Action Potentials in Neuromuscular Disease Models

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This paper, produced in collaboration by Scantox and iWorx Systems, highlights the application of action potential–based metrics to evaluate neuromuscular dysfunction in SOD1 mice, with emphasis on translational research applications.

Introduction

Electrophysiology enables direct assessment of neuromuscular function by recording the electrical activity of nerves and muscles. At its core is the action potential, a transient electrical impulse that reflects ion channel activity and governs communication between motor neurons and muscle fibers. The shape of an action potential provides information about the health and function of the tissue being studied, with a typical waveform consisting of a depolarization period, a repolarization period, and a hyperpolarization period. The shape and timing of these signals provide sensitive indicators of neuromuscular health and dysfunction.

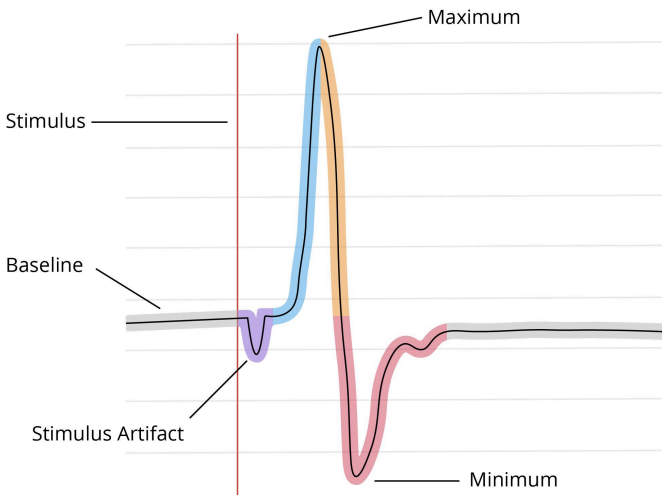


Figure 1: Action potential waveform. Depolarization (blue), repolarization (orange), and hyperpolarization period (red).

In preclinical research, electrophysiological measurements such as Compound Muscle Action Potential (CMAP), Motor Unit Number Estimation (MUNE), and repeated stimulation are powerful tools for quantifying disease progression and therapeutic effects in models of neurodegeneration. The SOD1 mouse, a widely used model of amyotrophic lateral sclerosis (ALS), exhibits progressive motor neuron loss and impaired neuromuscular transmission, making it well suited for longitudinal studies with these measurements.

Methods

Images, techniques, and procedures are courtesy of Aaron Fantina-Woblistin with Scantox.

Compound Muscle Action Potential (CMAP)

In standard analysis, CMAP recording is the simplest and most common approach for assessing neuromuscular function. In animal studies, CMAP recordings are acquired under anesthesia using a minimally invasive approach, allowing for precise placement of electrodes and control over stimulus delivery. Mice (B6.SOD1-G93A transgenic mice compared to wild type controls) are anesthetized using Isoflurane/O_2 inhalation including buprenorphine analgesia. CMAP can also be measured longitudinally in the same animal without detrimental effects if done correctly. Platinum needle electrodes are commonly used for stimulation and recording, although silver button electrodes may also be utilized depending on the application.

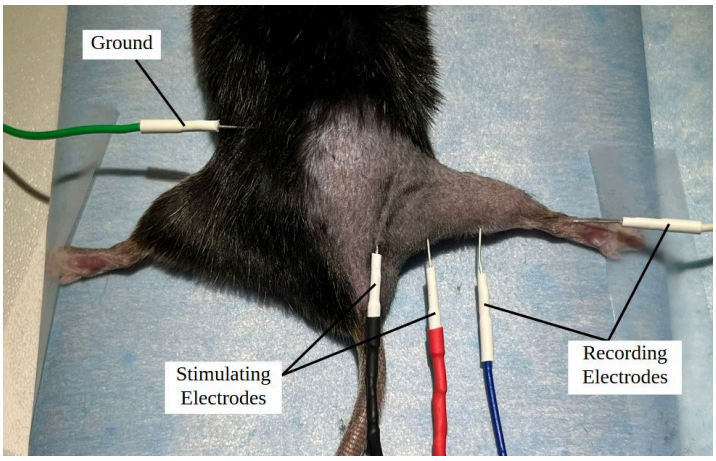


Figure 2: Platinum needle electrode placement for sciatic nerve stimulation.

