Tracking the roots of human mental retardation: cognitive impairments in gdi1 knockout mice are associated with anomalous synaptic vesicles.

D'Adamo P.[1], Meskenaite V.[2], Ziegler U.[1], Toniolo D.[3], Wolfer D.P.[1] and Lipp H.-P[1].

[1] Inst. of Anatomy, Univeristy of Zurich, Zurich, Switzerland; [2] Inst. of Biochemistry, Univeristy of Zurich, Zurich, Switzerland; [3] DIBIT, San Raffaele Scientific Institute, Milano, Italy

Abstract 318.23

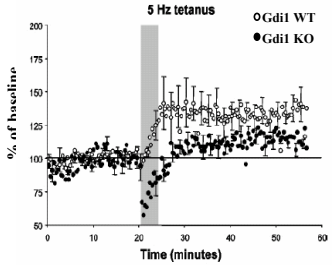
From phenotype to genotype in man

One of the genes responsible for human non-specific X-linked mental retardation is *Gdi1*, as found by pedigree and molecular analysis of affected families (D'Adamo et al., Nat. Genet. 19, 1998). It encodes alfaGDI, a protein controlling the activity of small GTPases of the Rab protein family known to mediate synaptic vesicle fusion and intracellular trafficking. Afflicted individuals show severe mental retardation without morphological anomalies.

From genotype to phenotype in mice

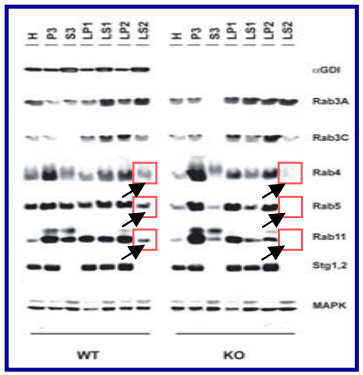
We then generated mice carrying a deletion of *Gdi1* and investigated their behavior, neurophysiology, synaptic biochemistry and neuroanatomy. Here we report our most recent finding: Gdi1 KO mice suffer from ultrastructural anomalies of synaptic vesicles, and there is evidence that this deficiency is at least partially linked to the observed phenotypic changes at the functional level.

Slow repetitive stimulation induces short-lasting impairments of synaptic transmission but no deficits in LTP



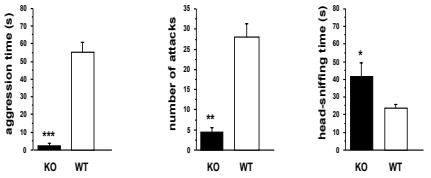
Stimulating for 30 seconds at 5Hz produced LTP in both genotypes, but the first 5 minutes after tetanus revealed a significant difference between the two groups. The field EPSP slope was significantly depressed (mean 78.6±4.5%) relative to baseline in KO mice in the first 5 minutes post tetanus (t=4.49, df=4, P<0.02), while WT mice were not different from baseline (mean 114.5±6.2%, t=2.13, P>0.05) in the first 5 minutes. By 25 minutes after tetanus, both groups are significantly potentiated (WT 133.4 ±9.8%, t =2.7, P <0.05; KO 112.3±4.3, t=2.9, P<0.05).

Gdi1 regulates expression of several Rab proteins involved in vesicle trafficking



Rab4 and Rab5 are the most reduced Rab GTPases in *Gdi1* KO mice. This suggests that the absence of alpha Gdi may affect specifically endocytic events. H: total homogenate, P3: cell body membrane, S3: cytosol, LP1: mitochondria, pre-and postsynaptic membranes, LS1: total synaptosomal fraction, LP2: synaptic vesicle fraction, LS2: synaptosol.

Aggressive behavior in mutant mice is impaired



Gdi1 KO mice show a significant reduction in aggressive behavior in the resident-intruder test, as shown by the total aggression time (F[1,37]=20.13, p<0.0001) and in the number of attacks (F[1,37]=9.47, p=0.003). KO mice have an atypical social approach toward the intruder, suggesting impaired ability in appropriate chaining of social behavioral elements.

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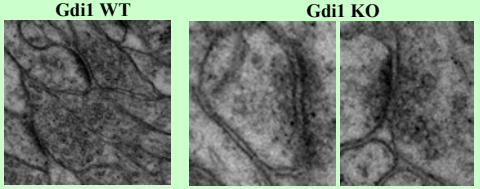
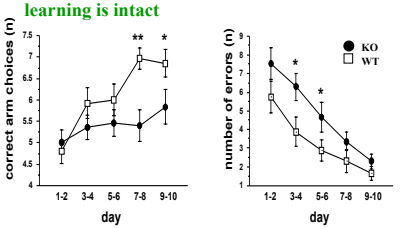
Mutant mice show less and lumped synaptic vesicles



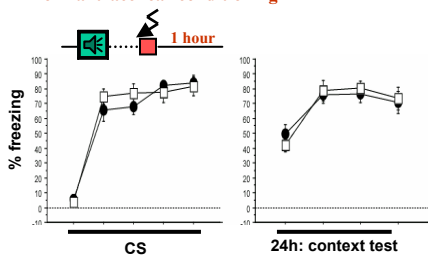
Gdi1 KO mice show a qualitative reduction in the number of synaptic vesicles. Electron microscopic analysis in P17 and 3 month old Gdi1 KO animals performed in several brain regions (hippocampus, somatosensory cortex and amygdala) showed that the synaptic vesicles are lumped at the presynaptic cleft. Also, there are indications of intrasynaptic degeneration.

Spatial short-term memory in the radial maze is impaired, procedural learning is intact

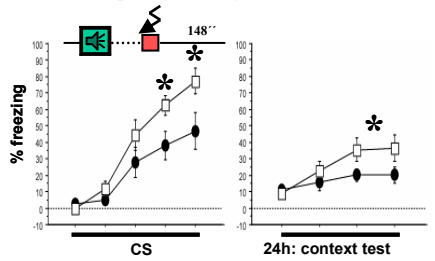


KO mice performed barely above chance level (5.5 correct arm choices), revealing a significant difference between genotypes (F[1,26]=0.015, p=0.0015). KO mice show slower acquisition than WT (F[1,26]=10.90, p=0.0028). However, mutants show an intact procedural learning (F[14,4]=9.98, p<0.0001), by significantly decreasing the number of errors at the end of the task.

Long intervals between delivery of shocks permit normal trace fear conditioning



Short intervals between delivery of shocks reveal impaired learning and recall



During trace fear conditioning, mice must associate an conditioning stimulus (CS) followed by a foot shock delivered 15 sec later. Learning this temporal association is reflected by increasing freezing scores during the training. Recall after 24h is reflected by freezing when placed in the test chamber after 24h. Note intact learning, short- and long-term memory after shock intervals of 1 h, but impaired learning and memory with short inter-trial intervals during training. This suggests that KO mice need much more time in order to restore synaptic transmission required for short-term memory and processing.

"Synaptic exhaustion": a cause for Gdi1-related mental retardation?

These data imply that the cognitive deficits in this mouse model reflect a temporary depletion of the immediately available synaptic vesicle pool. The normal performance of mutant mice in many other procedural tasks not shown here imply intact basic mechanisms for short- and long-term memory. Deficits in social behavior and conditioned taste aversion remain to be explained.

Further studies must use behavioral tests designed to challenge synaptic recycling, and analyze receptors and second-messenger systems. Finally, it should be studied whether individually different efficiency of synaptic vesicle recycling is contributing to the large variability of cognitive abilities in mice and men.

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