysis, or muscle and peripheral nerve biopsy examinations. The exact tests chosen for follow-up examination should be based on the results of the neurologic and physical examinations.

Conclusion
A properly conducted neurologic examination can provide a solid foundation for the assessment of deficits in animals induced by chemical agents. A well-documented examination provides a record of the current status of a wide variety of functions, and it can also provide information about how neurologic function changes over time if the examination is repeated at regular intervals. Using the results of the neurologic examination to localize the anatomical correlates of functional deficits may aid in understanding the pathogenesis of nervous system intoxication and complement neuropathologic investigations.

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