Stimulation electrodes are positioned subcutaneously on either side of the sciatic notch, with one placed near the spine and the other positioned distally, approximately 1 cm apart toward the hindlimb. The recording electrode is inserted subcutaneously at the gastrocnemius muscle, typically at the muscle belly, while the reference electrode is placed subcutaneously near the Achilles tendon. A ground electrode can be positioned subcutaneously on the contralateral side of the back relative to the measurement site.

The stimulation parameters used were one square wave pulse of 0.1 ms with an amplitude starting at 4 mA, increasing in small increments until the maximal CMAP response was achieved. These parameters are commonly used in standard CMAP studies. Stimulation was controlled using LabScribe software (iWorx Systems).

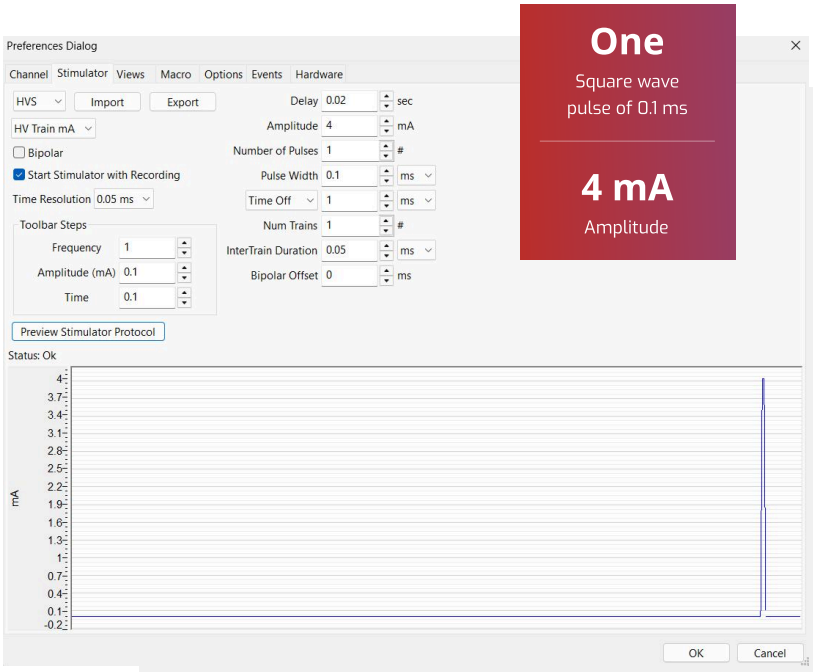
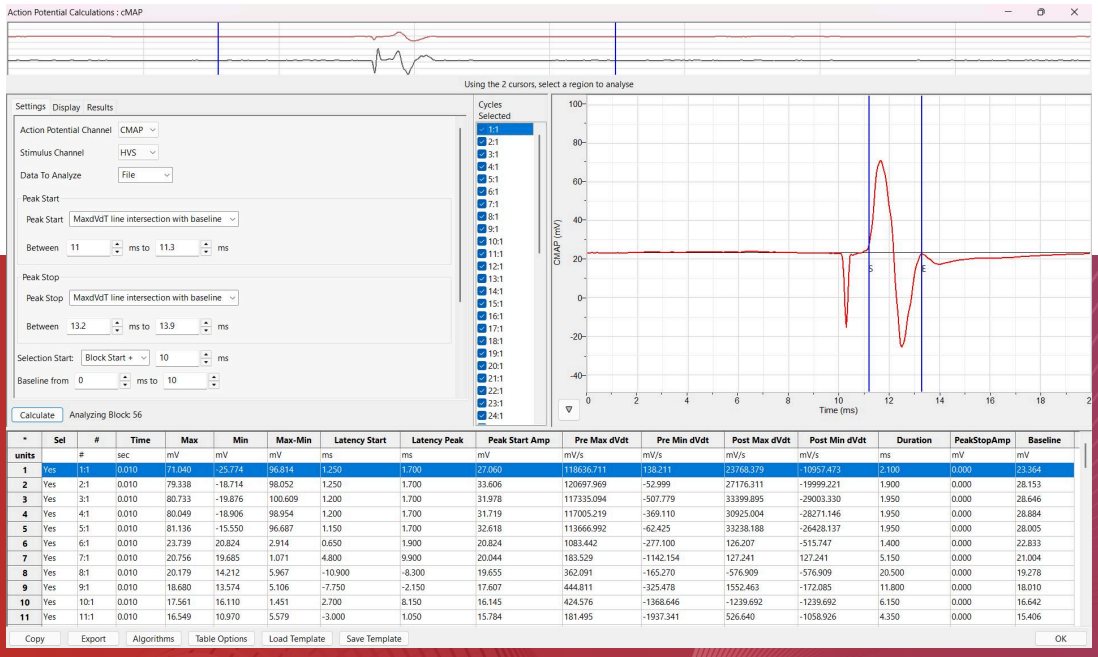


