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Application R231-2016-2144

Name Walden Bjørn-Yoshimoto
Position Assistant Professor

Institution University of Copenhagen
Institute Faculty of Health and Medical Sciences
Department Department of Drug Design and Pharmacology

Budget subtotals & total

Budget line	Year 1	Year 2	Year 3	Total
Salary - post doc	538.344,00 kr.	549.108,00 kr.	560.092,00 kr.	<u>1.647.544,00 kr.</u>
Running costs	161.000,00 kr.	150.000,00 kr.	139.000,00 kr.	<u>450.000,00 kr.</u>
Total	699.344,00 kr.	699.108,00 kr.	699.092,00 kr.	2.097.544,00 kr.

Project title

Novel tools for subtype-selective modulation of excitatory amino acid transporters

Summary of application

Background

Glutamate is involved in virtually all processes in the brain, including numerous disorders such as schizophrenia, Alzheimer's disease, epilepsy, bipolar disorder and obsessive-compulsive disorder. A family of excitatory amino acid transporters (EAATs) mediate the synaptic reuptake of glutamate in the brain. Five subtypes of EAATs exist in humans, but subtype-specific pharmacological tools have only been identified for EAAT1 (by our lab) and EAAT2, which limits studies of EAATs, both in the healthy brain and in pathological states associated with dysfunctional glutamatergic neurotransmission.

Aims and objectives

I aim to develop tools for subtype-specific modulation of EAATs and characterize the novel compounds to understand the molecular basis of their subtype preference in EAATs. To achieve this, I will pursue three objectives:

1. Optimize and characterize an EAAT3 preferring inhibitor that I recently identified.
2. Characterize molecular determinants for the subtype-selectivity profiles of recently identified EAAT1- and EAAT2-selective inhibitors.
3. Screen a small-compound library for modulatory activity at EAAT3 and EAAT4, and develop hits into more potent and/or selective modulators.

Methods

The project will use established methods in laboratories. This will include molecular biology, radio-substrate uptake, fluorescence based assays, and electrophysiological assays, primarily two electrode voltage clamp and patch clamp recordings.

Expected outcome and perspectives

We expect one or more novel tool compounds to be developed and characterized from objective (1). Objective (2) will yield insight into the modes of action of subtype-selective modulators at EAATs, thus facilitating rational design of future ligands. Finally, objective (3) will likely yield 1-3 lead compounds, and development of these could yield the first selective modulators of EAAT3 and EAAT4. Together these tools and insights will enable further research into glutamatergic neurotransmission.

Project start date**Project end date**

Personal information

First name	Walden
Last name	Bjørn-Yoshimoto
Email	waldenb@gmail.com
Date of birth	1983-06-27
Address	Rødvig Alle 27
Postal code	2650
City	Hvidovre
Country	Denmark
Phone number	+45 24494408
Academic degree	PhD
Position	Assistant Professor
Institution	University of Copenhagen

The project

Project title

Novel tools for subtype-selective modulation of excitatory amino acid transporters

Novelty & originality of the research

I aim to gain the following novel information and products pertaining to the excitatory amino acid transporters (EAATs):

- The first selective EAAT3 modulators
- The first selective EAAT4 modulators
- New insight into structure-function aspects of the mammalian EAATs:
- New insight into the molecular basis for the subtype-selectivity of ligands targeting the orthosteric site in the EAAT. Thought this:
- The basis for rational design of orthosteric subtype-selective EAAT ligands

Relevance to biomedicine

The glutamatergic system is involved in many different central disorders and thus holds great therapeutic potential. The subtype-selective pharmacological tools targeting EAAT3 and EAAT4 that will be pursued in this project can be used to elucidate the roles of these two glutamate transporters in health and disease. Moreover, the tools can also be used for studies of other EAATs, will thus make it possible to dissect the physiological functions of each subtype with relevant temporal resolution.

Applicant's CV

Web of Science

Results: 9
 Sum of Times Cited: 56
 Sum of Times Cited without self-citations: 47
 Citing Articles: 51
 Citing Articles without self-citations: 47
 Average Citations per item: 6.22
 h-index: 3

Name

Walden Emil Bjørn-Yoshimoto

Address

Sigynsgade 36, 4. TV
 2200 KBH N

email

waldenb@gmail.com/walden@sund.ku.dk

Education:

2010, June 8th

MSc in biochemistry

2007, August 27th

BSc in biochemistry

Current and most recently held positions

2013 –

PhD student at University of Copenhagen (Handing in thesis January 31st, 2017)

2011 – 2013

Research assistant (2011) and research scientist (2012-2013) at EVOLVA S/A

Academic awards

Master's thesis awarded 12 (highest grade possible).

Travel grant from Neurochemistry International to present at their conference in Cairns, Australia in 2015.

Grant to support a scientific visit at the NIH, 2015 (Augustinus).

Scientific focus area

I have worked with the dopamine transporter, and now with the excitatory amino acid transporters. Mainly in the cellular, and lately molecular aspects, where I am currently aiming at developing tools to further the field of glutamatergic neurotransmission and the excitatory amino acid transporters role in this.

