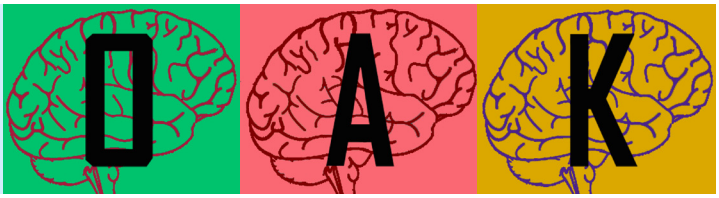

Trine Gjerløff

Visualising Parasympathetic Denervation in Parkinson's Disease - [11C]donepezil PET for Imaging Acetylcholinesterase Density in Peripheral Organs

Department of Nuclear Medicine and PET Centre, Aarhus University Hospital

Aim: Imaging of cholinergic processes outside the brain has received little attention. However, parasympathetic denervation is present in several disorders, including diabetes and Parkinson's disease (PD). Symptoms of parasympathetic dysfunction are often present in patients with PD. No *in vivo* methods for imaging the parasympathetic nervous system are currently available. We aimed to validate [11C]donepezil for imaging peripheral parasympathetic innervation in PD.

Materials and Methods: 12 PD patients were PET/CT scanned using [11C]donepezil and compared to 10 matched control subjects. Functional parasympathetic deficits were evaluated using gastric scintigraphies, salivary flow, HRV measurements, and questionnaires.

Preliminary results: The PD patients displayed a highly significant decrease in [11C]donepezil SUV values in the small intestines ($p=0.0007$) and pancreas ($p=0.0025$). Furthermore, patients with PD showed a trend towards decreased SUV values in myocardium ($p<0.2$) and salivary glands ($p<0.25$).
Conclusions: Our data suggests, that [11C]donepezil may be the first successful tracer for imaging parasympathetic deficit. Patients with PD exhibited significantly decreased PET signal in intestines and pancreas. A recent, influential hypothesis postulates that parasympathetic denervation could be present already at the pre-motor stage of PD. To examine this hypothesis, we are currently initiating further studies in *de novo*, untreated PD patients and patients with RBD.

Freja Bergholt Sørensen, Katrine Vogt Christensen, Lotte Vestergård and Anders Olsen

p25α Induces Neurodegeneration in C. elegans

Aarhus University, Department of Molecular Biology and Genetics, Gustav Wieds Vej 10, 8000 Aarhus C, Denmark

The brain specific protein Tubulin Polymerization Promoting Protein (p25α) was recently found to be a component of the Lewy bodies in PD patients and studies suggest potential influence of p25α in α-synuclein aggregation. To illuminate the possibility of p25α inducing neurodegeneration, we created a transgenic C. *elegans* worm expressing p25α::GFP under the dopamine transporter (*dat-1*) promoter. Interestingly, over-expression of p25α in the dopaminergic neurons changes the shape of soma from round and well defined to elongated and finally completely vanished in an age depended manner.

To find suppressors of p25α induced neurodegeneration, we performed an ethyl methanesulfonate (EMS) mutagenesis screen. EMS predominantly induces point mutations. A total of half a million F2 worms were screened and those with round and well defined neuron bodies were transferred to fresh plates. This way we identified 42 mutants suppressing the p25α-induced phenotype. For 7 of these mutants strong rescue was also observed in their progeny and after backcrossing to the parental strain. Western blot analysis revealed that these mutants express p25α at levels similar to the parental strain and genetic mapping showed that p25α is not mutated in these suppressor mutants. We are using next-generation sequencing to identify the mutated genes and transgenic rescue will be used to verify that the genes are indeed suppressing p25α.

We expect that the gene identities will teach us more about the molecular mechanisms underlying p25α induced neurodegeneration.

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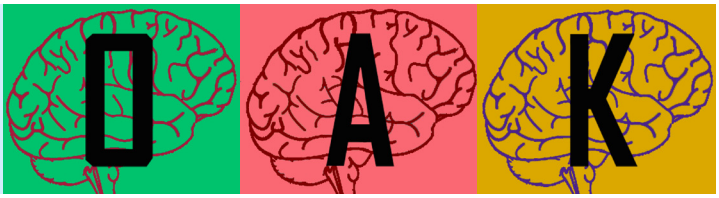

Jenny-A. Phan[1,2], Anne M. Landau[2,3], Albert Gjedde[2,3,4], Marina Romero-Ramos[1]

Neurodegeneration in an alpha-synuclein model of Parkinson's disease determined by PET imaging

1. Department of Biomedicine, Aarhus University
2. Department of Nuclear Medicine and PET Centre, Aarhus University Hospital
3. Center of Functionally Integrative Neuroscience, Aarhus University, Denmark
4. Department of Neuroscience and Pharmacology, University of Copenhagen, Denmark

Introduction
Parkinson's disease (PD) is a neurodegenerative disorder characterized by the accumulation of α-synuclein (α-syn), dopaminergic neuron death in substantia nigra (SN) and consequent decline of striatal dopamine. Here we investigate the early signs of degeneration in response to α-syn overexpression in the rat nigrostriatal system using *in vivo* PET imaging. PD was modeled by unilateral overexpression of the human wild type α-syn via recombinant adeno-associated viral vectors injected into the SN. In a parallel group, green fluorescent protein (GFP) was overexpressed as control. At 12 weeks after injection, dopaminergic cell death in SN was not observed by immunohistochemistry. However, clear accumulation of α-syn and dystrophic neurites were found in striatum. The early consequences of α-syn were addressed by PET imaging using a marker of the vesicular monoamine transporter-2, [^{11}C]DTBZ. To confirm *in vivo* data, *in vitro* autoradiography was carried out with [^{3}H]DTBZ. The degeneration of striatal dopaminergic projections was quantified as the binding in lesioned relative to intact side. At 12 weeks, the binding potential in the α-syn lesioned side of striatum was significantly lower than in the contralateral intact side (83±5% n=8) with no significant difference in GFP controls (98±7% n=5). Furthermore, the PET data was positively correlated with autoradiography (r^2=0.868, p=0.02). The data demonstrate that disruption of striatal projections occurs in response to α-syn overexpression.

Justyna Okarmus and Jan Bert Gramsbergen

Levodopa-induced dyskinesia in 6-hydroxydopamine lesioned rats – prevention and prediction

Department of Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark (SDU)

Levodopa (L-DOPA) is the most effective drug in the treatment of Parkinson's disease (PD), but after prolonged use, the majority of patients develop levodopa-induced dyskinesia (LID), highly disabling involuntary movements. As LID is associated with supersensitivity of dopamine D1 receptors, we studied whether early compensation of dopamine loss by early start of L-DOPA/benserazide treatment in 6-hydroxydopamine (6-OHDA) lesioned rats could delay onset of LID. Early treatment (from day 2-42 after 6-OHDA) postponed the onset and reduced severity of LID as compared to delayed treatment (from day 21-42 days after 6-OHDA), assessed 3, 4, 5 and 6 weeks after 6-OHDA lesioning. LID has also been associated with increased activation of mammalian target of rapamycin (mTOR) and therefore we studied the effect of rapamycine (inhibitor of mTOR) on LID. One week of co-treatment with rapamycine (15 mg/kg/day ip) could partly suppress established LID. Furthermore, we collected CSF from dyskinetic and non-dyskinetic rats for HPLC analysis of L-DOPA and dopamine metabolites and for analysis of total and phosphorylated extracellular signal-regulated kinase (ERK1/2) using a multispot assay from Mesoscale. We found significantly increased HVA/DOPA ratios and increased %phospho-ERK ratios in dyskinetic rats. These are promising results for future biomarker studies in CSF from PD patients.

