Supplementary Results:

- *CNF1 treatment does not affect activity levels and anxiety-like profile in MeCP2-308 mice during the open field test*

- *Motor coordination in the Dowel test is not affected by CNF1*

- *CNF1 treatment left unaffected the exploration of a novel object*

- *MeCP2-308 mice do not show abnormalities of neuronal dendritic tree or synapse*

Supplementary References

SUPPLEMENTARY RESULTS

CNF1 treatment does not affect activity levels and anxiety-like profile in MeCP2-308 mice during the open field test

During the 1-h open field test, neither genotype nor CNF1 treatment affected general activity (c*rossings frequency* and *inactivity*) and all groups showed a similar habituation profile (data not shown). Interestingly, CNF1 selectively increased the time wt mice spent in the center of the arena (wt control = 61.6 ± 4.4 s; wt CNF1 = 84.6 ± 6.5 s; hz control = 51.7 ± 4.6 s; hz CNF1 = 48.4 ± 4.3) ($p < .05$ after posthoc comparisons on the genotype*treatment interaction: $F(1, 27) = 5.24$; $p = .030$), thus suggesting an anxiolytic effect of the treatment in control mice. An effect of the treatment on explorative behaviors is also suggested, as CNF1 increased the number of *rearings* in both genotypes at late time intervals ($p < .01$ after posthoc comparisons on the treatment*repeated measures interaction: $F(2,54) = 3.34$; $p = .043$), in the absence of changes in *crossing frequency* (Fig. S1). No differences among groups were found for *grooming.*

CNF1 treatment left unaffected the exploration of a novel object

During the acquisition session, no genotype differences or CNF1 treatment effects were found in *Crossing* frequency and time spent exploring the objects (data not shown). During the retention test, all the experimental groups similarly explored the novel object (wt control: 11.5 ± 3.1 s; wt CNF1: 7.1 ± 2.3 s; hz control: 7.4 ± 3.1 s; hz CNF1: 8.1 ± 2.1 s). In line with a previous study (De Viti et al., 2011), CNF1 did not affect the ability of mice to recognize a novel object.

Motor coordination deficits in the Dowel test are not affected by CNF1

In line with a previous report (De Filippis *et al*, 2010), a main effect of genotype was evident in the Dowel test [$U = 47.5$; $p < .01$], with Mecp2-308 mice falling from the dowel significantly earlier than wt littermates (wt: 78.6 ± 12.1; hz: 2.6 ± 0.6). CNF1 treatment did not affect the

performance in this test in both genotypes. Thus, motor coordination deficits in mutants were not contrasted by the proposed treatment.

MeCP2-308 mice do not show abnormalities of neuronal dendritic tree or synapses.

An immunohistochemical analysis was conducted to characterize the neuronal dendritic tree and synapses in sections from hippocampus and motor cortex of MeCP2-308 mice and their wt littermates. SMI32 is a monoclonal antibody that labels non-phosphorylated neurofilaments mostly in dendrites. No differences were observed between the genotypes: dendrites showed similar growth and branching in wt and MeCP2-308 mice in both CA1 and motor cortex (Supplementary Fig. 2a). Similar results were obtained with antibodies against MAP2, a microtubule-associated protein compartmentalized in the somatodendritic area (data not shown). Dendritic spines contain the post-synapse apparatus, in absence of which synapses do not normally develop. To ascertain if synapses were affected we immunolabeled synaptophysin, an integral protein of synaptic vesicles considered a reliable synaptic marker, in cortical layer I. Again, no differences in synaptophysin immunoreactivity were found between genotypes, between treatments or within their interaction (Supplementary Fig. 2b). Treatments with the CNF1 also determined no changes in the immunoreactivity for the different antibodies in all analyzed regions (Supplementary Fig. 2). Based on qualitative evaluation, it does not appear that there is a significant difference in GFAP immunolabeling when wt animals received 2 µl of TRIS or received no infusion (see Supplementary Fig. 3)

SUPPLEMENTARY REFERENCES

De Filippis B, Ricceri L and Laviola G (2010). Early postnatal behavioral changes in the Mecp2-308 truncation mouse model of Rett syndrome. *Genes Brain Behav* **9**: 213-223.