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Review

Choosing the correct functional assay: A comprehensive assessment of functional tests in the rat

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Abstract

While there are several ways to quantify peripheral nerve regeneration; the true measure of successful outcome is functional recovery. Functional tests are relatively easily conducted in human subjects; however it is more difficult in a laboratory animal. The laboratory rat is an excellent animal model of peripheral nerve injury and has been used extensively in the field of peripheral nerve research. Due to the intense interest in the rat as an experimental model, functional assays have been reported. In an effort to provide a resource to which investigators can refer when considering the most appropriate functional assay for a given experiment, the authors have compiled and tabulated the available functional tests applicable to various models of rat nerve injury.

Keywords: Rat; Functional test; Nerve; Forelimb; Hind limb; Vibrissae; Sensory; Motor

Contents

1. Introduction

Although the process of peripheral nerve regeneration can be examined in a variety of ways ranging from electrophysiology to histomorphometry [78–81], the benchmark

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of successful reinnervation remains functional recovery. Nerve fibers may regenerate without making appropriate sensory/motor connections and axonal sprouting without pruning may similarly overestimate the number of functional connections. Thus, recovery of function does not necessarily correlate with histologic and electrophysiologic evidence of regeneration [49,114,65,16,29,1,94]. This stimulates interest in the development and utilization of functional tests as outcomes measures after nerve injury. Functional analysis may offer the best and most unequivocal method to demonstrate that a nerve has not only regenerated, but also made correct, working end organ connections. Functional assessments are easily conducted in human subjects, who can follow commands and communicate with the investigators. Many sensory and motor evaluation tools exist [48,95], as well as assessments that rely on the patient to report their own outcomes [96]. Unfortunately, these types of assessments are not easily translated to laboratory animals.

The rat is an invaluable model in peripheral nerve research: it is small, easily housed and handled, relatively inexpensive, and large numbers of genetically identical animals are readily available. Also, rat nerve fibers are similar in size and morphology to human nerves. Rat nerves are large enough to be readily manipulated with microsurgical techniques. Due to the fact that the rat is so well suited for research, numerous functional assays have been developed in order to better utilize these animals in investigations of nerve injury and functional deficit or recovery. These include motor and sensory tests ranging from the exactingly quantitative to the highly qualitative.

In an effort to provide a resource to which investigators can refer when seeking the most appropriate functional assay, the authors have compiled and tabulated the available functional tests applicable to various models of rat nerve injury. These tests have been categorized based on the anatomic region assessed as well as the equipment required, and the type of data yielded.

2. Forelimb tests

Although the sciatic nerve is the most commonly studied nerve, and lower extremity function most often evaluated, it is important to recognize that upper extremity tests of neurological function have also been described. The rat forelimb is innervated by nerve branches from the brachial plexus including the radial, ulnar, musculocutaneous and median nerves. Unlike in humans, however, where both the median and ulnar nerves are critical for grasp, in the rat it is the median nerve which is primarily involved in grasp [12]. The median nerve enters the forelimb as a branch of the brachial plexus originating from the fifth cervical through the first thoracic roots, and receives contributions from the posterior, medial, and lateral cords. It courses through the upper arm parallel to the brachial artery and then divides into a muscular branch and the volar interosseous nerve in the forearm [47]. The median nerve innervates all of the finger flexors and the flexor carpi radialis.

Rats possess prehensile forelimbs, forepaws, and digits. They naturally pick up and manipulate their food when hunting and foraging [63,64]. The dexterity of rat digits has been observed and quantified when seeking and manipulating various differently sized and shaped items [136]. Rats can also be trained to perform a wide variety of reaching and grasping tasks ranging from very simple to quite complex. This training can readily be achieved through operant conditioning with reward reinforcement.

Due to this dexterity and plasticity the rat is a useful model to evaluate forelimb function. Even in the rat, however, functional assessment of rat forelimb movement is complex, involving many factors including the trajectory, reach, grip, rate, frequency, and power of various upper limb motions [134]. The tests and parameters used to evaluate forelimb function in normal and nerve-injured animals are described in Table 1. The relative importance of each of these parameters is based, in part, on the function to be assayed, although there is little doubt that all of these parameters are interrelated. Careful dissection of all of these parameters, however, can be cumbersome, expensive, and resource-intensive. They may be unnecessary especially in less complex peripheral nerve injuries, or when the same functional assay is standardized between groups. The complexity of forelimb movement is evidenced by the multitude of assays that have been designed to examine the forelimb, and also by the intensive nature of some of the more intricate assays. These exhaustive tests are well suited to detecting some of the more subtle nuances of functional disorders arising from specific central nervous system lesions. However, in the peripheral nerve injury scenario, it may be appropriate to utilize some of the less meticulous assays which are not as descriptive, but simply measure and quantify the return of function. Upper extremity functional tests have been relatively under-utilized. With continuing problems of work-related upper extremity disorders these tests offer the potential to better study the issues of repetitive nerve injury and provide more relevant outcomes measurements than do the sciatic functional indices.