Acknowledgment: This study is supported by Region Syddanmark and Dansk Parkinsonforening.

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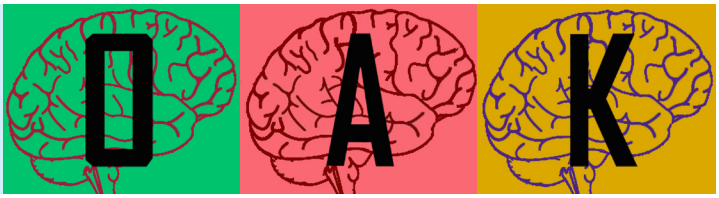

Thea Pinholt Lillethorup, Peter Iversen, Doris Doudet, Gregers Wegener, Anne M. Landau
Electroconvulsive Therapy Decreases α2A-adrenoceptor Levels in a Rat Model of Depression

Center of Functionally Integrative Neuroscience, Aarhus University/PET Center, Aarhus University Hospital

Electroconvulsive Therapy (ECT) is the most effective short-term treatment for severe depression. The exact therapeutic mechanism of action of ECT remains unresolved and we therefore tested the hypothesis that the beneficial effect of ECT in part could be the result of increased noradrenergic neurotransmission leading to a decrease in α_{2A} receptor binding.

Depressed (Flinders Sensitive Line (FSL)) and control (Sprague-Dawley (SD)) rats were treated with chronic ECT or sham-treatment for 10 days before brains were removed and cut into 20 µm thick sections. Density of α_{2A} was measured by quantitative autoradiography in 6 different brain regions using the α_{2A}-adrenoceptor antagonist, [^{3}H]RX 821002.

Four out of six regions showed no difference in α_{2A} levels between the sham treated FSL rats and the sham treated control rats, while two cortical regions gave a significant higher binding in the FSL rats compared with the controls, consistent with studies on brains from suicide victims showing increase of the cortical α_{2A} compared with age-matched controls.

Moreover, in both FSL and SD rats, we found an overall decrease in α_{2A} levels in the ECT treated rats compared with the sham treated rats suggesting that reduced α_{2A} density could be a potential therapeutic mechanism of action of ECT. These data suggest an importance of noradrenaline and the α_{2A}-adrenoceptor in depression and in the mechanism of antidepressant treatments.

Simon Bøggild[1,2,3], Simon Mølgaard[1,2,3], Simon Glerup[2,3], Jens R Nyengaard[1,2]
Investigation of the spatiotemporal expression patterns of Sortilin and SorCS2 in the murine hippocampal GABAergic system

1. Stereology and EM Laboratory, Aarhus University, Aarhus, Denmark
2. MIND Centre, Aarhus University, Aarhus, Denmark
3. Dept of Biomedicine, Aarhus University, Aarhus, Denmark

Sortilin and SorCS2 belong to the VPS10p-domain receptor family. They have been shown to serve widespread functions in different parts of the central nervous system (CNS) most notably in neurotrophin trafficking and signalling. Neurotrophins play an important role in an intact gamma-amino-butyric acid (GABA) system, the main source of inhibition in the CNS, but the possible roles of Sortilin and SorCS2 in this have not been investigated. Here we show that both receptors are expressed in the GABAergic system of the mouse hippocampus. Sortilin is highly expressed in embryonic structures of importance to GABAergic interneuron formation and migration, where SorCS2 is first expressed in the hippocampus postnatally. Hippocampal SorCS2(-/-) interneurons show defects in postnatal differentiation with a failure to increase complexity and GABAergic synapse number in response to brain derived neurotrophic factor (BDNF). Furthermore SorCS2(-/-) mice show increased susceptibility to seizures induced by the GABAA-receptor antagonist pentylenetetrazol with earlier onset of clonic and tonic seizures compared to C57bl/6j wildtype mice. In conclusion, both Sortilin and SorCS2 are likely to play a role in an appropriately functioning GABAergic system, which will be elaborated upon with future studies.

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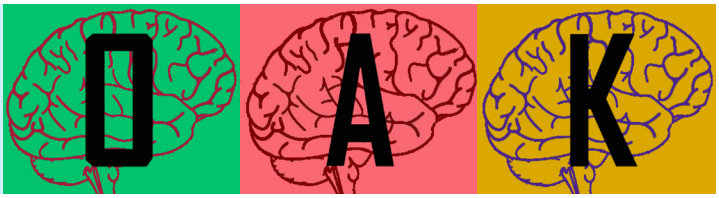

Freja Bertelsen[1,2], Davide Folloni[1], Annie Landau[1,2], Pia Weikop[3], Arne Møller[1,2], Jørgen Scheel-Krüger[1].

The Valproate Animal Model of Autism

1. Center of Functionally Integrative Neuroscience (CFIN), Aarhus University Hospital
2. PET-Center, Aarhus University Hospital
3. Laboratory of Neuropsychiatry, Psychiatric Centre Copenhagen

In the human clinic, prenatal exposure to the antiepileptic drug Valproate (VPA) is associated with Autism Spectrum Disorders. Our behavioural and biochemical rat model for autism based on prenatal, subchronic, clinical relevant doses of VPA exposure is presented.

Pregnant rats were exposed to daily IP injections of VPA (20, 60 and 100mg/kg) or saline from the 12th day of pregnancy until birth. The offspring were studied for alterations in behaviour and changes in the serotonin and oxytocin system of the brain.

The pups treated with 20 mg VPA/kg/day expressed significantly less play behaviour compared to the vehicle-treated animals and the pups receiving 100 mg VPA/kg/day ($p<0.05$). In line with the behavioral data, pups from the low VPA group had significantly lower levels of 5-HT in the striatum ($p<0.01$) compared with the other groups, a finding consistent with the involvement of serotonin in social play behavior. Furthermore the oxytocin receptor binding was descreased in amygdala ($p<0.05$). Data from the 60 mg/kg group are still under analysis.
The combination of behavioral and biochemical studies is necessary in the characterization and development of a novel model of autism. All parameters investigated here are relevant to the human condition and reinforce the use of the offspring of subchronic VPA-treated rats as a model of human autism.

M. M. Skovborg[1], F. Bertelsen[1,2], D. Follini[1], P. Weikop[3], J. Scheel-Krüger[1], A. Møller[1,2] and A. M. Landau[1,2]

Reduced striatal 5-HT_{2A} receptor but not serotonin transporter levels in the subchronic valproate model

1. Center of Functionally Integrative Neuroscience, Aarhus University, Denmark
2. PET-Center, Aarhus University Hospital, Denmark
3. Laboratory of Neuropsychiatry, Psychiatric Centre Copenhagen, Denmark

Alterations in the serotonin system are often detected in Autism Spectrum Disorder (ASD). We have previously found a decrease of 5-HT in striatum in our subchronic valproate (VPA) model of autism, and in light of this finding we want to further investigate the serotonin system focusing on the $5\text{-}HT^{2A}$ receptor and the serotonin transporter (SERT).

