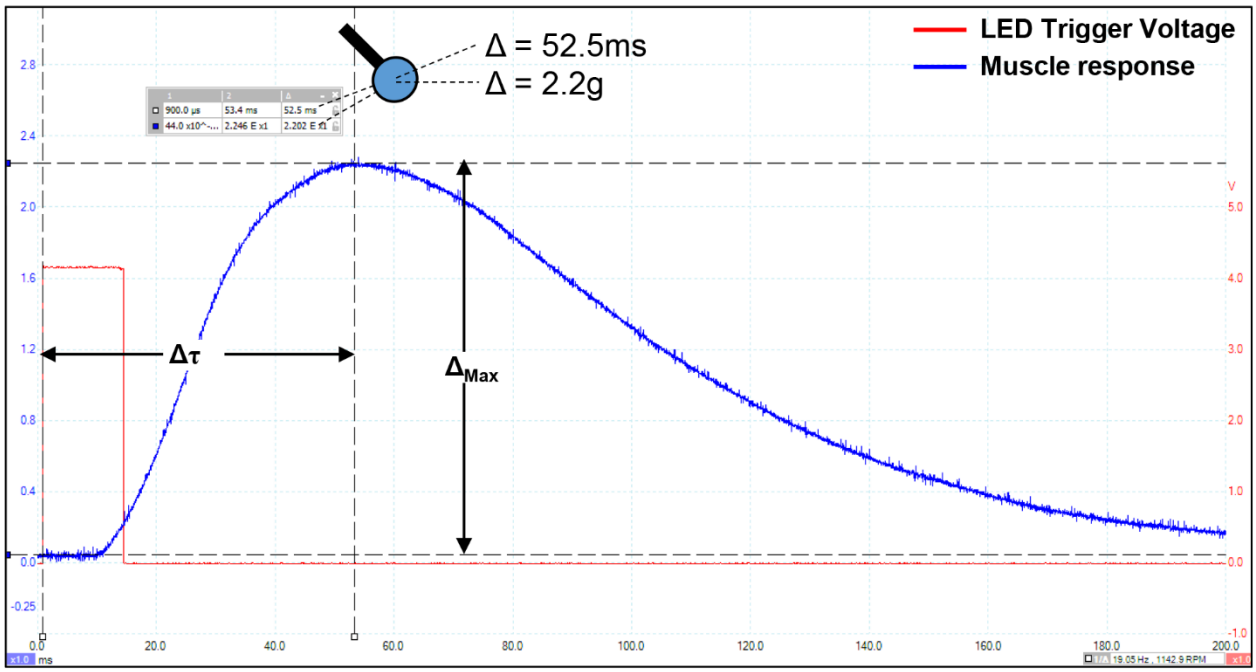
Student Activities to accompany "Optical Control of Muscle Function by Transplantation of Stem Cell–Derived Motor Neurons in Mice"

Data analysis exercise: Three representative recordings of triceps surae muscle contractions after optical stimulation (n=1) are provided below. Use the data in these tracings to complete the top row of following table. Average values for triceps surae muscle induced by optical stimulation (blue) and electrical stimulation (gray) are also presented.

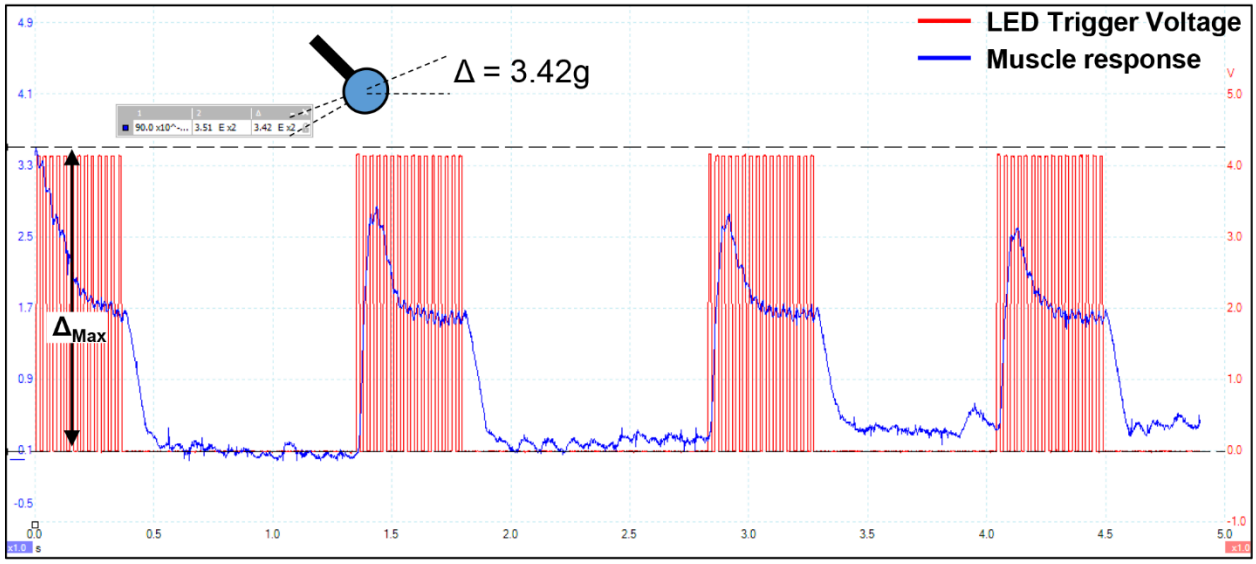
	Twitch (g)	Tetanus (g)	Tetanic : Twitch Ratio	Twitch Latency (ms)	TTP (ms)	1/2 RT (ms)	MU number estimate	MU Force (g)
Triceps Surae (*n=1*)					N/A	N/A	21	
Triceps Surae (*n =4*)	1.6 (+/- 0.5)	1.4 (+/- 0.4)	1.3 (+/- 0.2)	53.8 (+/- 1.3)	43.5 (+/- 3.2)	39.5 (+/- 6.0)	15 (+/- 5.0)*	0.1 (+/- 0.01)*
Triceps Surae (*n =3*)	8.3 (+/- 2.4)	27.8 (+/- 7.3)	3.6 (+/- 0.3)	55 (+/- 7.5)	37.7 (+/- 3.1)	39 (+/- 10.2)	N/A	N/A

