

S3 Table. Experiment Timeline. Step-by-step guide performing an experiment.

Steps	Time Duration	Procedure
1.	2–4 mins	Caged mouse C57BL10 is euthanized with CO ₂ .
2.	< 1 min	Mouse is placed in a weigh balance. Mass measurement
		is recorded.
3.	10–20 mins	Mouse is placed on surgical table to start dissection of
		the EDL muscle. The muscle is secured with suture at
		both ends close to the tendons. Only one EDL muscle is
		used per mouse. Due to euthanization, the other EDL muscle will not be responsive after the duration of the
		experiment (rigor mortis).
4.	2–3 mins	Isolated EDL muscle is mounted in the mechanical setup,
		moisten with $(95\%O_2, 5\%CO_2)$ physiological saline so-
		lution (PSS). Suture attached to the muscle is secured
		on both ends by nylon screws. The EDL muscle is sub-
		merged and placed parallel to the electrodes.
5.	10 mins	the EDL muscle is set at resting tension; that is 1gF.
		The optimum length, L_0 is recorded. During this time,
		the EDL muscle is at rest to acclimate to the PSS prior to the start of the trials. The EDL muscle will remain at
		resting tension throughout the entire experiment trials.
6.	< 1s	The EDL muscle is subjected to a twitch. At this mo-
		ment, the muscle is evaluated to determine whether we
		should continue with the various trials. If the muscle
		force produced is greater or equal to 5gF, then we start
		the trials. Force measurements lowered than 5 gF will
-	III ' O O '	not generate optimal isometric contractions.
7.	Wait 2-3 mins	Select the step trajectory from the Labview interface.
		Start tuning the control parameters (PI, MRAC, or ADP-PI) based on the EDL muscle response. Only one
		controller is used per muscle. Each trial lasts between
		9 to 10s. The order of the trajectories is always step,
		ramp, sine, and square. The control parameters for each
		trajectory are tuned accordingly. The number of trials
		per experiment relies on the muscle responses as muscle
	D + 20 1 +	decay in time.
8.	Rest 30s between	Every 5 to 6 trials, the EDL muscle is subjected to a
	trials	twitch to evaluate the quality of the muscle. After each twitch, the muscle will rest for a duration of 5 mins. The
		EDL muscle experiments last between 35-50 mins.
9.	After 35-50 mins	The EDL muscle is dismounted. Set on the surgical
		table to be dried, and for suture removal. Then, the
		EDL muscle is placed in a weigh balance and the mass
		measurement is recorded.
10.		Mouse carcass is prepared for disposal per outlined in
		Virginia Tech IACUC regulations.

PLOS 1/2