Pregnant Wistar rats were treated with VPA (20 or 100 mg/kg) or saline from day 12 until the end of pregnancy. Brains from the male offspring (n=7/group) were removed and fresh frozen at postnatal day 50 and then sliced into 20 µm thick sections. In vitro autoradiography was done using [^{3}H]Ketanserin and [^{3}H]DASB to asses 5-HT_{2A} receptors and SERT respectively.

The 5-HT_{2A} receptor binding was significantly decreased in dorsolateral and ventrolateral striatum in rats prenatally exposed to VPA compared to saline controls ($p<0.05$ and $p<0.01$ respectively). However, VPA did not induce changes in striatal [^{3}H]DASB binding.

The lower 5-HT_{2A} receptor binding combined with reduced levels of 5-HT in striatum indicate a down-regulation of the serotonin system in the VPA-exposed rats consistent with imaging studies in human in which 5-HT_{2A} receptor levels are altered. The changes at the receptor and not the transporter level in our study may suggest changes in serotonin metabolism and release coupled to 5-HT_{2A} receptor regulations.

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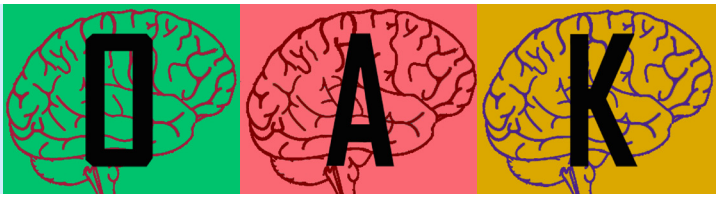


Thao Phuong Tran, Helle Lyng Christensen, Freja Bertelsen, Arne Møller and Ove Wiborg Cognitive assessment of depressed rats; iPADs for rodents

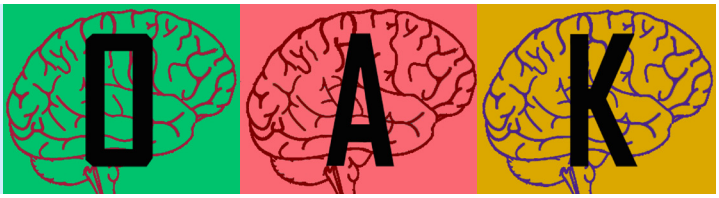
Translational Neuropsychiatry Unit, Dept of Clinical Medicine, AU

Major depressive disorder (MDD) is a disabling disease associated with significant mortality and health cost. Some of the main symptoms of MDD are anhedonia (loss of general interest) and devastating cognitive impairments or distortions. To identify new therapeutic targets, the focus is on cognitive dysfunctions like: 1) a cognitive bias or selective preference for negative inputs 2) cognitive deficits, which include impairments in attention, short-term memory and executive functioning. This project focuses on cognitive impairments associated with Chronic Mild Stress (CMS) induced anhedonia. After chronically exposure of rats to unpredictable stressors some of them will, like humans, become anhedonic, while others are able at coping and termed resilient. To assess the cognitive performance of the rats we use the Touch Screen Operant Platform which is highly similar to the human cognitive tests. One of the main tasks is Pairwise Discrimination (PD) learning and reversal. During the PD task, the rats are scored for their ability to acquire new associations (picture A=reward, picture B=punishment), functions known to depend upon the orbitofrontal region of prefrontal cortex and interconnected subcortical areas. After the task has been acquired, the rats have to do reversed learning (picture B=reward, picture A=punishment), addressing cognitive flexibility. With this project, we aim at getting new insights into cognitive impairments associated with stress-induced depression.

M. M. Busck[1,4], J. R. Nyengaard[2], B. Hansen[1], L.M. Rydtoft, J. Scheel-Krüger[1], O. Wiborg[5], A. Møller[1,3] The effect of chronic mild stress on pyramidal cell number in four subfields of the rat hippocampus – a stereology and MR spectroscopy study on the CMS model of depression

1. Center of Functionally Integrative Neuroscience (CFIN)
2. Stereology and EM Lab.
3. PET centre, Aarhus University Hospital, Aarhus, Denmark
4. Zoophysiology, Aarhus University, Aarhus, Denmark
5. Dept. of clinical medicine, Aarhus University, Risskov, Denmark

Depression is known to cause hippocampal atrophy. MRI studies have shown a marked decrease in hippocampal volume in humans following severe depression, and studies on rats exposed to chronic mild stress (a validated model of depression), have shown reduced neurogenesis in the dentate gyrus, and alterations in hippocampal shape, kurtosis parametres and metabolism. Remarkably, CMS resilient rats differ in several of these parameters from both CMS sensitive and non-stressed control rats. The aim of this study is to use stereology and magnetic resonance spectroscopy (MRS) to further elucidate the differential effect of chronic mild stress on the rat hippocampus. Using the optical fractionator the pyramidal neuron number is estimated in four subdivisions of CA and compared between the three experimental groups: CMS sensitive, CMS resilient and non-stressed controls. Neuron number estimates will then be compared to local concentrations of the neuronal marker N-acetyl-aspartate (NAA) as measured by MRS. A positive correlation could provide a non-invasive method for measuring neuronal densities, and thus allow longitudinal studies into the effect of stress and the mechanism of resilience. Preliminary stereology data shows approximately 20% more neurons in the ventral CA1 of the resilient rats compared to the other groups.



Nadja Bredo Rasmussen

Serotonin Receptor Alterations in a transgenic Mouse Model overexpressing human Alpha-synuclein and in post-mortem Frontal Cortex of Parkinson Disease patients

1. Research Laboratory for Stereology and Neuroscience, Bispebjerg University Hospital
2. Institute for Neuroscience and Pharmacology, Copenhagen University
3. Neuropsychiatric Laboratory, Rigshospitalet University Hospital
4. Lundbeck A/S, Otiliavej 9, Copenhagen, Denmark

Non-motor symptoms, e.g. executive dysfunction, have become a more prominent complication to Parkinson's Disease (PD). The serotonin system plays an important role in executive function through regulation of frontal cortex activation. In this study we want to look for serotonin receptor alterations in frontal cortex regions in a) a recognised PD human alpha-synuclein overexpression transgenic mouse model and in b) post-mortem brain tissue of PD patients.

We will focus primarily on the 5-HT2A receptor. In transgenic mice, changes in receptor functionality will be estimated by quantifying head-twitch-response after activation of the 5-HT2A receptor. Then, region-specific binding levels will be assessed by autoradiography using the [^{3}H]-MDL100.907 and [^{3}H]-WAY100.635 ligands specific for 5-HT2A and 5-HT1A receptors respectively, as an imbalance of the two affects 5-HT2A receptor output. Gene expression variances of both receptors will be investigated by qPCR. In the human tissue, differences will be assessed by in vitro receptor binding studies.

