# Stimulated Grip Strength Measurement: Validation of a Novel Method for Functional Assessment

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**ABSTRACT**

**Introduction:** Reliable measurement of functional recovery is critical in translational peripheral nerve regeneration research. Behavioral functional assessments such as volitional grip strength testing (vGST) are limited by inherent behavioral variability. Isometric tetanic force testing (ITFT) is highly reliable but precludes serial measurements. Combining elements of vGST and ITFT, stimulated grip strength testing (sGST) involves percutaneous median nerve stimulation to elicit maximal tetanic contraction of digital flexors, thereby allowing for consistent measurement of maximal grip strength.

**Methods:** We measured side-to-side equivalence of force using sGST, vGST and ITFT to determine relative reliability and repeatability. We also performed weekly force measurements following median nerve repair.

**Results:** sGST demonstrated greater reliability and inter-trial repeatability than vGST and similar reliability to ITFT, with the added benefit of serial measurements.

**Discussion:** sGST is a valid method for assessing functional recovery that addresses the limitations of the currently available modalities used in translational peripheral nerve regeneration research.

**Key words**: Peripheral nerve regeneration, muscle force testing, functional assessment, peripheral nerve injury, motor reinnervation



## INTRODUCTION

Reliable measurement of functional recovery is critical in translational research aimed at assessing peripheral nerve regeneration and end-organ reinnervation. Unfortunately, all of the currently available modalities used to measure motor functional recovery have considerable limitations.

The grasping test is a method for assessing function that measures the amount of grip strength a rodent can generate via the median nerve-innervated extrinsic digital flexor muscles.[1] To perform this test, the rodent is dangled by the tail to elicit instinctive grasping of a bar attached to a force transducer. Once grasp is initiated, the rodent is distracted away from the force transducer by the tail, and the maximum distraction force prior to loss of grip is recorded. Volitional grip strength testing (vGST, 'the grasping test') has a number of advantages in comparison to the sciatic functional index (SFI), which was the first and remains among the most commonly used methods for measuring functional recovery resulting from peripheral nerve regeneration. Following median nerve injury and repair, rodents do not exhibit autotomy or contractures[2-5], both of which occur frequently in the setting of sciatic nerve injury and repair

and limit use of the SFI[6-9]. Furthermore, gait is a more biomechanically complex, higher order behavior that is subject to more inherent variability as compared to grasping.[1,10,5] Lastly, it can be argued that grip strength is a more direct measure of motor function in comparison to using various parameters of the footprint during active gait to calculate the SFI. However, like the SFI and other behavioral tests[11-14], vGST suffers from some degree of behavioral variability, as well as from difficulty in controlling the speed and vector in which the tester distracts the animal away from the force meter.

Isometric tetanic force testing (ITFT) is the most reliable method for measuring motor function that is currently validated.[15-17] This method involves disinserting the tendon of the muscle being tested and securing it to a force transducer. The nerve innervating that muscle is then electrically stimulated to induce maximal tetanic muscle contraction, and the force generated is measured. The principal limitation of ITFT is that it can only be performed once at the time of sacrifice, precluding serial in vivo measurements to chart functional recovery over time. Additionally, this technique is difficult to learn, technically challenging and time consuming to perform, and requires expensive software and equipment.

We have developed a novel method for assessing functional recovery that combines elements of vGST and ITFT to take advantage of their respective benefits. The purpose of the study is to validate the use of stimulated grip strength testing (sGST) and compare the reliability of this

novel functional assessment modality to vGST and ITFT. Our hypothesis is that sGST provides greater reliability than vGST and similar reliability to ITFT with the added advantage of serial, in vivo measurements over time.



## MATERIALS AND METHODS

This study was carried out utilizing the *Guide for the Care and Use of Laboratory Animals* of the Nationals Institute of Health (No. 86-23). The protocol (no. RA18M74) was approved by the institutional Animal Care and Use Committee.