Figure 3: Example stimulation parameters set in the preferences dialog window of LabScribe software.

The LabScribe Action Potentials Module enables detailed analysis of Compound Muscle Action Potentials by using a stimulus marker and slope-based detection to identify the peak start and peak stop. Measured parameters include amplitude, latency to peak start and peak maximum, rise time, peak duration, and the maximum and minimum first derivatives (dV/dt) before and after the peak.



*	Sel	#	Time	Max	Min	Max-Min	Latency Start	Latency Peak	Peak Start Amp	Pre Max dVdt	Pre Min dVdt	Post Max dVdt	Post Min dVdt	Duration	PeakStopAmp	Baseline
units		#	sec	mV	mV	mV	ms	ms	mV	mV/s	mV/s	mV/s	mV/s	ms	mV	mV
1	Yes	1:1	0.010	71.040	-25.774	96.814	1.250	1.700	27.060	118636.711	138.211	23768.379	-10957.473	2.100	0.000	23.364
2	Yes	2:1	0.010	79.338	-18.714	98.052	1.250	1.700	33.606	120697.969	-52.999	27176.311	-19999.221	1.900	0.000	28.153
3	Yes	3:1	0.010	80.733	-19.876	100.609	1.200	1.700	31.978	117335.094	-507.779	33399.895	-29003.330	1.950	0.000	28.646
4	Yes	4:1	0.010	80.049	-18.906	98.954	1.200	1.700	31.719	117005.219	-369.110	30925.004	-28271.146	1.950	0.000	28.884
5	Yes	5:1	0.010	81.136	-15.550	96.687	1.150	1.700	32.618	113666.992	-62.425	33238.188	-26428.137	1.950	0.000	28.005
6	Yes	6:1	0.010	23.739	20.824	2.914	0.650	1.900	20.824	1083.442	-277.100	126.207	-515.747	1.400	0.000	22.833
7	Yes	7:1	0.010	20.756	19.685	1.071	4.800	9.900	20.044	183.529	-1142.154	127.241	127.241	5.150	0.000	21.004
8	Yes	8:1	0.010	20.179	14.212	5.967	-10.900	-8.300	19.655	362.091	-165.270	-576.909	-576.909	20.500	0.000	19.278
9	Yes	9:1	0.010	18.680	13.574	5.106	-7.750	-2.150	17.607	444.811	-325.478	1552.463	-172.085	11.800	0.000	18.010
10	Yes	10:1	0.010	17.561	16.110	1.451	2.700	8.150	16.145	424.576	-1368.646	-1239.692	-1239.692	6.150	0.000	16.642
11	Yes	11:1	0.010	16.549	10.970	5.579	-3.000	1.050	15.784	181.495	-1937.341	526.640	-1058.926	4.350	0.000	15.406

Figure 4: LabScribe Action Potentials Module for advanced analysis of CMAP data.