The first results show significantly lower 5-HT1A binding levels in Claustrum and significantly lower 5-HT2A binding levels in Frontal Association Cortex in the transgenic mice. Also, preliminary results suggest a difference in 5-HT2A receptor affinity and density in the Parkinson brains.
Our results point towards an involvement of the serotonergic system in PD that could be directly associated with the neuropathology behind this disease.

Carsten Tue Berg, Reza Khorooshi, Nasrin Asgari, Trevor Owens

Mechanisms underlying induction of antibody-mediated demyelination in MS

Department of Neurobiology Research, Institute for Molecular Medicine, University of Southern Denmark

The aim of this study is to investigate the role of antibody-mediated demyelination in Multiple Sclerosis (MS). Immunoglobulin (Ig) and activated complement (C') deposition in lesions, oligoclonal IgG bands in cerebrospinal fluid, and B cells and antibody-producing plasma cells in meninges and perivascular space have all been shown in MS (Lassmann et al, 2007). Pathology can be transferred to mice by intrathecal injection of IgG from Neuromyelitis Optica (NMO) + human C' (Asgari et al, 2013). However we were unable to transfer pathology using a mouse Mab against myelin oligodendrocyte glycoprotein (MOG) + mouse C'.

We hypothesized that endogenous C' inhibitors block mouse C' and thereby prevent antibody pathogenicity. Results show that intra-corpus callosum injection of anti-MOG Mab + mouse C' induced dose-dependent demyelination that was significantly enhanced by co-injection of a blocking Mab against the C' regulator CD59. Demyelination was C'-dependent. Astrocyte response showed similar C' dependence but was not enhanced by anti-CD59. Endogenous C' regulation therefore inhibits action of mouse Mab + C' and can be overcome by anti-CD59. This opens up the possibility for testing pathogenicity of mouse or human antibodies in future studies.

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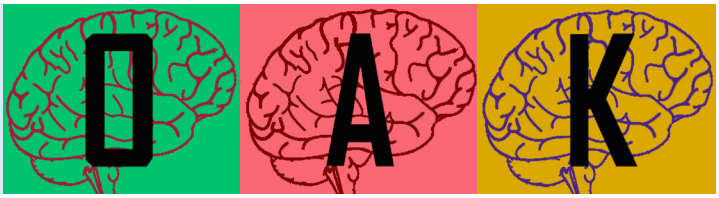

Camilla Hermansen[1,2], Reza Khorooshi[1], Ulrike M. Steckelings[2], Trevor Owens[1]

The involvement of the Angiotensin II type 2 receptor in Neuromyelitis Optica

1. Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark
2. Cardiovascular and Renal Research, Institute of Molecular Medicine, University of Southern Denmark

Neuromyelitis Optica (NMO) is an inflammatory demyelinating disease of the central nervous system. NMO involves IgG antibodies (NMO-IgG) directed against the water channel aquaporin 4 (AQP4) on astrocytes. The angiotensin AT2 receptor (AT2R) has been suggested to act anti-inflammatory, immune-modulatory and to have neuroprotective effects. Compound 21 (C21) is a non-peptide, orally active, agonist for the AT2R.

The aim of this project is to investigate the involvement of the AT2R in NMO. NMO-like pathology was compared between C57BL/6 and AT2R-deficient mice (AT2R-KO) by stereotactic intracerebral injection of NMO-IgG+human complement±C21. Mice then received C21 either by daily intraperitoneal injection, or by intrathecal injection at day 2. NMO-like pathology is assessed histologically by loss of AQP4, GFAP and myelin at 2, 4 and 7 days. We also measure expression of brain-derived-, glial cell-derived-, and ciliary neurotropic factors (BDNF, GDNF, CNTF) as well as CNTF-receptor, using qRT-PCR. Results show that whereas BDNF, GDNF and CNTF-receptor are down-regulated in the ipsilateral hemispheres after disease induction, CNTF is up-regulated. The project is still in progress and further results will be presented.

Katrine Tækker Jensen[1], Helle Hvilsted Nielsen[2], Manuela Grebing[1], Bettina Hjelm Clausen[1], Kate Lykke Lambertsen[1] and Bente Finsen[1]

T-cells effect post transcriptional regulation of TNF in areas of Wallerian degeneration

1. Department of Neurobiology, Institute of Molecular Medicine, University of Southern Denmark.
2. Department of Neurology, Odense University Hospital.

Multiple sclerosis is characterized by inflammation and demyelination leading to axonal degeneration. By combining an axonal lesion model with adoptive transfer of T-cells, we have previously shown that myelin basic protein specific T-cells enhance microglial activation in zones of axonal degeneration. In this study we report results showing that proteolipid protein specific T-cells (T_{PLP}) effect the transcriptional regulation of tumor necrosis factor (TNF) in microglial cells in zones of axonal degeneration.

Mice were prior to the axonal lesion adoptively transferred with 1) PLP specific T-cells (T_{PLP}), 2) ovalbumin specific T-cells (T_{OVA}), or 3) no T-cells. Mice were killed 2 and 7 days post lesion (dpl) and brains were isolated. Immunohistochemistry was used to detect T-cells and TNF protein, whereas *in situ* hybridization was used to detect TNF mRNA. q-PCR provided quantitative data and double immunofluorescent stainings were performed to locate TNF protein.

Results showed T-cell infiltration in T_{PLP} mice coinciding with an enhanced expression of TNF mRNA 2 and 7dpl. In the other groups of mice TNF mRNA was only expressed at 2dpl. Interestingly, expression of TNF protein was only observed in the TPLP mice, where TNF was shown to be expressed in CD11b+ microglia. These results indicate a role of autoreactive T-cells in the post transcriptional regulation of TNF expression in microglia, which is being further investigated.

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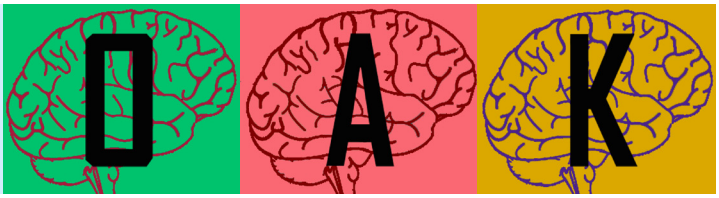

Fenger C[1], Thomassen M[2]*, Emery B[3]*, Kuhlmann T[4], Kruse T[2], and Finsen B[1].

Function of the Nkx2.2 transcription factor in oligodendrocytes and their progenitor cells

1. Neurobiology Research, University of Southern Denmark, Odense, Denmark
2. Human Microarray Centre, Odense University Hospital, Denmark
3. Centre of Neuroscience, University of Melbourne, Australia
4. Neuropathology, University Hospital of Münster, Germany.

*Contributed equally.