### Study Design

*Side-to-Side Equivalence*

Ten male Lewis rats aged 8-10 weeks and weighing approximately 250 g were used to determine the relative reliability of sGST, vGST and ITFT. Animals underwent bilateral ulnar nerve transection-without-repair to ensure that only median nerve-innervated muscles were contributing to the functional measurements. The median nerve was not manipulated. Force measurements from both the right and left forelimbs were obtained with vGST, sGST and ITFT for each animal. Results for each test were reported as a ratio of strength measured in the left arm versus the right arm and reported as a percentage of equivalence. Assuming that motor

function is approximately equivalent in bilateral forelimbs at baseline,[15,18] a perfect functional test should find a side-to-side equivalence approaching 100% with limited inter-trial variance.

*Serial In Vivo Functional Assessment*

To determine whether sGST can be used for serial *in vivo* assessments to monitor functional recovery over time, and to compare the quality of longitudinal functional data collected with sGST versus vGST, we analysed control data from ongoing studies using either sGST or vGST. The sGST data was collected from a group of eight animals undergoing median nerve transection followed by immediate proximal ulnar to distal median nerve transfer. The vGST data was collected from a group of 10 animals undergoing median nerve transection-and-repair and ulnar nerve transection-without-repair. Despite the differences in the surgical models, in both groups the ulnar-innervated muscles were denervated and regeneration took place through the median nerve pathway. Functional assessments were performed weekly until an endpoint of 15 weeks postoperatively.

**Surgical Procedures**

Under general anesthesia (2.5% inhaled isoflurane), rats were positioned supine with forelimbs fixed in 90 degrees of abduction. A 1 cm incision was made over the proximal bicipital groove. Using blunt dissection, the median and ulnar nerves were exposed within the mid-brachium. In

animals undergoing side-to-side functional testing, bilateral ulnar nerves were sharply transected and a 1 cm segment was excised, to prevent regeneration. The median nerve was left in continuity. In the animals undergoing serial sGST assessments, the left ulnar and median nerves were transected 0.75 cm proximal to the elbow, and the proximal ulnar nerve stump was coapted to the distal median nerve stump primarily with two 10-0 nylon epineural sutures (Ethicon, Sommerville, NJ). In the animals undergoing serial vGST assessments, the left median nerve was transected 0.75 cm proximal to the elbow and repaired primarily with two 10-0 nylon epineural sutures (Ethicon, Sommerville, NJ), and the ulnar nerve was transected and a portion excised to prevent regeneration.

In all animals, the skin was closed using 4-0 Vicryl (Ethicon, Sommerville, NJ) suture, and analgesia (buprenorphine 01 mg/kg) and antibiotics (enrofloxacin 1 mg/kg) were administered subcutaneously in the immediate post-operative period.

**Volitional Grip Strength Test**

Grip strength was measured in each forelimb using a Chatilon DE II force transducer (Ametek, Largo, FL). Pre-operatively rats were trained in vGST using positive reinforcement after successfully grasping the metal bar connected to the force transducer. The contralateral forepaw was wrapped in adhesive tape during testing to prevent grasping. Rats were held by the tail above a triangular metal bar connected to the force transducer and allowed to

reflexively grasp the bar. Once grasping was initiated, the investigator pulled the rat away from the force meter. The maximum force generated before loss of grip was recorded in Newtons (N). Three trials were performed in each tested forelimb.

**Stimulated Grip Strength Test**

General anesthesia was achieved with 2.5% inhaled isoflurane. In the supine position, the tested forelimb was abducted at 90 degrees with the elbow and wrist extended and secured in place with an elastic strap. Two stimulating needle point electrodes (ADInstruments, Dunedin, New Zealand) were placed 1 mm apart in the axilla just proximal to the insertion of pectoralis major on the humerus. Placement was most easily achieved by retracting the pectoralis major muscle superior-medially while inserting the electrodes in a slight caudal to cephalad direction. The median nerve was electrically stimulated at 10 V across multiple frequencies (50, 75, 100, 125, and 150 Hz) using the PowerLab 4/35 stimulator and LabChart software (ADInstruments) to induce maximal tetanic contraction of the extrinsic digital flexors. After induction of digital flexion, a stiff, looped wire attached to a force transducer (Chatilon DFE II) was placed in the clenched paw, and the force transducer was distracted away from the rat along a track in the vector of the long axis of the forelimb while attempting to maintain a constant speed of distraction (Supplementary Figure and Supplementary Video). The maximum force generated prior to loss of grip at each frequency was recorded. Three trials were performed at each frequency with a rest period of 2 minutes in-between trials. The three force measurements at

the frequency determined to elicit maximal digital flexion force ($F_o$) were averaged and used as a single value to determine the average maximal force generated.



