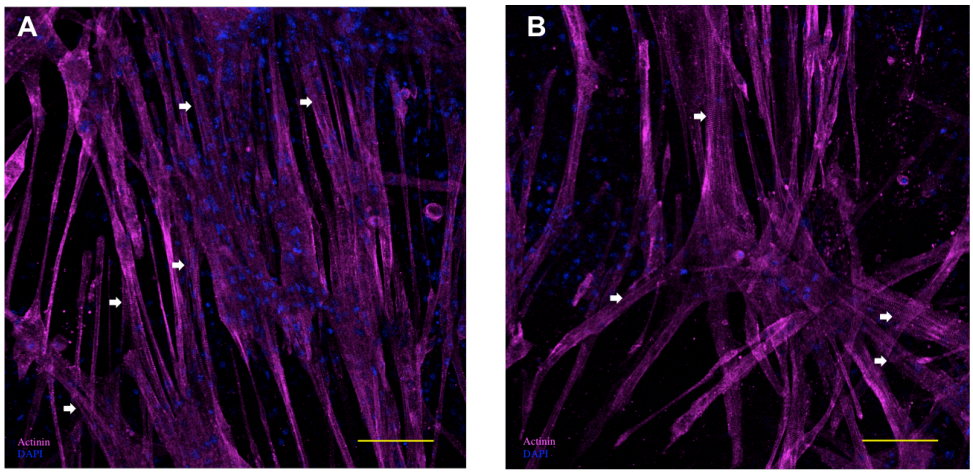
**SUPPLEMENTARY INFORMATION**

Surapon N. Charoensook, Damian J. Williams, Syandan Chakraborty, Kam Leong and Gordana Vunjak-Novakovic. Bioreactor Model of Neuromuscular Junction with Electrical Stimulation for Pharmacological Potency Testing

**Supplementary Figure**




**Supplementary Figure S1:** Stimulation resulted in an earlier formation of striations in myotubes. (A) Striations (white arrows) were commonly found in myotubes receiving electrical stimulation by as early as day 10 in the culture. (B) Striations of myotubes in unstimulated controls were observed at day 19.

**Supplementary Videos**

**Supplementary Video S1**: Calcium imaging of myotubes during electrical stimulation, showing a clear increase in intensity corresponding to the time of the stimuli. Myotubes were electrically stimulated every 10 seconds with 2 stimuli of 2 millisecond duration and a gap of 300 milliseconds between the two stimuli.

**Supplementary Video S2:** Calcium imaging of myotubes stimulated with nicotine, showing an increase in calcium activities in myotubes after adding 10 µM nicotine solution to the culture.

**Supplementary Video S3:** Early spontaneous contraction found in myotubes receiving electrical stimulation. Spontaneous contractions were commonly found in myotubes cultured with electrical stimulation by day 10.

**Supplementary Video S4:** Spontaneous contraction found in myotubes in control group. Spontaneous contractions myotubes in the unstimulated control group were commonly found by day 16.

**Supplementary Video S5:** Neuromuscular contraction in the coculture**,** showing the interaction between myotubes and axons of motoneurons via NMJ after 8 days of coculture.

**Supplementary Video S6:** Coculture contraction initiated by glutamate stimulation**.** Muscle contractions were observed shortly after adding glutamate, suggesting the formation of functional NMJs.

**Supplementary Video S7:** Glutamate triggered transient calcium flux in motoneurons, showing that glutamate can directly stimulate motoneurons.

**Supplementary Video S8:** Glutamate could not directly stimulate myotubes and trigger calcium flux, so that only spontaneous activity in the myotubes were observed.

**Supplementary Video S9:** Glutamate could not initiate contraction in monocultures of control myotubes and trigger muscle contraction.

**Supplementary Video S10:** Glutamate could not induce spontaneous muscle contractions in monocultures of unstimulated (control) myotubes.

