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Evaluation of Peripheral Nerve Injury and Regeneration in Animals: Clinical, Electrophysiological, and Functional Assessment Methods

Harsha Sahu[1], Chandana L[1], Aman Kumar Tiwari*[1], Rekha Pathak[1], Rohit Kumar[1], Noorulnissa Bandey[1]

[1]Division of Surgery, ICAR- IVRI, Izatnagar, Bareilly, U.P. India, 243122

*Corresponding author: vetaktiwari@gmail.com

Abstract

Peripheral nerve injuries are frequently encountered in veterinary clinical practice and experimental research, especially in cases of trauma, orthopedic surgeries, bite wounds, and iatrogenic injuries. Accurate evaluation of peripheral nerve damage and subsequent regeneration is essential for diagnosis, prognosis, selection of therapeutic strategies, and assessment of treatment outcomes. Unlike gross injuries that are easily visible, peripheral nerve dysfunction often requires a combination of clinical, functional, electrophysiological, histological, and behavioral assessment techniques. Over the years, numerous methods have been developed to evaluate axonal degeneration, regeneration, target organ reinnervation, and functional recovery in animals. This article presents a comprehensive and simplified overview of commonly used evaluation methods for peripheral nerve injury in animals, including axonal regeneration tests, nerve conduction studies, target reinnervation assessment, sensory and motor function tests, muscle evaluation techniques, gait analysis, and advanced electrophysiological and behavioral assessments.

Keywords: Peripheral nerve injury, axonal regeneration, nerve conduction, functional recovery, gait analysis, electromyography, veterinary neurology

Introduction

Peripheral nerves play a vital role in transmitting motor, sensory, and autonomic signals between the central nervous system and peripheral tissues. Injury to these nerves can result in loss of sensation, muscle weakness, atrophy or paralysis, autonomic dysfunction, and significant impairment of an animal's quality of life. In veterinary practice, peripheral nerve injuries may occur due to road traffic accidents, fractures, surgical trauma, injections, bite wounds, ischemia, or compression, injury due to prolonged recumbency, dystocia. Experimental peripheral nerve injury models are also widely used in laboratory animals to study nerve regeneration and to evaluate new surgical techniques, biomaterials, drugs, stem cell therapies, and rehabilitation protocols. Evaluation of peripheral nerve injury is challenging because nerve regeneration is a slow and complex biological process involving Wallerian degeneration, axonal sprouting, remyelination, and reinnervation of target organs. No single test can provide complete information about nerve structure and function. Therefore, a combination of clinical examination, functional tests, electrophysiological studies, and indirect indicators of regeneration is usually required. The choice of evaluation method depends on the species, nerve involved, type of injury, duration after injury, and purpose of assessment, whether clinical or experimental. This article discusses the major evaluation methods of peripheral nerve injury in animals in a systematic manner, focusing on axonal regeneration, nerve conduction, target reinnervation, sensory and motor recovery, muscle function, and overall functional outcome.

1. Evaluation of axonal regeneration

Assessment of axonal regeneration is fundamental in understanding the healing process of an injured peripheral nerve. Several classical and experimental methods are used to determine whether regenerating axons have crossed the injury site and reached the distal nerve segment.

a) The pinch test is one of the simplest and most widely used intraoperative or experimental methods. In this test, distal to the injury site is gently pinched using forceps. A positive response, such as muscle contraction, limb withdrawal, or vocalization, indicates that sensory or motor axons have regenerated to that level. The distance from the injury site to the point of positive response provides an estimate of regeneration length. Although simple, the pinch test is subjective and mainly useful in experimental settings.

b) Nerve conduction in situ provides more objective information. In this method, the nerve is electrically stimulated at one point, and the evoked response is recorded from a muscle or another point along the nerve without removing it from the body. The presence, latency, and amplitude of the response reflect axonal continuity and functional regeneration. This technique is particularly useful in longitudinal studies.

c) Retrograde tracing is an advanced experimental technique used to confirm axonal regeneration and neuronal survival. A tracer substance is applied to the distal part of the nerve or target organ. The tracer is taken up by regenerating axons and transported back to the neuronal cell bodies in the spinal cord or dorsal root ganglia. The presence of labeled neurons confirms successful regeneration. Although highly accurate, this method is mainly limited to research laboratories.

