Botox Potency Evaluation Using a 3D Human Neuromuscular Junction Model

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Highlights

- Easy creation of 3D iPSC-derived Neuromuscular Junction model with automated functional output.
- NMJ-specific inhibition of muscular contractility through BoNT activity.
- Dose-dependent response to BoNT with EC_{50} calculation for potency assay applications.

Summary

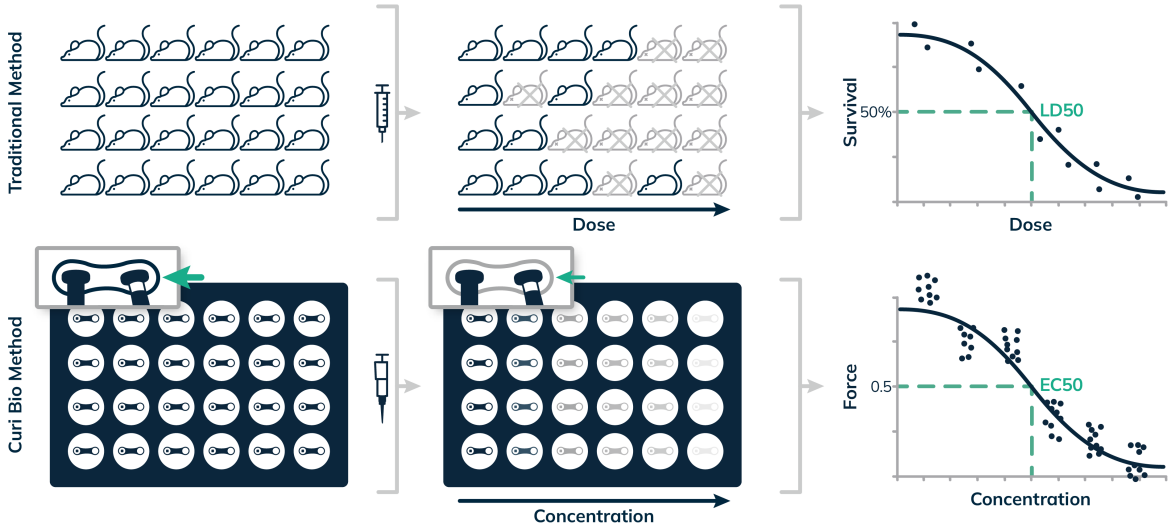

Figure 1: Advancing beyond traditional methods. *The mouse lethality bioassay (top) is the golden standard for evaluating the toxicity of substances and uses the LD50 metric. Our novel in vitro approach (bottom) quantifies the functional effects of substances, utilizing the EC50 as the potency metric. A single 24-well plate facilitates a 5-point dose-response curve with four technical replicates and control tissues per experiment. In this study, BoNT is administered in a range from 10 to 0.001 µg of BoNT complex A per tissue.*

Engineered human tissue models are advancing preclinical testing by more accurately reflecting in tandem human biology and tissue-level physiology compared to traditional in vitro and animal models. These models are particularly crucial for the evaluation of complex biologics, where consistent potency characterization is vital for ensuring compound safety. Given increasing ethical concerns, legislative pressure, and the need for human biology that animal models fail to satisfy, the development of specific tissue models for potency testing of therapeutic products is essential. We present a 3D human tissue model of the neuromuscular junction (NMJ) as an alternative to the mouse lethality bioassay (MLB) for Botulinum Toxin (BoNT) (Fig. 1).

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This novel NMJ model is set apart from similar models [1–5] due to the easy integration iPSC-derived motor neurons and myoblasts in a scalable 3D approach. Furthermore, the model can be functionally characterized in a non-invasive manner using the commercial Mantarray platform, shows biological relevance with specific inhibition of the NMJ in response to BoNT, and proves its utility as a potency assay through a dose-dependent response for EC_{50} identification.

