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Evaluation of *Clostridium botulinum* A5 neurotoxin actions in vivo and ex vivo at the mouse skeletal neuromuscular junction

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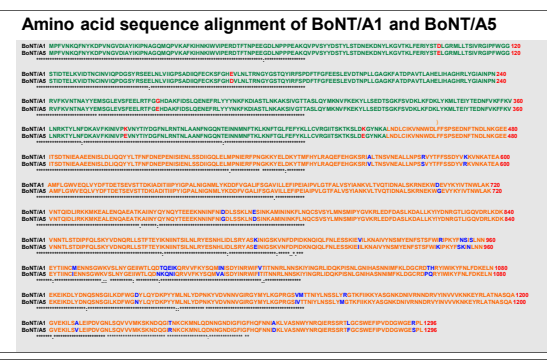
Evelyne BENOIT¹, Aurélie COUESNON², Bogdan I. IORGA³, Michel R. POPOFF⁴, Jordi MOLGÓ^{1,2*}



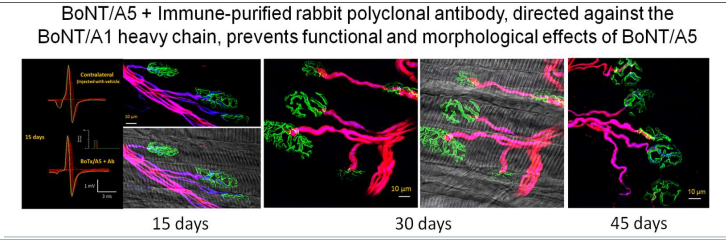
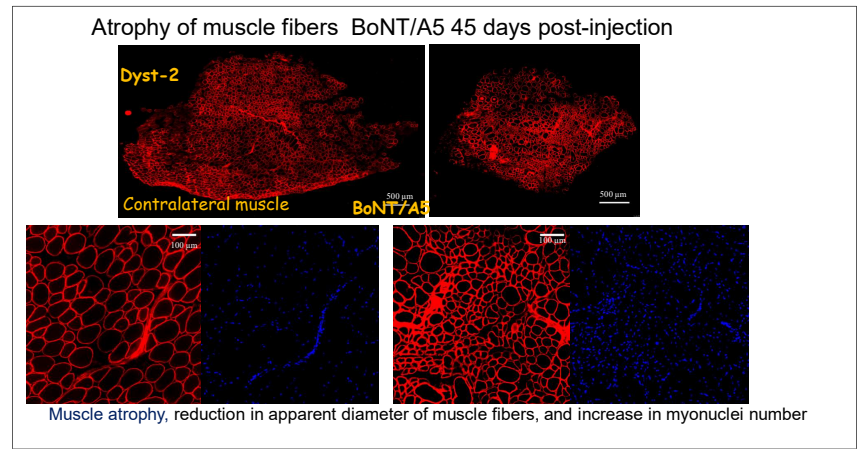
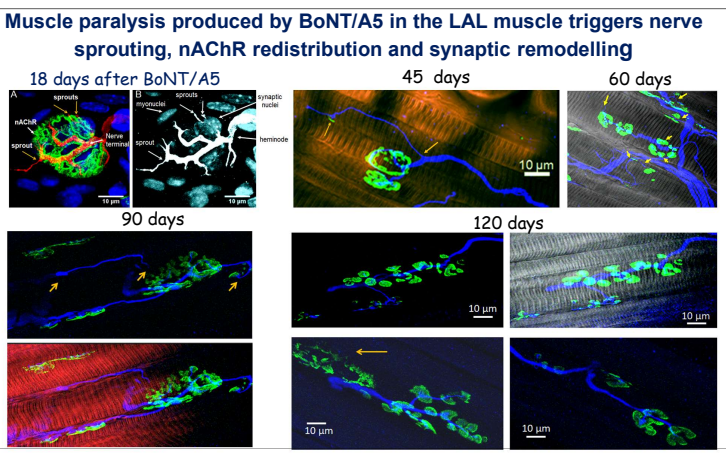
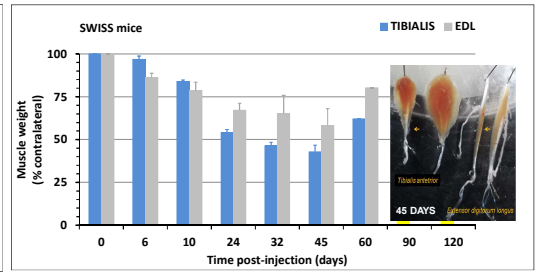
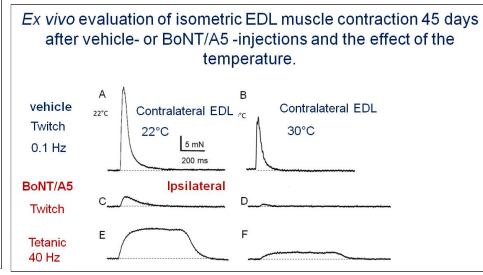
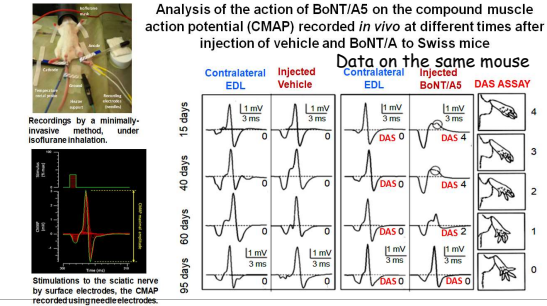
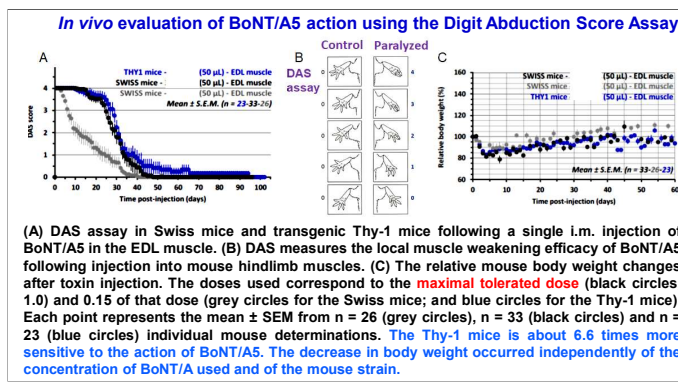
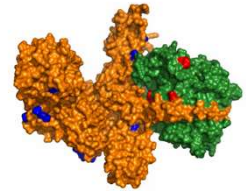
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Clostridium botulinum neurotoxins (BoNTs), the most potent toxins known, are the cause of a worldwide life-threatening disease in humans and animals known as botulism. This disease usually manifests as descending symmetrical flaccid paralysis of skeletal muscles together with autonomic dysfunction. BoNTs include a large family of zinc metalloproteases that can be immunologically distinguished by homologous antitoxins into seven primary serotypes, designated A to G. Additionally, for BoNT/A, at least 8 subtypes (/A1-/A8) have been identified from gene sequence analysis. In France, several cases of human botulism due to BoNT/A5, /A6 or /A7 have been reported, though BoNT/A1 and /A2 are the