Posters

P.1.001 Neuropeptide S activates extracellular-signal regulated kinase 1/2 within the amygdala and interacts with the oxytocinergic system

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Anxiety disorders are highly prevalent and despite substantial research novel therapeutics are still required. Neuropeptides represent potential candidates, such as Neuropeptide S (NPS), which exerts a strong anxiolytic activity in rodents [1]. To date little is known about NPS receptor (NPSR)-mediated intracellular signaling pathways and interactions with other neuropeptide systems that are responsible for the physiological and behavioral effects of NPS. In order to extend our understanding of the molecular and cellular mechanisms of action of NPS we studied (i) NPSR-coupled neuronal pathways in the amygdala and (ii) the effects of NPS on the anxiolytic nonapeptide oxytocin (OXT) within the hypothalamic paraventricular nucleus (PVN) of male Wistar rats.

Western blot analysis revealed that central NPS (1 nmol, 5 µl, icv) induces phosphorylation of both ERK1 ($t_{19} = -2.817$, $p < 0.05$) and ERK2 ($t_{19} = -2.776$, $p < 0.05$) in the cytosolic, but not nuclear, fraction of amygdala tissue in relation to total ERK1/2 within 15 min. Similar activation patterns were found for both pERK1 ($t_{18} = -2.123$, $p < 0.05$) and pERK2 ($t_{18} = -2.179$, $p < 0.05$) in relation to the reference protein β-tubulin. Total ERK1/2 levels were not altered following NPS application.

Next, we assessed the effect of intra-PVN administration of NPS (0.2 nmol, 0.5 µl) on anxiety-related behavior on the elevated plus maze. Here we observed that rats treated with NPS displayed an increased percentage of time spent on the open arms, indicative of an anxiolytic-like effect ($t_{10} = -2.667$, $p < 0.05$), whereas closed arm entries, as a measure of locomotion, were not affected ($t_{10} = -0.833$, $p = 0.424$). Further, qRT-PCR analysis revealed that central NPS (1 nmol, 5 µl, icv) induces the expression of OXT mRNA within the PVN after 3h in relation to the reference gene GAPDH ($t_{17} = -3.212$, $p < 0.01$). A potential direct interaction between these systems was indicated by immunohistochemical studies, which revealed strong, but not exclusive NPSR expression on oxytocinergic neurons within the PVN by means of confocal laser scanning microscopy ($n = 4$). The specificity of the NPSR antibody was tested by Western Blot analysis with tissue from a NPSR knockout mouse vs. wild type (generous gift from Dr. Chiara Ruzza, University of Ferrara, Italy).

In conclusion, our results indicate a role for the MAP kinase cascade in the behavioral effect of NPS. Based on these findings we currently investigate the behavioral consequences of pharmacological inhibition of MEK1/2 by the MEK1/2 inhibitor U0126 (0.5 nmol, 0.5 µl) prior to local NPS infusion (0.2 nmol, 0.5 µl) within the medial amygdala, a region known to express high levels of the NPSR [2]. These experiments will reveal an important link between the ERK1/2 activation and its involvement in the anxiolytic activity of NPS. Moreover, central NPS induced OXT mRNA expression, and, additionally, we demonstrated a co-localization of NPSR and OXT. These results lead us to study whether NPS induces local OXT release using microdialysis and a highly sensitive radioimmunoassay. Taken together, these data fill an important gap in our knowledge of the underlying mechanisms of the anxiolytic-like effects of NPS.

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P.1.002 The developmental role and function of Ntrk2b in the zebrafish model

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Rationale: Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, plays an important role in neuronal network plasticity by promoting differentiation and survival of neurons during development and in adult brain. BDNF signals by activating its cognate receptor tyrosine kinase B, TrkB and has trophic effects on the serotonergic system [1]. The exact role of TrkB in the behavioral effects of serotonin remains poorly understood. Alterations in the BDNF-TrkB signaling and/or in 5-HT neurons have been implicated in the pathophysiology of psychiatric or mood disorders [2,3]. This study pursues to interrogate the role of TrkB in brain development and more specifically the effects on the serotonergic system. To study the developmental effects of TrkB in brain, we have used the zebrafish model. The TrkB receptor has two

forms in zebrafish Ntrk2a and Ntrk2b. The Ntrk2a is not found in significant amounts therefore the focus has been mostly on Ntrk2b.

