Peer reviewed publications


Abstract: Multiple sclerosis (MS) is an inflammatory demyelinating disease of the CNS. Activated microglia/macrophages play a key role in the immunopathogenesis of MS and its corresponding animal models, experimental autoimmune encephalomyelitis (EAE). Microglia activation begins at early stages of the disease and is associated with elevated expression of the 18 kDa mitochondrial translocator protein (TSPO). Thus, positron emission tomography (PET) imaging of microglial activation using TSPO-specific radioligands could be valuable for monitoring disease-associated neuroinflammatory processes. EAE was induced in rats using a fragment of myelin basic protein, yielding acute clinical disease that reflects extensive spinal cord inflammation. Enhanced TSPO expression in spinal cords of EAE rats versus those of controls was confirmed by Western blot and immunohistochemistry. Biodistribution studies in control and EAE rats were performed using the TSPO radioligand $[^{18}F]DPA-714$ [N,N-diethyl-2-(2-(4-(2-fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide]. At 1 h after injection, almost fivefold higher levels of $[^{18}F]DPA-714$ were measured in spinal cords of EAE rats versus controls. The specific binding of $[^{18}F]DPA-714$ to TSPO in spinal cords was confirmed in competition studies, using unlabeled (R,S)-PK11195 [(R,S)-N-methyl-N-(1-methylpropyl)-1-(2-chlorophenyl)isoquinoline-3-carboxamide] or DPA-714 in excess. MicroPET studies affirm that this differential radioactivity uptake in spinal cords of EAE versus control rats could be detected and quantified. Using $[^{18}F]DPA-714$, neuroinflammation in spinal cords of EAE-induced rats could be visualized by PET, offering a sensitive technique for monitoring neuroinflammatory lesions in the CNS and particularly in the spinal cord. In addition to current MRI protocols, this approach could provide molecular images of neuroinflammation for detection, monitoring, and research in MS.

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Abstract: Partitioning of polypeptides between protein folding and amyloid formation is of outstanding pathophysiological importance. Using yeast phosphoglycerate kinase as model, here we identify the features of the energy landscape that decide the fate of the protein: folding or amyloidogenesis. Structure formation was initiated from the acid-unfolded state, and monitored by fluorescence from 10 ms to 20 days. Solvent conditions were gradually shifted between folding and amyloidogenesis, and the properties of the energy landscape governing structure formation were reconstructed. A gradual transition of the energy landscape between folding and amyloid formation was observed. In the early steps of both folding and misfolding, the protein searches through a hierarchically structured energy landscape to form a molten globule in a few seconds. Depending on the
conditions, this intermediate either folds to the native state in a few minutes, or forms amyloid fibers in several days. As conditions are changed from folding to misfolding, the barrier separating the molten globule and native states increases, although the barrier to the amyloid does not change. In the meantime, the native state also becomes more unstable and the amyloid more stable. We conclude that the lower region of the energy landscape determines the final protein structure.


Abstract: PURPOSE: The type 2 cannabinoid receptor (CB2R) is part of the human endocannabinoid system and is involved in central and peripheral inflammatory processes. In vivo imaging of the CB2R would allow study of several (neuro)inflammatory disorders. In this study we have investigated the safety and tolerability of [11C]-NE40, a CB2R positron emission tomography (PET) ligand, in healthy human male subjects and determined its biodistribution and radiation dosimetry.

PROCEDURE: Six healthy male subjects (age 20-65 years) underwent a dynamic series of nine whole-body PET/CT scans for up to 140 min, after injection of an average bolus of 286 MBq of [11C]-NE40. Organ absorbed and total effective doses were calculated through OLINDA.

RESULTS: [11C]-NE40 showed high initial uptake in the spleen and a predominant hepatobiliary excretion. In the brain, rapid uptake and swift washout were seen. Organ absorbed doses were largest for the small intestine and liver, with 15.6 and 11.5 μGy/MBq, respectively. The mean effective dose was 3.64 ± 0.81 μSv/MBq. There were no changes with aging observed. No adverse events were encountered.

CONCLUSIONS: This first-in-man study of [11C]-NE40 showed an expected biodistribution compatible with lymphoid tissue uptake and appropriate fast brain kinetics in the healthy human brain, underscoring the potential of this tracer for further application in central and peripheral inflammation imaging. The effective dose is within the typical expected range for 11C ligands.


Abstract: On the one hand, the translocator protein (TSPO) radioligand N,N-diethyl-2-(2-(4-(2-(18)F-fluoroethoxy)phenyl)-5,7-dimethylpyrazolo[1,5-a]pyrimidin-3-yl)acetamide (18F-DPA-714) has been suggested to serve as an alternative radiotracer to image human glioma, and on the other hand the alkylphosphocholine erufosine (ErPC3) has been reported to induce apoptosis in otherwise highly apoptosis-resistant glioma cell lines. The induction of apoptosis by ErPC3 requires TSPO, a mitochondrial membrane protein highly
expressed in malignant gliomas. In this preclinical study, we monitored the effect of ErPC3 treatment in vivo using (18)F-DPA-714 PET.

METHODS: In vitro studies investigated the antitumor effect of ErPC3 in 9L rat gliosarcoma cells. In vivo, glioma-bearing rats were imaged with (18)F-DPA-714 for the time of treatment.

RESULTS: A significant decrease in 9L cell proliferation and viability and a significant increase in apoptosis and caspase-3 activation were demonstrated on ErPC3 treatment in cell culture. In the rat model, ErPC3 administration resulted in significant changes in (18)F-DPA-714 tumor uptake over the course of the treatment. Immunohistochemistry revealed reduced tumor volume and increased cell death in ErPC3-treated animals accompanied by infiltration of the tumor core by CD11b-positive microglia/macrophages and glial fibrillary acidic protein-positive astrocytes.

CONCLUSION: Our findings demonstrate a potent antitumor effect of ErPC3 in vitro, in vivo, and ex vivo. PET imaging of TSPO expression using (18)F-DPA-714 allows effective monitoring and quantification of disease progression and response to ErPC3 therapy in intracranial 9L gliomas.


Abstract: A novel method is presented to perform material map segmentation from preclinical MRI for corresponding PET attenuation correction. MRI does not provide attenuation ratio, hence segmenting a material map from it is challenging. Furthermore the MRI images often suffer from ghost artifacts. On the contrary MRI has no radiation dose. Our method operated with fast spin echo scout pairs that had perpendicular frequency directions. This way the direction of the ghost artifacts were perpendicular as well. Our body-air segmentation method built on this a priori information and successfully erased the ghost artifacts from the final binary mask. Visual and quantitative validation was performed by two preclinical specialists. Results indicate that our method is effective against MRI scout ghost artifacts and that PET attenuation correction based on MRI makes sense even on preclinical images.


Abstract: Two novel adamantane derivatives, adamantan-1-yl(1-pentyl-1H-indol-3-yl)methanone (AB-001) and N-(adamantan-1-yl)-1-pentyl-1H-indole-3-carboxamide (SDB-001), were recently identified as cannabimimetic indoles of abuse. Conflicting anecdotal reports of the psychoactivity of AB-001 in humans, and a complete dearth of information about the bioactivity of SDB-001, prompted the preparation of AB-001, SDB-001, and several analogues intended to explore preliminary structure-activity relationships within
this class. This study sought to elucidate which structural features of AB-001, SDB-001, and their analogues govern the cannabimimetic potency of these chemotypes in vitro and in vivo. All compounds showed similar full agonist profiles at CB1 (EC50 = 16-43 nM) and CB2 (EC50 = 29-216 nM) receptors in vitro using a FLIPR membrane potential assay, with the exception of SDB-002, which demonstrated partial agonist activity at CB2 receptors. The activity of AB-001, AB-002, and SDB-001 in rats was compared to that of Δ(9)-tetrahydrocannabinol (Δ(9)-THC) and cannabimimetic indole JWH-018 using biotelemetry. SDB-001 dose-dependently induced hypothermia and reduced heart rate (maximal dose 10 mg/kg) with potency comparable to that of Δ(9)-tetrahydrocannabinol (Δ(9)-THC, maximal dose 10 mg/kg), and lower than that of JWH-018 (maximal dose 3 mg/kg). Additionally, the changes in body temperature and heart rate affected by SDB-001 are of longer duration than those of Δ(9)-THC or JWH-018, suggesting a different pharmacokinetic profile. In contrast, AB-001, and its homologue, AB-002, did not produce significant hypothermic and bradycardic effects, even at relatively higher doses (up to 30 mg/kg), indicating greatly reduced potency compared to Δ(9)-THC, JWH-018, and SDB-001.


Abstract: Recent evidence highlights the peroxisome proliferator-activated receptors (PPARs) as critical neuroprotective factors in several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). To gain new mechanistic insights into the role of these receptors in the context of ALS, here we investigated how PPAR transcriptional activity varies in hSOD1(G93A) ALS transgenic mice. We demonstrate that PPARγ-driven transcription selectively increases in the spinal cord of symptomatic hSOD1(G93A) mice. This phenomenon correlates with the up-regulation of target genes, such as lipoprotein lipase and glutathione S-transferase α-2, which are implicated in scavenging lipid peroxidation by-products. Such events are associated with enhanced PPARγ immunoreactivity within motor neuronal nuclei. This observation, and the fact that PPARγ displays increased responsiveness in cultured hSOD1(G93A) motor neurons, points to a role for this receptor in neutralizing deleterious lipoperoxidation derivatives within the motor cells. Consistently, in both motor neuron-like cultures and animal models, we report that PPARγ is activated by lipid peroxidation end products, such as 4-hydroxynonenal, whose levels are elevated in the cerebrospinal fluid and spinal cord from ALS patients. We propose that the accumulation of critical concentrations of lipid peroxidation adducts during ALS progression leads to the activation of PPARγ in motor neurons. This in turn triggers self-protective mechanisms that involve the up-regulation of lipid detoxification enzymes, such as lipoprotein lipase and glutathione S-transferase α-2. Our findings indicate that anticipating natural protective reactions by pharmacologically modulating PPARγ transcriptional activity may attenuate neurodegeneration by limiting the damage induced by lipid peroxidation derivatives.

Abstract: PURPOSE: Neuroinflammation is involved in several brain disorders and can be monitored through expression of the translocator protein 18 kDa (TSPO) on activated microglia. In recent years, several new PET radioligands for TSPO have been evaluated in disease models. [(18)F]DPA-714 is a TSPO radiotracer with great promise; however results vary between different experimental models of neuroinflammation. To further examine the potential of [(18)F]DPA-714, it was compared directly to [(11)C]PK11195 in experimental cerebral ischemia in rats.

