resting state networks in (transgenic) mice: differential effects of genetic background, sensory stimulation, and pharmacological intervention

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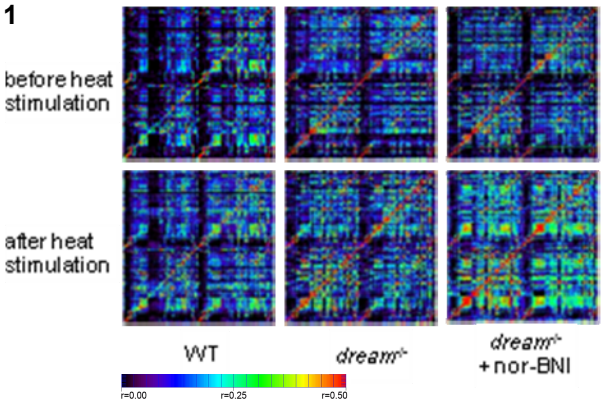
Introduction: Resting state or "default-mode" networks (1) refer to brain regions that are correlated and active without any external physical stimulation. These spontaneous low-frequency fluctuations of the resting brain are used to investigate functional connectivity. The "default-mode" network has been hypothesized to play an essential role in creativity, reflect particular thoughts and presents malfunctions for certain pathologies like Alzheimer's disease, autism, and schizophrenia (2). As the "default network" seems to be conserved across mammalian species, resting state functional connectivity studies were extended recently to other species, i.e. monkeys (3) and rats (4). In this study, we used, to our knowledge for the first time, functional connectivity analysis to investigate changes of cerebral resting state processing before and after heat-induced nociception in mice. We compared the resting state network of pain associated brain structures of dynorphin-overexpressing *dream*$^{-/-}$ transgenic and wild-type mice. Dynorphin is an opioid neuropeptide which inhibits pain transmission in the spinal cord (5). The transcriptional repressor DREAM (downstream regulatory element antagonistic modulator) acts to suppress the expression of the precursor of dynorphin, prodynorphin, in the spinal cord neurons. Consequently, knocking out DREAM results in dynorphin overexpression producing a strong reduction in generalized pain behavior as well as in nociception (5) and already shown by us with fMRI (6). The selective κ-opioid receptor antagonist nor-binaltorphimine (nor-BNI) is known to reverse this reduction to wild-type levels (5,6).

Material and Methods: Experiments were performed on 10 male wild-type and 8 *dream*$^{-/-}$ transgenic mice (10-14 weeks old). Animals were anesthetized with 1.2% isoflurane in medical air. Body temperature was maintained at 37°C by warm water circulating throughout the holding cradle. The very same *dream*$^{-/-}$ mice were measured two times with and without subcutaneous injection of nor-BNI (2 mg/kg) 24h before the fMRI experiment. *Image data acquisition*: fMRI experiments were performed with a 4.7 T/40 cm horizontal bore actively shielded magnet BioSpec (BRUKER, Germany). Gradient system (200 mT/m) and whole-body birdcage resonator enabled homogenous excitation. An actively RF-decoupled quadrature head coil was used as a receiver coil. Functional BOLD MRI scans were performed using a T2*-weighted single-shot gradient echo EPI sequence (22 axial slices, 64 x 64 matrix, TR= 2000 ms, k-space averaging of 2, TEef= 24.4 ms, field of view 15 x 15 mm, in-plane spatial resolution 234 x 234 µm, slice thickness 500 µm). *Experimental protocol*: The first 4 min of the fMRI scan without stimulation were used for resting state analysis. Afterwards a set of single thermal stimuli (45°,50°,55° and 60° ± 1°C) was applied repeatedly in 3 min 25 sec intervals for 41 min by a Peltier element on the right hindpaw. After 15 min rest another 4 min "resting state" scan was acquired. *Data processing*: Functional data were analyzed using our custom developed software MagnAn in IDL. After spatial smoothing in-plane using a Gaussian filter with a FWHM of 3 pixel the resting BOLD signal time courses were low-pass filtered at 0.1 Hz. Time courses were corrected for global signal fluctuations by linear regression using the global mean as regressor. Residual time courses were used for further analysis. Functional connectivity of the fMRI time courses was assessed by temporal correlating the average time course of a seed region of a given brain structure to the time courses of all other brain voxels. Seed regions were 6 voxels located around the center of mass of 162 nociception associated brain structures. The correlation coefficients obtained were FDR thresholded (q<0.05, dependent, two sided) and further analysis was restricted to those significant correlations. Registering each volume of significant correlations to a 3D version of the Paxinos rat brain atlas allowed to obtain average correlation coefficients of all 162 brain structures incorporated in that atlas for a given seed region. Consequently, full cross-correlation maps per animal were obtained as the mean correlation coefficients (r) of all 162 brain structures for all 162 seed regions. Next, these matrices were averaged over all animals per group. Connection numbers was calculated as the sum of all connections that occur in 80 % of all animals per group.

Results: Resting state connectivities for wild-type increased after peripheral heat stimulation in strength and number of connections. Contrary, *dream*$^{-/-}$ mice without nor-BNI injection, whose nociceptive sensitivity remains reduced, showed no difference in the connectivity strength or number of connections of the resting state network before and after heat stimulation indicating that the stimulation had no impact on the resting state network. Interestingly, *dream*$^{-/-}$ mice after nor-BNI injection show highly similar networks before the heat stimulation. However, they had an increase of the number of connections (Fig.2b) as well as in connectivity strength leading to a higher r-values (Fig. 2a) after heat stimulation. The stimulation increased the efficacy of the resting state network. Further analysis may reveal structure specific effects. Additionally more significant connections can be observed in *dream*$^{-/-}$ mice before heat stimulation compared to wild-type mice indicating an influence of this genetic modification on the resting state network.

Discussion and Conclusion: Resting state networks can be demonstrated in wild-type and (transgenic) mice. They differ according to physiological (before and after nociceptive stimulation) and genetic background (*dream*$^{-/-}$). Especially nociceptive sensations, novel for *dream*$^{-/-}$ mice after nor-BNI injection, increase the connectivity of pain related brain structures during rest. These results demonstrate the usefulness of non invasive fMRI in transgenic mice and molecular defined pharmacological intervention to further investigation of functional connectivity in pain research.



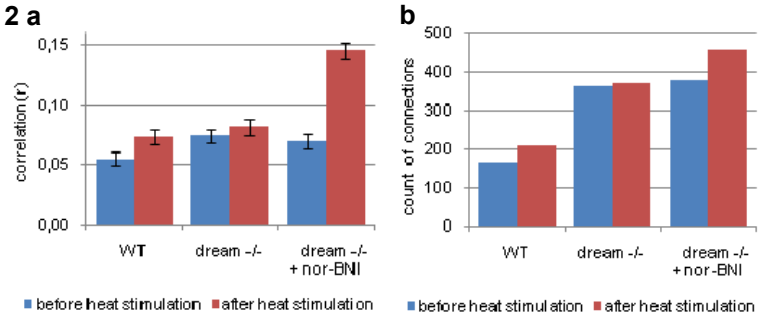


Fig.1: Averaged correlation matrices. **Fig. 2a:** average correlation strength ± SEM, **Fig.2b:** Number of connections above 80 % probability across animals

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