Measuring the latency period is a critical component of CMAP analysis. Latency refers to the time interval between the onset of the electrical stimulus and the initial deflection of the CMAP waveform (peak start), as well as the time to the waveform's maximum (peak maximum). These values reflect the speed of signal conduction along the motor nerve and across the neuromuscular junction. Prolonged latencies may indicate demyelination, nerve compression, or synaptic transmission abnormalities, making latency a key diagnostic and research parameter in studies of neuromuscular function.

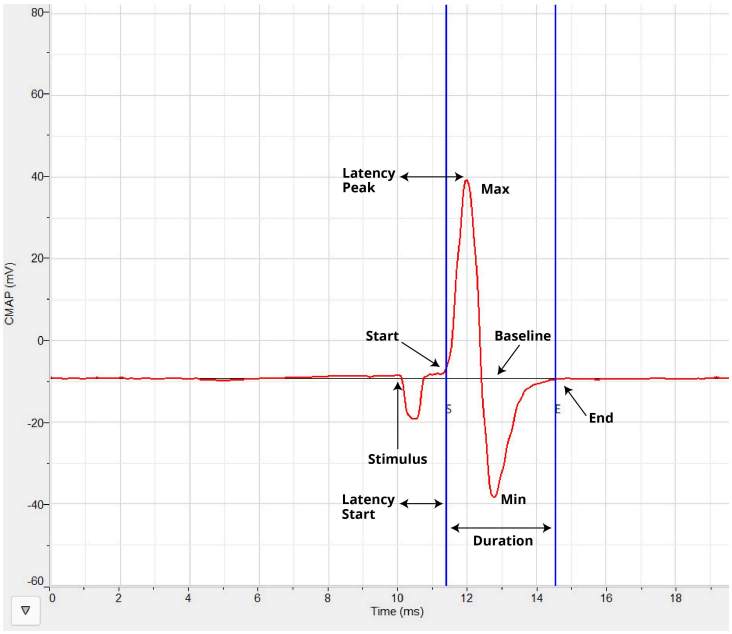


Figure 5: Action potential measurements defined in the software.

Indications

CMAP is highly indicative of neuromuscular disorders and is used to quantify disease severity, track progression, and assess responses to therapy. It is commonly used in studies of several conditions:

- **Amyotrophic Lateral Sclerosis (ALS)** is a neurodegenerative disease that affects nerve cells in the brain and spinal cord, resulting in muscle weakness and atrophy.[1]
- **Lambert-Eaton Myasthenic Syndrome (LEMS)** is an autoimmune disorder where antibodies attack the neuromuscular junction, causing muscle weakness.[2]
- **Guillain-Barré Syndrome (GBS)** is a condition where the body's immune system attacks the nerves. This can cause weakness, numbness, or paralysis.[3]
- **Pompe Disease (glycogen storage disease)** is a genetic condition where there is a deficiency of a digestive enzyme called α-glucosidase. Glycogen builds up in the body's cells, resulting in severe muscle weakness.[4]