The importance of the Nkx2.2 transcription factor during oligodendrogenesis has been demonstrated by findings of impaired oligodendrogenesis in perinatal Nkx2.2 deficient mice. However, only few Nkx2.2 target genes have been identified so far in oligodendrocyte (Ol)-lineage cells. Our hypothesis is that Nkx2.2 promotes oligodendrogenesis and myelination of CNS by acting as activator and repressor depending on the presence of other transcription factors/co-factors at different stages of the oligodendrogenesis. To further elucidate the function of Nkx2.2, primary Nkx2.2 target genes were identified in murine Ol and their progenitors (OPC) by chromatin immunoprecipitation combined with DNA sequencing. Furthermore, the transcriptomes of OPC/Ol-containing CNS structures were investigated in postnatal Nkx2.2 deficient and wild type mice by microarray, qPCR, and in situ hybridization. In total, we identified 513 putative primary Nkx2.2 target genes in OPC/Ol. These genes included known target genes (Mbp, Pdgfra, Plp1, Sirt2) and several unknown genes with known or possible roles in cell division, myelination, myelin compaction, and axonal protection suggesting that Nkx2.2 regulates the expression of these genes in OPC/Ol. Since many of these genes appeared to be target genes in OPC and Ol, although they were not expressed at both developmental stages, the results of this study support the hypothesis that Nkx2.2 acts as activator and repressor at different stages of the oligodendrogenesis.

Pernille M. Madsen[1,2], David E. Szymkowski[3], John R. Bethea[4], Kate Lykke Lambertsen[2], and Roberta Brambilla[1]

Oligodendroglial TNFR2 promotes remyelination in experimental autoimmune encephalomyelitis

1. The Miami Project To Cure Paralysis, Miller School of Medicine, University of Miami, Miami, FL 33136, USA
2. Department of Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark
3. Xencor Inc., Monrovia, CA 91016, USA
4. Department of Biology, Drexel University, Philadelphia, PA 19104, USA.

Tumor necrosis factor (TNF) has been associated with the pathology of multiple sclerosis (MS), as MS patients have elevated concentrations of TNF in cerebrospinal fluid and active lesions. TNF exists in two forms, transmembrane (tmTNF) and soluble (solTNF), whose functions are mediated by TNFR1 and TNFR2. We and others have demonstrated that solTNF-TNFR1 signaling is detrimental in EAE/MS whereas tmTNF-TNFR2 signaling is protective and important for repair and remyelination. TNFR2 is expressed throughout the oligodendrocyte lineage and we therefore hypothesized that activation of TNFR2-dependent cascades in oligodendrocytes is associated with the protective functions of tmTNF in EAE. This hypothesis was tested with a genetic/pharmacological approach using oligodendrocyte-specific TNFR2 conditional KO (CNPcreTNFR2$^{fl/fl}$ mice) in combination with XPro1595 treatment, a selective solTNF inhibitor. Following EAE, CNPcreTNFR2$^{fl/fl}$ mice show a significantly worse clinical outcome compared to TNFR2$^{fl/fl}$ littermates, and did not improve by XPro1595 treatment, which suppresses EAE in TNFR2$^{fl/fl}$ mice. Furthermore, CNPcreTNFR2$^{fl/fl}$ mice have decreased axon and myelin preservation, as well as remyelination compared to TNFR2$^{fl/fl}$ mice. Collectively, our data demonstrate that TNFR2 activation in oligodendrocytes is key to the protective effect of tmTNF in EAE. A better understanding of how oligodendroglial TNFR2 signaling regulates remyelination may lead to new therapeutic strategies for MS.

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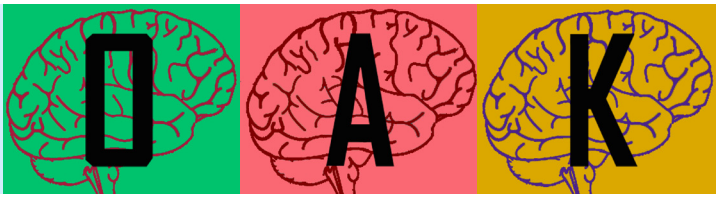


Trine Schütt[1], Mette Berendt[1], Lone Helboe[2] & Jan T. Pedersen[2]

Immunohistochemical characterization of Aβ and tau pathology in the brain from aged dogs with cognitive dysfunction and comparison to human patients with Alzheimer's disease.

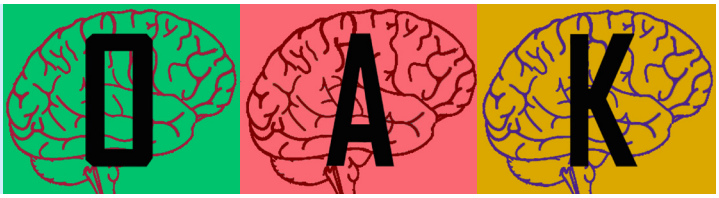
1. Department of Veterinary Clinical and Animal Science, Faculty of Health and Medical Sciences, 1870 Frederiksberg, Denmark
2. Neuroscience Drug Discovery, H. Lundbeck, 2500 Valby, Denmark

Introduction: In the aging canine brain Aβ plaques are of the diffuse subtype has been demonstrated, whereas neuritic plaques and neurofibrillary tangles (NFT), the hallmarks of AD, has not consistently been described in this species. Neurofibrillary tangles have never been detected with silver staining methods in the canine brain and immunohistochemical staining for tau has been described with conflicting results for some of the antibodies directed against human tau species.

Materials and methods: Paraffin sections from frontal cortex from canine and human brain samples were subjected to immunohistochemical staining for tau and Aβ pathology with the following primary antibodies; clone PC1C6, AT-8, 6E10 plus Gallyas silver staining.

Results: 4 types of plaque deposition can be demonstrated in the canine sections. Brain sections from frontal cortex and hippocampus from human patients with AD exhibited extensive evidence of tau pathology with both of the applied antibodies and with Gallyas silver stain. In the sections of frontal cortex from dogs, few neurons with heavy accumulation of hyperphosphorylated tau in the cell body and neurites could be found focally. These pathological changes could not be found in hippocampus or in the young dog. No NFT could be detected with the AT-8 antibody or with the Gallyas staining in the canine sections.

Conclusion: The brain sections from human AD patients had the typical pathological characteristics. In this study we found evidence of hyperphosphorylated tau but no NFT's in the canine sections. The changes in the aged dog brain seem to have a different phenotype with regard to tau pathology as NFT's cannot be demonstrated even though these dogs exhibit signs of cognitive dysfunction.



Mona El-Sayed (MSc. Humanbiology, PhD student)
The involvement of 5-HT2ARs activation in the neuronal projection from the amygdala to the ventral striatum in mice exposed to a novel environment

Research laboratory for Stereology and Neuroscience, Bispebjerg Hospital

Atypical antipsychotics have a high affinity for the serotonin 2A receptor (5-HT2AR) and activation of this receptor in animals has a hallucinogenic effect mediated by the medial prefrontal cortex (mPFC) in which the 5-HT2AR is highly expressed. Further, level changes, binding differences and functional dysregulation of PFC 5HT2ARs have been associated with psychiatric disorders. The PFC plays a major role in working memory, attention and decision-making and is activated following exposure to a novel environment. We have previously shown novelty-induced PFC activation to be 5-HT2A-dependent, and we want to further investigate the activation of striatal-projecting neurons from the amygdala, since the amygdala-striatum connectivity integrates emotion-regulation and the reward system in the process of emotion-based decision-making in the PFC. In order to label striatal-projecting neurons from the amygdala we injected a retrograde tracer (ChB) in the ventral striatum of all mice. To differentiate the mPFC-mediated effect from a local effect of ketanserin (5-HT2AR antagonist) on amygdala activation, an mPFC-lesioned control group was included. All mice were novelty-exposed following ketanserin or vehicle treatment. The brains were processed for immunohistochemical analysis and we will quantify c-Fos immunoreactivity (IR) (as a marker of neuronal activation) in neurons projecting from amygdala to striatum (ChB IR neurons) and c-Fos IR in ventral striatum. Analysis is in progress.