2. Evaluation of target reinnervation

Regeneration of axons alone is not sufficient for functional recovery unless the regenerating fibers successfully reinnervate their target organs such as skin, muscles, or glands. Therefore, assessment of target reinnervation is an essential component of nerve injury evaluation.

a) The pinprick test is commonly used to assess sensory reinnervation. Gentle pricking of the skin supplied by the injured nerve is performed using a needle or pin. A withdrawal response, vocalization, or behavioral reaction indicates return of sensory function. The test is simple but depends on animal cooperation and observer experience.
b) The foot flick test is another sensory evaluation method, particularly used in small animals. A mild stimulus is applied to the plantar surface of the paw, and the speed and intensity of limb withdrawal are observed. Improved response over time suggests sensory reinnervation.
c) Evans blue extravasation test is an experimental method used to evaluate sensory nerve function and neurogenic inflammation. Evans blue dye is injected intravenously, and a noxious stimulus is applied to the skin. Increased vascular permeability caused by sensory nerve activation leads to dye leakage, which can be visually or quantitatively assessed.
d) Toe spreading is a simple but reliable indicator of motor reinnervation of intrinsic foot muscles, especially in sciatic nerve injury models. Normally, when an animal is lifted by the tail, the toes spread outward. Loss of toe spreading indicates denervation, while gradual return suggests motor reinnervation.

3. Sensory and motor nerve conduction studies

Nerve conduction studies provide quantitative and objective information about nerve function. Sensory nerve conduction involves stimulation of a sensory nerve and recording the sensory nerve action potential. Parameters such as conduction velocity and amplitude reflect myelination status and axonal integrity.

a) Motor nerve conduction studies involve stimulation of a motor nerve and recording the compound muscle action potential from the target muscle. Prolonged latency, reduced amplitude, or absence of response indicates nerve damage. Improvement in these parameters over time reflects regeneration and remyelination.
b) Motor unit number estimation is an advanced electrophysiological technique used to estimate the number of functional motor units supplying a muscle. Reduction in motor unit number occurs after nerve injury, while gradual increase indicates reinnervation through axonal sprouting.

4. Evaluation of muscle function and integrity

Muscle changes are important indirect indicators of peripheral nerve injury. Denervated muscles undergo atrophy, loss of strength, and changes in fiber composition.

a) Muscle tension measurement assesses the force generated by a muscle during contraction. Reduced tension indicates denervation or incomplete reinnervation, while increasing tension suggests functional recovery.

b) Muscle weight is a simple and commonly used parameter in experimental studies. Denervated muscles show significant reduction in weight compared to the contralateral normal side. Restoration of muscle weight reflects successful reinnervation.

5. Evaluation of functional recovery

Functional recovery is the ultimate goal of peripheral nerve regeneration and is best assessed by observing the animal's ability to perform normal activities.

a) The grasping test is frequently used in forelimb nerve injury models. The animal is allowed to grasp a bar or grid, and grip strength or ability to hold is evaluated. Improvement indicates motor recovery.

b) The whisker motion test is particularly useful in facial nerve injury models. Symmetry, frequency, and coordination of whisker movement are observed. Return of synchronized whisker motion indicates facial nerve regeneration.

c) Walking track analysis is a widely accepted method for evaluating sciatic nerve function. Footprints are recorded as the animal walks along a corridor. Various parameters are measured to calculate the Sciatic Functional Index (SFI), which provides a numerical value representing nerve function.

d) The Sciatic Static Index is a modification of walking track analysis that uses static footprints, making it useful in animals that do not walk consistently.

e) Video analysis of gait allows detailed observation of limb movement, posture, and coordination. Modern software enables slow-motion and frame-by-frame analysis, improving accuracy.

f) Kinematic analysis involves measurement of joint angles, stride length, and limb trajectories during movement. This technique provides detailed quantitative data on locomotor recovery.

