

Supplemental Materials

The $\alpha 4$ Nicotinic Acetylcholine Receptor Is Necessary for the Initiation of Organophosphate-Induced Neuronal Hyperexcitability

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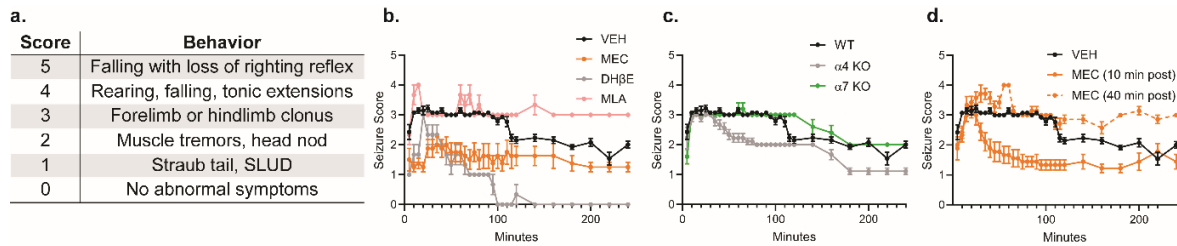
$\alpha 4$ / $\alpha 7$ genotyping protocol details:

Reaction Mix:

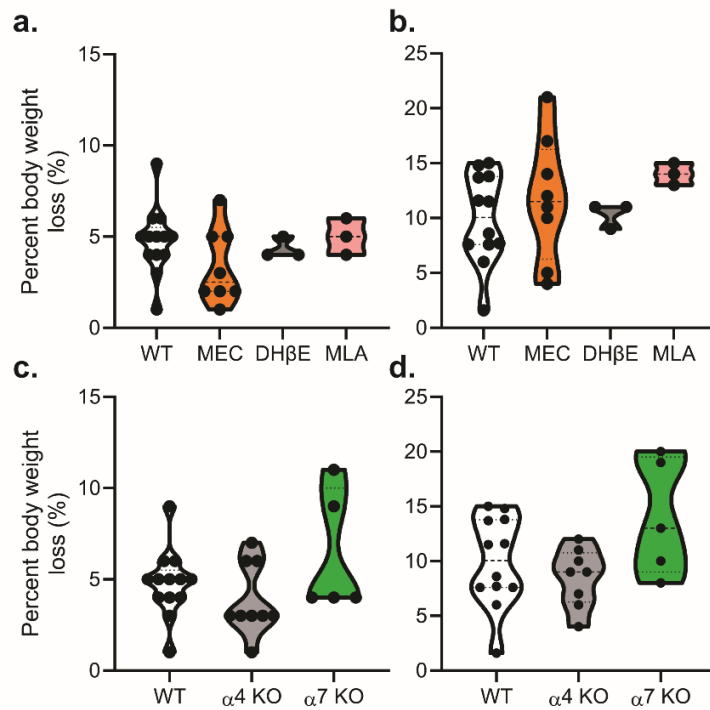
Reagent	Volume (μ l)
DNA Sample	2
Go Taq Green, Promega #M7123	12.5
Primer 1 – stock concentration 50 μ M	0.25
Primer 2 – stock concentration 50 μ M	0.25
Primer 3 – stock concentration 50 μ M	0.25
Primer 4 – stock concentration 50 μ M	0.25
Double-distilled H ₂ O	9.5

Thermocycler Program:

Steps	Temp (°C)	Time	# of Cycles
1. Initiation/Melting	94	3 min	1
2. Denaturation	94	45 sec	1
3. Annealing	60	30 sec	1
4. Elongation	72	1 min	1 – return to step 2 x 35 iterations
5. Amplification	72	10 min	1
6. Finish	4	n/a	n/a



Supplemental Figure S1. (a) Behavioral scoring scale used to evaluate seizures following DFP administration. SLUD = salivation, lacrimation, urination, defecation. (b) Average behavioral seizure scores over time following acute DFP intoxication. Mice were pretreated with vehicle (VEH), the nonselective nAChR antagonist mecamylamine (MEC), the $\alpha 4$ -selective nAChR antagonist dihydro- β -erythroidine hydrobromide (DH β E), or the $\alpha 7$ -selective nAChR antagonist methyllycaconitine citrate (MLA) 10 min prior to injection of DFP (9.5 mg/kg, s.c.), followed 1 min later by a combined injection of atropine sulfate (AS) (0.1 mg/kg, i.m.) and 2-pralidoxime (2-PAM) (25 mg/kg, i.m.). Data are presented as mean \pm SEM (n= 3 – 14). (c) Average behavioral seizure score over time following DFP intoxication in wildtype (WT), $\alpha 4$ nAChR knockout ($\alpha 4$ KO), and $\alpha 7$ nAChR knockout ($\alpha 7$ KO) animals. Data are presented as mean \pm SEM (n= 5 – 14). WT animals are the same as VEH animals presented in panel (b). (d) Average behavioral seizure score over time following DFP intoxication in VEH animals (same animals as shown in panels b and c) or mice treated with MEC 10- or 40-min post-DFP intoxication. Data are presented as mean \pm SEM (n= 9-14).



Supplemental Figure S2. (a, b) The impact of pretreatment with nAChR antagonists on body weight loss at 4 (a) and 24 hours (b) post-DFP intoxication. (c, d) Percent body weight loss at 4 (c) and 24 hours (d) post-DFP intoxication in WT, $\alpha 4$ KO, and $\alpha 7$ KO animals. Data are presented as violin plots in which each point represents an individual animal, and the horizontal lines represent the minimum, quartile, median, and maximum values (n= 3 – 14). WT animals are the same as VEH animals presented in panels (a) and (b).