

Investigation of Pharmacological Manipulation on Brain Connectivity in Rats and Humans for Improvement of Drug Development

The aim of the current PhD project was to investigate the influence of pharmacological manipulations on brain connectivity with the overall goal to advance the discovery of new pharmaceutical drugs for treating mental disorders. Psychoactive drugs may be used as pharmacological models of mental disorder in animals, and the effect of potential new treatments may be tested in animals using biomarkers (surrogate measures) of disease states. Biomarkers could be based on measures of brain connectivity in neurophysiological signals including local field potentials (LFP), electrocorticograms (ECoG), electroencephalography (EEG), and magnetoencephalography (MEG) and should be translational. A translational biomarker can be used and interpreted similarly in humans and test animals.

Main focuses of the project were on the effects of ketamine on brain connectivity and on the translation of drug-effects between animal and human studies. Ketamine has traditionally been used in research to model symptoms of schizophrenia in both animals and humans besides clinical uses of ketamine as an anesthetic and analgesic. More recently, ketamine was discovered to produce antidepressant effect in treatment-resistant unipolar and bipolar depressed patients. As ketamine has undesirable side effects, extensive research has been made to discover the mechanisms underlying the antidepressant effect. The neuronal network activity of the brain is commonly investigated in terms of frequency content and cross-frequency-couplings of neurophysiological signals, both of which have been found altered after ketamine administration. In rats, one of the most pronounced effects of ketamine is observed in the range of high frequency oscillations (HFO). It has not previously been investigated whether ketamine also induced HFO in humans though the induced HFO may reflect disease-relevant alterations in brain activity. In humans, ketamine has shown highly consistent effects on frequency content across studies, however, in rats many conflicting findings have been made in the lower frequency bands. This could indicate that influential factors are not adequately accounted for in the rat studies. During recording, movement is generally avoided in human studies while rats are freely moving. Locomotor activity in the rat has been found to largely affect the frequency content of electrophysiological recordings and could thus 1) influence the reproducibility of research findings and 2) reduce the validity of animal studies for predicting treatment efficacy in humans. The overall objective of this PhD project was to investigate the influence of psychoactive drugs on brain connectivity measures in rats and humans with a particular focus on the translational value of the ketamine model of schizophrenia and biomarker potential. The goal was achieved through two studies. In the first study, an algorithm was developed to detect states of activity and inactivity of the rat. The detection method was used to assess effects of a broad range of commonly investigated drugs on electrophysiological recordings specifically in each state. Locomotion had large impact on the frequency content of the signals, and several drug-effects were found state-dependent. Ketamine-induced delta (1-4 Hz) activity, forming part of the evidence for the N-methyl-D-aspartate receptor (NMDAR) hypofunction hypothesis of schizophrenia (de la Salle et al., 2016), was found related to locomotor activity in the rat. Importantly, HFO was found induced by ketamine in both states, suggesting that locomotion-independent mechanisms underlie the profound HFO increase. Finally, ketamine-induced changes in the power spectrum of neurophysiological recordings were more similar to those found in humans when signals were obtained specifically during rat inactivity. In the second study, effects of ketamine in humans were investigated to reveal changes in frequency content up to 150 Hz and in phase-amplitude coupling (PAC). The study was based on magnetoencephalographic recordings from healthy subjects from

which estimates of neurophysiological activity throughout the brain were obtained using beamforming methods (spatial filtering). Ketamine was found to increase activity above 100 Hz also in humans. The change occurred as a broadband power increase with no clear indications of a peak. The HFO increase in the temporal pole (showing a unique pattern in the power spectrum) was found to correlate with PANSS ratings of depression with $p=0.043$. Ketamine was furthermore found to induce PAC between low beta activity (14 Hz) and broadband gamma activity (55-135 Hz) most pronounced in supramarginal and temporal gyri. Collectively, the current PhD project has resulted in the exemplification that taking rat locomotor activity into account in preclinical pharmac-EEG studies may 1) affect research conclusions and 2) enhance the validity of preclinical studies in predicting effects in humans. Responses to ketamine treatment were found most similar in humans and rats when recordings in rats were obtained during inactivity. For the first time, ketamine was found to induce HFO in humans, previously only shown in rats. Results indicated an association between ketamine-induced HFO in the temporal pole and induced depressive symptoms suggesting that the biomarker potential of HFO should be further investigated e.g. in depressed patients. The discovered coupling of high gamma activity to specific phases of low beta activity induced by ketamine should be investigated in future studies to assess the replicability of the finding.

Following two publications/manuscripts form part of the PhD-thesis:

Pharmac-Electroencephalographic Responses in the Rat Differ Between Active and Inactive Locomotor States

Ingeborg H. Hansen, Claus Agerskov, Lars Arvastson, Jesper F. Bastlund, Helge B. D. Sørensen, Kjartan F. Herrik

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The Effects of Ketamine on High-Frequency Oscillations (HFO, 100-150 Hz) and Cross-Frequency Coupling in Humans: A MEG study

Ingeborg H. Hansen, Tineke Grent-'T-Jong, Frederic Roux, Davide Rivolta, Tonio Heidegger, Michael Wibral, Wolf Singer, Andreas Sauer, Bertram Scheller, Kjartan F. Herrik, Helge B. D. Sørensen, Jesper F. Bastlund, Peter Uhlhaas.

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