prevalent forms of type-A botulism. The characterization of the C. botulinum strains involved in two cases of BoNT/A5-poisoning revealed that they possess the gene encoding for BoNT/A5 identical to the one previously reported.

In the present study, we produced the BoNT/A5 crude complex (3 x 10⁷ LD₅₀/mg) from the C. botulinum A5 strain 126.07 [1], and studied in vivo and ex vivo, in murine models, the skeletal neuromuscular block caused by BoNT/A5, at different times after a single local toxin injection into the hind-limbs or the head.



BoNT/A5 differed from BoNT/A1 by 5 identified amino acids in the Light Chain (LC) domain, and by 32 amino acids in the Heavy Chain (HC) domain.



CONCLUSIONS: (i) the duration and degree of paralysis depends on the BoNT/A5, and the mouse strain; (ii) DAS assay allowed evaluating the BoNT/A5-induced paralysis, a better information was obtained by measuring the CMAP on the "same mouse" at various times after toxin injection. (iii) Ex vivo studies revealed: (a) the presence of axonal outgrowths (nodal and nerve terminal sprouts), (b) an extension of nicotinic acetylcholine receptor clusters, (c) a reduction in muscle weight and apparent diameter of fibers, and the prolonged atrophy of muscle fibers; (iv) Pre- and postsynaptic remodeling was completely abolished by an immune-purified rabbit polyclonal antibody directed against the BoNT/A1-Hc1

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