3. Hind limb tests

The rat sciatic nerve is the most commonly utilized nerve for evaluating nerve injury and regeneration and the greatest experience with functional outcome measurements is reported with the sciatic nerve. The rat hind limb is primarily innervated by the sciatic nerve and its branches. The sciatic nerve is formed from the fusion of the fourth and fifth lumbar spinal nerves, with some variable contributions from the third lumbar spinal nerve [3]. The sciatic nerve then passes through the sciatic notch and enters the hind limb. At this point it gives off branches to the hip extensors and leg flexors and continues its course beneath the gluteus medius muscle into the thigh. In the mid-thigh the nerve trifurcates into

Table 1
Forelimb tests

Test	Description	Primary source	Additional references	Equipment	Data	Sensory–motor
Modification*						
Reaching dish	Rats reached between cage bars to obtain food from a canary dish. Handedness was assessed based on reaching preference	[100]		–	+	M
Reaching tray*	Rats were tested in a cage fitted with a glass tray with multiple alcoves containing food pellets. Slots were designed to admit only one limb and had an opening on the bottom to force the rats to grasp and lift, rather than scrape the pellets into the cage. Rats were rated on ratio of touches to successful placement of the pellet in their mouths	[18]	[17]	–	R	M
Bracelet*	The test was modified by restraining one limb with a plaster/rubber bracelet that would not allow the limb to reach through a slot; this allowed selection of reaching limb	[140]	[39,40,91,129, 131–133,138,139, 142,144]	–	R	M
Scrape bar*	The test was modified by adding a bar at the front of the tray to prevent rats from scraping pellets into the cage without grasping them	[140]		–	R	M
Spacer*	The test was modified by adding a spacer so that pellets would fall through the space if the rats tried to scrape pellets into the cage without actually grasping them	[40]	[39,91,129,131–133, 138–140,142,144]	–	R	M
Local anesthetic*	The test was modified by using injection of local anesthetic instead of a bracelet to restrain one limb	[40]	–	R	M	
Pellet array	Rats were trained and subsequently tested in a cage with 21 spaces between the cage bars which led to slots containing food pellets. Food pellets of seven different sizes were used in randomly varying locations. Rats were graded on latency of reaching, successful reaches, and number and accuracy of grasp attempts via a computer assisted analysis system	[145]	–	#	M	
Single-pellet reaching shelf	Rats were trained to reach through a slot onto a shelf to retrieve a food pellet steadied in one of two indentations on the shelf. The indentations were placed at an oblique angle to the opening so that the rat could only reach with the chosen paw. Animals were videotaped and scored on successful vs. failed reaches	[141]	[83,88,91,120,129, 134–137,141,143]	V	R	M
Kinematic analysis	Rats were filmed and analyzed based on frame by frame analysis of selected components of reaching and grasping	[145]	[19,141,142]	V	K	M
Eshkol–Wachman movement notation (EWMN)*	System of movement notation originally developed to describe dance as a common standard of notation for all styles. This system allows for the notation of any visually discernable movement of the body. The notation system shows the rate, size, direction, force, shape, etc., of body part movement in relation to other parts of the body	[41]	–	K	M	
EWMN (five component)*	Using a single pellet reaching box made of clear Plexiglas, the rats were filmed from several perspectives while performing a reaching task. The reaching movement was subdivided in to five components which were analyzed frame by frame and graded using Eshkol–Wachman movement notation (EWMN)	[141]	[142]	V	K	M
EWMN (seven component)*	Reach/grasping movements were further subdivided into seven components and analyzed as above	[144]	V	K	M	
EWMN (10 component)*	Reach/grasping movements were further subdivided into 10 components and analyzed as above	[143]	[83,88,91,129, 130,137,138]	V	K	M
EWMN (pasta)	Reach/grasping movements while handling and eating segments of pasta were subdivided into six components and analyzed as above	[8]	[86]	V	K	M
Arpeggio*	Rats were filmed in a single pellet reaching box, detailed analysis of the hand movements were undertaken as the rat felt for and located the food item and initiated grasp	[136]	[83,134]	V	K	SM
Postural adjustments*	Rats were filmed during reaching, and their postural adjustments were broken down into five components and scored on each component	[91]	[92,138]	V	K	M

Table 1 (*Continued*)

Test	Description	Primary source	Additional references	Equipment	Data	Sensory–motor
Grooming assessment	Grooming was elicited by misting fur with water. Grooming behavior was video recorded and analyzed based on multiple parameters including order, structure and yntactic chain completion	[11]	V	K	M	
Free grasping analysis	Rats were filmed freely moving about their cage. Spontaneous grasping was assessed by placing a food pellet on the floor. The rat was then videotaped and analyzed as it handled and ate the food	[91]	[130]	V	K	M
Pasta or other*	Same as above except the rat was given various food items (grapes, sunflower seeds, peanuts, pastas)	[134]	[137]	V	K	M
Predation*	Same as above except the rat was analyzed catching, handling and eating prey animals (crickets)	[63]	V	K	M	
Grasp test (with hind limb)	Rats were tested on a grip strength apparatus consisting of a ring anterior, and a bar posterior; both connected to strain gauges. The animal grasped the anterior ring and was pulled by the tail until its grip is broken; this force was measured by the strain gauge. This procedure was repeated for the hind limbs with the posteriorly located bar (see Table 2)	[90]	F	#	M	
Forepaw*	Rats were held by the pelvis and lowered toward a horizontal bar from above, they were rated on whether they grasped the bar or not	[89]	–	+	M	
Horizontal bar*	Rats' forepaws were rested on a horizontal bar above a flat surface and time to replacement of the forepaws on the ground was recorded	[89]	–	T	M	
Vertical cling*	Rats were made to hang from a wire grid by their forepaws, the time to their release of grasp was measured	[89]	–	T	M	
Grid + balance*	Rats were held by the tail over a wire grid attached to an electronic balance and allowed to grasp the grid with a forepaw and lifted until their grip was broken. The reading at moment of release recorded. The other paw was either restrained or denervated	[12]	[4]	F	#	M
Reduced grid + tape + median nerve*	Rats were suspended by the tail and allowed to grasp a three wire grid attached to a balance. The weight at which grip was broken was recorded. Tape was placed along the bar to keep them from using their wrists to hold the bar. The grid was reduced to three wires to prevent the rat form walking on it. The contralateral median nerve was divided to force the rat to use only the experimental limb	[48]	F	#	M	
Staircase test	Rats were placed in an apparatus with an elevated platform from which they could reach down the successive steps of staircases on either side of them to retrieve food pellets from recessed wells in each step. They were graded on number of pellets retrieved	[93]	[38]	–	#	M
Staircase + EWMN*	Rats were filmed in a clear Plexiglas reaching box fitted with a raised platform with a staircase down along either side. Rats reached down from the platform to retrieve food pellets located in indentations on each successive step. They were graded on the number of food pellets retrieved in a 15 min session. Movement analysis using EWMN was used to provide detailed analysis of the reaching actions performed during this task	[146]	V	#K	M	
Pasta reaching	Rats were filmed reaching through an aperture for a single piece of pasta arranged in various orientations, or tested to differentiate between pasta and similarly or differently textured objects. Reach/grasp was assessed by force transduction and video analysis of components of the movement	[9]	[7]	VF	#K	SM
Pasta matrix*	Similar to the above test except that rats reached for pasta arranged in a 13 × 20 row matrix. Reach/grasp was assessed by EMWN rating, and the number and location of the pieces of pasta retrieved	[8]	V	#K	SM	

