Supporting Information

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SI Text

Further Description of the Electrophysiological Correlates of Botulinum Toxin Action, with a Focus on the Differences Between Botulinum Toxins Type E (Botx/E) and Type A (Botx/A). Treatments with Boxt/E led to the elimination of all detectable evoked acetylcholine (Ach) release after 40–90 min of motor nerve stimulation (1 Hz) in either the wild-type or the Rab3A$^{-/-}$ mouse (n = 5 experiments for each). In contrast, when preparations were subjected to the same protocol without botulinum toxins (see *Materials and Methods*), stimulation at 1Hz for as long as 20 h failed to impair neuromuscular transmission. SI Fig. S1*A* shows such a control experiment, in which after 20 h of 1-Hz stimulation, end-plate potentials (EPPs) that were suprathreshold for action potential (AP) generation were still present.

The electrophysiological correlates of Ach release differed after treatment with Botx/E and Botx/A, but not between the Rab3A mutant and wild-type mice for each toxin fraction. After Ach release is eliminated with Botx/E (by 1-Hz stimulation in normal physiological salt solutions), only a very transient recovery of Ach release (<5 min) was produced by the potassium channel blocker solution used to record Ca^{2+} currents (see *Materials and Methods*). This brief recovery occurred only in the deepest muscle fibers of the preparation, not the surface fibers used for electrophysiological recordings. In contrast, after Botx/A treatment, the potassium channel blocking solutions produced such a large increase in Ach release (by prolonging the nerve terminal Ca^{2+} currents) that twitches were immediately restored in the entire skeletal muscle in response to nerve stimulation. Subsequently, suprathreshold EPPs in either strain of mouse, were observed. Fig. S1*B* shows EPPs recorded 1 h after the beginning of superfusion with high potassium solutions in a Botx/A treated preparation. These EPPs retrieved by increasing Ca^{2+} entry were not stable and gradually declined over time until they were completely eliminated from all fibers 40 min after the experiment shown in Fig. S1*B*. No electrophysiological correlates of Ach release were observed in preparations treated with Botx/C or Botx/D after the addition of the solution used to record Ca^{2+} currents in either strain of mouse. However, after Botx/D treatment, raising the Ca^{2+} concentration to 8 mM in the presence of 100 μM tetraethylammonium and stimulating the motor nerve repetitively at 5–10 Hz generated miniature EPPs (MEPPs) with frequencies ranging from 0.92–1.9 s $^{-1}$ (n = 4 preparations). Individual experiments revealed a dependence of MEPP frequency on the frequency of nerve stimulation (0.5–10 Hz), with MEPP frequencies being stable for as long as 40 min of nerve stimulation in either strain of mouse.

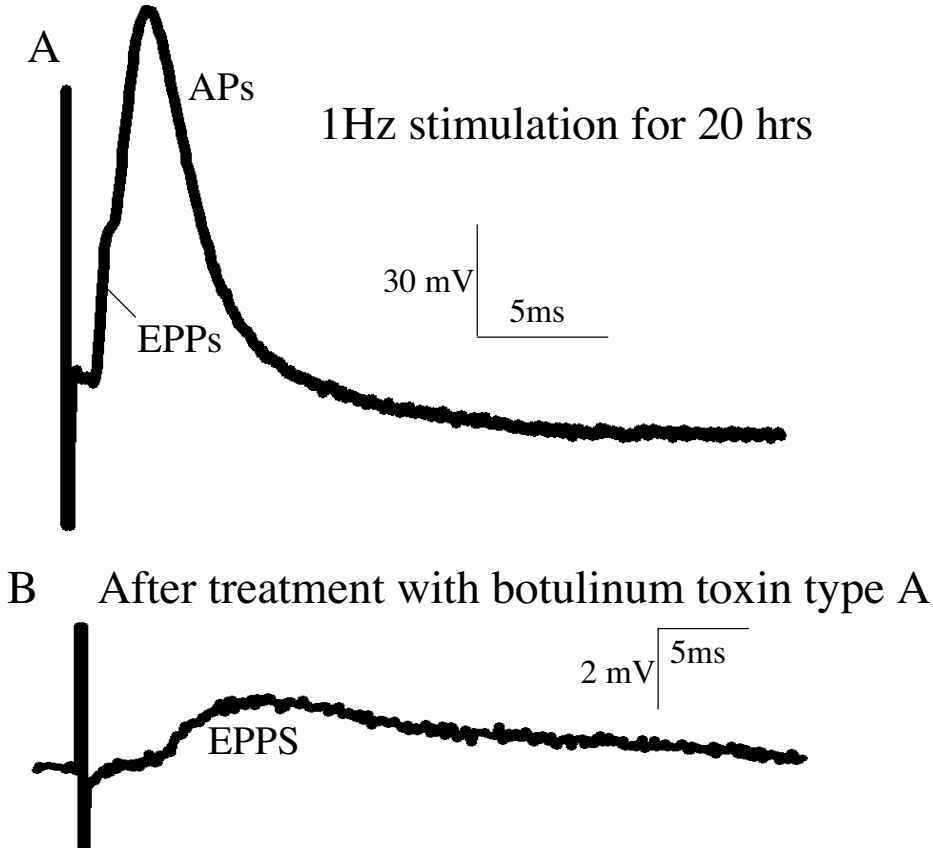

Fig. S1. Electrophysiological correlates of neuromuscular transmission after 1-Hz stimulation for 20 h (*A*) and after treatment with botulinum toxin type A (*B*). (*A*) Suprathreshold EPPs (constituting an end-plate step on a superimposed action potential) and action potentials (APs) are observed even after such extensive stimulation. The trace is the average response to four stimuli. Resting potential = −60 mV. (*B*) EPPs retrieved by superfusion with the solution used to record calcium currents (normal solutions with potassium channel blockers) in a preparation treated with Botx/A ($n = 11$ stimuli averaged, 0.1 Hz). The trace was recorded 80 min after the switch from normal solutions to the solutions containing potassium channel blockers. Resting potential = −70 mV.