METHODS: Under anaesthesia, the middle cerebral artery of adult rats was occluded for 60 min using the filament model. Rats were allowed recovery for 5 to 7 days before one hour dynamic PET scans with [(11)C]PK11195 and/or [(18)F]DPA-714 under anaesthesia.

RESULTS: Uptake of [(11)C]PK11195 vs [(18)F]DPA-714 in the ischemic lesion was similar (core/contralateral ratio: 2.84±0.67 vs 2.28±0.34 respectively), but severity of the brain ischemia and hence ligand uptake in the lesion appeared to vary greatly between animals scanned with [(11)C]PK11195 or with [(18)F]DPA-714. To solve this issue of inter-individual variability, we performed a direct comparison of [(11)C]PK11195 and [(18)F]DPA-714 by scanning the same animals sequentially with both tracers within 24 h. In this direct comparison, the core/contralateral ratio (3.35±1.21 vs 4.66±2.50 for [(11)C]PK11195 vs [(18)F]DPA-714 respectively) showed a significantly better signal-to-noise ratio (1.6 (1.3-1.9, 95%CI) fold by linear regression) for [(18)F]DPA-714.

CONCLUSIONS: In a clinically relevant model of neuroinflammation, uptake for both radiotracers appeared to be similar at first, but a high variability was observed in our model. Therefore, to truly compare tracers in such models, we performed scans with both tracers in the same animals. By doing so, our result demonstrated that [(18)F]DPA-714 displayed a higher signal-to-noise ratio than [(11)C]PK11195. Our results suggest that, with the longer half-life of [(18)F] which facilitates distribution of the tracer across PET centre, [(18)F]DPA-714 is a good alternative for TSPO imaging.

METHODS: Twenty-three adult C57BL6 mice were scanned with [(18)F]FDG and [(18)F]FPECT. Registrations and affine spatial normalizations were performed using SPM8. [(18)F]FDG data were quantified using (1) an image-derived-input function obtained from the liver (cMRglc), using (2) standardized uptake values (SUVglc) corrected for blood glucose levels and by (3) normalizing counts to the whole-brain uptake. Parametric [(18)F]FPECT binding images were constructed by reference to the cerebellum. Registration accuracy was determined using random simulated misalignments and vectorial mismatch determination.

RESULTS: Registration accuracy was between 0.21-1.11 mm. Regional intersubject variabilities of cMRglc ranged from 15.4% to 19.2%, while test-retest values were between 5.0% and 13.0%. For [(18)F]FPECT uptake in the caudate-putamen, these values were 13.0% and 10.3%, respectively. Regional values of cMRglc positively correlated to SUVglc measured within the 45-60 min time frame (spearman r=0.71). Next, SPM analysis of 6-OHDA-lesioned mice showed hypometabolism in the bilateral caudate-putamen and cerebellum, and an unilateral striatal decrease in DAT availability.

CONCLUSION: MRM-based small-animal PET templates facilitate accurate assessment and spatial localization of mouse brain function using VOI or voxel-based analysis. Regional intersubject- and test-retest variations indicate that for these targets accuracy comparable to humans can be achieved.


Abstract: Neuroinflammation is a complex biological response to any injury occurring to the central nervous system. It is mainly characterized by the recruitment of immune system cells, namely the microglial cells, in the site of injury. Once activated, microglia expresses a cholesterol transporter protein (TPSO), previously also known as peripheral type benzodiazepine receptor. PK11195 is a ligand for TPSO and, labelled with a positron emitter, it is also the most used tracer for PET molecular imaging to in vivo map the microglia activation in various neurological disorders, including ischemic stroke. Recent [11C]PK11195 PET studies proved activated microglia both locally in the area of the infarct and at distance along the affected fibre tracts, suggesting the presence of two different microglia subtypes with peculiar functions in disease progression. The aim of this review is to discuss the most recent knowledge about imaging neuroinflammation in ischemic stroke and in the atherosclerotic and vascular inflammatory disorders, trying to elucidate the interplay between the clinical course and the activation of a microglial response.


Abstract: DPA-713 is the lead compound of a recently reported pyrazolo[1,5-a]pyrimidineacetamide series, targeting the translocator protein (TSPO 18 kDa), and as such, this structure, as well as closely related derivatives, have been already successfully
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used as positron emission tomography radioligands. On the basis of the pharmacological core of this ligands series, a new magnetic resonance imaging probe, coded DPA-C(6) - (Gd)DOTAMA was designed and successfully synthesized in six steps and 13% overall yield from DPA-713. The Gd-DOTA monoamide cage (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) represents the magnetic resonance imaging reporter, which is spaced from the phenylpyrazolo[1,5-a]pyrimidineacetamide moiety (DPA-713 motif) by a six carbon-atom chain. DPA-C(6) - (Gd)DOTAMA relaxometric characterization showed the typical behavior of a small-sized molecule (relaxivity value: 6.02 mM(-1) s(-1) at 20 MHz). The good hydrophilicity of the metal chelate makes DPA-C(6) - (Gd)DOTAMA soluble in water, affecting thus its biodistribution with respect to the parent lipophilic DPA-713 molecule. For this reason, it was deemed of interest to load the probe to a large carrier in order to increase its residence lifetime in blood. Whereas DPA-C(6) - (Gd)DOTAMA binds to serum albumin with a low affinity constant, it can be entrapped into liposomes (both in the membrane and in the inner aqueous cavity). The stability of the supramolecular adduct formed by the Gd-complex and liposomes was assessed by a competition test with albumin.


Abstract: There is growing evidence of activated microglia and inflammatory processes in the cerebral cortex in amyotrophic lateral sclerosis (ALS). Activated microglia is characterized by increased expression of the 18 kDa translocator protein (TSPO) in the brain and may be a useful biomarker of inflammation. In this study, we evaluated neuroinflammation in ALS patients using a radioligand of TSPO, (18)F-DPA-714. Ten patients with probable or definite ALS (all right-handed, without dementia, and untreated by riluzole or other medication that might bias the binding on the TSPO), were enrolled prospectively and eight healthy controls matched for age underwent a PET study. Comparison of the distribution volume ratios between both groups were performed using a Mann-Whitney’s test. Significant increase of distribution of volume ratios values corresponding to microglial activation was found in the ALS sample in primary motor, supplementary motor and temporal cortex (p=0.009, p=0.001 and p=0.004, respectively). These results suggested that the cortical uptake of (18)F-DPA-714 was increased in ALS patients during the “time of diagnosis” phase of the disease. This finding might improve our understanding of the pathophysiology of ALS and might be a surrogate marker of efficacy of treatment on microglial activation.


Abstract: Although significant inconsistencies remain to be clarified, a role for neurogenesis in hippocampal functions, such as cognition, has been suggested by several reports. Yet, investigation in various species of mammals, including humans, revealed that
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rates of hippocampal neurogenesis are steadily declining with age. The very low levels of hippocampal neurogenesis persisting in the aged brain have been suspected to underlie the cognitive deficits observed in elderly. However, current evidence fails to support the hypothesis that decrease of neurogenesis along normal ageing leads to hippocampal dysfunction. Nevertheless, current studies are suggestive for a distinct role of hippocampal neurogenesis in young versus adult and old brain.


Abstract: The TSPO (translocator protein), also known as the peripheral benzodiazepine receptor, is upregulated in the brain of subjects suffering from neurodegenerative disorders such as Alzheimer’s, Parkinson’s and Huntington’s disease. Moreover, this overexpression has been proved to be linked to microglia activation making thus the TSPO a marker of choice of neuroinflammatory processes and therefore a potential target for the development of radioligands for positron emission tomography imaging. The discovery of selective TSPO ligands and their labelling with the short-lived positron-emitter isotopes carbon-11 and fluorine-18 emerged in the mid-1980s with the preparation of the 3-isoquinolinecarboxamide [(11) C]PK11195. To date, an impressive number of promising compounds-[(11) C]PK11195-challengers-have been developed; some radioligands-for example, [(11) C]PBR28, [(11) C]DPA-713, [(18) F]FEDAA1106 and [(18) F]DPA-714-are currently used in clinical trials. As illustrated in this review, the methodologies applied for the preparation of these compounds remain mainly [(11) C]methyllations using [(11) C]MeI or [(11) C]MeOTf and SN 2-type nucleophilic aliphatic [(18) F]fluorinations-two processes illustrating the state-of-the-art arsenal of reactions that involves these two short-lived radioisotopes-but alternative processes, such as [(11) C]carbonylations using [(11) C]CO and [(11) C]COCl2 as well as SN Ar-type nucleophilic [(18) F]fluorinations, have also been reported and as such, reviewed herein.


Abstract: Over the past decade a lot of research has been performed towards the therapeutic use of mesenchymal stem cells (MSCs) in neurodegenerative and neuroinflammatory diseases. MSCs have shown to be beneficial in different preclinical studies of central nervous system (CNS) disorders due to their immunomodulatory properties and their capacity to secrete various growth factors. Nevertheless, most of the transplanted cells die within the first hours after transplantation and induce a neuroinflammatory response. In order to increase the efficacy of MSC transplantation, it is thus imperative to completely characterise the mechanisms mediating neuroinflammation and cell death following MSC transplantation into the CNS. Consequently, different
components of these cell death- and neuroinflammation-inducing pathways can be targeted in an attempt to improve the therapeutic potential of MSCs for CNS disorders.


Abstract: Perfluorocarbon (PFC) particles are currently on the rise as cell labeling agents for $^{19}$F-MRI tracking of dendritic cell (DC)-based vaccines. In this work, we design theranostic PFC particles for single-step loading of DCs with both antigenic protein and with a liquid PFC for $^{19}$F-MRI detection of the antigen-loaded cells. Upon addition to DCs in vitro, the antigen-loaded PFC particles are efficiently internalized, resulting in intracellular presence of up to 40 pmol $^{19}$F atoms per cell. At the same time, the DCs become loaded with antigenic proteins, that can be efficiently processed, without important effects on cell viability or altering the DC’s phenotype and the cell’s capacity to respond to danger signals. In addition, antigen-loaded PFC particle containing DCs are capable of inducing extensive proliferation of antigen-specific CD8$^+$ T cells in vitro. Importantly, the antigen-coated PFC particles allow in vitro $^{19}$F-MRI-based detection of the antigen-containing DCs with detection limits as low as $10^3$ cells μL$^{-1}$. The dual-modality characteristics of the designed particles could assure that only those DCs that have taken up the antigen, and hence are responsible for an immune response, are traceable via $^{19}$F-MRI. Taken together, these novel dual-modality particles represent an interesting strategy in the development of a traceable DC vaccine.