Table 1 (*Continued*)

Test	Description	Primary source	Additional references	Equipment	Data	Sensory–motor
Haptic discrimination*	Rats reached through a slot and were presented a piece of pasta and either a smooth non-food item, or a serrated non-food item of equivalent diameter. They were rated on their ability to correctly select the pasta	[7]	–	+	SM	
Conveyor belt	Rats were trained to reach through a slot onto a moving conveyor belt to retrieve food pellets. Animals were graded on percent of pellets retrieved vs. percent omitted and were analyzed for paw preference	[42]		*	R	M
Rotating table	Rats were trained in a skilled reaching task that consisted of reaching through a slot to grasp a food pellet from a rotating table (6 rpm) 1 cm away from cage. Rats were graded based on the ratio of reaches to successful retrievals	[69]	–	R	M	
Latch test	Rats were trained to press a latch to open a box of food. Handedness was assessed based on preferred paw	[100]	[69]	–	+	M
Force transducer plate*	Rats were trained to reach into a chambers designed to admit only the right or left limb and depress a plate attached to a force transducer to receive a water reward. Rate/number of attempts as well as force (including sub-threshold attempts) were recorded by a computer	[101]	FB	#	M	
Force transducer lever*	Rats were trained to press isometric levers located in a slot outside of their cage, levers were positioned to force use of only the left or right forelimb. The velocity, force, and duration of lever pressing were recorded. Results were correlated with implanted cortical microstimulation maps	[19]	FB	#C	M	
Vertical paw placing	Rats were placed in a clear Plexiglas cylinder and videotaped for 5 min, the number of paw contacts with the wall were counted to assess whether one limb was favored	[7]	V	#	M	
Adhesive dot removal	Rats had small adhesive dots affixed to the radial aspect of their forelimbs; the time it took for them to contact and remove the dot was recorded	[7]	–	T	SM	
Ladder rung walking	Rats were trained to walk across a ladder (with sidewalls to keep them from falling). Rung placement was varied so that they could not learn the pattern. They were videotaped and graded based on successful steps and misses, as well as placement of their feet on the rungs	[87]	V	#K	SM	
Variable height shelf test	Rats were trained to retrieve food pellets by reaching through a slot onto a shelf that could be placed at different heights. Rats were filmed from multiple angles including from below using a reflectance technique to highlight the contact points of their feet. Posture, foot placement, and limb use at different reach heights was analyzed, as well as successful reaches at each shelf height	[92]	V	#K	M	
Cortical mapping	Rats were trained in a single pellet reaching box. They were videotaped and analyzed based on successful reaches as well as kinematic and movement notation analysis. Differences between strains were compared and correlated with cortical mapping done by cortical microelectrode stimulation	[120]	[19,62,69]	VB	KC	M

Data—+: the parameter is measured as present or absent, R: the data is yielded as a ratio or percentage of normal, #: discreet numeric data points are produced, T: temporal data are produced, C: central nervous system excitation recording, K: kinematic analysis. Equipment—V: video recording equipment, S: strain/force recording device, C: computer, B: brain/CNS implanted recording electrodes, E: electrical stimulation equipment, *: other specialized equipment.

the peroneal, tibial, and sural branches; the peroneal nerve subsequently innervates the tibialis anterior and the extensor digitorum longus, while the tibial nerve supplies the plantar flexors, toe flexors, and the tibialis posterior [47].

At constant speed, rats walk with a very consistent and quantifiable gait and postural pattern. Their steps are regular and symmetrical, yielding highly reproducible pawprint patterns. The rats' stride is also quite regular with marked consistency in values for paw placement in relationship to the other limbs, length of stride, and time spent in the stance and swing phases of gait [59]. Normal function of the rat hind limb is essential to regular gait and posture and nerve

lesions will affect the function of the leg, foot, and toes. Functional deficit of the hind limb is apparent through changes in the established patterns of paw placement and stepping [34], changes in the phases of gait [126], as well as direct changes in limb strength [67,75].

