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Supplemental Material

Influence of Nanomolar Deltamethrin on the Hallmarks of Primary Cultured Cortical Neuronal Network and the Role of Ryanodine Receptors

Jing Zheng, Yiyi Yu, Wei Feng, Jing Li, Ju Liu, Chunlei Zhang, Yao Dong, Isaac N. Pessah, and Zhengyu Cao

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Figure S3. Subchronic effect of DMSO on axonal and dendritic outgrowth in cortical neurons. (A) Representative images of neurons immunostained with MAP-2B antibody at 2 DIV in the absence and presence of Veh (0.1% DMSO). (B) Quantification of the axon length after Veh (0.1% DMSO) exposure. (C) Representative Image J processed images of neuronal morphology in 7 DIV. (D) Quantification of numbers of intersections at different radius in the absence and presence of Veh treatment. Arrowheads indicate the radius of 30 pixel and 60 pixels, respectively. (E) Quantification of the number of intersections at radius 30 and radius 60, respectively. The total number of neurons tested was inserted in the bar graph. Axonal length and number of intersections of each neuron was used as an analysis unit. Each data point represented the Mean \pm SEM, from 3 independent cultures. T-test was used to compare the statistical significance between Lockes' control (CT)-treated and Veh-treated neurons. No statistical significance was observed. Bar = 50 μ m.

Figure S4. The power test of p value curve in sub-sampling comparisons. An equal number of samples, i.e. from 5 to 900, were randomly chosen from each groups with 100 times permutation for power test using R package software (version 3.4.1). The p value from each comparison was calculated from ANOVA with *post hoc* Bonferroni correction and presented as $-\log_{10}(p)$ format in the curves. The solid lines with error bar represent different concentrations of DM compared to Veh group with 95% confidence interval. The dashed line in the panel represent the threshold of p value (0.05). Veh, vehicle (0.1%DMSO); DM, deltamethrin.

Figure S5. Influence of DM on [3 H]Ry binding to RyR1, RyR2 and RyRs. The average value obtained from each preparation was used as analysis unit. Each data point represented the Mean \pm SEM from 3 independent preparations. The concentrationresponse curves of DM SCO response were fitted with a nonlinear logistic equation using Prism GraphPad software (version 7.0). The EC_{50} values and 95% confidence intervals were summarized in **Table S2**. RyR1, type 1 ryanodine receptor, prepared from mice skeletal muscle; RyR2, type 2 ryanodine receptor, prepared from mice cardiac muscle; RyRs, mixed ryanodine receptor isoforms prepared from mice braincortex; Veh, vehicle (0.1% DMSO); DM, deltamethrin.

Table S1 List of Chemicals and Reagents Used in This Study

Chemicals and Reagent	Source	Catalog number
Cytosine-D-arabinofuraboside (Ara-C)	Sigma-Aldrich (St. Louis, MO)	C1768-100MG
3-phenoxybenzoic acid (3-PBA)	Rhawan (Shanghai, China)	R050063-5g
DC TM protein assay kit	Bio-Rad (Hercules, CA)	5000111
Deltamethrin (DM)	Chem Service (West Chester, PA)	N-11579-250MG; 99.5%
DNase I	Sigma-Aldrich (St. Louis, MO)	D5025-150KU
DPBS	Life Technology (Grand Island, NY)	14040224
Fetal bovine serum	Atlanta Biologicals (Norcross, GA)	A93E-500ml
Fluo-4/AM	Life Technology (Grand Island, NY)	F14202
GlutaMax	Life Technology (Grand Island, NY)	35050061
Goat anti-chicken secondary antibody (Alexa Fluor [®] 488)	Thermo Fisher (San Diego, CA)	A-11042
GS21 supplement	MTI-GlobalStem (Gaithersburg, MD)	GSM-3100
Leupeptin hydrochloride	Sigma-Aldrich (St. Louis, MO)	L9783-5MG
Lipid	Avanti Polar Lipids, Inc. (Alabaster, AL)	790304P
Microtubule-Associated Protein 2B (MAP-2B)	Thermo Fisher (San Diego, CA)	APPA6751
Neurobasal medium	Life Technology (Grand Island, NY)	12348017
Phenylmethylsulfonyl fluoride (PMSF)	Sigma-Aldrich (St. Louis, MO)	10837091001
Poly-L-lysine (PLL)	Peptides International Inc. (Louisville, KE)	P2636-100MG
[³ H]ryanodine ([³ H]Ry)	PerkinElmer (Santa Clara, CA)	NET950250UC; 56.6 Ci/mmol
Ryanodine	Abcam (Cambridge, MA)	Ab120083
Soybean trypsin inhibitor (SBTI)	Atlanta Biologicals (Norcross, GA)	T9128-1G
Trypsin	Atlanta Biologicals (Norcross, GA)	I56201