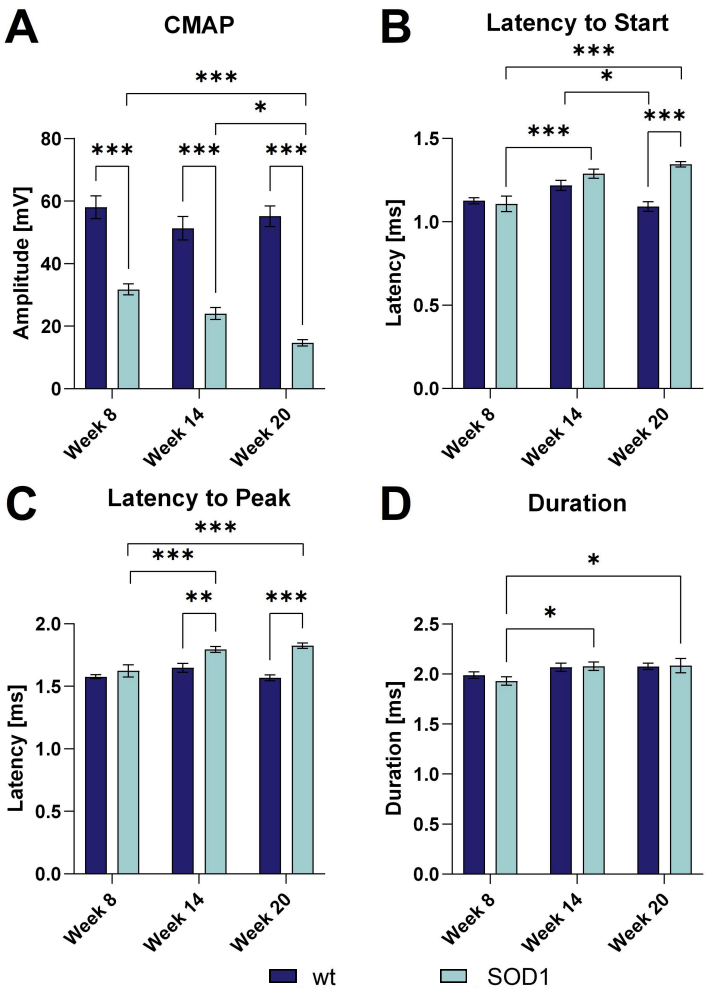


Figure 5: Longitudinal CMAP Analysis. Displayed are max amplitude (A), latency to start (B), latency to peak (C), and duration (D) repeatedly measured by stimulation of the sciatic nerve and measurement in the gastrocnemius of B6.SOD1-G93A transgenic (tg) mice compared to wild type (wt) controls at 8, 14, and 20 weeks of age. Two-way ANOVA with genotype as main factor, followed by Bonferroni's *post hoc* test. Mean ± SEM. *p <0.05; **p <0.01; ***p <0.001. n= 16 per group.

Motor Unit Number Estimation (MUNE)

Motor Unit Number Estimation (MUNE) is an electrophysiological technique developed in the early 1970's to estimate the number of functional motor units in a muscle. It provides a way to quantify motor neuron loss in neuromuscular disorders.[5] Accurate electrode placement is critical in MUNE studies, as the technique relies on clearly visible signals with low noise. Studies start with low amplitude stimulation (typically around 0.2 mA) to identify the smallest detectable motor unit potential above baseline. The stimulation amplitude is gradually increased in fine increments to observe corresponding changes in response, each reflecting the recruitment of an additional motor unit. This process is time-consuming and requires careful observation and technical expertise, particularly in models where motor unit activity may be inconsistent. The averaged difference in resulting amplitudes is referred to as the Motor Unit Action Potential (MUAP), which represents the electrical activity of the muscle fibers innervated by a single motor neuron.

Indications

MUNE is an early diagnostic tool by design. It can be more insightful than standard nerve conduction studies by detecting motor unit loss when muscle pathology might still be masked by re-innervation by other motor units. It is used in studies of neurodegenerative disorders including ALS, spinal muscular atrophy (SMA), and diabetic neuropathy. However, if pathology is already affecting CMAP amplitudes, motor unit estimation becomes inaccurate. Here, MUAP can be used to highlight the pathological decrease in motor unit size itself, rather than the estimated number of motor units.

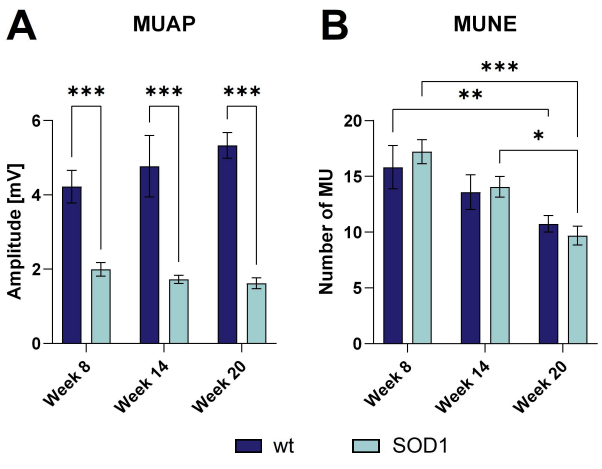


Figure 6: Longitudinal motor unit analysis. Displayed are average motor unit action potential (A) and total motor unit number estimation (B), repeatedly measured by stimulation of the sciatic nerve and measurement in the gastrocnemius of B6.SOD1-G93A tg mice compared to wt controls at 8, 14, and 20 weeks of age. Two-way ANOVA with genotype as main factor, followed by Bonferroni's *post hoc* test. Mean ± SEM. *p <0.05; **p <0.01; ***p <0.001. n= 16 per group.