Because rat hind limb nerves are the most commonly used model in peripheral nerve research, de Medinaceli et al. [34,32] and then others developed numerous tests to assess toe, foot, and leg function, as well as gait and posture characteristics. These tests are described in Table 2. The validity of many of these tests as a measure of neurologic recovery, however, can be compromised by the development of muscle fibrosis and joint stiffness [30]. The effects of motor and sensory recovery can be affected by the adverse mechanical sequelae of these processes. These issues have been addressed, to some extent, by providing rats with meshed-wire screens to provide a mechanism for physical therapy [112]. However, it is unclear if physical therapy affects the experimental results, or augments compensation for injury-induced gait changes. The relevance of post-surgical changes is also poorly controlled in most functional assays of recovery following peripheral nerve or spinal cord injury. Immediately after surgery, pain, skin, muscle or bony damage and the effects of post-operative analgesics can have a profound effect on many parameters of functional recovery. Presumably, these effects become less important the longer time rats are from surgery. Ideally, the effects of surgery could be differentiated from those of the nerve injury itself by evaluating animals sustaining placebo operations, or by waiting at least 2–3 weeks after surgery before functional evaluations are started. We would recommend using placebo-operated animals as controls since delaying functional assessment would prevent the detection of important changes occurring in the acute post-operative period. The Bain–Mackinnon modifications of de Medinaceli's functional indices which have been based on multiple linear regressions can be used to demonstrate neuromuscular recovery from specific nerve injuries [6].

When footprints of the nerve-injured rat are assessed with standardized functional indices (Fig. 1), the peroneal nerve recovers full function, the tibial nerve has been shown to recover 54% of baseline function, while the sciatic nerve recovers only 41% of its function [55]. This discrepancy is likely due to the higher density of fibers in the sciatic nerve leading to a higher likelihood of motor–motor or motor–sensory pathway mismatches during nerve regeneration. Of the sciatic nerve branches, the tibial nerve contains the largest proportion of motor and sensory fibers and contributes most significantly to lower extremity function. Thus, a tibial nerve injury pattern is very similar to that obtained from a sciatic nerve injury. Therefore, when choosing a peripheral nerve injury model to study functional recovery in the lower limb, the tibial nerve provides a dependable unifasicular alternative to the sciatic nerve [55,45]. In comparison, peroneal nerve injury alone can recover completely after transection injury and causes a loss of dorsiflexion and eversion of the foot, and decreases toe spread [55]. Moreover, because the peroneal nerve remains intact with a tibial nerve injury model the animal is able to dorsiflex the ankle thus plant its foot without a foot drop. This assures that all walking tracks are "measurable" [29,133]. With a total sciatic nerve injury or with an isolated peroneal nerve injury the animal will drag the ankle until the anterior compartment musculature is innervated. As well, functional recovery

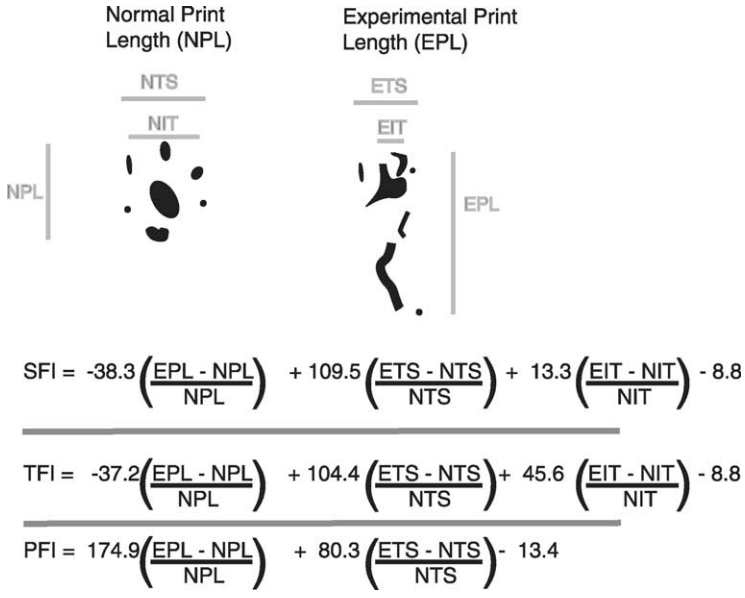

Fig. 1. Walking track analysis relates the injured, experimental limb to the uninjured, normal side. EPL = experimental print length; NPL = normal print length; NIT = normal intermediate toe spread (between second and fourth toes); EIT = experimental toe spread; ETS = experimental toe spread (between first and fifth toes); NTS = normal toe spread. Functional indices specific to distinct peripheral nerve injuries are also listed.