Table S2 Potency and efficacy of DM towards RyR1, RyR2, and cortical RyR mixed isoforms in homogenate membrane preparations

Type	EC ₅₀ (μM) (95% CI)	Efficacy (% Veh) (95% CI)
RyR1	6.5 (4.3, 10.1)	258.2 (240.4, 279.9)
RyR2	8.9 (3.1, 28.2)	164.8 (147.7, 199.6)
RyRs	1.6 (0.2, 8.0)	239.5 (198.6, 294.8)

Note: The EC₅₀ values and efficacy were obtained by fitting the data in Figure 3B with a nonlinear logistic equation using Prism GraphPad software (version 7.0). RyR1, type 1 ryanodine receptor, prepared from rabbit skeletal muscle; RyR2, type 2 ryanodine receptor, prepared from mice cardiac muscle; RyRs, mixed ryanodine receptor isoforms prepared from mice brain cortex; DM, deltamethrin; Veh, vehicle, 0.1% DMSO.

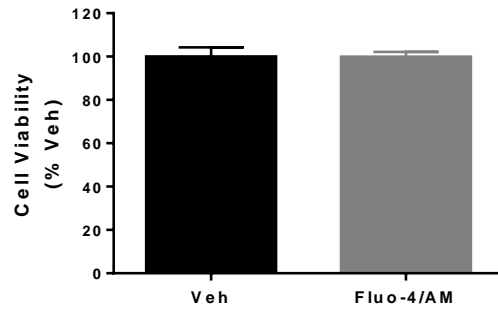


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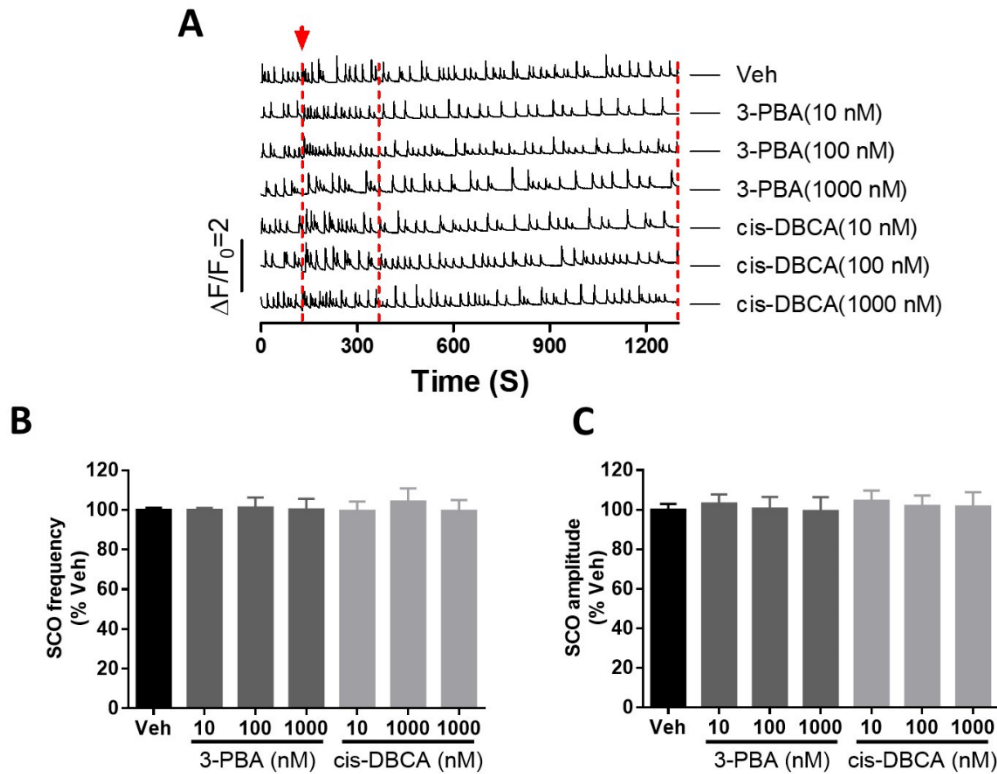