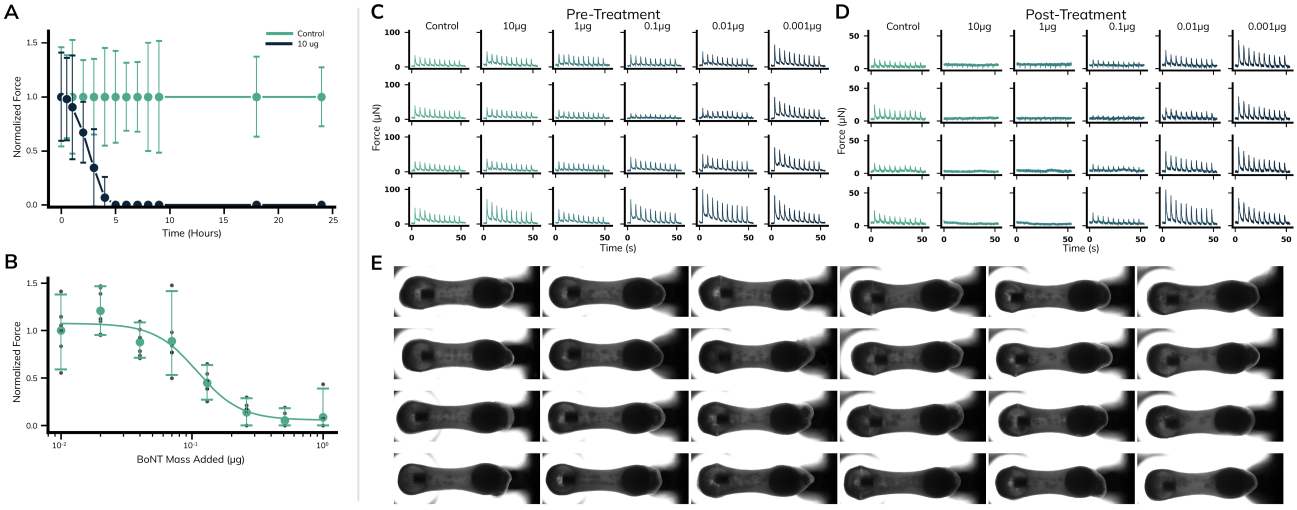

Figure 2: BoNT application to engineered NMJ tissues causes a dose-dependent loss of neuronally evoked force generation. (A) *Neuronally evoked skeletal muscle force normalized by control mean at the associated timepoint and pre-treatment force generation for each given tissue. Graph displays group means ± SD for a minimum of n=3 tissues and 10 contractions per tissue. Datapoints represent mean of individual tissues.* ***(B)*** *4 parameter logistic fit of normalized functional output following 24 hours of BoNT complex A exposure* ***(C-D)*** *Force traces in response to blue light stimulation before* ***(C)*** *and after* ***(D)*** *24 hours of BoNT-A exposure at 5 decreasing (left to right) concentrations.* ***(E)*** *Tiled micrograph 2x micrograph showing gross tissue morphology of 24 individual NMJ engineered tissues from a single 24 well plate.*

Methods & Results

3D engineered neuromuscular tissues were assembled from iPSC-derived myoblasts (CAT# MRC-SKM-WT), primary stromal cells, and blue-light sensitive iPSC-derived neurons using the Mantarray Mini Plate Kits (CAT# MA-MINI-12X-24-2), as described in our previous application note (link here) [6]. Following 21 days of co-culture, a series of dose response experiments were carried out by adding BoNT complex A at increasing masses. Application of BoNT complex A resulted in a dose- and time-dependent loss of NMJ function in engineered NMJ tissues. Fig 2A. shows the normalized functional response of tissues across the 24 hour period following BoNT application. Addition of 10 µg BoNT-A resulted in complete functional loss across approximately 5 hours, consistent with the mechanism of action of BoNT-A. A sigmoidal dose fit of tissue function at 24 hours was carried out to produce an EC_{50} value, similar to the approach used in the MLB.

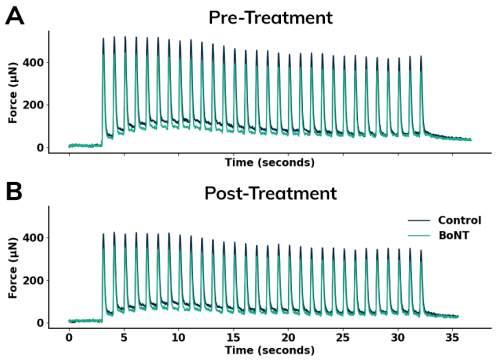

Figure 3: BoNT treatment does not reduce muscle contractility in response to electrical field stimulation, indicating NMJ specific mechanism of action of BoNT. *Representative waveforms from 1 Hz electrical stimulation of control and BoNT treated (10µg) from (A) pre and (B) post treatment (24 hours) time point.*

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This value yielded a potency for this experiment of 0.114 ± 0.039 µg (Fig 2B), while the corresponding MLB value of the batch is reported at $3.7x10^4$ LD_{50}/µg. Individual traces from a full 24-well plate (Fig 2C-D) showed that BoNT reduced peak height without significantly affecting the fidelity of NMJ transmission. The underlying skeletal muscle function, driven through electrical stimulation, was unaffected after treatment with BoNT (Fig 3), highlighting the specific, potent and selective action of BoNT at the NMJ alone and not a broad-spectrum loss of muscle function [6].

Conclusions

The demonstration of a dose dependent loss of function following BoNT complex A confirms that the model contains NMJs, as the mechanism of BoNT potency and NMJ specificity is well understood. The time period of action engenders confidence that the BoNT is acting by the canonical uptake and activation mechanisms associated with this toxin. As additional batches of BoNT are tested, an accurate conversion factor can be regressed to convert the EC_{50} of this model to MLB-derived potency. We therefore present this model both as a step towards a widely usable model of the NMJ for disease modeling, but also as potential replacement of the MLB for BoNT potency testing. Achieving regulatory approval for this method will pave the way for future potency models for novel therapeutics aimed at treating musculoskeletal disorders.

References

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