Repeated Stimulation Techniques

Repeated stimulation is used to assess neuromuscular transmission. It involves delivering high frequency repetitive stimulation to the motor nerve and recording the resulting muscle responses. This method places stress on the neuromuscular junction (NMJ), challenging its ability to maintain consistent neurotransmitter release over time. Repeated stimulation can be useful for uncovering subtle defects in synaptic transmission that might not be apparent during single-pulse stimulation.

Indications

These stimulation techniques are applied in models of neurodegenerative disease, such as ALS, and neuromuscular junction disorders, such as LEMS, and botulism studies. In ALS models, comparing CMAP amplitude of transgenic mice to wild type controls reflects an impaired ability to sustain neurotransmission over time.

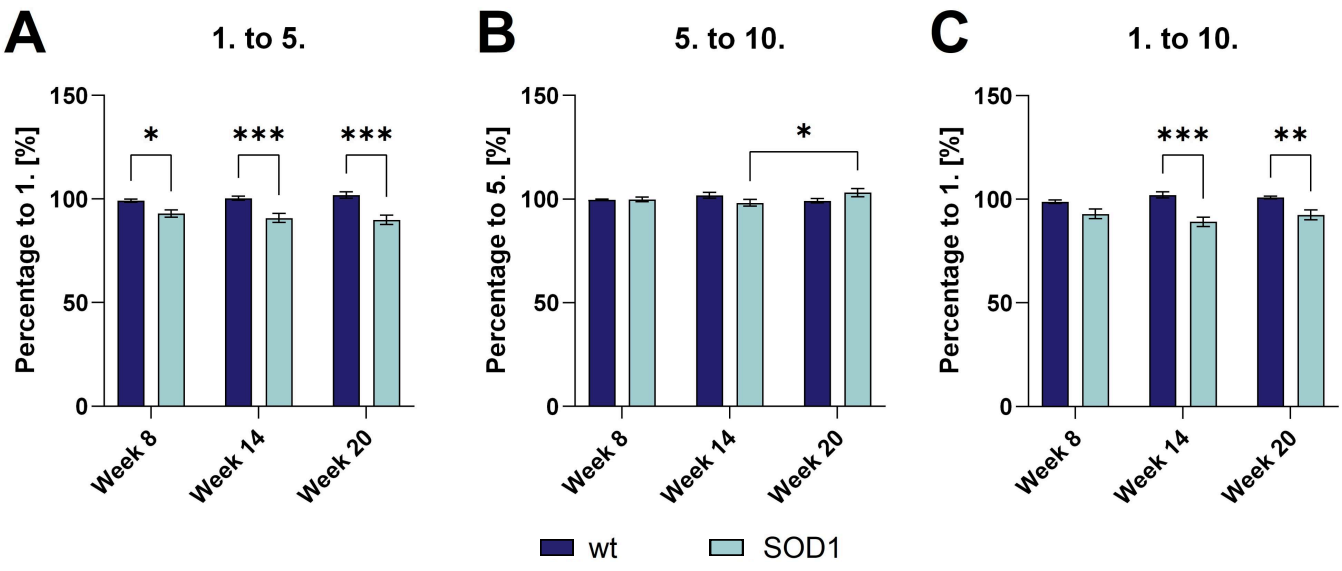
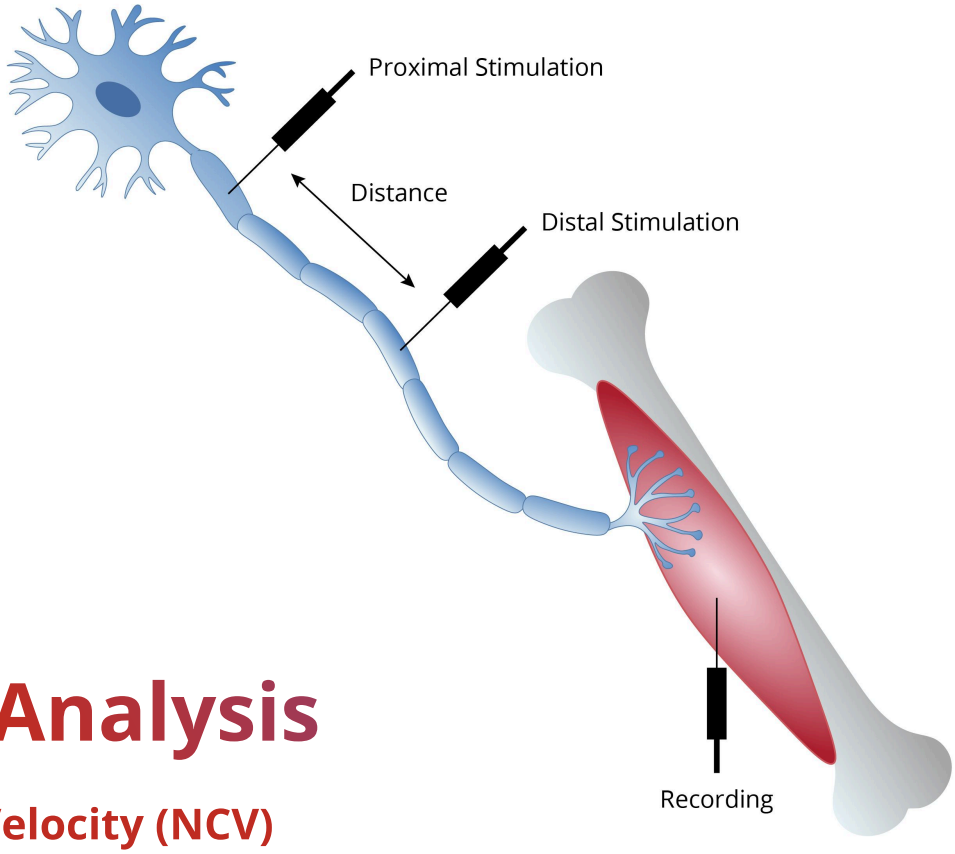