1. Single twitch contraction in response to optical stimulation



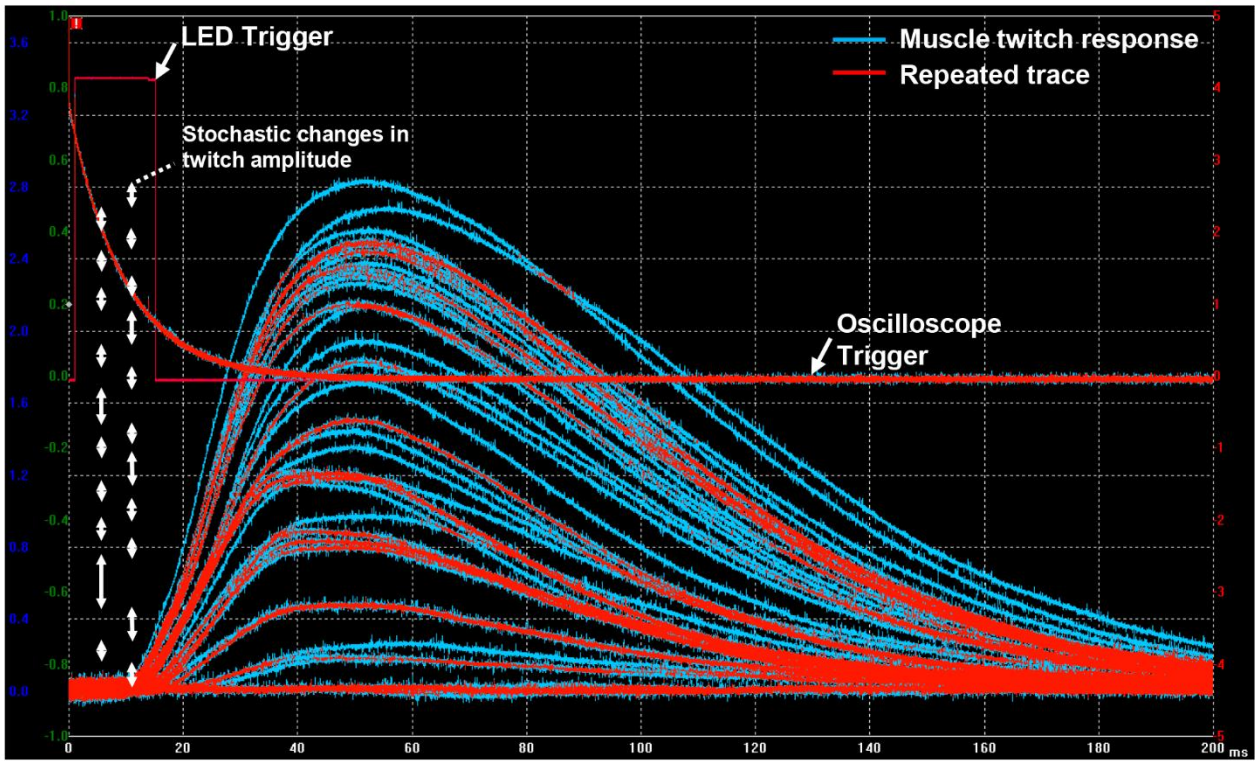
- *Peak twitch contractile force = 2.2 g*
- *Latency to peak twitch response = 52.5 ms*
- *Latency to response = approx. 13 ms*

2. Repetitive tetanic contractions induced by optical stimulation



- *Maximum tetanic contractile force = 3.42 g. (Calculated from first tetanic contraction.)*
- *Together with the twitch contraction recording above, calculate the tetanic to twitch ratio. This is the ratio of supramaximal to submaximal contractile force in muscles innervated by engrafted ChR2 motor neurons. Tetanic/twitch = 3.42 g/2.22 g = 1.55 g*

3. Motor unit trace from triceps surae muscle following repetitive optical stimulation at increasing intensity.



- *The graph shows 21 motor units. Calculate the average force per motor unit. Maximal tetanic force / number of motor units = 3.42/21 = 0.16 g.*

4. Additional discussion for data analysis exercise:

- What is the likely cause of the decline in contractile force in the second and subsequent tetanic contractions?
  Answer: Fatigue

- Stochastic changes in twitch amplitude are indicated on the graph. What does each stochastic increase in contractile force represent?
  Answer: Recruitment of additional motor units, which generates an increase in total contractile force.

- Which neuron populations are activated by optical vs. electrical stimulation? What factors may have contributed to the differences in muscle contractile force induced by optical vs. electrical stimulation?
  Answer: Optical stimulation activates engrafted ChR2 motor neurons, whereas electrical stimulation activates both endogenous regenerated motor neurons and engrafted ChR2 motor neurons. Inactivity of ChR2 motor neurons for 35 days before controlled optical stimulation, while endogenous regenerated motor axons are active, may be one contributor to the difference in muscle activity associated with each motor neuron population.