Aim 1: Mapping of HDAC isoforms

We have successfully mapped the basal gene expression of the class I HDAC isoforms HDAC1, HDAC2, HDAC3 and HDAC8 by in-situ hybridization in the brains of Bl6 and S1 mice. Expression patterns were quantified and compared between Bl6 and S1 mice in areas of the fear circuitry including the prefrontal cortex, hippocampus, amygdala and PAG. Basal expression of HDAC1, HDAC2, HDAC3 and HDAC8 mRNA does not seem to differ between Bl6 and S1 mice with the exception of a subtle reduction of HDAC1 and HDAC2 mRNA in the hippocampus of S1 as compared with Bl6 mice. Investigation of potential expression differences of different HATs is ongoing.

Aim 2: Role of histone acetylation in the therapeutic normalisation of aberrant fear extinction

We have revealed that HDAC inhibitors (e.g. MS-275 and valproic acid) rescue impaired fear extinction in non-extinguishing 129S1/SvImJ mice (Whittle et al, Neuropharmacology, 2013). We now provide novel data showing that HDAC inhibitors and a multi-target approach involving HDAC inhibition, NMDA and GABA-A receptor activation (Zn-restricted diet) can induce long term protection against the return of fear, which is of particular clinical relevance. Progress towards revealing the underlying molecular changes associated with reversal of impaired fear extinction has been achieved. We can demonstrate that rescue of impaired fear extinction increases protein acetylation in extinction-relevant brain regions (e.g. amygdala and medial prefrontal cortex). We are currently assessing whether these proteins include histone proteins (Collaboration with Lusser, P08 and Herbert Lindner). To establish a causal relationship between HDAC inhibition and sustained inhibition of fear we have commenced experiments using shRNA to genetically knock-down specific HDAC isoforms during an extinction training session (Collaboration with Flucher/Obermair, P06). It is anticipated that results of these experiments will aid in the impetus for the development of HDAC-isoform specific inhibitors (which are currently unavailable for the isoforms currently targeted).

Aim 3: Identification of target genes regulated by fear extinction-related change in histone acetylation.

Using unbiased gene chip analysis, current results revealed that reversal of impaired fear extinction dynamically regulates the expression of a restricted number of amygdala-localised genes (Collaboration with Striessnig, P02 and external collaborator Reinhard Kofler) in a time-dependent manner. Amongst these genes, we identified novel genes as well as a subset known to be linked to either cognitive performance or associated with human anxiety. We are now confirming these changes using biased rtPCR methodology and also confirming the anatomical locus of these changes within specific amygdala subunits using in-situ hybridisation. We are also assessing whether the expression of these genes is under epigenetic regulation (Collaboration with Lusser, P08).

Aim 4: Role of ncRNAs is aberrant extinction and its therapeutic normalisation.

Using unbiased non-coding(nc)RNA chip, current results reveal that reversal of impaired fear extinction dynamically regulates a restricted number of non-coding RNAs (Collaboration with Hüttenhofer, P11). Bioinformatical analysis has provided first evidence for a functional role as predicted targets of these ncRNAs exhibit altered gene expression following successful rescue of impaired fear extinction (see ‘Aim 3’). We are now confirming these changes using biased rtPCR methodology and also confirming the anatomical locus of these changes within specific amygdala subunits using in-situ hybridisation.

Papers published (SFB support mentioned):

- Neuropharmacology 64 (2013) 414-423
- Dobi A, Sartori SB, Busti D, Van der Putten H, Singewald N, Shigemoto R, Ferraguti F.
- Neural substrates for the distinct effects of presynaptic group III metabotropic glutamate receptors on extinction of contextual fear conditioning in mice.
- Anxiety- rather than depression-like behavior is associated with adult neurogenesis in a female mouse model of higher trait anxiety- and comorbid depression-like behavior
- Transl Psychiatry (2012) 2; e171; doi:10.1038/tp.2012.94
- Genetic Strain Differences in Learned Fear Inhibition Associated with Variation in Neuroendocrine, Autonomic, and Amygdala Dendritic Phenotypes
- Neuropsychopharmacology 37: 1534–1547 (2012)
- Sartori SB, Landgraf R, Singewald N
- The clinical implications of mouse models of enhanced anxiety.
- Future Neurol. 6: 531-571 (2011).
- Papers submitted:
  - MacPherson K, Whittle N, Camp M, Gunduz-Cinar O, Singewald N, Holmes A
  - Procedural modifiers of fear extinction in extinction-impaired and extinction-intact genetic mouse strains
  - Biology of Mood and Anxiety Disorders (under revision)
- Current SFB collaborations:
  - Striessnig (P02), G. Wenning, (P04), Flucher/Obermair (P06), Lusser (P08), Hütenhofer (P11), Aigner/Couillar-Despres (P13): Nicolas Singewald und Nigel Whittle