Figure 7: Longitudinal repeated stimulation. Displayed are the percent change in amplitude during a 10-pulse simulation between 1st and 5th (A), 5th and 10th (B), and 1st and 10th stimulation, repeatedly measured by stimulation of the sciatic nerve and measurement in the gastrocnemius of B6.SOD1-G93A tg mice compared to wt controls at 8, 14, and 20 weeks of age. Two-way ANOVA with genotype as main factor, followed by Bonferroni's *post hoc* test. Mean ± SEM. *p <0.05; **p <0.01; ***p <0.001. n= 16 per group.



Advanced Analysis

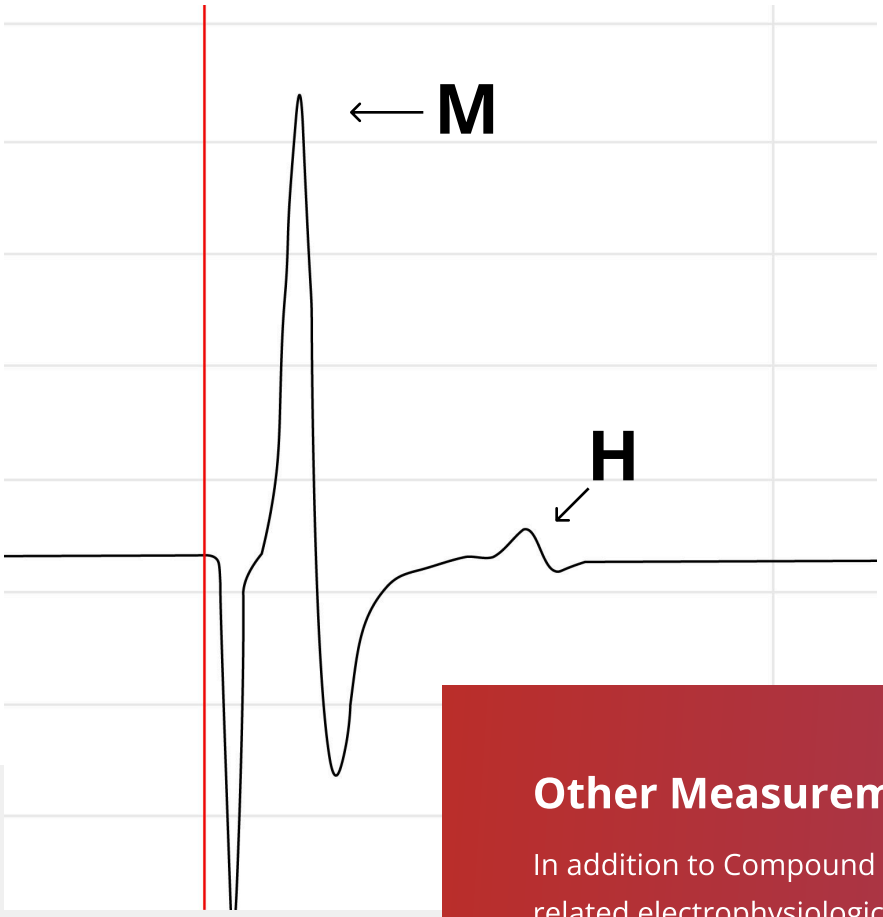
Nerve Conduction Velocity (NCV)

Nerve Conduction Velocity (NCV) quantifies how quickly an action potential travels between two points on a nerve. In motor NCV studies, a mixed or motor nerve is stimulated at two separate sites (proximal and distal), and the resulting CMAPs are recorded from the corresponding muscle. The distance between the stimulation sites is divided by the difference between the proximal and distal latencies to calculate conduction velocity. In sensory NCV studies, such as those performed on the tail nerve in mice, the response is recorded directly from the sensory nerve rather than the muscle. In this case, latency can be directly converted into conduction velocity based on the distance between the stimulation and recording electrodes. Assessing both motor and sensory conduction provides complementary information, as motor NCV primarily reflects motor neuron and neuromuscular junction integrity, whereas sensory NCV evaluates axonal and myelin health in afferent pathways. Slower than normal conduction suggests myelin damage or loss, whereas preserved conduction with reduced CMAP amplitudes may indicate axon degeneration.[6]

$$\text{Nerve Conduction Velocity (m/s)} = \frac{\text{(distance between stimulation sites [mm])}}{\text{(proximal latency – distal latency [ms])}}$$

Hoffman Reflex

The **Hoffman Reflex (H-reflex)** is commonly used to test reflex responses, assessing the excitability of the spinal cord reflex pathway, particularly the dorsal root ganglion. It is an indicator of spasticity, which is an involuntary muscle stiffness or tightness caused by disrupted communication between the brain and spinal cord. The H-reflex is elicited by electrically stimulating a mixed peripheral nerve, causing both afferent (sensory) and efferent (motor) action potentials. The resulting M-wave represents direct activation of motor fibers, while the H-wave reflects afferent stimulation of sensory neurons that synapse onto motor neurons within the spinal cord, resulting in a motor reflex.



The amplitudes of the M-wave and H-wave vary significantly with the level of stimulus intensity.[7] In healthy animals, the H-wave is suppressed during high-frequency stimulation. However, in cases of spasticity, such as in models of peripheral neuropathy, nerve lesions, or Parkinson's disease, the H-wave is apparent, indicating abnormal reflex pathway excitability.

Other Measurements

In addition to Compound Muscle Action Potentials and related electrophysiological assessments, supplemental measurements can provide further insight into neuromuscular and cardiac function. These measurements include basal activity, electrocardiography, assessment of other nerves and muscles, sensory nerve action potential (SNAP) and conduction velocity, and evoked action potentials.

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