Table 2
Hind limb tests

Test (modification*)	Description	Primary source	Additional references	Equipment	Data	Sensory–motor
Pawprint analysis	Rats were placed in a Y-shaped runway and allowed to explore freely. Paws were smeared with Vaseline and powdered charcoal dust was used to demonstrate prints. Stride length and variability (splay) were measured	[105]		–	#	M
Fingerprint dust, video recording, gait analysis*	Rats had feet coated with grease and were then allowed to walk down a corridor. Footprints were revealed with fingerprint dust and analyzed. A lateral view of the rats was videotaped as they walked; footfall sequence and gait analysis was done by correlating the prints with the video	[59]		V	#K	M
Toe spread*	Rats were held by the hips with paws pressed slightly toward the floor. Distances between first and fifth as well as second and fourth digits were measured with callipers	[57]		–	#	M
Walking tracks, sciatic function index (SFI)*	Rats with unilateral sciatic nerve injuries were walked down a narrow walkway with a dark shelter at the end. The floor of the corridor was covered with a piece of x-ray paper, and the rats hind feet were dipped in developer. The reaction of the developer with the film created prints. These prints were assessed for: distance to opposite foot (TOF), print length (PL), toe spread between toes 1 and 5 (TS), and toe spread between toes 2 and 4 (IT). A formula was developed to compare the injured to uninjured side, yielding a combined sciatic function index (SFI) for these values	[34]	[49,31,33,35]	–	#	M
Digital data input*	Digital measuring of pawprints was assessed and compared to conventional measuring methods	[32]	[97]	C	#	M
Modified SFI, tibial function index (TFI), peroneal function index (PFI)*	Pawprints of rats with sciatic as well a solely peroneal or tibial nerve injury were analyzed. PL, TS, IT were used to develop function indices for tibial (TFI) and peroneal (PFI) nerve injuries	[20]	[6]	–	#	M
Bromphenol blue*	Compared walking track results with X-ray paper and developer to those on copy paper impregnated with bromphenol blue and water	[77]	[97]	–	#	M
Modified SFI, TFI, PFI*	Prints from sciatic, tibial, and peroneal lesions were analyzed based on PL, TS, IT, TOF, and print angle (PA). Modified the SFI, TFI, and PFI were computed based on multiple linear regression analysis of these factors	[6]		C	#	M
SFI tutorial*	Provides an example for SFI calculation and assessment of one's own SFI results	[15]		–	#	M
Ink and paper, stride analysis*	Walking track with ink and paper, stride length and stride width were measured as well as consecutive print length (S1) for gait symmetry. Different strains were compared	[99]		–	#	M
Outward rotation angle*	Walking track with dye, glue, water, and paper. Analyzed toe spread (1–5), outward rotation angle of the foot (the line between toes 1 and 5 and the straight line trajectory of the rat), and print length	[148]		–	#	M
Reflectance, walking speed, computer analysis*	Rats were filmed ventrally walking down a darkened Plexiglas runway, via a 45° mirror below. The glass floor of the runway was internally illuminated with fluorescent light. The contact points of the paws with the glass would scatter the light and were recorded as bright areas. The forward speed of the rat as it crossed the runway was also calculated by the time it took to break sequential infrared beams across the runway. The data was sent to a microcomputer and footfall patterns, foot pad contact points, contact area, and speed were calculated	[25]	[26,54]	VC*	#	M
Long-term assessment*	SFI, TFI, PFI were followed long term (1-year) for evaluation of time of maximal functional recovery	[55]		–	#	M
Sciatic stance index (SSI)*	Rats were filmed from below in a Perspex enclosure; footprints during the static stance phase of gait were analyzed frame by frame. TS, ITS, and PL were measured and used to create a sciatic stance index (SSI)	[14]	[13]	V	#	M

Table 2 (*Continued*)

Test (modification*)	Description	Primary source	Additional references	Equipment	Data	Sensory–motor
Video analysis*	Rat walking tracks were videotaped; a frame by frame analysis was used to calculate SFI	[76]	[85]	V	#	M
Toe out angle (TOA)*	Rats were filmed from below using a mirror and pawprints were analyzed from captured video images. Toe out angle (TOA) was measured as the angle between the direction of progression and the midline of the foot. SFI was also calculated from these images and correlated to TOA	[121]		V	#	M
Toe twitch tension	Rats were anaesthetized and their foot was secured to a vertical bar with a rubber band. Needles or alligator clips were used to deliver an electric current (2 Hz) across the sciatic nerve to induce maximal muscular contraction. A thread was attached to the distal phalanx of the third toe and the force developed was measured on a strain gauge. The injured side was compared to the uninjured limb	[67]		F	#	M
Ankle stance angle	Rats were filmed walking in a Perspex tunnel. Their legs were shaved and marked at predefined points on the lateral malleolus and fourth metatarsal; these points were used to form two lines to calculate the ankle angle during the stance phase of gait	[75]		V	#	M
Modified reference points*	Legs were shaved and marked at the tibia, lateral malleolus, calcaneus, and fifth metatarsal and these points were used to calculate ankle angle	[123]	[122]	V	#	M
Grasp test (hind limb)	Animals are tested on a grip strength apparatus consisting of a ring anterior, and a bar posterior; both connected to strain gauges. The animal grasps the posterior bar and is pulled by the tail until its grip is broken; this force is measured by the strain gauge. This procedure is also conducted with the forelimb on the anteriorly located ring	[90]		S	#	M
Grasping motion*	Rat lifted by scruff and evaluated for the presence of the ability to make a grasping motion with the hind limb	[74]		–	+	
Tactile placing response (TPR)	Rats were held and their toes were manually flexed so that the dorsal surfaces were curled under in contact with the walking surface, they were evaluated on their ability to correctly reposition their toes	[115]	[60]	-	+	M
Hopping response	Rats were placed with their hind paws only on a surface. One hind leg was lifted, and the animal was moved laterally until it either hopped or fell over	[115]	[60]	–	+	M
Extensor postural thrust	Rats were held upright with their distal metatarsals and toes on a balance, they were made to bear weight until the heel contacted balance. This force was recorded	[115]	[60]	F	#	M
Inclined plane test	Rats were placed transversely on a textured pad attached to an angled board which could be raised and lowered to increase the angle. They were raised until they could no longer maintain their position for 5 s. This angle was recorded	[103]	[89,43]	–	#	SM
Swimming test	Rats were thrown into a tank of water with two exits. The direction they swam in (left or right was recorded)	[100]		–	+	M
Swimming test*	Rats were placed in a swimming tank and videotaped as they swam across to an inclined ramp. Graded on number of fore and hind limb kicks, body angle, and time to cross to the ramp	[46]		V	T#K	M
Cortical stimulation/treadmill	Rats were anesthetized and placed in a stereotactic frame suspended over a moving treadmill. Stepping patterns elicited by stimulating different portions of the CNS were recorded	[104]		B	K	M
BBB locomotor rating scale	Rats were placed in a circular enclosure and encouraged to locomote continuously. They were tested for 4–5 min and examined by two separate examiners who then scored them on a 21 point scale based on limb movement, weight bearing, foot drop, etc.	[10]		#	K	M