Abstract: It remains unclear how different translocator protein (TSPO) ligands reflect the spatial extent of astrocyte or microglial activation in various neuroinflammatory conditions. Here, we use a reproducible lipopolysaccharide (LPS)-induced model of acute central nervous system inflammation to compare the binding performance of a new TSPO ligand (18)F-GE-180 with (11)C-(R)-PK11195. Using immunohistochemistry, we also explore the ability of the TSPO ligands to detect activated microglial cells and astrocytes.

METHODS: Lewis rats (n = 30) were microinjected with LPS (1 or 10 μg) or saline (1 μL) into the left striatum. The animals were imaged in vivo at 16 h after the injection using PET radiotracers (18)F-GE-180 or (11)C-(R)-PK11195 (n = 3 in each group) and were killed afterward for autoradiography of the brain. Immunohistochemical assessment of OX-42 and glial fibrillary acidic protein (GFAP) was performed to identify activated microglial cells and reactive astrocytes.

RESULTS: In vivo PET imaging revealed an increase in the ipsilateral TSPO binding, compared with binding in the contralateral hemisphere, after the microinjection of 10 μg
of LPS. No increase was observed with vehicle. By autoradiography, the TSPO radiotracer binding potential in the injected hemisphere was increased after striatal injection of 1 or 10 μg of LPS. However, the significant increase was observed only when using (18)F-GE-180. The area of CD11b-expressing microglial cells extended beyond that of enhanced GFAP staining and mapped more closely to the extent of (18)F-GE-180 binding than to (11)C-(R)-PK11195 binding. The signal from either PET ligand was significantly increased in regions of increased GFAP immunoreactivity and OX-42 colocalization, meaning that the presence of both activated microglia and astrocytes in a given area leads to increased binding of the TSPO radiotracers.

CONCLUSION: (18)F-GE-180 is able to reveal sites of activated microglia in both gray and white matter. However, the signal is increased by the presence of activated astrocytes. Therefore, (18)F-GE-180 is a promising new fluorinated longer-half-life tracer that reveals the presence of activated microglia in a manner that is superior to (11)C-(R)-PK11195 due to the higher binding potential observed for this ligand.


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**Abstract:** PURPOSE: This study is aimed at demonstrating the in vivo potential of Gd(III)- loaded glucan particles (Gd-GPs) as magnetic resonance imaging (MRI)-positive agents for labeling and tracking phagocytic cells.

PROCEDURE: GPs were obtained from Saccharomyces cerevisiae and loaded with the water-insoluble complex Gd-DOTAMA(C(18))(2). The uptake kinetics of Gd-GPs by murine macrophages was studied in vitro and the internalization mechanism was assessed by competition assays. The in vivo performance of Gd-GPs was tested at 7.05 T on a mouse model of acute liver inflammation.

RESULTS: The minimum number of Gd-GPs-labeled J774.A1 macrophages detected in vitro by MRI was ca. 300 cells/μl of agar, which is the lowest number ever reported for cells labeled with a positive T(1) agent. Intravenous injection of macrophages labeled with Gd-GPs in a mouse model of liver inflammation enabled the MRI visualization of the cellular infiltration in the diseased area.

CONCLUSIONS: Gd-GPs represent a promising platform for tracking macrophages by MRI as a T(1) alternative to the golden standard T(2)-based iron oxide particles.


**Abstract:** A new class of nanovesicles formed by the self-assembly of amphphilic Janus dendrimers, dendrimersomes, loaded with hydrophilic or amphphilic magnetic resonance imaging chelates shows promising properties as a novel, efficient and versatile nanoplatform for biomedical imaging.

Abstract: The signaling molecule histamine plays a key role in the mediation of immune reactions, in gastric secretion, and in the sensory system. In addition, it has an important function as a neurotransmitter in the central nervous system, acting in pituitary hormone secretion, wakefulness, motor and cognitive functions, as well as in itch and nociception. This has raised interest in the role of the histaminergic system for the treatment and diagnosis of various pathologies such as allergy, sleeping and eating disorders, neurodegeneration, neuroinflammation, mood disorders, and pruritus.

In the past 20 years, several ligands targeting the four different histamine receptor subtypes have been explored as potential radiotracers for positron emission tomography (PET). This contribution provides an overview of the developments of subtype-selective carbon-11-labeled and fluorine-18-labeled compounds for imaging in the brain.

Using specific radioligands, the $H_1R$ expression in human brain could be examined in diseases such as schizophrenia, depression, and anorexia nervosa. In addition, the sedative effects of antihistamines could be investigated in terms of $H_1R$ occupancy. The $H_3R$ is of special interest because of its regulatory role in the release of various other neurotransmitters, and initial $H_3R$ PET imaging studies in humans have been reported. The $H_4R$ is the youngest member of the histamine receptor family and is involved in neuroinflammation and various sensory pathways. To date, two $H_4R$-specific $^{11}$C-labeled ligands have been synthesized, and the imaging of the $H_4R$ in vivo is in the early stage.


Abstract: Microglial cell function receives increasing interest. To date, the majority of experiments are performed by using immortalized microglia-like cells or primary microglia prepared from pre- or postnatal rodent brain. As those may not adequately reflect the microglial biology in the adult brain, this protocol advocates a procedure which allows for the isolation, purification, and subsequent analysis of microglial cells. Once isolated, the principal state of activation, M1 or M2, can be determined in adult microglia using fluorescence-activated cell sorting, quantitative PCR, and/or Western blotting. Likewise, adult microglia generated by this protocol can be used for functional analysis through cell cultivation for a limited time.

Abstract: Multiple sclerosis is a devastating demyelinating disease of the central nervous system (CNS) in which endogenous remyelination, and thus recovery, often fails. Although the cuprizone mouse model allowed elucidation of many molecular factors governing remyelination, currently very little is known about the spatial origin of the oligodendrocyte progenitor cells that initiate remyelination in this model. Therefore, we here investigated in this model whether subventricular zone (SVZ) neural stem/progenitor cells (NSPCs) contribute to remyelination of the splenium following cuprizone-induced demyelination. Experimentally, from the day of in situ NSPC labeling, C57BL/6J mice were fed a 0.2% cuprizone diet during a 4-week period and then left to recover on a normal diet for 8 weeks. Two in situ labeling strategies were employed: (i) NSPCs were labeled by intraventricular injection of micron-sized iron oxide particles and then followed up longitudinally by means of magnetic resonance imaging (MRI), and (ii) SVZ NSPCs were transduced with a lentiviral vector encoding the eGFP and Luciferase reporter proteins for longitudinal monitoring by means of in vivo bioluminescence imaging (BLI). In contrast to preceding suggestions, no migration of SVZ NSPC towards the demyelinated splenium was observed using both MRI and BLI, and further validated by histological analysis, thereby demonstrating that SVZ NSPCs are unable to contribute directly to remyelination of the splenium in the cuprizone model. Interestingly, using longitudinal BLI analysis and confirmed by histological analysis, an increased migration of SVZ NSPC-derived neuroblasts towards the olfactory bulb was observed following cuprizone treatment, indicative for a potential link between CNS inflammation and increased neurogenesis.

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Abstract: The peripartum period is a time of high susceptibility for mood and anxiety disorders, some of which have recently been associated with alterations in hippocampal neurogenesis. Several factors including stress, aging, and, perhaps unexpectedly, lactation have been shown to decrease hippocampal neurogenesis. Intriguingly, lactation is also a time of reduced stress responsivity suggesting that the effect of stress on neurogenic processes may differ during this period. Therefore, the aim of the present study was to assess the effect of repeated stress during lactation [2 h restraint stress from lactation day (LD) 2 to LD13] on brain weight, hippocampal volume, cell proliferation and survival, and on neuronal and astroglial differentiation. In addition to confirming the known lactation-associated decrease in cell proliferation and survival, we could reveal that stress reversed the lactation-induced decrease in cell proliferation, while it did not affect survival of newly born cells, nor the number of mature neurons, nor did it alter immature neuron production or the number of astroglial cells in lactation. Stress exposure increased relative brain weight and hippocampal volume mirroring the observed changes in neurogenesis. Interestingly, hippocampal volume and relative brain weight were lower in lactation as compared to nulliparous females under nonstressed conditions. This study assessed the effect of stress during lactation on hippocampal neurogenesis and indicates that stress interferes with important peripartum adaptations at the level of the hippocampus.

Abstract: Sex differences in basal as well as in stress-induced hippocampal neurogenesis processes have been reported in the literature. However, studies directly comparing sex differences on multiple neurogenesis processes under such conditions are lacking to date. Therefore, the aim of the present study was to directly compare cell proliferation and survival, neuronal and astroglial differentiation as well as stem cells quiescence in male and female Wistar rats under both basal and chronic stress conditions (12 days of 2 h restraint stress (RS)). In addition, corticosterone (CORT) levels and spatial working memory were assessed. Under baseline conditions, only the number of immature neurons within the hippocampal dentate gyrus was higher in males compared with females. In contrast, chronic stress resulted in a number of sex-specific alterations. Thus, stress exposure reduced cell proliferation in males with a concurrent increase in stem cell quiescence, while it did not alter either parameter in females but decreased cell survival. Analysis of astroglial and neuronal differentiation patterns revealed that chronic stress specifically diminished the number of mature neurons in females, with no effect in males. Despite the observed sex differences in adult hippocampal neurogenesis, spatial working memory was not altered by stress exposure in either sex. While basal CORT levels were higher, chronic stress exposure did not affect this parameter in either sex across the initial stress period. This study presents the first direct and detailed evaluation of sex-dependent and chronic stress-induced changes in adult hippocampal neurogenesis not only showing changes in cell proliferation and survival, but moreover immature neuron production, differentiation patterns, stem cell quiescence and therefore contributes to a better understanding of sex differences in neurogenesis processes.


Abstract: Reactive microgliosis, hallmark of neuroinflammation, may contribute to neuronal degeneration, as shown in several neurodegenerative diseases. We in vivo evaluated microglia activation in early dementia with Lewy bodies, still not reported, and compared with early Parkinson's disease, to assess possible differential pathological patterns.

METHODS: We measured the [(11)C]-PK11195 binding potentials with Positron Emission Tomography, using a simplified reference tissue model, as marker of microglia activation, and cerebral spinal fluid protein carbonylation levels, as marker of oxidative stress. Six dementia with Lewy bodies and 6 Parkinson's disease patients within a year from the onset, and eleven healthy controls were included. Clinical diagnosis was confirmed at a 4-year follow-up.

