Ubiquitin ligase E3a role in axonal contact guidance: rescue strategies in Ube3a-deficient hippocampal neurons

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@ Neuroguide

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Background

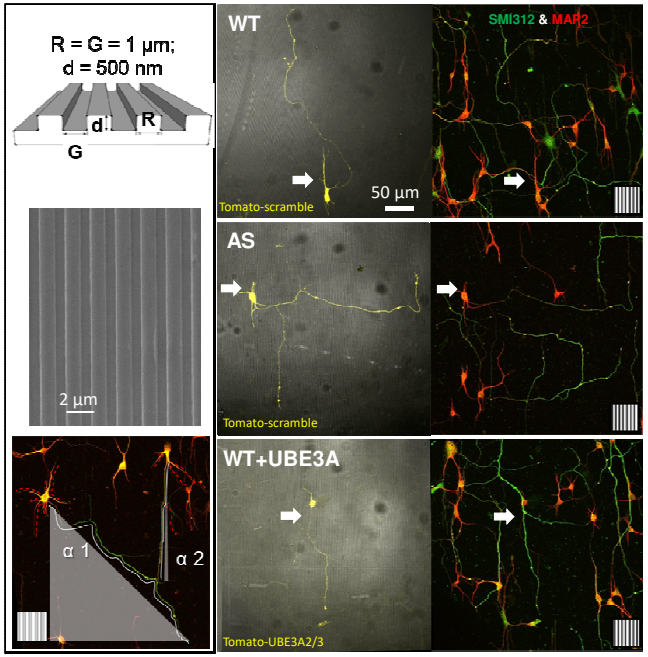
Although neuronal extracellular sensing is emerging as crucial for brain wiring, little is known about these processes in pathological conditions. Ubiquitin E3A ligase (**UBE3A**) has crucial functions in the brain, being its unbalance the most prevalent genetic origin for autisms. Changes in its expression levels indeed lead to neurodevelopmental disorders [to Angelman Syndrome (**AS**) or 15duplication-autism]. We previously investigated neuronal guidance in UBE3A-deficient neurons, model of AS, by using ***micro-grooved substrates*** that can induce specific directional stimuli to cells. We found deficient polarization and topographical contact guidance in AS neurons, linked to a dysregulated focal adhesion (**FA**) pathway activation (*Tonazzini I, Adv Healthcare Mat 2016*).

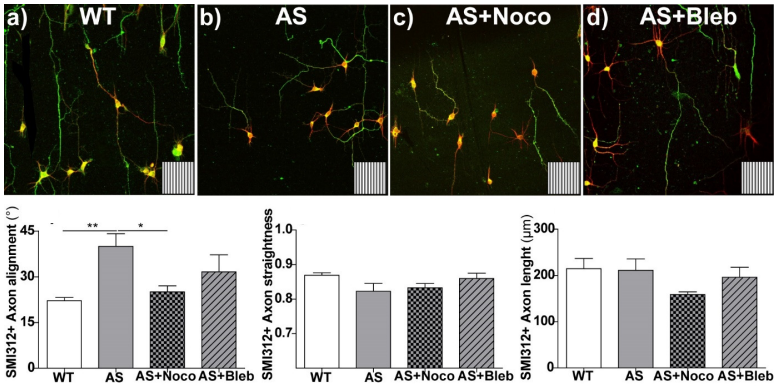
Aims

Here, we selectively study **axon** and dendrite **contact guidance** and neuronal morphological features of Wild-Type (**WT**), UBE3A-deficient (**AS**) and UBE3A-overexpressing (**WT+UBE3A**) hippocampal neurons on *micro-grating substrates* (**GRs;** 1 µm ridge, 1 µm groove, and 500 nm depth), with the aim to further clarify the UBE3A role in neuronal contact guidance. Moreover, we test the effect of pharmacological treatments acting on ***cytoskeleton contractility*** for restoring a correct topographical guidance in UBE3A-deficient neurons. We finally analyze the molecular processes regulating the **FA pathway**.

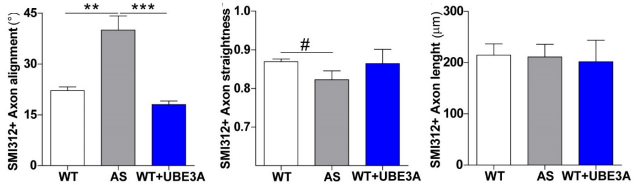
Results

- We demonstrate that the loss of axonal topographical guidance is specific for AS neurons while UBE3A overexpression does not impair axonal directional polarization and growth.
- Deficits at level of growth-cone guidance on GRs, and focal adhesion assembly are revealed in AS hippocampal neurons.
- Finally, low dose of nocodazole (40nM), a drug that increases cell contractility, is shown to improve neurite alignment by restoring the FA maturation and signaling pathway activation.



