Results: As compared to the CNT fibroblasts (75 affected microRNAs), the D samples showed almost three times more microRNA expression changes (214 microRNAs) caused by both GAL and NL metabolic stress treatments. The microRNA profile alterations were more remarkable under NL circumstances. Although higher overlap was detected between the GAL and NL induced microRNA changes in the D group (47 microRNAs, while only 4 microRNAs in the CNT), Pearson correlation showed that the two different stress treatments resulted in very similar microRNA expression pattern in the CNT ($r^2 = 0.51$ and 0.43) but not in the D group. Nearly 10% of the suspected target mRNAs of the most significantly affected microRNAs changed due to metabolic stress treatments in the CNT group.

Conclusion: We suppose that the metabolic stress induced microRNA changes in dermal fibroblasts of CNT subjects are part of the normal cell adaptation, while those found in the D group could be the sign of a genetically determined, pathologic stress response presumed in depression. These results could facilitate the understanding of microRNAs' role in the development of depression and contribute to the mapping of the stress related physiologic and degenerative gene expression changes by setting up a potential new in vitro metabolic stress related depression model and diagnostic tool.

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P.3.008 S-Adenosyl-methionine impairs forced swimming-induced behavioural immobility by inhibiting gene expression in dentate gyrus neurons

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The consolidation of stress-induced adaptive behaviours, such as the learned immobility response in the forced swim (FS) test, depends on specific epigenetic modifications underlying gene transcriptional responses in dentate gyrus (DG) granule neurons of the hippocampus. In these neurons FS evokes the activation of two interacting signalling pathways, i.e. the glucocorticoid receptor (GR) and the NMDAR/ERK1/2/MSK1-Elk-1 pathways, resulting in phosphorylation of serine10 and acetylation of lysine14 at histone H3 (H3S10p-K14ac) which leads to induction of immediate early genes (IEGs) c-Fos and Egr-1 [1,2]. These molecular responses are critical for the consolidation of the behavioural immobility response [1,2].

The drug and endogenous methyl-donor S-adenosyl-methionine (SAM) impairs the consolidation of the behavioural immobility response [3] suggesting the involvement of histone methylation and/or DNA methylation in gene transcriptional control underlying the behavioural response but this is unknown. Therefore, to understand the mechanism of action of SAM and to gain insight into the involvement of histone/DNA methylation, rats were injected with SAM (100 mg/kg s.c.) 30 minutes before FS (15 min, 25ºC). Twenty-four hours later they were subjected to FS again and immobility behaviour were scored in 10 s bins. SAM had no effect on immobility in the initial FS test (mean±SEM; vehicle: 15.8±1.6 bins, $n = 8$; SAM: 15.0±1.9 bins, $n = 9$; $P > 0.05$ post-hoc Bonferroni test). However, in the retest, immobility in the SAM group was significantly lower (11.4±3.3 bins, $n = 9$) than that in the vehicle group (20.6±2.3 bins, $n = 8$; $P < 0.05$), confirming that SAM indeed impairs the consolidation of the behavioural immobility response [3].

To study whether SAM's effects on behaviour could be explained through effects on FS-induced epigenetic and transcriptional responses in DG neurons, rats were pre-treated with SAM or vehicle and submitted to FS or not (baseline control) and killed 1 h later. Compared to vehicle, SAM evoked a significant decrease in FS-induced c-Fos and Egr-1in DG neurons (Vehicle: c-Fos: 127.1±4.6 neurons $n = 5$, Egr-1: 18.4±2.5 $n = 5$; SAM: c-Fos 82.6±5.0 neurons $n = 6$, Egr-1 8.5±1.2 $n = 5$; both c-Fos and Egr-1: $P < 0.01$). However, SAM had no effect on stress-induced H3S10p-K14ac in DG neurons suggesting that the drug effect was independent of this dual epigenetic mark. Next, we investigated the effect of SAM and FS on the methylation status of histone H3 lysine residues. Chromatin immuno-precipitation (ChIP) analysis revealed that SAM and FS had no significant effects on H3K4me3, H3K9me3 and H3K27me3 at the c-Fos and Egr-1 promoters.

Recently, we have started investigating DNA methylation using bisulfite pyrosequencing and methyl-DNA IP (MeDIP) to assess the cytosine methylation status of CpG islands within the c-fos and egr-1 gene promoters. We found that cytosine methylation of both promoters was very low (<5%) in the DG, the rest of hippocampus, and the neocortex and did not change after FS. Presently we are

studying the effects of SAM on de novo DNA methylation of the gene promoters.

We conclude that SAM impairs the FS-induced behavioural immobility response through inhibition of gene transcription in DG neurons. This drug effect appears to be independent of histone modifications.

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P.3.009 Differential effects of prenatal stress on serotonin transporter deficient mice: the role of epigenetic programming

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Prenatal stress (PS) exposure in early life is an environmental risk factor that has been shown to affect fetal brain development and to increase the risk for adult psychopathology. Furthermore, a length-polymorphism in the serotonin transporter gene (5-HTTLPR) has been suggested to modulate the association between stress exposure in early life and the development of psychiatric disorders in later life by rendering short allele carriers more vulnerable to stress. The exact molecular mechanisms underlying this gene–environment (G×E) interaction remain to be elucidated though.

Recently, using a maternal restraint stress paradigm of PS in wild-type (WT) and heterozygous (+/–) 5-Htt deficient mice, we have shown that the long-term behavioural effects of PS are partly dependent on the 5-Htt genotype [1]. In our study, 5-Htt$^{+/-}$ mice, particularly females, appeared to be more vulnerable to PS exposure when compared to WT offspring. Additionally, hippocampal gene expression profiles of the females indicated that the effects of the 5-Htt$^{+/-}$ genotype, PS exposure, and their interaction were mediated by distinct molecular mechanisms. More specifically, MAPK and neurotrophin signalling were regulated by both the 5-Htt$^{+/-}$ genotype and PS exposure, whereas cytokine and Wnt signalling were affected in a 5-Htt genotype × PS manner.

Epigenetic mechanisms such as DNA methylation and histone modifications build the interface between environment and genome and thus have been recently heavily discussed to play a role in the development of psychiatric disorders.

In light of recent findings concerning the role of epigenetic mechanism in the regulation of signalling pathways that mediate depression-like behaviour in rodents, the present study aims to examine the role of DNA methylation in mediating the changes in gene expression observed in our 5-Htt × PS paradigm. For this purpose, genome-wide promoter methylation was assessed in the hippocampus using methylated DNA immunoprecipitation followed by Affymetrix Mouse Promoter 1.0R Array analysis (MeDIP-on-chip). Probe signals from MeDIP and input samples were normalised and MeDIP-input signal log2 ratios calculated. A sliding-window approach was applied to decrease the noise in the experiment readout. Genomic regions enriched by MeDIP were detected by the CMARRT algorithm.

We found that the genotype, the PS exposure and an interaction of both caused changes in the DNA methylation of a number of genes that showed also differential expression in our previous study indicating a possible correlation between DNA methylation and gene expression for those genes. Among them were genes as Fgfr4, Map3k1, Nos1, Mbp1 and Cabin1. The promoter array results are currently investigated in detail using bisulfite treatment and pyrosequencing.

In conclusion, hippocampal gene expression and DNA-methylation profiles of female prenatally stressed 5-Htt$^{+/-}$ mice suggest that distinct molecular mechanisms, including epigenetic programming, might mediate the behavioural effects of the 5-Htt genotype, PS exposure, and their interaction.

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