RESULTS: In dementia with Lewy bodies as well as in Parkinson's disease, we found significant (p < 0.001) [(11)C]-PK11195 binding potential increases in the substantia nigra and putamen. Patients with Lewy bodies dementia had extensive additional microgli
activation in several associative cortices. This was evident also at a single subject level. Significant increase of Cerebral Spinal Fluid protein carbonylation was shown in both patients’ groups.

CONCLUSIONS: [(11)C]-PK11195 Positron Emission Tomography imaging revealed neuroinflammation in dementia with Lewy bodies and Parkinson’s disease, mirroring, even at a single subject level, the common and the different topographical distribution of neuropathological changes, yet in the earliest stages of the disease process. Focusing on those events that characterize parkinsonisms and Parkinson’s disease may be the key to further advancing the understanding of pathogenesis and to taking these mechanisms forward as a means of defining targets for neuroprotection.


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Abstract: Neurons generated from pluripotent stem cells (PSCs) self-organize into functional neuronal assemblies in vitro, generating synchronous network activities. Intriguingly, PSC-derived neuronal assemblies develop spontaneous activities that are independent of external stimulation, suggesting the presence of thus far undetected intrinsically active neurons (IANs). Here, by using mouse embryonic stem cells, we provide evidence for the existence of IANs in PSC-neuronal networks based on extracellular multielectrode array and intracellular patch-clamp recordings. IANs remain active after pharmacological inhibition of fast synaptic communication and possess intrinsic mechanisms required for autonomous neuronal activity. PSC-derived IANs are functionally integrated in PSC-neuronal populations, contribute to synchronous network bursting, and exhibit pacemaker properties. The intrinsic activity and pacemaker properties of the neuronal subpopulation identified herein may be particularly relevant for interventions involving transplantation of neural tissues. IANs may be a key element in the regulation of the functional activity of grafted as well as preexisting host neuronal networks.


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Abstract: Inflammation is a highly dynamic and complex adaptive process to preserve and restore tissue homeostasis. Originally viewed as an immune-privileged organ, the central nervous system (CNS) is now recognized to have a constant interplay with the innate and the adaptive immune systems, where resident microglia and infiltrating immune cells from the periphery have important roles. Common diseases of the CNS, such as stroke, multiple sclerosis (MS), and neurodegeneration, elicit a neuroinflammatory response with the goal to limit the extent of the disease and to support repair and regeneration. However, various disease mechanisms lead to neuroinflammation (NI) contributing to the disease process itself. Molecular imaging is the method of choice to try to decipher key aspects of the dynamic interplay of various inducers, sensors, transducers, and effectors of the
orchestrated inflammatory response in vivo in animal models and patients. Here, we review the basic principles of NI with emphasis on microglia and common neurologic disease mechanisms, the molecular targets which are being used and explored for imaging, and molecular imaging of NI in frequent neurologic diseases, such as stroke, MS, neurodegeneration, epilepsy, encephalitis, and gliomas.


Abstract: PURPOSE: The use of resting-state functional MRI (rsfMRI) in preclinical research is expanding progressively, with the majority of resting-state imaging performed in anesthetized animals. Since anesthesia may change the physiology and, in particular, the neuronal activity of an animal considerably, it may also affect rsfMRI findings. Therefore, this study compared rsfMRI data from awake mice with rsfMRI results obtained from mice anesthetized with α-chloralose (120 mg/kg), urethane (2.5 g/kg), or isoflurane (1%).

METHODS: Functional connectivity (FC) was estimated using both independent component analysis (40 components) and ROI-based analysis to zoom in on the effect of different anesthetics on inter-hemispheric FC.

RESULTS: The data revealed an important diminishment of cortical interhemispheric FC in both the α-chloralose and urethane groups in comparison with the isoflurane and awake groups.

CONCLUSION: When performing FC analysis in anesthetized mice, the impact of anesthetics must be taken into account. The required doses for stable anesthesia during MRI significantly decrease interhemispheric FC.


Abstract: Amyloid β25–35 (Aβ25–35) is a toxic fragment of Alzheimer’s beta peptide. We have previously shown that Aβ25–35 fibrils form a trigonally oriented network on mica by epitaxial growth mechanisms. Chemical reactivity can be furnished to the fibril by introducing a cysteine residue (Aβ25–35_N27C) while maintaining oriented assembly properties. Previously we have shown that fibril binding to mica is strongly influenced by KCl concentration. In the present work we explored the kinetics of epitaxial assembly of the mutant fibrils at different peptide and KCl concentrations by using in situ time-resolved AFM. We measured the length of Aβ25–35_N27C fibrils as a function of time. Increasing free peptide concentration enhanced fibril growth rate, and the critical peptide concentration of fibril assembly was 3.92 μM. Increasing KCl concentration decreased the number of fibrils bound to the mica surface, and above 20 mM KCl fibril formation was completely abolished even at high peptide concentrations. By modulating peptide and KCl concentrations in the optimal ranges established here the complexity of the Aβ25–35_N27C network can be finely tuned.

**Abstract:** Nuclear autoantibodies have been found in patients with autoimmune diseases. One possible source for nuclear antigens are apoptotic cells. However, the mechanism of how apoptotic cells make nuclear factors accessible to the immune system is still elusive. In the present study, we investigated the redistribution of nuclear components after UV irradiation in the microglial cell line BV-2 and in primary mouse microglia at the ultrastructural level. We used transmission electron microscopy-coupled electron energy loss spectroscopy (EELS) to measure phosphorus as an indicator for nucleic acids and immunogold labeling to detect histone H3 and lamin B1 in apoptotic cells. EELS revealed elevated concentrations of phosphorus in nuclear and cytoplasmic condensed chromatin compared to the remaining cytoplasm. Furthermore, immunolabeling of lamin B1 and histone H3 was detected in apoptotic microglia not only in the nucleus, but also in the cytoplasm, and even at the plasma membrane. Confocal images of apoptotic microglia, which were not previously permeabilized, showed patches of histone H3 and lamin B1 labeling at the cell surface. The pan-caspase inhibitor Z-VAD-FMK (carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone) prevented the occurrence of cytoplasmic condensed chromatin in apoptotic microglia. Our findings indicate that nuclear components leak from the nucleus into the cytoplasm in apoptotic microglia. At least histone H3 and lamin B1 reach the cell surface, this may promote autoreactive processes.


**Abstract:** Since the first report of amyloid imaging with Pittsburgh compound B (PiB), special attention has been given to the individuals who, though cognitively normal by testing, have substantial amounts of fibrillar amyloid-β (Aβ) pathology in their brain.(1) This state, previously predicted by several postmortem studies,(2) is also the focus of research criteria for preclinical Alzheimer disease (AD).(3) With these criteria, the pathophysiologic spectrum of AD was stretched past the earliest clinically detectable stages such as mild cognitive impairment (MCI)(4) to include cognitively normal individuals who show evidence of brain amyloid deposition by CSF analysis or amyloid PET. This concept of preclinical AD currently forms the foundation of a trial aimed at preventing the emergence of AD in amyloid-positive individuals—the Anti-Amyloid Treatment of Asymptomatic Alzheimer’s Disease (A4) trial.(S) Clearly, it is important to understand this state of asymptomatic β-amyloidosis as fully as possible.

Abstract: Brain pericytes (BrPCs) are essential cellular components of the central nervous system neurovascular unit involved in the regulation of blood flow, blood-brain barrier function, as well as in the stabilization of the vessel architecture. More recently, it became evident that BrPCs, besides their regulatory activities in brain vessel function and homeostasis, have pleiotropic functions in the adult CNS ranging from stromal and regeneration promoting activities to stem cell properties. This special characteristic confers BrPC cell plasticity, being able to display features of other cells within the organism. BrPCs might also be causally involved in certain brain diseases. Due to these properties BrPCs might be potential drug targets for future therapies of neurological disorders. This review summarizes BrPC properties, disorders in which this cell type might be involved, and provides suggestions for future therapeutic developments targeting BrPCs.


Abstract: Pathological heterogeneity within patients with Frontotemporal lobar degeneration (FTLD) in general precludes the accurate assignment of diagnostic subtype in life. The aim of this study was to assess the extent of microglial cell activation in FTLD in order to determine whether it might be possible to employ this as a diagnostic marker in vivo using PET ligand [11C](R)-PK11195 in order to differentiate cases of FTLD according to histological subtype.

METHODS: The distribution and extent of microglial cell activation was assessed semi-quantitatively in cortical grey and subcortical white matter of CD68 immunostained sections of frontal and temporal cortex from 78 pathologically confirmed cases of FTLD, 13 of Alzheimer’s disease (AD) and 13 controls.

RESULTS: Significantly higher levels of microglial cell activation than controls occurred in all 4 regions in FTLD, and in 3 of the 4 regions in AD. Microglial activation was greater in frontal subcortical white matter in FTLD than AD, whereas it was higher in temporal cortical grey matter in AD than FTLD. Microglial cell activation was significantly higher in temporal subcortical white matter in FTLD-MAPT than in other genetic (GRN, C9ORF72) or non-genetic forms of FTLD.

CONCLUSIONS: The present study suggests that high levels of microglial cell involvement in temporal lobe (subcortical white matter) might serve as a marker of inherited FTLD associated with intronic mutations in MAPT, with a relatively intense signal in this region in PET studies using [11C](R)-PK11195 as microglial cell marker could indicate the presence of MAPT mutation in vivo.


Abstract: The objective of this study was to investigate the effects of modulating brain amyloid-β (Aβ) levels at different stages of amyloid pathology on synaptic function,
inflammatory cell changes and hippocampal neurogenesis, i.e. processes perturbed in Alzheimer’s disease (AD). Young (4- to 6-month-old) and older (15- to 18-month-old) APPSWE transgenic (Tg2576) mice were treated with the AD candidate drug (+)-phenserine for 16 consecutive days. We found significant reductions in insoluble Aβ1-42 levels in the cortices of both young and older transgenic mice, while significant reductions in soluble Aβ1-42 levels and insoluble Aβ1-40 levels were only found in animals aged 15-18 months. Autoradiography binding with the amyloid ligand Pittsburgh Compound B ((3)H-PIB) revealed a trend for reduced fibrillar Aβ deposition in the brains of older phenserine-treated Tg2576 mice. Phenserine treatment increased cortical synaptophysin levels in younger mice, while decreased interleukin-1β and increased monocyte chemoattractant protein-1 and tumor necrosis factor-alpha levels were detected in the cortices of older mice. The reduction in Aβ1-42 levels was associated with an increased number of bromodeoxyuridine-positive proliferating cells in the hippocampi of both young and older Tg2576 mice. To determine whether the increased cell proliferation was accompanied by increased neuronal production, the endogenous early neuronal marker doublecortin (DCX) was examined in the dentate gyrus (DG) using immunohistochemical detection. Although no changes in the total number of DCX(+) expressing neurons were detected in the DG in Tg2576 mice at either age following (+)-phenserine treatment, dendritic arborization was increased in differentiating neurons in young Tg2576 mice. Collectively, these findings indicate that reducing Aβ1-42 levels in Tg2576 mice at an early pathological stage affects synaptic function by modulating the maturation and plasticity of newborn neurons in the brain. In contrast, lowering Aβ plaque pathology is prominent mainly alters the levels of proinflammatory cytokines and chemokines.


