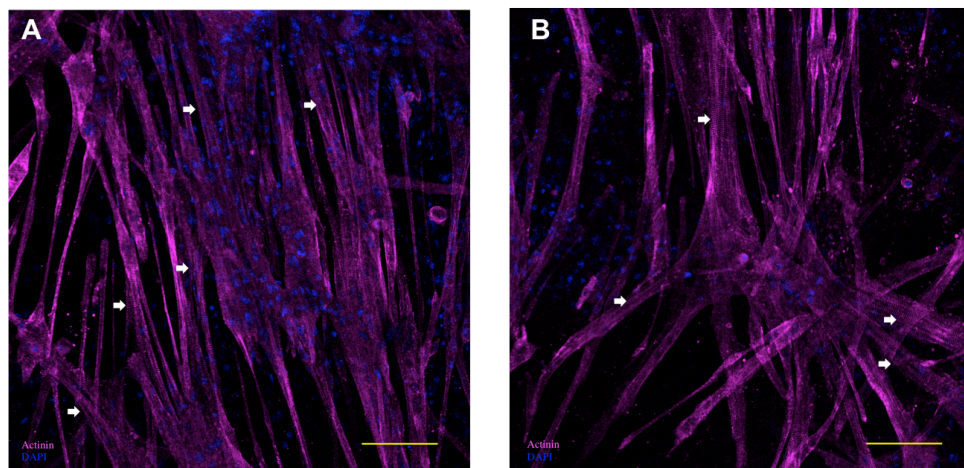


SUPPLEMENTARY INFORMATION

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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 μ M 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 μ M	IC ₅₀ = 1.33 μ M
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 μ M	IC ₅₀ = 5.337 μ M
Neostigmine	EC ₅₀ = 0.357 μ M	EC ₅₀ = 0.461 μ M

Specification of culture media

Coculture Medium (CM)	DFK5 medium	Growth Medium (GM)	Coculture Medium (CMHS)
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	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	DMEM, high glucose 12.5 mM HEPES 10% FBS 1% P/S	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
Differentiation Medium (DM)	ES cell medium	Mix	
DMEM, high glucose 12.5 mM HEPES 2% horse serum 1% P/S 1X ITS 20 ng/mL IGF-1	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	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	