Marianne Sparre Lippert
Characterization and comparison of the inflammatory response in peripheral blood, cerebrospinal fluid, cervical lymph nodes and medulla of Lewis rats with EAE

MSc. at Flemming Fryd Johansen's group

Multiple sclerosis is a chronic, autoimmune disease affecting the central nervous system. The first sign of multiple sclerosis is accumulation of lymphocytes and fluid in the tissue which causes inflammation. The inflammation leads to demyelination, which eventually leads to paresis of the limbs. The animal model of human multiple sclerosis is called experimental autoimmune encephalomyelitis and is widely used in studies trying to understand multiple sclerosis and the inflammatory response during the attacks of the disease. In this study female Lewis rats are induced with experimental autoimmune encephalomyelitis through injections of myelin basic protein emulsified in incomplete Freund's adjuvant and additional mycobacteria. The inflammatory response in cerebrospinal fluid, blood and cervical lymph nodes are characterized via flow cytometry and compared with the inflammatory response in medulla in order to find, which kind of peripheral sample that is the most representative for the inflammatory response in the central nervous system. Samples are taken from the animals before induction of experimental autoimmune encephalomyelitis, just before the relapsing phase, at peak of disease and in the remission phase.

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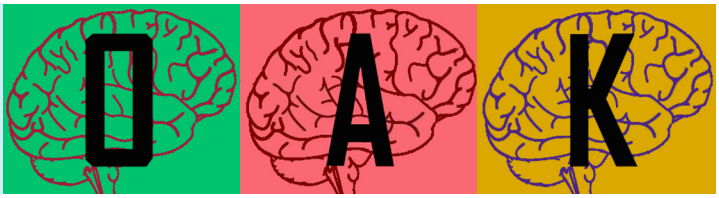

Eugenio Gutiérrez-Jiménez[1,3], Changsi Cai[1,3], Irene K. Mikkelsen[1,3], Nina K. Iversen[1,3], Peter Rasmussen[1,3], Kim Mouridsen[1,3], Mads Merrid[1],Leif Østergaard[1,2,3]

Capillary transit time heterogeneity in mice under electrical stimulation

1. CFIN, NeuroCampus Aarhus, Aarhus University,
2. Institute of Clinical Medicine, Aarhus University,
3. MIND*Lab*, NeuroCampus Aarhus, Aarhus University.

Introduction: Neurovascular coupling is a phenomenon that adjusts cerebral blood flow according to the brain metabolic needs. It is suggested that not only cerebral blood flow, but also capillary transit time heterogeneity (CTH) which originates from blood flow variations in the capillary network, can determines oxygen availability. We used two-photon microscopy (TPM) to measure changes in CTH under electrical stimulation.

Methods: Surgery was performed under isoflurane. Vital signs were monitored, catheters placed into femoral vessels, and mechanical ventilation was performed. A cranial window was be made over the S1 cortex and bolus tracking was performed by TPM. Three trials of baseline and electrical stimuli were performed. Boluses of dextran-dye were injected at second 15 of each scan. The transport function of capillary network between artery and vein was modeled as the γ-variate function. MTT was calculated as mean in the transport function; while CTH as standard deviation.

Results: In our preliminary analysis, electrical stimulation produced a reduction of 13.77% on MTT ($p=0.025$) and 23.59% on CTH ($p=0.006$) from artery to vein, which implies that capillary network can functionally adjust flow distribution in response to electrical and chemical stimulation.

Discussion: This study suggests a new dimension of neurovascular coupling – CTTH, which affects oxygen availability in capillary network. Neuronal activation will decrease CTTH and increase oxygen availability.

Nina Kerting Iversen

Acute cerebral ischemia and reperfusion injury: the role of capillary transit time heterogenity

CFIN, Institute of Clinical medicine, Aarhus University

Nitrite, which is naturally occurring in green vegetables and fruits, has been shown to significantly reduce infarct volume in animal stroke models. This effect, which have been shown to be very dose-specific, might be due to an improved capillary flow pattern, and there as enhanced tissue oxygen availability. However, despite the promising effect of nitrite, how it acts on single capillaries and the velocity of erythrocytes are unknown. Healthy male Sprague Dawley rats were anaesthetized with Hypnorm-Dormicum (1.8 ml/kg), intubated for normal ventilation, and blood pressure as well as arterial blood gasses were monitored throughout the experiment to ensure normal physiological state. A cranial window was made over the somatosensory cortex, and the animal was moved to a Two Photon Florescence Microscope. Line- and diameters scans was performed in rat single cerebral capillaries before and after injecting different doses of nitrite. We demonstrate a dose-dependent increase in both diameter and erythrocytes velocity. In the future, chosen doses of nitrite will be tested in rat middle cerebral artery occlusion stroke model by comparing changes in capillary flow patterns in penumbral tissue during ischemia and after reperfusion to parallel measurements of tissue oxygen tension. The results may improve our understanding of the pathophysiology of cerebral ischemia, and unveil new therapeutic strategies for its treatment.

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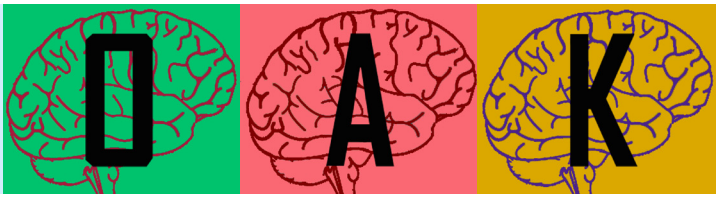


Nicole Elisabeth Carstens

Influence of statins on inflammatory response in stroke patients

BRIC

The aim of this Clinical trial is to examine the relationship between the T-cells' Rho GTPase, cytokines and stroke outcome – pre vs. post statin intake.

The Rho GTPase regulates cell adhesion and cell migration, which is part of the inflammatory response. Post stoke, an inflammatory response will occur. The size of this response seems to play and important role when patients are scored by the NIHSS, post stroke. When statins a taken pre stroke – the inflammatory response will decrease which at the end, will give the patients a better prognosis.

This can hopefully led us a step further in the search for a new treatment to stroke without time limitations.