Abstract: In recent years several studies demonstrated the presence of estrogen receptors in mammalian tissues and significantly improved our understanding of their ability to control biological processes in reproductive as well as non-reproductive organs. Considering the manifold mechanisms and organs that are involved in estrogen action and the implication of estrogens in human female physiology, innovative approaches are required to shed light on the widespread activities of estrogen receptors in woman physiology. This is particularly relevant for the definition of novel, more efficacious hormonal replacement therapies or for the evaluation of the risk associated with the exposure to endocrine disruptors. The introduction of genetic engineering and the development and application of in vivo imaging techniques offer new tools for pre-clinical studies. The generation of the ERE-Luc mouse, a reporter animal developed for in vivo studies of the estrogen receptor activity, allows assessing the activity state of the ER signaling pathway in all target tissues and organs at once, under physiological stimuli or as a result of a pharmacological treatment. This review summarizes the main steps in the generation and appraisal of the estrogen receptor reporter mouse ERE-Luc, designed for in vivo molecular imaging studies, and describes examples demonstrating the suitability of the ERE-Luc model for drug development and for the investigation of the effects of endogenous, environmental, and dietary estrogens in vivo. This article is part of a Special Issue entitled 'Phytoestrogens'. 

**Abstract:** Titin is a giant elastomeric muscle protein that has been suggested to function as a sensor of sarcomeric stress and strain, but the mechanisms by which it does so are unresolved. To gain insight into its mechanosensory function we manipulated single titin molecules with high-resolution optical tweezers. Discrete, step-wise transitions, with rates faster than canonical Ig domain unfolding occurred during stretch at forces as low as 5 pN. Multiple mechanisms and molecular regions (PEVK, proximal tandem-Ig, N2A) are likely to be involved. The pattern of transitions is sensitive to the history of contractile events. Monte-Carlo simulations of our experimental results predicted that structural transitions begin before the complete extension of the PEVK domain. High-resolution atomic force microscopy (AFM) supported this prediction. Addition of glutamate-rich PEVK domain fragments competitively inhibited the viscoelastic response in both single titin molecules and muscle fibers, indicating that PEVK domain interactions contribute significantly to sarcomere mechanics. Thus, under non-equilibrium conditions across the physiological force range, titin extends by a complex pattern of history-dependent discrete conformational transitions, which, by dynamically exposing ligand-binding sites, could set the stage for the biochemical sensing of the mechanical status of the sarcomere.


**Abstract:** Titin is a giant elastomeric protein responsible for the generation of passive muscle force. Mechanical force unfolds titin's globular domains, but the exact structure of the overstretched titin molecule is not known. Here we analyzed, by using high-resolution atomic force microscopy, the structure of titin molecules overstretched with receding meniscus. The axial contour of the molecules was interrupted by topographical gaps with a mean width of 27.7 nm that corresponds well to the length of an unfolded globular (immunoglobulin and fibronectin) domain. The wide gap-width distribution suggests, however, that additional mechanisms such as partial domain unfolding and the unfolding of neighboring domain multimers may also be present. In the folded regions we resolved globules with an average spacing of 5.9 nm, which is consistent with a titin chain composed globular domains with extended interdomain linker regions. Topographical analysis allowed us to allocate the most distal unfolded titin region to the kinase domain, suggesting that this domain systematically unfolds when the molecule is exposed to overstretching forces. The observations support the prediction that upon the action of stretching forces the N-terminal β-sheet of the titin kinase unfolds, thus exposing the enzyme's ATP-binding site and hence contributing to the molecule's mechanosensory function.

**Abstract:** BACKGROUND: The pathological features in Alzheimer’s disease (AD) brain include the accumulation and deposition of β-amyloid (Aβ), activation of astrocytes and microglia and disruption of cholinergic neurotransmission. Since the topographical characteristics of these different pathological processes in AD brain and how these relate to each other is not clear, this motivated further exploration using binding studies in postmortem brain with molecular imaging tracers. This information could aid the development of specific biomarkers to accurately chart disease progression.

RESULTS: In vitro binding assays demonstrated increased [\(^{3}H\)]-PIB (fibrillar Aβ) and [\(^{3}H\)]-PK11195 (activated microglia) binding in the frontal cortex (FC) and hippocampus (HIP), as well as increased binding of [\(^{3}H\)]-L-deprenyl (activated astrocytes) in the HIP, but a decreased [\(^{3}H\)]-nicotine (α4β2 nicotinic acetylcholine receptor (nAChR)) binding in the FC of AD cases compared to age-matched controls. Quantitative autoradiography binding studies were also performed to investigate the regional laminar distributions of [\(^{3}H\)]-L-deprenyl, [\(^{3}H\)]-PIB as well as [\(^{125}I\)]-α-bungarotoxin (α7 nAChRs) and [\(^{3}H\)]-nicotine in hemisphere brain of a typical AD case. A clear lamination pattern was observed with high [\(^{3}H\)]-PIB binding in all layers and [\(^{3}H\)]-deprenyl in superficial layers of the FC. In contrast, [\(^{3}H\)]-PIB showed low binding to fibrillar Aβ, but [\(^{3}H\)]-deprenyl high binding to activated astrocytes throughout the HIP. The [\(^{3}H\)]-PIB binding was also low and the [\(^{3}H\)]-deprenyl binding high in all layers of the medial temporal gyrus and insular cortex in comparison to the frontal cortex. Low [\(^{3}H\)]-nicotine binding was observed in all layers of the frontal cortex in comparison to layers in the medial temporal gyrus, insular cortex and hippocampus. Immunohistochemical detection in the AD case revealed abundant glial fibrillary acidic protein positive (GFAP+) reactive astrocytes and α7 nAChR expressing GFAP+ astrocytes both in the vicinity and surrounding Aβ neuritic plaques in the FC and HIP. Although fewer Aβ plaques were observed in the HIP, some hippocampal GFAP+ astrocytes contained Aβ-positive (6 F/3D) granules within their somata.

CONCLUSIONS: Astrocytosis shows a distinct regional pattern in AD brain compared to fibrillar Aβ, suggesting that different types of astrocytes may be associated with the pathophysiological processes in AD.


**Abstract:** We aimed to evaluate the novel high-affinity and relatively lipophilic CB(1) receptor (CB(1)R) antagonist radioligand [(125)I]SD7015 for SPECT imaging of CB(1)Rs in vivo using the multiplexed multipinhole dedicated small animal SPECT/CT system, NanoSPECT/CT(PLUS) (Mediso, Budapest, Hungary), in knock-out CB(1) receptor knock-out (CB(1)R/-) and wildtype mice. In order to exclude possible differences in cerebral blood
flow between the two types of animals, HMPAO SPECT scans were performed, whereas in
order to confirm the brain uptake differences of the radioligand between knock-out mice
and wildtype mice, in vivo scans were complemented with ex vivo autoradiographic
measurements using the brains of the same animals. With SPECT/CT imaging, we
measured the brain uptake of radioactivity, using %SUV (% standardised uptake values) in
CB(1)R-/- mice (n=3) and C57BL6 wildtype mice (n=7) under urethane anaesthesia after
injecting [(125)I]SD7015 intravenously or intraperitoneally. The Brookhaven Laboratory
mouse MRI atlas was fused to the SPECT/CT images by using a combination of rigid and
non-rigid algorithms in the Mediso Fusion™ (Mediso, Budapest, Hungary) and VivoQuant
(inviCRO, Boston, MA, USA) softwares. Phosphor imager plate autoradiography (ARG) was
performed on 4μm-thin cryostat sections of the excised brains. %SUV was 8.6±3.6
(average±SD) in CB(1)R-/- mice and 22.1±12.4 in wildtype mice between 2 and 4h after
injection (p<0.05). ARG of identically taken sections from wildtype mouse brain showed
moderate radioactivity uptake when compared with the in vivo images, with a clear
difference between grey matter and white matter, whereas ARG in CB(1)R(-/-) mouse showed
practically no radioactivity uptake. [(125)I]SD7015 enters the mouse brain in sufficient
amount to enable SPECT imaging. Brain radioactivity distribution largely coincides with
that of the known CB(1)R expression pattern in rodent brain. We conclude that
[(125)I]SD7015 should be a useful SPECT radioligand for studying brain CB(1)R in mouse
and rat disease models.

40. Mouysset S, Zbib H, Stute S, Girault JM, Charara J, Noailles J, Chalon S, Buvat I,
Tauber C. Segmentation of dynamic PET images with kinetic spectral clustering.
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Abstract: Segmentation is often required for the analysis of dynamic positron emission
tomography (PET) images. However, noise and low spatial resolution make it a difficult task
and several supervised and unsupervised methods have been proposed in the literature to
perform the segmentation based on semi-automatic clustering of the time activity curves
of voxels. In this paper we propose a new method based on spectral clustering that does
not require any prior information on the shape of clusters in the space in which they are
identified. In our approach, the p-dimensional data, where p is the number of time frames,
is first mapped into a high dimensional space and then clustering is performed in a low-
dimensional space of the Laplacian matrix. An estimation of the bounds for the scale
parameter involved in the spectral clustering is derived. The method is assessed using
dynamic brain PET images simulated with GATE and results on real images are presented.
We demonstrate the usefulness of the method and its superior performance over three
other clustering methods from the literature. The proposed approach appears as a
promising pre-processing tool before parametric map calculation or ROI-based
quantification tasks.