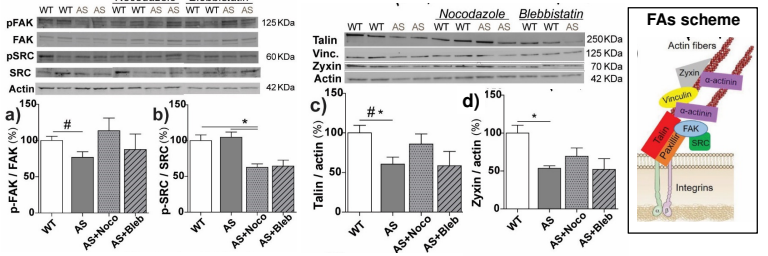


WT (**a**) and AS (**b**) HNs were cultured on GRs (div4) in the presence of nocodazole (**Noco**; 40nM) (**c**), a microtubule depolymerizing agent that activates the RhoA-ROCK-MLC pathway, or Blebbistatin (**Bleb**; 25µM) (**d**), a myosin-II-contractility inhibiting drug (*Tonazzini I, Biomaterials 2013*). In AS neurons, the axonal alignment along GR pattern is ***improved*** **by** a low dose of **Noco** (40nM) (*P<0.05, AS vs. AS+Noco; Tukey's test). Inset: GR direction. Mean ± SEM, n ≥ 3.



During neuronal polarization (div4), there is a specific *deficit in axonal topographical guidance* along GRs in AS neurons (i.e. higher alignment angle α; **/*** Tukey's test). This defective axonal alignment is due selectively to the UBE3A loss while UBE3A increase does not impair axonal contact guidance. Other morphological aspects do not change in different neurons, except axonal straightness in AS (# Student *t*-test).
Inset: GR direction; mean ± SEM, n > 4.

Mean ± SEM, n > 3; * 1-way ANOVA Tukey's test; # Student *t*-test.

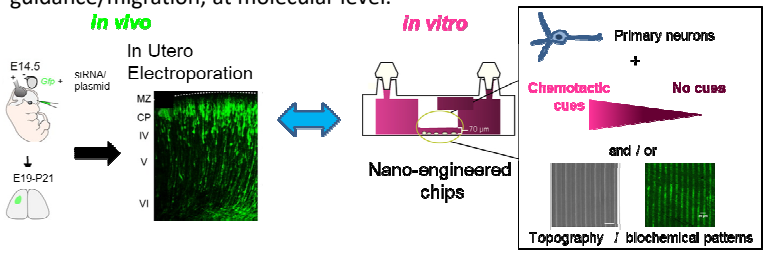


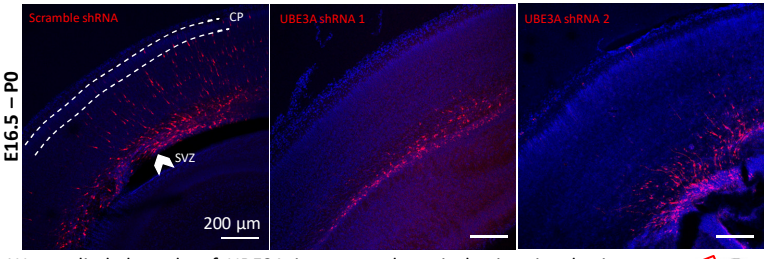
We tested the effect of Noco and Bleb on FA pathway at molecular level. Results confirm the impaired activation of FAK in AS (**a**: #P<0.05 vs. WT). Importantly, Noco exposure restores FAK activation (**a**: P>0.05 vs. WT). Talin is reduced in AS neurons (**c**: *P<0.05 WT vs. AS) and interestingly restored by Noco. Zyxin, which is present only in mature FAs, is reduced in AS (**d**: *P<0.05 WT vs. AS). Noco and Bleb treatments had not major effects in WT neurons (*not shown*). Overall, the maturation and anchorage of FAs maybe be altered in AS hippocampal neurons, and Noco can restore it at certain extend.

NEUROGUIDE

*"Study of **neuro**nal sensing and migration/**guid**ance dynamics in neuro-developmental disorders by nano-engineered chips"*

Neuroguide project is focused on the study of neuronal migration and extracellular sensing in neuropatho-physiological conditions, in particular in UBE3A-mutant models, with a *multidisciplinary* approach: *in vivo*, by in utero electroporation; *in vi*tro, by micro/nano-engineered devices (i.e. nano-structured substrates and microfluidic chips). These platforms will provide an enriched environment (bio-mimicking condition) to tune and test neuronal guidance/migration, at molecular level.





We studied the role of UBE3A in neuronal cortical migration by in utero electroporation. The knock-down of UBE3A, by two different shRNAs, induces a delay in neuronal migration (somatosensory cortex) at P0.