## Supplementary tables

**Supplementary Table S1:** Pharmacological potency of the drugs studied using the NMJ model. Experiments were performed using motoneurons derived from mouse ESCs and skeletal muscle from mouse myoblast cell line, MEF-transdifferentiated myocytes and mouse ESC-derive motoneurons. Cocultures from both models exhibited dose-dependent drug responses. Potency values were comparable for different cell sources, except for pancuronium.

| Pharmacological agents | Pharmacological potency in C2C12/motoneurons NMJ model | Pharmacological potency in transdifferentiated myocytes/motoneurons NMJ model |
|---|---|---|
| AF-64A | $IC_{50}$ = 1.29 µM | $IC_{50}$ = 1.33 µM |
| Vesamicol | $IC_{50}$ = 113.6 nM | $IC_{50}$ = 98.08 nM |
| BoT-A | $IC_{50}$ = 69.07 pM | $IC_{50}$ = 50.01 pM |
| Pancuronium | $IC_{50}$ = 10.71 µM | $IC_{50}$ = 5.337 µM |
| Neostigmine | $EC_{50}$ = 0.357 µM | $EC_{50}$ = 0.461 µM |

**Specification of culture media**

| Coculture Medium (CM) |
| --- |
| Advanced DMEM/F12 |
| Neurobasal medium |
| 1% P/S |
| 1X B-27 |
| 1X GlutaMAX |
| 12.5 mM HEPES |
| 100 µM IBMX |
| 10 µM forskolin |
| 10 ng/mL BDNF |
| 10 ng/mL GDNF |
| 10 ng/mL CNTF |
| 20 ng/mL NT-3 |
| 20 ng/mL NT-4 |
| 2 µg/mL laminin |

| Differentiation Medium (DM) |
| --- |
| DMEM, high glucose |
| 12.5 mM HEPES |
| 2% horse serum |
| 1% P/S |
| 1X ITS |
| 20 ng/mL IGF-1 |

| DFK5 medium |
| --- |
| Advanced DMEM/F12 |
| 1X nonessential amino acids |
| 1X nucleosides |
| 0.1 mM 2-mercaptoethanol |
| 2 mM L-glutamine |
| 1% P/S |
| 60 µM Putrescine |
| 20 nM Progesterone |
| 1X ITS |
| 10% Knockout Serum Replacement |

| ES cell medium |
| --- |
| DMEM, high glucose |
| 1X nonessential amino acids |
| 1X nucleosides |
| 0.1 mM 2-mercaptoethanol |
| 2 mM L-glutamine |
| 1% P/S |
| 15% FBS |
| 1000 u/mL LIF |

| Growth Medium (GM) |
| --- |
| DMEM, high glucose |
| 12.5 mM HEPES |
| 10% FBS |
| 1% P/S |

| Mix |
| --- |
| DMEM, high glucose |
| Advanced DMEM/F12 |
| Neurobasal Medium |
| 1% P/S |
| 2% horse serum |
| 1X ITS |
| 1X B-27 |
| 1X GlutaMAX |
| 12.5 mM HEPES |
| 100 µM IBMX |
| 10 µM Forskolin |
| 20 ng/mL IGF-1 |
| 10 ng/mL BDNF |
| 10 ng/mL GDNF |
| 10 ng/mL CNTF |
| 20 ng/mL NT-3 |
| 20 ng/mL NT-4 |
| 2 µg/mL Laminin |

| Coculture Medium (CMHS) |
| --- |
| DMEM, high glucose |
| Neurobasal Medium |
| 1% P/S |
| 2% horse serum |
| 1X ITS |
| 1X B-27 |
| 1X cholesterol |
| 1X GlutaMAX |
| 12.5 mM HEPES |
| 100 µM IBMX |
| 10 µM Forskolin |
| 20 ng/mL IGF-1 |
| 10 ng/mL BDNF |
| 10 ng/mL GDNF |
| 10 ng/mL CNTF |
| 20 ng/mL NT-3 |
| 20 ng/mL NT-4 |
| 100 ng/mL Vitronectin |
| 20 ng/mL VEGF |
| 25 ng/mL acidic FGF |
| 2 µg/mL Laminin |