Table 2 (*Continued*)

Test (modification*)	Description	Primary source	Additional references	Equipment	Data	Sensory–motor
Gait analysis (mirror)	Rats were recorded walking in a Perspex runway and via a mirror angled at 45° beneath them, thus yielding a split screen side and ventral view image. They were evaluated based on quantitative gait parameters (stride length, displacement, duration, stance vs. swing phase) as well as kinematic reconstructions of paw excursion	[128]		V	K	M
Gait stance duration*	Rats were filmed from below and pawprints were analyzed based on the amount of time spent in the stance portion of the gait cycle. A ratio was established with the uninjured side. This was compared to toe spread as well as SFI. The effect of morphine analgesia on this parameter was also measured to evaluate if incisional pain would affect it	[126]	[85]	V	R#	M
Gait analysis*	Rats were filmed walking in a clear Plexiglas tunnel. Gait analysis was performed based on ankle angle, back height, stride length, step length, and stance vs. swing phase duration	[106]		V	T#	M
Walking speed*	Video analysis (lateral, ventral, and posterior views) of rat gait was used to observe: (1) walking speed; (2) ratio of stance to swing phase (injured vs. uninjured side); (3) ratio of step length (injured vs. uninjured side); (4) ankle joint angle (terminal stance and mid-swing); (5) tail height from the floor at terminal stance; (6) tail deviation from midline of the body axis at terminal stance; (7) angle of body lean from horizontal through the hip joints	[150]		V	R#	M
Stance/swing ratio*						
Step length ratio*						
Ankle stance angle*						
Tail height*						
Tail deviation*						
Midline deviation*						

For legends see Table 1.

can be measured with the print length factor ((experimental print length − normal print length)/normal print length) making the more complex sciatic or tibial functional indices unnecessary.

Another factor, which may confound analyses based on parameters that assess rat walking tracks, is the issue of post-surgical autotomy. When a limb is rendered insensate, rats have the tendency to chew off the insensate foot depending upon the extent of autotomy. This behavior may render the limb useless to subsequent analysis of function. Not all rats harbor this behavior and autotomy can be avoided by selection of the appropriate strain of animals. Lewis rats are noted to have little to no self-mutilation after denervating injury, while Brown–Norway and Sprague–Dawley rats are the most notorious for this behavior [21].

4. Vibrissal tests

The facial nerve is commonly injured in humans in trauma as well as in head and neck cancer surgery. This injury poses a very challenging reconstructive dilemma based upon its complex, multiple branched neuroanatomy, thus it is an area of great clinical interest. The rat facial nerve emerges from the skull through the stylomastoid foramen; it then supplies multiple branches to the muscles of the face and neck, the lacrimal gland, and sensory branches to the ear and tongue. It possesses three major branches to the facial musculature, the largest of which is the buccal branch, which supplies the superficial muscles of the upper lip, nose, face, and provides efferent innervation to the vibrissae (whiskers) [47]. Based upon its caliber and length, the buccal branch has been the principle branch of experimental interest. Afferent innervation of the vibrissae is derived from the infraorbital branch of the maxillary division of the trigeminal nerve.

The rat vibrissal system provides an excellent model for the study of facial nerve injury and facial reanimation, and several types of analysis of vibrissal function have been devised in order to quantify facial nerve function and recovery from injury. Vibrissae are typically arranged in an array five horizontal rows and seven vertical rows on the lateral aspect of the snout, which has corresponding representation on the somatosensory cortex in the central nervous system. Normally rats use their vibrissae, whose piloerector muscles are innervated by the buccal branch of the facial nerve, in a characteristic whisking and sniffing behavior by which they protract and retract the vibrissae in order to explore their environment [127,82,22,70]. Studies describing and employ-

Table 3
Vibrissal tests

Test (modification*)	Description	Primary source	Additional references	Equipment	Data	Sensory–motor
Video analysis	Cinematographic analysis was used to record and analyze the sniffing behavior of rats. Protraction and retraction of the vibrissae was assessed	[127]		V	#	M
Twitch rate, EEG, EMG*	Slow motion video analysis of vibrissal twitch rate during normal sniffing was used to correlate twitches with EEG and EMG recordings	[75]		V	#	M
Air stream, licking rate*	Rats were trained to lick a water source at a constant rate. Once trained they were filmed to monitor changes in licking rate from vibrissal stimulation. A vibrissa was stimulated by a modulated air stream at various frequencies which were calibrated to degree of vibrissal deflection. Stimulation was also modulated to provide a 'safe' period and a 'warning' period after which an electrical shock was administered. Rats were monitored for cessation of licking in conjunction with the 'warning' frequency	[61]		VE*	+	S
Quantitative geometric analysis*	Rats were initially filmed during free, spontaneous whisking/sniffing behavior. Vibrissal movement compared to facial reference points was analyzed in two-dimensions. Parameters assessed were vibrissal protraction and amplitude, whisking frequency, angular velocity, and acceleration. The test was later modified by restraining the animals	[51]	[52,119]	V	#	M
Cortical monitoring	Responses to vibrissal manipulation and stimulation in chemically paralyzed rats were monitored via cortical electrodes. Vibrissae were manipulated with hand held probes, as well as a mechanical vibrissal deflection apparatus	[111]	[110,108]	B	C	S
Jump test	Blinded rats were trained to jump across a variable distance gap for a food reward. They would feel for the other side of the gap with their vibrissae and would not jump if it could not be palpated. They were scored on their jump/no jump decisions	[61]		–	+	S
Texture discrimination*	Blinded rats (via eyelid suture) were trained to distinguish between smooth and rough sandpaper that could only be reached with their vibrissae and jump to a corresponding goal area for a food reward. Once trained, rats were graded on percent of correct choices	[50]		–	R	S
Video analysis of vibrissal function*	Rats were trained to feel across a gap using their vibrissae to discriminate between smooth and rough plates. They would jump across to the appropriate plate and be rewarded with food. Video analysis of vibrissal velocity, amplitude, and whisking pattern were obtained as the rats performed this task (some vibrissae were trimmed to optimize image clarity)	[23]		V	#K	S
Qualitative assessment	Following the initial nerve lesion, rats were observed for return of vibrissal motor function until the vibrissae returned to the level of the mouth, regained a posterior orientation, and resumed a rhythmical whisking pattern. The post-op times at which these events occurred were noted	[2]		–	TK	M
Angle of arcing	Whisking movements post-facial nerve crush injury was measured. Observations of the angle of vibrissal arcing were measured, and recovery determined by the return of symmetrical vibrissal whisking	[147]		V	R	M
Laser array	Rats' heads and bodies were restrained to align vibrissae with an optoelectronic laser array bilaterally. Whiskers were marked with a foam marker and whisking parameters were recorded as each whisker moved through the array successively interrupting the laser beams. This data was analyzed to determine whisking frequency, duration, amplitude, velocity, and synchrony between the two sides	[44]		C*	#	M