Lotte Kellemann Kristensen[1], Andreas Ettrup Clemmensen[2], Bettina Hjelm Clausen[3], Carsten Haagen Nielsen[3], Andreas Kjær[3], Anders Bach[4], Kristian Strømgaard[4], Kate Lykke Lambertsen[3], Flemming Fryd Johansen[1]

Implications for PSD-95 inhibitors in stroke therapy

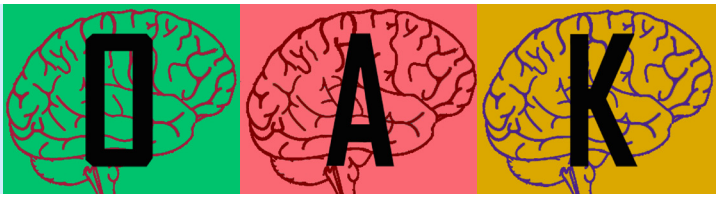
1. Department of Biomedical Sciences and Biotech Research & Innovation Centre (BRIC), University of Copenhagen
2. Cluster for Molecular Imaging, Rigshospitalet, University of Copenhagen
3. Department of Neurobiology Research, Institute of Molecular Medicine, University of Southern Denmark
4. Department of Drug Design and Pharmacology, University of Copenhagen.

Objectives Tat-N-dimer is a new promising drug in stroke therapy, targeting the interaction between postsynaptic density protein 95 (PSD-95) and neuronal nitric oxide synthase (nNOS) downstream of the *N*-methyl-D-aspartate receptor (NMDAR). We have previously shown a significant neuroprotective effect of Tat-N-dimer in the acute phase after experimental stroke (Bach et al., PNAS 2012, 109:3317), and with these extended studies we wish to evaluate the long-term outcome by magnetic resonance imaging (MRI).

Methods C57Bl/6 mice were subjected to permanent middle cerebral artery occlusion (pMCAO) and randomly assigned to receive either physiological saline or a 3 nmol/g Tat-N-dimer dose *i.v.* 30 min post-injury. Over a time-course of 28 days mice were exposed to repeated MRI at 6 hours, 48 hours, 7 days and 28 days after insult on a Bruker BioSpec 700/20 pre-clinical scanner. After ended scan a small group of mice were randomly selected for histological assessment of infarct volumes.

Results MRI revealed the evolution of brain damage caused by ischemia over 28 days with maximal lesion volumes at 6 and 48 hours, followed by a reduction towards 7 and 28 days. A single 30 min post surgery *i.v.* injection of Tat-N-dimer reduced lesion volumes at 6 hours. Despite a tendency towards beneficial effects of Tat-N-dimer beyond the acute phase, we did not identify any effect on the outcome at 48 hours, 7 days and 28 days. A correlation between infarct volumes obtained from histology and MR lesion volumes revealed T_2-weighted MRI as a powerful predictor of tissue destined to infarction.

Conclusions Our results indicate Tat-N-dimer as an efficacious neuroprotective agent in the very early events following cerebral ischemia.



Thomas Krøigård[1], Henrik Daa Schrøder[2], Camilla Qvortrup[3], Lise Eckhoff[3], Per Pfeiffer[3], David Gaist[1], and Søren H Sindrup[1]

Characterisation and diagnostic evaluation of chronic polyneuropathies induced by oxaliplatin and docetaxel comparing skin biopsy to quantitative sensory testing and nerve conduction studies

1. Department of Neurology, Odense University Hospital & Institute of Clinical Research, University of Southern Denmark
2. Department of Clinical Pathology, Odense University Hospital
3. Department of Oncology, Odense University Hospital.

Background: Chemotherapy-induced peripheral neuropathy (CIPN) negatively affects the quality of life for many patients treated with oxaliplatin or docetaxel for gastrointestinal cancer or breast cancer. Our objective was to characterise the neuropathies with regard to symptoms, neurological signs, and objective evidence of damage to peripheral nerves. We also compared the diagnostic values of skin biopsy, quantitative sensory testing (QST), and nerve conduction studies (NCS).

Methods: Patients complaining of neuropathy symptoms at least three months after completion of treatment with oxaliplatin (n=20) or docetaxel (n=20) were recruited from the Department of Oncology. Neuropathy scores were determined along with the intraepidermal nerve fibre density in skin biopsies from the leg, QST, and NCS.
Results: Clinically only sensory functions were affected. Both sensory and motor fibres were affected in the nerve conduction studies, showing predominantly signs of axonal damage. Mechanical detection threshold was most often affected in the quantitative sensory testing. NCS, QTS, and skin biopsy were abnormal in 11, 13, and 17 and 7, 11, and 15 of the oxaliplatin treated patients and docetaxel treated patients, respectively.

Conclusions: CIPN after oxaliplatin or docetaxel treatment is clinically a sensory, axonal neuropathy affecting only small nerve fibres in some patients. NCS are often normal, whereas QST and skin biopsy have a higher diagnostic sensitivity.

Mikkel Vestergaard Olesen

Neuronal alterations in the hippocampal following stress-induced depression and ECS

Forskningslaboratorium for Stereologi og Neuroscience, Bispebjerg Hospital

The neurobiological mechanisms underlying depression are not fully understood. In this study, we used a rat model of depression in combination with a clinically relevant schedule of electroconvulsive stimulation to study whether depression and/or electroconvulsive therapy induces structural changes in hippocampal subregions as well as alters behavior. Using a stereological counting method, the optical fractionator, and point-counting, we quantified the total number of newly formed neurons as a measure of neurogenesis in the subgranular zone as well as the total number of neurons and the volume of the hippocampal granule cell layer. Rat behavior was evaluated using the forced swim test. Our results show that chronic restraint stress induced depressive-like behavior, without a significant change in neurogenesis or changes in the total number of neurons. In contrast, electroconvulsive stimulation prevented stress-induced depression-like behavior and increased neurogenesis. The total number of neurons and granule cell layer volume was not affected by electroconvulsive stimulation. These results indicate that total cell numbers and volume in subregions of the hippocampus are not significantly altered by stress, whereas electroconvulsive stimulation induces a significant increase in neurogenesis.

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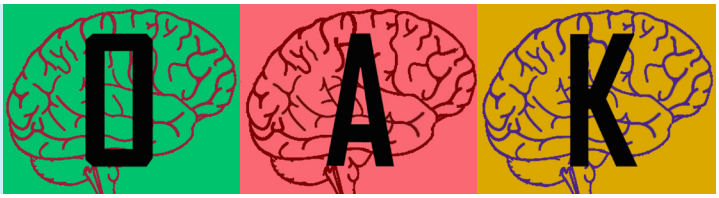

Maryam Ardalan[1,2], Gregers Wegener[2,4], Torsten M. Madsen[5], Jens R. Nyengaard[1,3]

Neuroplasticity Alteration in Hippocampus and Sustained Antidepressant Effect of Ketamine

1. Stereology and Electron Microscopy Laboratory, Aarhus University Hospital, Aarhus, Denmark
2. Translational Neuropsychiatry Unit, Aarhus University Hospital, Risskov, Denmark
3. Centre for Stochastic Geometry and Advanced Bioimaging (CSGB), Aarhus University, Aarhus, Denmark
4. Pharmaceutical Research Center of Excellence, School of Pharmacy (Pharmacology), North-West University, Potchefstroom, South Africa
5. Lundbeck A/S, International Clinical Research, Valby, Denmark

Background: Major Depressive Disorder (MDD) is a common medical impairment associated with a huge economic problem in public health. The glutamatergic system and the morphological basis related to neuronal plasticity hypothesis for a rapid and sustained antidepressant effect are principal components of novel antidepressant therapeutics. In this study, the unbiased stereological methods are used to test neuronal plasticity hypothesis in hippocampus (neuronal and synaptic plasticity) as one of the main underlying mechanisms for sustained anti-depressive effect of Ketamine.