**Isometric Tetanic Force Test**

*Surgical Procedure*

Rats were anesthetized using continuous 2.5% isoflurane and positioned supine with the forelimb abducted at 90 degrees. The prior bicipital groove incision was re-incised and the median nerve was exposed in the mid-brachium using blunt dissection. The incision was then carried distally into the volar antebrachium to expose the flexor compartment. The flexor digitorum sublimus (FDS) tendon slips were identified and secured to each other *in situ* with a 3-0 silk suture at the level of the carpal tunnel to maintain physiologic relative tension. The tendons were detached distally and attached to the adjustable lever arm of the Aurora Scientific Inc Dual Mode Lever System (Ontario, Canada). The remaining forearm flexor tendons were carefully transected and reflected proximally to avoid interference during testing. The forelimb temperature was maintained at 37$^{o}$C using an overhead heating lamp and warm saline irrigation.

*Force Measurements*

The median nerve was stimulated at the axilla using shielded bipolar hook electrodes connected to the PowerLab 4/35 stimulator. Single twitch contractions were induced at varying muscle lengths to plot a force-intensity curve using LabChart software, and thereby determine the optimal resting muscle length ($L_o$) at which maximal twitch force was generated. Single twitch contraction forces with varying voltages at $L_o$ were then measured to the determine the optimal voltage ($V_o$). Then, with $L_o$ and $V_o$ held constant, the force-frequency relationship was assessed by stimulating tetanic contraction of FDS for a maximum of 5 seconds or until a force peak was clearly seen, at the following frequencies; 40, 50, 80, 100, 120, 150, and 180 Hz. A two-minute rest period separated tetanic contractions. The surgical exposure and testing procedure were repeated in the contralateral forelimb prior to sacrifice.

**Statistical Analysis**

Prior studies found less than 5% variation in side-to-side force measurements;[18,15] thus, side-to-side equivalencies closer to 100% were deemed to be more reliable. The absolute difference from 100% was used to compare the reliability between testing modalities using a mixed effects model, with testing modalities as fixed effects and random intercepts for each animal.

To evaluate the inter-trial repeatability of testing modalities, intraclass correlation coefficients (ICC) were calculated for vGST and sGST using a one-way random-effects model. This

correlation index generates values ranging between 0 and 1, with values closer to 1 representing stronger reliability. Index values are traditionally classified as poor (<0.40), fair (0.40–0.59), good (0.60–0.74), or excellent (≥0.75) reliability.[19] ITFT was precluded from evaluation as only one maximal tetanic force measurement was recorded per limb in accordance with previous reported methodologies.[15,20,16]

Non-parametric Mann-Whitney U testing was used to compare continuous force variables and were reported in Newtons ± the coefficient of variation. Statistical analyses were performed using Stata (College Station, TX) software.

## RESULTS

### *Side-to-Side Equivalence*

The mean side-to-side equivalence was 82.8 ± 6.4%, 100 ± 2.0% and 96.7 ± 8.4% for vGST, sGST and ITFT, respectively (Figure 1). Outputs from the mixed effects analytical model found sGST to be significantly more reliable than vGST ($p<0.001$). There was no difference in reliability between sGST and ITFT ($p=0.062$). The average grip strength was greater with sGST than with vGST ($p<0.001$). Table 1 lists the average values measured for each side with the three tests.

### *Inter-Trial Repeatability*

When comparing variability between trials for individual animals, vGST was found to be a poor measurement tool, with an estimated correlation coefficient of 0% (95% CI: -31%, 41%). Comparatively, sGST was found to have fair inter-trial repeatability with a correlation coefficient of 59% (95% CI: 22%, 86%). Of note, during vGST we observed behavioral fatigue and diminishing cooperation and effort with subsequent trials during a testing session.