Table 3 (*Continued*)

Test (modification*)	Description	Primary source	Additional references	Equipment	Data	Sensory–motor
Aperture width	Rats were trained to poke their nose into an infrared sensor and feel the width of a surrounding aperture with their vibrissae. Based on differences in the width of the aperture they would receive a water reward by activating another nose sensor either to their left or right. Rats were rated on their ability to detect fine differences in aperture width	[73]		*	+	S

For legends see Table 1.

ing measures of vibrissal function are detailed in Table 3. Because the vibrissae comprise a sensitive sensory organ as well as a mobile motor system, they can be assessed for changes in either modality. They are well suited to both movement analysis and tests of sensitivity and detection thresholds (Fig. 2). While analysis of the vibrissal system is used to assess reinnervation through the buccal branches of the facial nerve [37,119], reinnervation of the orbicularis oculi through the zygomatic branches has been evaluated using a blink reflex described by Terzis and colleagues [113,116–118]. The authors show that the orbicularis oculi sphincter is dually innervated in both rats and humans, thus enabling a reliable

Table 4
Sensory tests

Test	Description	Primary source	Additional references	Equipment	Data	Sensory–motor
Modification*						
Nociceptive pressure stimulation	Rat's pain threshold to pressure was measured using a pneumatic pressure recording device. Pain threshold was described as the pressure at which the rat began to struggle	[102]	[56]	F	#	S
von Frey hair test*	Rats were placed in a wire mesh bottomed cage. Feet were tested by applying von Frey hairs of increasing size to the plantar surface of the foot. The test was scored as the ratio of withdrawals to stimuli	[68]	[4,24]	*	R	SM
Forceps*	The rat's withdrawal to pressure on first and fifth toe was tested with calibrated forceps attached to a recorder that recorded the intensity and duration of the stimulus	[115]	[74,60]	F	#	SM
Vocalization*	Pressure was applied to the rat's hind paw with an analgesimeter until the rat vocalized; the pressure measurement at this time was recorded	[4]		F	#	S
Nociceptive electric stimulation	Electric current (0.1 mA) was applied to several points on the rat's foot pads and withdrawal response was evaluated	[28]		E	+	SM
Incremental*	Rats underwent electric stimulation of the foot sole to evaluate for withdrawal, evaluated in 0.1 mA increments	[36]	[84]	E	#	SM
Nociceptive caloric stimulation	The rat was held while its paw was placed on heated surface and withdrawal latency was measured	[56]	[4,60,68]	–	T	SM
Unrestrained*	Unrestrained rats had radiant heat source shined through a glass floor onto their paw and withdrawal latency was recorded using a microcomputer. They were also graded on time that they held the paw up and the presence of licking of the paw	[56]		–	+T	SM
Heated probe*	The rat's withdrawal latency to a hot probe on the medial margin of the metatarsus was evaluated	[115]		–	T	SM
Tail caloric test	Rats had their tails placed in hot water and rate of discharge of dorsal horn cells was recorded	[58]		C	C	S
Withdrawal latency*	Rats had their tails placed in hot water and the time until withdrawal of the tail was measured	[4]		–	T	SM
Myotactic reflexes	Myotactic reflexes were elicited by tapping over the tendon or muscle belly with a pediatric plexor; evaluated as present or absent	[115]		–	+	SM
Autonomic function	Rats' autonomic response was monitored through changes in vasomotor tone by measuring skin temperature at the base of the heel	[115]		*	#	Sy

For legends see Table 1.