Halldin C. Synthesis and biological evaluation of novel propargyl amines as
potential fluorine-18 labeled radioligands for detection of MAO-B activity.
Abstract: The aim of this project was to synthesize and evaluate three novel fluorine-18 labeled derivatives of propargyl amine as potential PET radioligands to visualize monoamine oxidase B (MAO-B) activity. The three fluorinated derivatives of propargyl amine ((S)-1-fluoro-N,4-dimethyl-N-(prop-2-ynyl)-pent-4-en-2-amine (5), (S)-N-(1-fluoro-3-(furan-2-yl)propan-2-yl)-N-methylprop-2-y1-amine (10) and (S)-1-fluoro-N,4-dimethyl-N-(prop-2-ynyl)pentan-2-amine (15)) were synthesized in multi-step organic syntheses. IC(50) values for inhibition were determined for compounds 5, 10 and 15 in order to determine their specificity for binding to MAO-B. Compound 5 inhibited MAO-B with an IC(50) of 664 ± 48.08 nM. No further investigation was carried out with this compound. Compound 10 inhibited MAO-B with an IC(50) of 208.5 ± 13.44 nM and compound 15 featured an IC(50) of 131.5 ± 0.71 nM for its MAO-B inhibitory activity. None of the compounds inhibited MAO-A activity (IC(50) > 2 μM). The fluorine-18 labeled analogues of the two higher binding affinity compounds (10 and 15) (S)-N-(1-[(18)F]fluoro-3-(furan-2-yl)propan-2-yl)-N-methylprop-2-y1-amine (16) and (S)-1-[(18)F]fluoro-N,4-dimethyl-N-(prop-2-ynyl)pentan-2-amine (18) were both prepared from the corresponding precursors 9A, 9B and 14A, 14B by a one-step fluorine-18 nucleophilic substitution reaction. Autoradiography experiments on human postmortem brain tissue sections were performed with 16 and 18. Only compound 18 demonstrated a high selectivity for MAO-B over MAO-A and was, therefore, chosen for further examination by PET in a cynomolgus monkey. The initial uptake of 18 in the monkey brain was 250% SUV at 4 min post injection. The highest uptake of radioactivity was observed in the striatum and thalamus, regions with high MAO-B activity, whereas lower levels of radioactivity were detected in the cortex and cerebellum. The percentage of unchanged radioligand 18 was 30% in plasma at 90min post injection. In conclusion, compound 18 is a selective inhibitor of MAO-B in vitro and demonstrated a MAO-B specific binding pattern in vivo by PET in monkey. It can, therefore, be considered as a candidate for further investigation in human by PET.


Abstract: The objective of this study was to synthesize and evaluate a novel fluorine-18 labeled deuterium substituted analogue of rasagiline (9, [18F]fluororasagiline-D2) as a potential PET radioligand for studies of monoamine oxidase B (MAO-B). The precursor compound (6) and reference standard (7) were synthesized in multi-step syntheses. Radiolabeling of 9 was accomplished by a two-step synthesis, compromising a nucleophilic substitution followed by hydrolysis of the sulfamidate group. The incorporation radiochemical yield from fluorine-18 fluoride was higher than 30%, the radiochemical purity was >99% and the specific radioactivity was >160GBq/μmol at the time of administration. In vitro compound 7 inhibited the MAO-B activity with an IC50 of 173.0±13.6nM. The MAO-A activity was inhibited with an IC50 of 9.9±1.1μM. The fluorine-18 version 9 was characterized in the cynomolgus monkey brain where a high brain uptake was found (275% SUV at 4min). There was a higher uptake in the striatum and thalamus compared to the cortex and cerebellum. A pronounced blocking effect (50% decrease) was observed in the specific brain regions after administration of l-deprenyl (0.5mg/kg) 30min prior to the administration of 9. Radiometabolite studies demonstrated 40% of unchanged
radioligand at 90min post injection. An efficient radiolabeling of 9 was successfully established and in the monkey brain 9 binds to MAO-B rich regions and its binding is blocked by the selective MAO-B compound l-deprenyl. The radioligand 9 is a potential candidate for human PET studies.


Abstract: Imaging fibrillar amyloid-β deposition in the human brain in vivo by positron emission tomography has improved our understanding of the time course of amyloid-β pathology in Alzheimer’s disease. The most widely used amyloid-β imaging tracer so far is (11)C-Pittsburgh compound B, a thioflavin derivative but other (11)C- and (18)F-labelled amyloid-β tracers have been studied in patients with Alzheimer’s disease and cognitively normal control subjects. However, it has not yet been established whether different amyloid tracers bind to identical sites on amyloid-β fibrils, offering the same ability to detect the regional amyloid-β burden in the brains. In this study, we characterized (3)H-Pittsburgh compound B binding in autopsied brain regions from 23 patients with Alzheimer’s disease and 20 control subjects (aged 50 to 88 years). The binding properties of the amyloid tracers FDDNP, AV-45, AV-1 and BF-227 were also compared with those of (3)H-Pittsburgh compound B in the frontal cortices of patients with Alzheimer’s disease. Saturation binding studies revealed the presence of high- and low-affinity (3)H-Pittsburgh compound B binding sites in the frontal cortex (K(d1): 3.5 ± 1.6 nM; K(d2): 133 ± 30 nM) and hippocampus (K(d1):5.6 ± 2.2 nM; K(d2): 181 ± 132 nM) of Alzheimer’s disease brains. The relative proportion of high-affinity to low-affinity sites was 6:1 in the frontal cortex and 3:1 in the hippocampus. One control showed both high- and low-affinity (3)H-Pittsburgh compound B binding sites (K(d1): 1.6 nM; K(d2): 330 nM) in the cortex while the others only had a low-affinity site (K(d2): 191 ± 70 nM). (3)H-Pittsburgh compound B binding in Alzheimer’s disease brains was higher in the frontal and parietal cortices than in the caudate nucleus and hippocampus, and negligible in the cerebellum. Competitive binding studies with (3)H-Pittsburgh compound B in the frontal cortices of Alzheimer’s disease brains revealed high- and low-affinity binding sites for BTA-1 (Ki: 0.2 nM, 70 nM), florbetapir (1.8 nM, 53 nM) and florbetaben (1.0 nM, 65 nM). BF-227 displaced 83% of (3)H-Pittsburgh compound B binding, mainly at a low-affinity site (311 nM), whereas FDDNP only partly displaced (40%). We propose a multiple binding site model for the amyloid tracers (binding sites 1, 2 and 3), where AV-45 (florbetapir), AV-1 (florbetaben), and Pittsburgh compound B, all show nanomolar affinity for the high-affinity site (binding site 1), as visualized by positron emission tomography. BF-227 shows mainly binding to site 3 and FDDNP shows only some binding to site 2. Different amyloid tracers may provide new insight into the pathophysiological mechanisms in the progression of Alzheimer’s disease.

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Abstract: The time course and order of the pathological-physiological processes in Alzheimer’s disease (AD) are still under investigation and it is expected that molecular imaging will provide important insight into early brain pathology. Multi-tracer positron emission tomography studies visualizing fibrillar amyloid, inflammatory changes including astrocytosis and activation of microglia as well as cerebral glucose metabolism indicate that AD pathological processes are initiated and ongoing decades before the onset of cognitive symptoms. Therefore, prevention might be a new promising target for AD therapy.


Abstract: Flavonoids target a variety of pathophysiological mechanisms and are therefore increasingly considered as compounds encompassed with therapeutic potentials in diseases such as cancer, diabetes, arteriosclerosis, and neurodegenerative diseases and mood disorders. Hops (Humulus lupulus L.) is rich in flavonoids such as the flavanone 8-prenylnaringenin, which is the most potent phytoestrogen identified so far, and the prenylchalcone xanthohumol, which has potent tumor-preventive, anti-inflammatory and antiviral activities. In the present study, we questioned whether hops-derived prenylflavonoids and synthetic derivatives thereof act on neuronal precursor cells and neuronal cell lines to induce neuronal differentiation, neurite outgrowth and neuroprotection. Therefore, mouse embryonic forebrain-derived neural precursors and Neuro2a neuroblastoma-derived cells were stimulated with the prenylflavonoids of interest, and their potential to activate the promoter of the neuronal fate-specific doublecortin gene and to stimulate neuronal differentiation and neurite outgrowth was analyzed. In this screening, we identified highly “neuroactive” compounds, which we termed “enhancement of neuronal differentiation factors” (ENDFs). The most potent molecule, ENDF1, was demonstrated to promote neuronal differentiation of neural stem cells and neurite outgrowth of cultured dorsal root ganglion neurons and protected neuronal PC12 cells from cobalt chloride-induced as well as cholinergic neurons of the nucleus basalis of Meynert from deafferentation-induced cell death. The results indicate that hops-derived prenylflavonoids such as ENDFs might be powerful molecules to promote neurogenesis, neuroregeneration and neuroprotection in cases of chronic neurodegenerative diseases, acute brain and spinal cord lesion and age-associated cognitive impairments.

**Abstract:** Background: Alpha-synuclein is a key protein implicated in the pathogenesis of Parkinson's disease (PD). It is the main component of the Lewy bodies, a cardinal neuropathological feature in the disease. In addition, whole locus multiplications and point mutations in the gene coding for alpha-synuclein lead to autosomal dominant monogenic PD. Over the past decade, research on PD has impelled the development of new animal models based on alpha-synuclein. In this context, transgenic mouse lines have failed to reproduce several hallmarks of PD, especially the strong and progressive dopaminergic neurodegeneration over time that occurs in the patients. In contrast, viral vector-based models in rats and non-human primates display prominent, although highly variable, nigral dopaminergic neuron loss. However, the few studies available on viral vector-mediated overexpression of alpha-synuclein in mice report a weak neurodegenerative process and no clear Lewy body-like pathology. To address this issue, we performed a comprehensive comparative study of alpha-synuclein overexpression by means of recombinant adeno-associated viral vectors serotype 2/7 (rAAV2/7) at different doses in adult mouse substantia nigra.

**Results:** We noted a significant and dose-dependent alpha-synucleinopathy over time upon nigral viral vector-mediated alpha-synuclein overexpression. We obtained a strong, progressive and dose-dependent loss of dopaminergic neurons in the substantia nigra, reaching a maximum of 82% after 8 weeks. This effect correlated with a reduction in tyrosine hydroxylase immunoreactivity in the striatum. Moreover, behavioural analysis revealed significant motor impairments from 12 weeks after injection on. In addition, we detected the presence of alpha-synuclein-positive aggregates in the remaining surviving neurons. When comparing wild-type to mutant A53T alpha-synuclein at the same vector dose, both induced a similar degree of cell death. These data were supported by a biochemical analysis that showed a net increase in soluble and insoluble alpha-synuclein expression over time 25to the same extent for both alpha-synuclein variants.

**Conclusions:** In conclusion, our in vivo data provide evidence that strong and significant alpha-synuclein-induced neuropathology and progressive dopaminergic neurodegeneration can be achieved in mouse brain by means of rAAV2/7.