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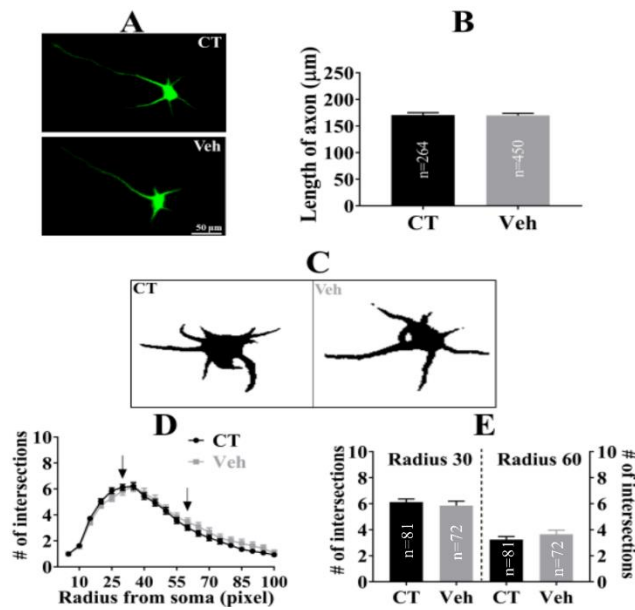


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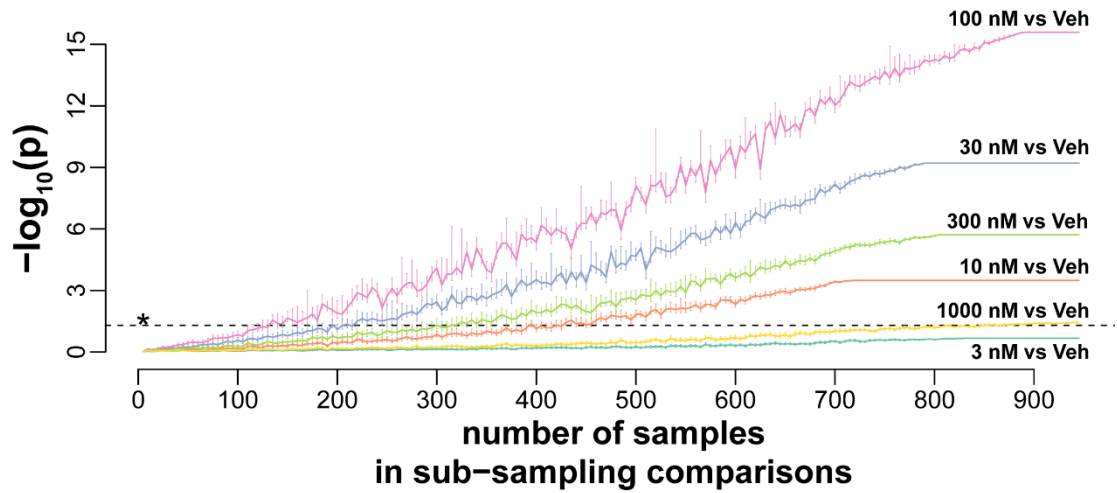


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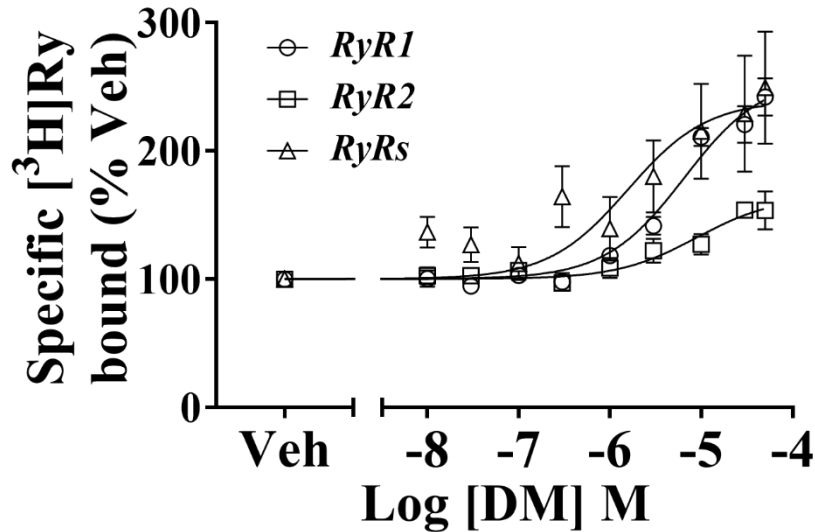


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