*Longitudinal Functional Recovery*

Longitudinal functional recovery was recorded for 15 weeks in two cohorts of rats (Figure 2). The functional recovery curve generated using weekly sGST measurements demonstrated consistent week-to-week improvements until reaching a plateau at week 15. Conversely, measurements obtained with vGST revealed decreases in force at weeks 7, 8, 14, and 15, although these changes were not statistically significant. Measurements obtained using the sGST method were more precise than vGST measurements, as measured by the coefficient of variation (19.5% vs. 51.3%, respectively; p=0.001).

## DISCUSSION

The results from this study confirm that sGST is a valid method for measuring motor function in rats. This novel functional test incorporates elements of both vGST and ITFT to take advantage of their respective benefits. Stimulated grip strength testing demonstrated a greater

degree of side-to-side equivalence and inter-trial repeatability than vGST, and should therefore be considered a more reliable and precise method for measuring motor function. Whereas sGST uses direct nerve stimulation to consistently induce maximal grip force, vGST requires cooperation from the animal being tested, introducing the potential for sub-maximal exertion on any given trial. The impact of sub-maximal exertion on vGST is supported by the finding of greater force values measured with sGST than with vGST (Table 1). The greater degree of variability observed with vGST is likely due in part to the observed variability in the degree of effort exerted by animals being tested. While dangling tends to elicit the grasping response, this is not always the case. It can be difficult at times to coax the animals to participate in vGST, making the testing process both tedious and time-consuming. It should be noted that there are modifications to vGST that incorporate more elaborate training protocols and rewards systems in an effort to limit this behavioral variability.[21-23] However, these more intensive methods are still subject to behavioral variability, and it is not clear that they provide the same degree of reliability that can be achieved with direct nerve stimulation. In addition to behavioral variability, vGST is also affected by variability introduced by the method in which the test is administered, given the inability to standardize the speed and vector of distraction.[5] In contrast, the vector of distraction is controlled during sGST by placing the force transducer on a unidirectional track. While the speed of distraction is still subject to some degree of user-dependence, sGST is performed on a fully-anesthetized rodent that is secured in a stationary position, which likely provides a greater degree of control in comparison to vGST, in which the

awake and moving animal is pulled by the tail away from force transducer. That being said, incorporating a motorized platform to standardize the speed at which the force transducer moves away from the animal might further improve the reliability of sGST.

There was no statistically significant difference between sGST and ITFT in the measured side-to-side equivalence. Prior studies found similar side-to-side equivalence values with ITFT: 100.0 ± 4.4% in a rat tibialis anterior model,[18] 102.7 ± 6.0% in a rabbit tibialis anterior model,[16] and 99.7 ± 19.5% in a rabbit biceps model.[15] The results of our study support the conclusion that sGST is equally reliable as ITFT in measuring motor function. In contrast to ITFT, sGST allows for serial in vivo measurements, thereby providing critical information regarding the progression of functional return over time. In comparison to ITFT, we find sGST to be easier and less time-intensive to learn and perform. ITFT requires extensive surgical dissection and multiple additional steps to determine the optimal muscle length prior to beginning tetanic force testing, all while maintaining a constant temperature in the exposed nerve and muscle. In contrast, sGST requires no dissection and the muscle is kept at physiologic length *in situ*, thereby eliminating this variable.

In order to isolate the effects of median nerve regeneration on grip strength recovery, the ulnar nerve is transected and left in discontinuity to facilitate vGST and sGST. While the ulnar nerve does not innervate the extrinsic digital flexors in rats, and therefore provides minimal if any

contribution to grip strength,[1,24,25] it does innervate the intrinsic muscles of the forepaw.[25] Therefore, transecting the ulnar nerve to facilitate vGST and sGST precludes other behavioral functional tests that make use of fine digital movements,.[25,2]

In this validation study, the median nerve was acutely transected and repaired primarily. However, sGST can also be utilized with other median nerve injury models, including nerve gap and chronic denervation models.[26]

## CONCLUSIONS

Stimulated grip strength testing (sGST) is a novel method for assessing motor function in rats that is more reliable than the traditional method of vGST. Stimulated grip strength testing provides the same degree of reliability as ITFT, with the added benefit of allowing for serial *in vivo* measurements. It provides valid measurement of functional recovery in a translational model of nerve injury and repair that addresses the limitations of other methods in use.