6. Electrophysiological and reflex assessments

Electrophysiological and reflex assessments provide objective and semi-objective information about the functional status of peripheral nerves, neuromuscular junctions, muscles, and associated spinal reflex pathways. These methods are valuable both in clinical diagnosis and in experimental models to monitor nerve degeneration and regeneration over time.

a) Electromyography is performed by inserting a fine needle electrode into the muscle supplied by the injured nerve. The animal is usually restrained gently or sedated, depending on species and temperament. Electrical activity is recorded at rest and during voluntary or induced muscle contraction. In normal muscles, there is electrical silence at rest and organized motor unit action potentials during contraction. Following denervation, spontaneous abnormal activities such as fibrillation potentials and positive sharp waves appear within 1–3 weeks, indicating loss of neural input. During reinnervation, these spontaneous activities gradually disappear, and polyphasic motor unit potentials with increasing amplitude and duration are observed, reflecting collateral sprouting and restoration of neuromuscular transmission. Serial electromyographic recordings are particularly useful for monitoring the progression of nerve recovery.

b) Reflex tests are simple bedside or laboratory procedures used to assess the integrity of sensory afferent fibers, motor efferent fibers, neuromuscular junctions, muscles, and spinal cord segments. The withdrawal reflex is tested by applying a mild noxious stimulus, such as pinching or pricking the skin of the limb, and observing flexion of the limb. Absence or reduction of the response suggests sensory or motor pathway disruption. The patellar reflex is elicited by tapping the patellar tendon and observing extension of the stifle joint, indicating integrity of the femoral nerve and corresponding spinal segments. The panniculus reflex is tested by pinching the skin along the trunk and observing contraction of the cutaneous trunci muscle; loss of this reflex caudal to a lesion helps localize nerve or spinal cord damage. These reflexes are easy to perform and provide rapid functional information.

c) Evoked potentials are recorded by electrically stimulating a peripheral nerve and recording the resulting electrical responses at different levels of the nervous system, such as the nerve itself, spinal cord, or brain. The animal is usually anesthetized lightly to reduce movement artifacts. Parameters such as latency, amplitude, and waveform morphology are analyzed. Delayed or absent responses indicate impaired conduction across the injury site, while gradual normalization over time reflects axonal regeneration and remyelination. Evoked potential studies are especially useful for assessing conduction across repaired nerves and for correlating peripheral nerve recovery with central nervous system integration.

d) The staircase test is a behavioral and functional assessment mainly used in research animals, particularly rodents, to evaluate skilled forelimb function and fine motor control. The test apparatus consists of a narrow chamber with a series of steps on both sides, each step holding a small food pellet. Before nerve injury, animals are trained to retrieve pellets using their forelimbs, establishing baseline performance. After nerve injury, the animal is placed in the chamber, and its ability to reach, grasp, and retrieve pellets from different step heights is observed and recorded over a fixed period. The number of pellets retrieved, the highest step reached, and the precision of grasping are noted. Impaired performance indicates motor and sensory deficits, while gradual improvement over time reflects functional reinnervation, coordination, and recovery of skilled movements.

Conclusion

Evaluation of peripheral nerve injury in animals requires a multidisciplinary approach combining clinical observation, functional testing, electrophysiology, muscle assessment, and behavioral analysis. Each method provides unique information, and no single test can fully describe the extent of nerve damage or recovery. Understanding the principles, advantages, and limitations of these evaluation techniques is essential for accurate diagnosis, monitoring of regeneration, and assessment of therapeutic interventions. For veterinary clinicians and researchers, a systematic and integrated evaluation strategy ensures better clinical outcomes and advances in the field of peripheral nerve repair and regeneration.