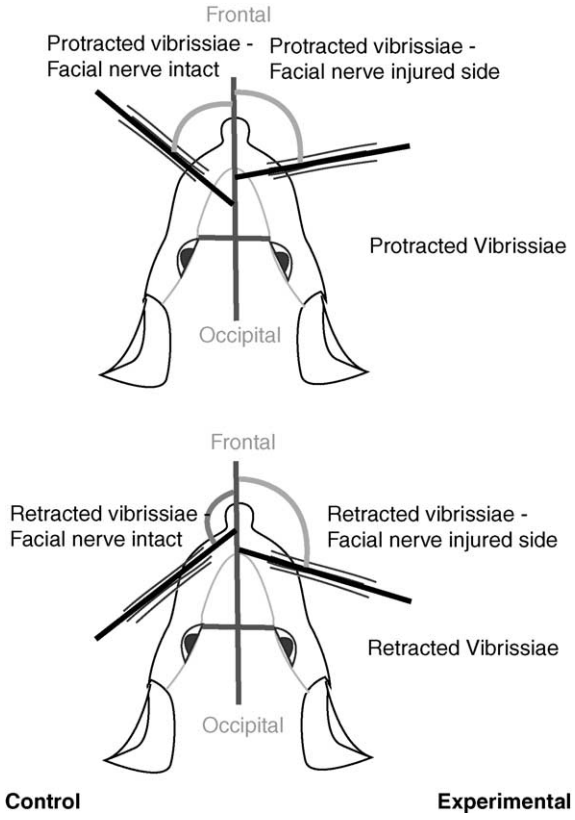

Fig. 2. Vibrissal function is determined by comparing protraction and retraction between experimental and normal sides. A line connecting the medial canthi serves as a consistent landmark, and a perpendicular to this line marks the frontal-occipital midline. The angle formed between the midline and the mid-point of the vibrissae determines the angles of protraction and retraction and enables comparison between sides. Figure adapted from Tomov et al. [119].

rodent model for studying nerve grafting procedures for re-establishing blink in facial nerve-injured animals.

5. Sensory tests

Sensory perception is also an important measure to consider in recovery from nerve injury. The return of sensory nerve function allows for protective sensibility and proprioception. Also, pathologic syndromes of increased sensibility (tactile allodynia, thermal hyperalgesia) may occur after peripheral nerve lesions [24]. In order to better address questions about the function of the sensory nervous system in an experimental setting, various tests to quantify nociceptive function have been developed for rats. These tests are listed in Table 4.

It is very difficult to describe a *pure* sensory test. Since the animals cannot express sensory perception, the majority of these tests rely on a motor response to a sensory stimulus. Thus, it becomes impossible to separate sensory and motor function in most of these assays. This motor dependency can be circumvented if the response to the stimulus can be elicited from and area of the body which was unaffected by the initial intervention being tested, such as in the Nociceptive pressure stimulation test [102] or the vocalization test [4] (Table 4). Pure sensory function can also be determined if the animal can be trained in such a manner as to respond predictably to sensory cues as is demonstrated by several of the tests of vibrissal function (Table 3).

Although not designed to test function, a novel model has recently been developed to recreate chronic regional pain syndrome-1 (CRPS-I) or reflex sympathetic dystrophy—a clinical problem that is difficult to reproduce in animal models [27,149]. In this model, a tight-fitting O-ring is applied for three hours to the ankle to produce chronic post-ischemic

Table 5
Comparisons/reviews

Test	Description	Primary source	Sensory–motor
Modification*			
Methods compared	Walking tracks were recorded using X-ray film/developer, paint/paper, diluted paint/paper, and compared.	[66]	M
	Pawprints recorded on photographic paper with developer, or on plain paper with finger-paint to determine SFI. Video analysis of pawprints was also conducted. Gait analysis (stance duration) was also assed via video analysis	[36]	M
	Compared walking track analysis to extensor postural thrust (EPT) as methods of evaluating functional impairment	[71]	M
	Reviewed and compared SFI, stance duration, and ankle index	[124]	M
	Reviewed and compared walking track to neurobehavioral tests: extensor postural thrust (EPT), tactile placing response (TPR), hopping response (HR), and withdrawal reflex latency (WRL)	[53]	M
	Discussed, compared and contrasted methods of hind limb functional assessment including: walking track/SFI, TS, EPT, nociceptive withdrawal, gastrocnemius and soleus mass, kinematic assessment, gait stance duration, ankle angle, and toe out angle. Recommended a battery of tests as the most accurate assessment of function	[125]	SM
	The Basso, Bresnahan and Beattie (BBB) locomotor scale was compared to walking track analysis and calculation of the SFI	[107]	M
Correlation to other assays*	SFI was compared to muscle strength, evoked potentials, and morphometry to determine correlation	[109]	M

Equipment—E: electrical stimulation equipment; *: other specialized equipment.

pain. The affected foot initially demonstrates painful edema and hyperalgesic responses to modestly noxious stimuli are noticed as early as eight hours later and last for four weeks. The application of free radical scavengers reduces the observed pain response in these animals as measured by hindpaw withdrawal to von Frey filament stimulation [27].

6. Comparisons and reviews

Several reports have compared specific functional tests' results, reliability, and reproducibility in order to determine which tests are better suited to detect differences in certain parameters. Other studies have compared the data from functional tests with electrophysiological and histologic assays in an attempt to correlate specific histologic and nerve conduction findings with a certain level of functional recovery. A summary of these reports is listed in Table 5. These comparisons of different functional assay are useful in determining which of a group of similar tests will provide the most reliable data for a specific parameter of interest, and are invaluable to investigators in choosing between specific functional tests for an experiment.

7. Conclusions

Each functional test that has been developed has different strengths and weaknesses. These are based on the parameters which they are designed to examine, the type of outcome data that they produce, the technical difficulty and the time required to administer (or potentially train the animals to perform), and the need for expensive or highly specialized equipment. Each assay is designed to test a specific hypothesis and provide data to illustrate differences between groups. In choosing the correct functional assay for a specific experiment, the investigator must choose the test that best answers the question and produces the type of data that is suited to that experiment, i.e. quantitative, qualitative, numeric, temporal, percent of normal function, etc. In this way, the investigator can choose simplest, least expensive, least time intensive test to suit a particular experiment. In compiling this review, the authors have attempted to produce a comprehensive resource for investigators when choosing a functional assay for a specific experiment.

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