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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

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Table S1. List of Chemicals and Reagents Used in This Study.

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

Figure S1. Influence of Fluo-4/AM (4 μ M) on cell viability in 8 DIV cortical neurons. Each data point represented the Mean \pm SEM (n=4). No statistical significance was observed between Fluo-4/AM and Veh groups (T-test). Veh, vehicle (0.1% DMSO).

Figure S2. Influence of DM hydrolytic metabolites 3-PBA and *cis*-DBCA on SCOs in 8 DIV cortical neurons. (A) Representative SCO traces after 3-PBA or *cis*-DBCA exposure. The arrowhead together with the dashed line at 120 s indicate the additions of Veh (0.1% DMSO), 3-PBA or *cis*-DBCA. Two additional dashed lines indicate the time frame that was used to quantify the SCO frequency and amplitude. (B) Quantification of SCO frequency in the presence of Veh, 3-PBA or *cis*-DBCA. (C) Quantification of SCO amplitude in the presence of Veh, 3-PBA or *cis*-DBCA. Each data point represented the Mean \pm SEM (n=3). One-way ANOVA followed by *post hoc* Bonferroni comparison was used to compare the statistical significance between 3-PBA or *cis*-DBCA exposed group and Veh group. No statistical significance was observed. Veh, vehicle (0.1% DMSO); 3-PBA, 3-phenoxybenzoic acid; *cis*-DBCA, *cis*-(2,2-dibromovinyl)-2,2 dimethylcyclopropane-1-carboxylic acid (*cis*-DBCA).

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 EC₅₀ 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.