**Abstract:** Stroke induces inflammation that can aggravate brain damage. This work examines whether interleukin-10 (IL-10) deficiency exacerbates inflammation and worsens the outcome of permanent middle cerebral artery occlusion (pMCAO). Expression of IL-10 and IL-10 receptor (IL-10R) increased after ischemia. From day 4, reactive astrocytes showed strong IL-10R immunoreactivity. Interleukin-10 knockout (IL-10 KO) mice kept in conventional housing showed more mortality after pMCAO than the wild type (WT). This effect was associated with the presence of signs of colitis in the IL-10 KO mice, suggesting that ongoing systemic inflammation was a confounding factor. In a pathogen-free environment, IL-10 deficiency slightly increased infarct volume and neurologic deficits. Induction of proinflammatory molecules in the IL-10 KO brain was similar to that in the WT 6 hours after ischemia, but was higher at day 4, while differences decreased at day 7. Deficiency of IL-10 promoted the presence of more mature phagocytic cells in the ischemic
tissue, and enhanced the expression of M2 markers and the T-cell inhibitory molecule CTLA-4. These findings agree with a role of IL-10 in attenuating local inflammatory reactions, but do not support an essential function of IL-10 in lesion resolution. Upregulation of alternative immunosuppressive molecules after brain ischemia can compensate, at least in part, the absence of IL-10.

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Abstract: no abstract available

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Abstract: Stem cells offer great potential for regenerative medicine because they regenerate damaged tissue by cell replacement and/or by stimulating endogenous repair mechanisms. Although stem cells are defined by their functional properties, such as the potential to proliferate, to self-renew, and to differentiate into specific cell types, their identification based on the expression of specific markers remains vague. Here, profiles of stem cell metabolism might highlight stem cell function more than the expression of single genes/markers. Thus, systematic approaches including spectroscopy might yield insight into stem cell function, identity, and stemness. We review the findings gained by means of metabolic and spectroscopic profiling methodologies, for example, nuclear magnetic resonance spectroscopy (NMRS), mass spectrometry (MS), and Raman spectroscopy (RS), with a focus on neural stem cells and neurogenesis.


Abstract: In Alzheimer’s disease (AD), persistent microglial activation as sign of chronic neuroinflammation contributes to disease progression. Our study aimed to in vivo visualize and quantify microglial activation in 13- to 15-month-old AD mice using [(11)C]-(R)-PK11195 and positron emission tomography (PET). We attempted to modulate neuroinflammation by subjecting the animals to an anti-inflammatory treatment with pioglitazone (5-weeks’ treatment, 5-week wash-out period). [(11)C]-(R)-PK11195 distribution volume values in AD mice were significantly higher compared with control mice after the wash-out period at 15 months, which was supported by immunohistochemistry data. However, [(11)C]-(R)-PK11195 μPET could not demonstrate genotype- or treatment-dependent differences in the 13- to 14-month-old animals, suggesting that microglial activation in AD mice at this age and disease stage is too mild to be detected by this imaging method.

**Abstract:** INTRODUCTION: Transplantation of neural stem cells (NSCs) is increasingly suggested to become part of future therapeutic approaches to improve functional outcome of various central nervous system disorders. However, recently it has become clear that only a small fraction of grafted NSCs display long-term survival in the (injured) adult mouse brain. Given the clinical invasiveness of NSC grafting into brain tissue, profound characterisation and understanding of early post-transplantation events is imperative to claim safety and efficacy of cell-based interventions.

METHODS: Here, we applied in vivo bioluminescence imaging (BLI) and post-mortem quantitative histological analysis to determine the localisation and survival of grafted NSCs at early time points post-transplantation.

RESULTS: An initial dramatic cell loss (up to 80% of grafted cells) due to apoptosis could be observed within the first 24 hours post-implantation, coinciding with a highly hypoxic NSC graft environment. Subsequently, strong spatiotemporal microglial and astroglial cell responses were initiated, which stabilised by day 5 post-implantation and remained present during the whole observation period. Moreover, the increase in astrocyte density was associated with a high degree of astroglial scarring within and surrounding the graft site. During the two-week follow up in this study, the NSC graft site underwent extensive remodelling with NSC graft survival further declining to around 1% of the initial number of grafted cells.

CONCLUSIONS: The present study quantitatively describes the early post-transplantation events following NSC grafting in the adult mouse brain and warrants that such intervention is directly associated with a high degree of cell loss, subsequently followed by strong glial cell responses.


**Abstract:** Multiple sclerosis (MS) is a demyelinating immune-mediated disease of the central nervous system (CNS). It is the most frequent neurological disease in young adults and affects over 2 million people worldwide. Current treatments reduce the relapse rate and the formation of inflammatory lesions in the CNS, but with only temporary and limited success. Despite the presence of endogenous oligodendroglial progenitors (OPCs) and of spontaneous remyelination, at least in early MS its levels and its qualities are apparently insufficient for a sustained endogenous functional repair. Therefore, novel MS therapies should consider not only immunemodulatory but also myelin repair activities. Mesenchymal stem cells (MSCs) represent an attractive alternative to develop a cell-based therapy for MS. MSCs display stromal features and exert bystander immunemodulatory and neuroprotective activities. Importantly, MSCs induce oligodendrocyte fate decision and differentiation/maturation of adult neural progenitors, suggesting the existence of
MSC-derived remyelination activity. Moreover, transplanted MSCs promote functional recovery and myelin repair in different MS animal models. Here, we summarize the current knowledge on endogenous mechanisms for remyelination and proposed autologous MSC therapy as a promising strategy for MS treatment.


Abstract: Neurogenesis in the adult central nervous system has been well documented in several mammals including humans. By now, a plethora of data has been generated with the aim of understanding the molecular and cellular events governing neurogenesis. This growing comprehension will provide the basis for modulation of neurogenesis for therapeutic purposes, in particular in neurodegenerative diseases. Herein, we review the current knowledge on neurogenesis, in particular in the frame of epilepsy, since seizures have massive effects on neurogenesis. Conversely, some studies have suggested that aberrant neurogenesis might contribute to the development or manifestation of epilepsy and, moreover, chronic inhibition of neurogenesis in epilepsy might contribute to comorbidities of epilepsy such as cognitive deficits. Therefore, a better understanding of neurogenesis in the context of epilepsy is still required for future therapeutic purposes.


Abstract: Spinocerebellar ataxia type 2 (SCA2) is an autosomal-dominant degenerative disorder that is neuropathologically characterized primarily by infratentorial damage, although less severe supratentorial involvement may contribute to the clinical manifestation. Diffusion-weighted imaging (DWI)-Magnetic Resonance Imaging (MRI) studies of SCA2 have enabled in vivo quantification of neurodegeneration in infratentorial regions, whereas supratentorial regions have been explored less thoroughly. We measured microstructural changes in both infratentorial and supratentorial regions in 13 SCA2 patients (9 men, 4 women; mean age, 50 ± 12 years) and 15 controls (10 men, 5 women; mean age, 49 ± 14 years) using DWI-MRI and correlated the DWI changes with disease severity and duration. Disease severity was evaluated using the International Cooperative Ataxia Rating Scale and the Inherited Ataxia Clinical Rating Scale. Cerebral diffusion trace (D̂) values were generated, and regions of interest (ROIs) and voxel-based analysis with Statistical Parametric Mapping (SPM) were used for data analysis. In SCA2 patients, ROI analysis and SPM confirmed significant increases in D̂ values in the pons, cerebellar white matter (CWM) and middle cerebellar peduncles. Moreover, SPM analysis revealed increased D̂ values in the right thalamus, bilateral temporal cortex/white matter, and motor cortex/pyramidal tract regions. Increased diffusivity in the frontal white matter (FWM) and the CWM was significantly correlated with ataxia severity. DWI-MRI revealed
that both infratentorial and supratentorial microstructural changes may characterize SCA2 patients in the course of the disease and might contribute to the severity of the symptoms.


**Abstract:** BACKGROUND: An effective NMDA antagonist imaging model may find key utility in advancing schizophrenia drug discovery research. We investigated effects of subchronic treatment with the NMDA antagonist memantine by using behavioural observation and multimodal MRI.

METHODS: Pharmacological MRI (phMRI) was used to map the neuroanatomical binding sites of memantine after acute and subchronic treatment. Resting state fMRI (rs-fMRI) and diffusion MRI were used to study the changes in functional connectivity (FC) and ultra-structural tissue integrity before and after subchronic memantine treatment. Further corroborating behavioural evidences were documented.

RESULTS: Dose-dependent phMRI activation was observed in the prelimbic cortex following acute doses of memantine. Subchronic treatment revealed significant effects in the hippocampus, cingulate, prelimbic and retrosplenial cortices. Decreases in FC amongst the hippocampal and frontal cortical structures (prelimbic, cingulate) were apparent through rs-fMRI investigation, indicating a loss of connectivity. Diffusion kurtosis MRI showed decreases in fractional anisotropy and mean diffusivity changes, suggesting ultra-structural changes in the hippocampus and cingulate cortex. Limited behavioural assessment suggested that memantine induced behavioural effects comparable to other NMDA antagonists as measured by locomotor hyperactivity and that the effects could be reversed by antipsychotic drugs.

CONCLUSION: Our findings substantiate the hypothesis that repeated NMDA receptor blockade with nonspecific, noncompetitive NMDA antagonists may lead to functional and ultra-structural alterations, particularly in the hippocampus and cingulate cortex. These changes may underlie the behavioural effects. Furthermore, the present findings underscore the utility and the translational potential of multimodal MR imaging and acute/subchronic memantine model in the search for novel disease-modifying treatments for schizophrenia.


**Abstract:** INTRODUCTION: We examined whether [(18)F]LBT-999 ((E)-N-(4-fluorobut-2-enyl)2β-carbomethoxy-3β-(4’-tolyl)nortropane) is an efficient positron emission tomography (PET) tracer for the quantification of the dopamine transporter (DAT) in the healthy rat brain.
METHODS: PET studies were performed using several experimental designs, i.e. test-retest, co-injection with different doses of unlabelled LBT, displacement with GBR12909 and pre-injection of amphetamine.

RESULTS: The uptake of $[(18)F]LBT-999$ confirmed its specific binding to the DAT. The non-displaceable uptake (BP(ND)) in the striatum, between 5.37 and 4.39, was highly reproducible and reliable, and was decreased by 90% by acute injection of GBR12909. In the substantia nigra/ventral tegmental area (SN/VTA), the variability was higher and the reliability was lower. Pre-injection of amphetamine induced decrease of $[(18)F]LBT-999$ BP(ND) of 50% in the striatum.

CONCLUSIONS: $[(18)F]LBT-999$ allows the quantification of the DAT in living rat brain with high reproducibility, sensitivity and specificity. It could be used to quantify the DAT in rodent models, thereby allowing to study neurodegenerative and neuropsychiatric diseases.