**ABBREVIATIONS**

vGST: volitional grip strength testing

ITFT: isometric tetanic force testing

sGST: stimulated grip strength testing

SFI: sciatic functional index

N: Newtons

Hz: Hertz

$F_o$: maximal digital flexion force

FDS: flexor digitorum sublimis

$L_O$: optimal resting muscle length

$V_O$: optimal voltage

ICC: interclass correlation coefficient

CI: confidence interval

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## FIGURE LEGENDS

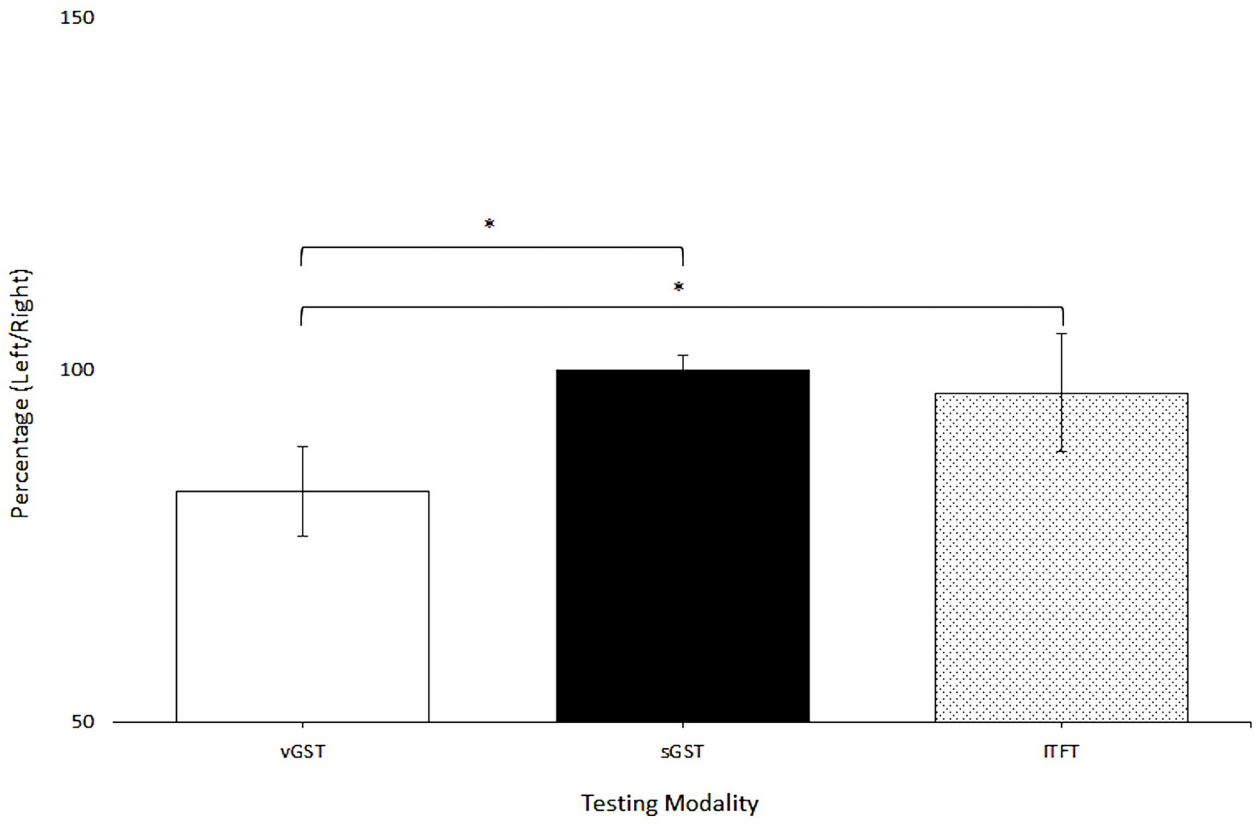
**Figure 1**. The average side-to-side equivalence values are depicted for each testing modality. Values closer to 100% represent stronger test reliability. Error bars depict the standard error. vGST, volitional grip strength testing; sGST, stimulated grip strength testing; ITF, isometric tetanic force testing. * denotes p value < 0.05.