Material & Method: Flinders Sensitive Line rats (a highly validated genetic animal model of depression) and Flinders Resistant Line rats are selected. A single intraperitoneal injection of Ketamine (15mg/kg) or saline is given to the rats and then euthanized and perfused 7 days after treatment. Hippocampus is sectioned on a vibratome perpendicular to its longest axis at a thickness of 65-µm. Two sets of sections are chosen based on a systematic sampling principle and a section sampling fraction of 1/12. One set was used for light microscopy (LM) to quantify the total number of neurons in dentate gyrus(DG) and the volume of total and subregions of hippocampus by using stereological methods. The second set of sections was used for counting synapse number and type of the synapses (perforated and non-perforated) in the CA1 stratum radiatum of rat hippocampus on electron microscopy (EM) by using physical disector method.

Results: The results showed that the volume of hippocampal subfields (CA1SR, GCL and molecular layer of DG) are larger in the FRL-Vehicle rats comparing to FSL-vehicle rats and FSL-Ket versus FSL-Vehicle rats($p<0.05$). Significantly more synapses were found in FSL-Ket rats in comparing with FSL-Veh rats. We found no significant changes in the number of perforated and shaft synapses between FSL-Ket and FSL-Veh groups, ($p>0.05$) while the number of non-perforated synapses was significantly higher in the FSL-Ket versus FSL-Veh group, ($p=0.01$). Significant sustained effect of ketamine on the enhancement of the number of neurons in DG of FSL rats was observed ($p=0.01$).

Conclusion: The neuroplasticity is one of the mechanisms underlying the sustained antidepressant effect of ketamine as a novel antidepressant drug by reversing the alteration of the volume, number of synapses and neurons in the hippocampus.

Key words: Depression, Ketamine, Neuronal Plasticity, Stereology, Synaptic Plasticity

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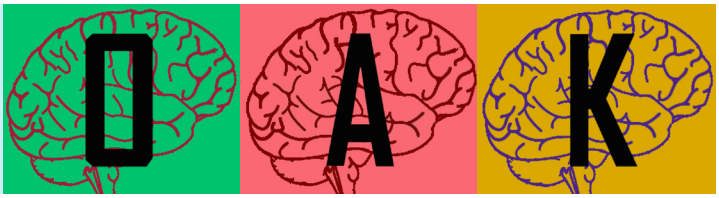

Ali Khalidan Vibholm

In vivo detection of glutamate NMDA ion channel activation - a GE179-PET study in epilepsy and Deep Brain Stimulation – from animal studies to human studies

Danish Neuroscience Centre and PET-Centre at Aarhus University Hospital

Late stage paroxysmal and degenerative neurological disorders e.g. Epilepsy and Parkinson's disease are known to be difficult to treat. Activation of ion-channels plays a major role in the mechanism of Deep Brain Stimulation (DBS) and in the detection of Fokal Epilepsy.

We will study Focal Epilepsy and DBS in Parkinsons Disease with the PET radioligand GE179.
In Epilepsy, we will validate GE179 as a marker for the affected brain region. Hereby visualising the affected site more precise before e.g. epilepsy-surgery.
In DBS, we want to investigate the NMDA ion-channel modulation - with GE179 as a detector.
We have developed both a rat-model and a porcine-model of focal epilepsy and DBS function.
Kindling one hippocampus with electrical stimulation visualizes side-differences in activity - either during spontaneous unilateral active seizures or seizures induced by DBS.
With DBS electrodes implanted - then turned on and off during PET scan, we might also consider the functional/ behavioural effects of High Frequency DBS on ion-channel activation in both the porcine model and in Parkinson's patients with a DBS implanted.

Patients with focal temporal or frontal lobe epilepsy will be scanned and studied. During the scan, we will measure the focal uptake of GE179, provided that it is possible to scan the patient during attack - either spontaneous or induced. Thus, we wish to validate GE179 as a biomarker and learn more about ion-channel activity.

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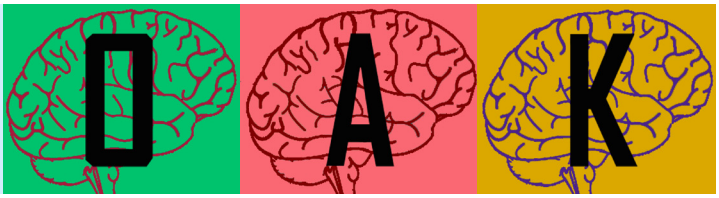

Nava N[1,2], Treccani G[2], Chen F[1,2], Liebenberg N[2], Elfving B[2], Müeller HK[2], Popoli M[3], Wegener G[2], Nyengaard JR[1]

Disconnecting and reconnecting brain: structural adaptations to stress

1. Stereology and Electron Microscopy Laboratory, Danish Neuroscience Centre, Aarhus, Denmark
2. Translational Neuropsychiatry Unit, Aarhus University, Denmark
3. Laboratory of Neuropsychopharmacology and Functional Neurogenomics, University of Milano, Italy

Stressful events are associated with increased risk of developing mood disorders, such as depression and anxiety. A number of reports have shown significant structural alterations in the prefrontal cortex (PFC), a limbic region mediating the stress response (McEwen and Morrison, 2013). Strong evidence for an association between stress and altered structural plasticity within PFC also derives from preclinical studies in animal models of stress. While a unidirectional negative influence of chronic stress has been repeatedly demonstrated, the effects of acute stress on mPFC structural remodeling and cognitive functions remain largely elusive. Thus, in the present study we aimed at evaluating 1) the immediate effects of acute stress on mPFC synaptic remodeling (including synapse and glutamate vesicle number) and mPFC-mediated cognitive functions 2) the time-dependent effects of acute stress on dendritic remodeling (including dendrite length and spines) and 3) how chronic antidepressant treatment affects any such changes. By mean of serial section electron microscopy, acute stress was found to rapidly induce *ex novo* sprouting of small synapses and increased number of docked vesicles. Chronic desipramine (DMI) partially prevented such stress-induced changes. When tested for mPFC-working memory function, animals showed short-term increased attention 5 h after stress, followed by impaired working-memory 24 h after stress cessation. Three-dimensional reconstruction of prelimbic pyramidal neurons showed stress-induced increased spine density 1 day after stress and significant apical dendritic atrophy 14 days after stress. DMI alone produced strong elaboration of the apical dendritic tree 7 days after treatment termination.

In conclusion, we found that the morphological correlates of synaptic strength potentiated up to 24 h after stress cessation, as shown by increased synapse and vesicle number, and spine density. These effects were followed by a long-term negative influence of acute stress, as shown by dendritic retraction and atrophy 14 days after stress. In addition, a preventive role of DMI on stress-induced structural plasticity and, in turn, over-excitation was identified.

References: McEwen BS, Morrison JH (2013) The brain on stress: vulnerability and plasticity of the prefrontal cortex over the life course. Neuron 79:16-29.