Materials and Methods: Zebrafish of the Turku line, used for more than a decade in experimental work, were used in these experiments because of their ease of manipulation, transparent embryos and ex-utero development. Ntrk2b gene knockdown was performed using antisense morpholino oligonucleotides for studying the transient effects during development in larval zebrafish. The efficiency of knockdown was characterized using TrkB specific antibodies. The gene expression was analyzed by in-situ hybridization and localization by immunohistochemistry. Cell death studies were performed using TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) staining method. Monoamine levels were measured by HPLC. Locomotor activity was measured by Ethovision software.

Results: The mRNA expression of Ntrk2b was assessed by in-situ hybridization in the larval stages and adult brain of zebrafish. Wide distribution of the mRNA was observed in the CNS such as dorsal telencephalon, diencephalon, in the parvocellular pre-optic nucleus, hypothalamus, positive radial glial cells lining the mesencephalic ventricle, cerebellum and dispersed positive fibers in the medulla oblongata. The expression of mRNA for BDNF coincided with specific Ntrk2b expression pattern. The morpholino oligonucleotides against Ntrk2b was designed and the dose was validated by western blotting using TrkB specific antibody. The Ntrk2b morphants had no major gross phenotype and the motor behavior did not change significantly as compared to the controls. There was a significant difference in the monoamines levels of dopamine and serotonin observed in the TrkB morphants. Immunoreactivity using antibodies for serotonin, GABA1H and tyrosine hydroxylase was reduced in the morphants as compared to the controls. No change in Histamine immunoreactivity was observed. The expression level of serotonin transporter, serta, and tyrosine hydroxylase, th1, declined in the morphants. No major change in cell death in the Ntrk2b morphants was observed by TUNEL staining method.

Conclusions: These results reveal potential role of TrkB during development and its effect on the monoaminergic system. The results establish that zebrafish Ntrk2b has wide spread distribution throughout development and loss of Ntrk2b could possibly play an important role in neurotrophins mode of neuronal plasticity.

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P.1.003 Docosahexaenoic acid as a novel strategy for stress-related disorders: reversal of corticosterone-induced changes in cortical neurons

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Chronic exposure to stress can exert long-lasting changes on the brain increasing vulnerability to mental illness. Corticosterone (CORT) is the main rodent stress hormone that has been shown to exert negative effects on neuronal morphology and viability across a number of brain regions. Understanding the molecular and cellular basis of susceptibility and resilience to stress may open up novel therapeutic strategies for disorders such as depression and anxiety. Indeed, current treatments for psychiatric disorders are of limited efficacy in a considerable proportion of patients, and are often associated with troublesome side-effects that reduce compliance.

Growing evidence suggests that omega-3 polyunsaturated fatty acids (PUFAs) may have a beneficial effect on health including mental health. Indeed, being constituents of the cellular membrane, they might play a critical role in the development and function of the central nervous system [1]. However, the ability of PUFAs to abrogate the stress-induced toxic effects on neurons has not been well investigated. To this end, we studied the protective effect of two different omega-3 PUFA, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), against CORT-induced cellular changes in a mixed cortical primary culture.

We first characterized the effect of CORT (75, 100, 150, 200 uM) at different time points (24, 48, 72 hours) in a mixed cortical primary culture over 10 days *in vitro* (DIV), prepared from rats at postnatal day 1–2. Cells were then pretreated with either DHA or EPA (3.6 uM) at 1 DIV.

CORT (72 hours) induced a dose-dependent reduction in cellular viability as assessed by methylthiazol tetrazolium