Abstract Introduction: Functional connectivity (FC) studies have gained immense popularity in the evaluation of several neurological disorders, such as Alzheimer’s disease (AD). AD is a complex disorder, characterised by several pathological features. The problem with FC studies in patients is that it is not straightforward to focus on a specific aspect of pathology. In the current study, resting state functional magnetic resonance imaging (rsfMRI) is applied in a mouse model of amyloidosis to assess the effects of amyloid pathology on FC in the mouse brain. Methods: Nine APP/PS1 transgenic and nine wild-type mice (average age 18.9 months) were imaged on a 7T MRI system. The mice were anesthetized with medetomidine and rsfMRI data were acquired using a gradient echo EPI sequence. The data were analysed using a whole brain seed correlation analysis and interhemispheric FC was evaluated using a pairwise seed analysis. Qualitative histological analyses were performed to assess amyloid pathology, inflammation and synaptic deficits. Results: The whole brain seed analysis revealed an overall decrease in FC in the brains of transgenic mice compared to wild-type mice. The results showed that interhemispheric FC was relatively preserved in the motor cortex of the transgenic mice, but decreased in the somatosensory cortex and the hippocampus when compared to the wild-type mice. The pairwise seed analysis confirmed these results. Histological analyses confirmed the presence of amyloid pathology, inflammation and synaptic deficits in the transgenic mice. Conclusions: In the current study, rsfMRI demonstrated decreased FC in APP/PS1 transgenic mice compared to wild-type mice in several brain regions. The APP/PS1 transgenic mice had advanced amyloid pathology across the brain, as well as inflammation and synaptic deficits surrounding the amyloid plaques. Future studies should longitudinally evaluate APP/PS1 transgenic mice and correlate the rsfMRI findings to specific stages of amyloid pathology.
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Abstract: PURPOSE: Translocator protein (TSPO) is a biomarker of neuroinflammation that can be imaged by PET using [(11)C]-((R)PK11195. We sought to characterize the [(11)C]-((R)PK11195 kinetics in gliomas of different histotypes and grades, and to compare two reference tissue input functions (supervised cluster analysis versus cerebellar grey matter) for the estimation of [(11)C]-((R)PK11195 binding in gliomas and surrounding brain structures.

METHODS: Twenty-three glioma patients and ten age-matched controls underwent structural MRI and dynamic [(11)C]-((R)PK11195 PET scans. Tissue time-activity curves (TACs) were extracted from tumour regions as well as grey matter (GM) and white matter (WM) of the brains. Parametric maps of binding potential (BPND) were generated with the simplified reference tissue model using the two input functions, and were compared with each other. TSPO expression was assessed in tumour tissue sections by immunohistochemistry.

RESULTS: Three types of regional kinetics were observed in individual tumour TACs: GM-like kinetics (n = 6, clearance of the tracer similar to that in cerebellar GM), WM-like kinetics (n = 8, clearance of the tracer similar to that in cerebral WM) and a form of mixed kinetics (n = 9, intermediate rate of clearance). Such kinetic patterns differed between low-grade astrocytomas (WM-like kinetics) and oligodendrogliomas (GM-like and mixed kinetics), but were independent of tumour grade. There was good agreement between parametric maps of BPND derived from the two input functions in all controls and 10 of 23 glioma patients. In 13 of the 23 patients, BPND values derived from the supervised cluster input were systematically smaller than those using the cerebellar input. Immunohistochemistry confirmed that TSPO expression increased with tumour grade.

CONCLUSION: The three types of [(11)C]-((R)PK11195 kinetics in gliomas are determined in part by tracer delivery, and indicated that kinetic analysis is a valuable tool in the study of gliomas with the potential for in vivo discrimination between low-grade astrocytomas and oligodendrogliomas. Supervised cluster and cerebellar input functions produced consistent BPND estimates in approximately half of the gliomas investigated, but had a systematic difference in the remainder. The cerebellar input is preferred based on theoretical and practical considerations.

Abstract: Background: Neurodegenerative diseases are characterized by key features such as loss of neurons, astrocitosis, and microglial activation/proliferation. These changes cause differences in the density of cell types between control and disease subjects, confounding results from gene expression studies. Chromosome X (ChrX) is known to be specifically important in the brain. We hypothesized the existence of a chromosomal signature of gene expression associated with the X-chromosome for neurological conditions not normally associated with that chromosome. The hypothesis was investigated using publicly available microarray datasets from studies on Parkinson’s disease, Alzheimer’s disease, and Huntington’s disease. Data were analyzed using Chromowave, an analytical tool for detecting spatially extended expression changes along chromosomes. To examine associations with neuronal density and astrocitosis, the expression of cell specific reporter genes was extracted. The association between these genes and the expression patterns extracted by Chromowave was then analyzed. Further analyses of the X:Autosome ratios for laser dissected neurons, microglia cultures and whole tissue were performed to detect cell specific differences. Results: We observed an extended pattern of low expression of ChrX consistent in all the neurodegenerative disease brain datasets. There was a strong correlation between mean ChrX expression and the pattern extracted from the autosomal genes representing neurons, but not with mean autosomal expression. No chromosomal patterns associated with the neuron specific genes were found on other chromosomes. The chromosomal expression pattern was not present in datasets from blood cells. The X:Autosome expression ratio was also higher in neuronal cells than in tissues with a mix of cell types. Conclusions: The results suggest that neurological disorders show as a reduction in mean expression of many genes along ChrX. The most likely explanation for this finding relates to the documented general up-regulation of ChrX in brain tissue which, this work suggests, occurs primarily in neurons. If validated, this cell specific ChrX expression warrants further research as understanding the biological reasons and mechanisms for this expression, may help to elucidate a connection with the development of neurodegenerative disorders.


Abstract: Endogenous neural stem cells (eNSCs) reside in defined regions of the adult brain and have the potential to generate new brain cells, including neurons. Stimulation of adult neurogenesis presents an enormous potential for regenerative therapies in the central nervous system. However, the methods used to monitor the proliferation, migration, differentiation, and functional integration of eNSCs and their progeny are often invasive and limited in studying dynamic processes. To overcome this limitation, novel techniques and contrast mechanisms for in vivo imaging of neurogenesis have recently been developed and successfully applied. In vivo labeling of endogenous neuronal progenitor cells in situ with contrast agents or tracers enables longitudinal visualization of their proliferation and/or migration. Labeling of these cells with magnetic nanoparticles has proven to be very useful for tracking neuroblast migration with MRI. Alternatively, genetic labeling using reporter gene technology has been demonstrated for optical and MR imaging, leading to the development of powerful tools for in vivo optical imaging of
In recent years, the iron storage protein ferritin has been used as an endogenously produced MRI contrast agent to monitor neuroblast migration. The use of specific promoters for neuronal progenitor cell imaging increases the specificity for visualizing neurogenesis. Further improvements of detection sensitivity and neurogenesis-specific contrast are nevertheless required for each of these imaging techniques to further improve the already high utility of this toolbox for preclinical neurogenesis research.


Abstract: Purpose: Amyloid deposition in the brain is considered an initial event in the progression of Alzheimer's disease. We hypothesized that the presence of amyloid plaques in the brain of APP/PS1 mice leads to higher diffusion kurtosis measures due to increased microstructural complexity. As such, our purpose was to provide an in vivo proof of principle for detection of amyloidosis by diffusion kurtosis imaging (DKI).

Methods: APP/PS1 (n=5) mice were imaged by DKI at the age of 16 months. After normalization of absolute values to the cerebellum, a nearly plaque-free region, mean, radial, and axial diffusion kurtosis were significantly higher in the hippocampus, thalamus, and cortex than in the brain of wild-type mice. This finding suggests increased DKI metrics in the hippocampus, thalamus, and cortex in APP/PS1 mice compared to wild-type mice.

Results: Histograms of the frequency distribution of the DKI parameters tended to shift to higher values. After normalization of absolute values to the cerebellum, a nearly plaque-free region, mean, radial, and axial diffusion kurtosis were significantly higher in the hippocampus, thalamus, and cortex than in the brain of wild-type mice. This finding suggests increased DKI metrics in the hippocampus, thalamus, and cortex in APP/PS1 mice compared to wild-type mice.

Conclusion: The current study, although small-scale, suggests increased DKI metrics in the hippocampus, thalamus, and cortex of APP/PS1 mice compared to wild-type mice. These results warrant further investigations on the potential of DKI as a sensitive marker for Alzheimer's disease.
METHODS: We examined a healthy control group (BALB/C mice, n = 6) and group of induced hepatocellular carcinoma (HCC, matrilin-2 transgenic KO mice, n = 9), where hepatocellular carcinoma was induced by diethylnitrosamine. We used [99mTc]-MAA as radiopharmaceutical for liver SPECT imaging in a small animal SPECT/CT system. A liver radioactivity overview map was generated. Segmentation of the liver was calculated by Otsu thresholding method. Based on the segmentation the radioactivity volume and the summarized liver activity were determined.

RESULTS: Tumor burden of the livers was quantitatively determined by creating parametric data from the resulting volumetric maps. Ex vivo liver mass data were applied for the validation of in vivo measurements. An uptake with cold spots as tumors was observed in all diseased animals in SPECT/CT scans. Isotope-labeled particle uptake (standardized uptake concentration) of control (median 0.33) and HCC (median 0.18) groups was significantly different (p = 0.0015, Mann Whitney U test).

CONCLUSION: A new potential application of [99mTc]-MAA was developed and presents a simple and very effective means to quantitatively characterize liver cold spot lesions resulting from Kupffer cell dysfunctions as a consequence of tumor burden.


Abstract: Performance of two supervised cluster analysis (SVCA) algorithms for extracting reference tissue curves was evaluated to improve quantification of dynamic (R)-[(11)C]PK11195 brain positron emission tomography (PET) studies. Reference tissues were extracted from images using both a manually defined cerebellum and SVCA algorithms based on either four (SVCA4) or six (SVCA6) kinetic classes. Data from controls, mild cognitive impairment patients, and patients with Alzheimer’s disease were analyzed using various kinetic models including plasma input, the simplified reference tissue model (RPM) and RPM with vascular correction (RPMV(b)). In all subject groups, SVCA-based reference tissue curves showed lower blood volume fractions (V(b)) and volume of distributions than those based on cerebellum time-activity curve. Probably resulting from the presence of specific signal from the vessel walls that contains in normal condition a significant concentration of the 18 kDa translocation protein. Best contrast between subject groups was seen using SVCA4-based reference tissues as the result of a lower number of kinetic classes and the prior removal of extracerebral tissues. In addition, incorporation of V(b) in RPM improved both parametric images and binding potential contrast between groups. Incorporation of V(b) within RPM, together with SVCA4, appears to be the method of choice for analyzing cerebral (R)-[(11)C]PK11195 neurodegeneration studies.
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