**Figure 2**. Curves demonstrating longitudinal functional recovery in two separate cohorts. The solid bar corresponds to animals that underwent ulnar-to-median nerve transfer followed by weekly functional assessments using stimulated grip strength testing (sGST). The dashed bar corresponds to animals that underwent median nerve transection-and-repair and ulnar nerve transection-without-repair followed by weekly functional assessments using grip strength testing (vGST). The sGST curve demonstrates steady gradual improvement in function until plateau, while the vGST curve demonstrates some fluctuations. Error bars depict the coefficient of variation.

**Table 1**. Force measurements

| | Left (N) | Right (N) |
|---|---|---|
| vGST | 2.4 ± 15% | 3.0 ± 19% |
| sGST | 4.4 ± 12% | 4.4 ± 10% |
| ITFT | 0.9 ± 20% | 1.0 ± 13% |

N, Newtons; vGST, volitional grip strength testing; sGST, stimulated grip strength testing; ITFT, isometric tetanic force testing.

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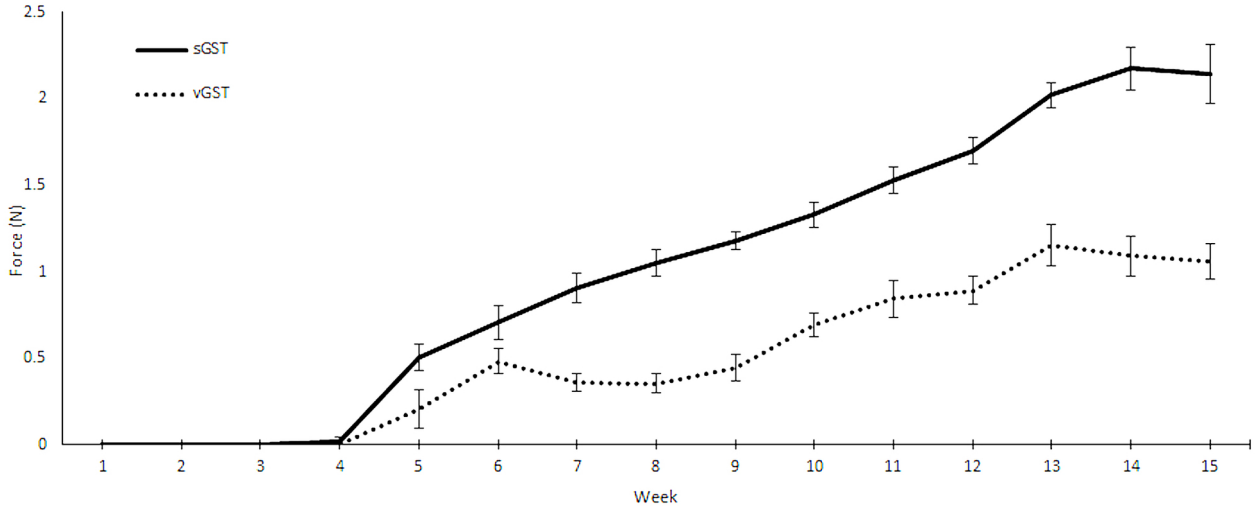


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**Table 1**. Force measurements for each testing modality

| | Left (N) | Right (N) |
|---|---|---|
| vGST | 2.4 ± 15% | 3.0 ± 19% |
| sGST | 4.4 ± 12% | 4.4 ± 10% |
| ITFT | 0.9 ± 20% | 1.0 ± 13% |