International relations

Harvard Medical School, Boston, MA, USA

The ICCB-Longwood and the lab of Dr. Clifford Woolf. Here I performed the screen identifying "Compound X" that I will pursue in objective 1.

National Institute of Mental Health (NIH), Bethesda, MD, USA

I did a 7-month stay in the lab of Dr. Susan Amara, one of the world leaders in the neurotransmitter transporter field and scientific director of the National Institute of Mental Health (NIMH/NIH). It was a pleasure to work with her, and she is high on my list of people to contact for expertise and collaborations.

Supervision of PhDs and post docs

As PhD student, I have not supervised PhD students or post docs, but I have supervised a student doing research in our lab.

Management expertise

Project management from master's thesis, PhD, and from time as research scientist in EVOLVA S/A (mainly last months, where I was the only scientist on my projects). Management of people from EVOLVA S/A where I was responsible for 1/3 of a technician and ½ of a student helper doing research. Organization management as founding board member for our student house and from various student political bodies, including some where I was assisting the director of studies in managing the structure of the biochemistry study.

Full publication list for applicant

2016

1. Hansen, J. C., Bjorn-Yoshimoto, W. E., Bisballe, N., Nielsen, B., Jensen, A. A., and Bunch, L. (2016) beta-Sulfonamido Functionalized Aspartate Analogues as Excitatory Amino Acid Transporter Inhibitors: Distinct Subtype Selectivity Profiles Arising from Subtle Structural Differences. *Journal of medicinal chemistry* 59, 8771-8786

I (together with my PI, Dr. Jensen) was the only non-chemists on in this study, and as such I was responsible for all the EAAT-related work and pharmacological experiments, and deeply involved in determining the structure-activity relationships. Thereby I was also, together with authors Hansen, Jensen and Bunch, responsible for determining the compounds to pursue.

2. Huynh, T. H., Erichsen, M. N., Tora, A. S., Goudet, C., Sagot, E., Assaf, Z., Thomsen, C., Brodbeck, R., Stensbol, T. B., Bjorn-Yoshimoto, W. E., Nielsen, B., Pin, J. P., Gefflaut, T., and Bunch, L. (2016) New 4-Functionalized Glutamate Analogues Are Selective Agonists at Metabotropic Glutamate Receptor Subtype 2 or Selective Agonists at Metabotropic Glutamate Receptor Group III. *Journal of medicinal chemistry* 59, 914-924

In this study, my main contribution was testing the compounds in [3H]-D-Asp uptake assays on EAAT1-3. We thought some of the compounds could be EAAT selective, which was my initial interest in this project. After realizing that this was not the case, my involvement became reduced in the later parts of this project.

3. Haym, I., Huynh, T. H., Hansen, S. W., Pedersen, M. H., Ruiz, J. A., Erichsen, M. N., Gynther, M., Bjorn-Yoshimoto, W. E., Abrahamsen, B., Bastlund, J. F., Bundgaard, C., Eriksen, A. L., Jensen, A. A., and Bunch, L. (2016) Bioavailability Studies and in vitro Profiling of the Selective Excitatory Amino Acid Transporter Subtype 1 (EAAT1) Inhibitor UCPH-102. *ChemMedChem* 11, 403-419

My role in this study was primarily the [3H]-D-Asp uptake assays, as indicated as IC50 values in this paper. I also had a role in assessing structure-activity relationships based on this data.

4. Hansen, S. W., Erichsen, M. N., Huynh, T. H., Ruiz, J. A., Haym, I., Bjorn-Yoshimoto, W. E., Abrahamsen, B., Hansen, J., Storgaard, M., Eriksen, A. L., Jensen, A. A., and Bunch, L. (2016) New Insight into the Structure-Activity Relationships of the Selective Excitatory Amino Acid Transporter Subtype 1 (EAAT1) Inhibitors UCPH-101 and UCPH-102. *ChemMedChem* 11, 382-402

My main contributions to this manuscript was evaluating the structure-activity relationship to determine this, and as the basis for deciding on new compounds to synthesize. This was of course in collaboration with the chemists and my supervisor, Dr. Jensen. Besides this, I did a large part of the [3H]-D-Asp uptake assays for testing analogs (all the authors with higher priority than me are chemists, so I was the main contributor in the in vitro pharmacology department).

5. Hansen, S. W., Erichsen, M. N., Fu, B., Bjorn-Yoshimoto, W. E., Abrahamsen, B., Hansen, J. C., Jensen, A. A., and Bunch, L. (2016) Identification of a New Class of Selective Excitatory Amino Acid Transporter Subtype 1 (EAAT1) Inhibitors Followed by a Structure-Activity Relationship Study. *Journal of medicinal chemistry* 59, 8757-8770

For this study I was deeply involved in the structure-activity relationship elucidation, and the majority of in vitro profiling of compounds at EAATs.

6. Bjørn-Yoshimoto, W. E., and Underhill, S. M. (2016) The importance of the excitatory amino acid transporter 3 (EAAT3). *Neurochemistry international* 98, 4-18

This is a review article, which I wrote with input from Dr. Suzanne Underhill at the National Institute of Mental Health, Bethesda, USA.

2015

7. Jensen, A. A., Fahlke, C., Bjorn-Yoshimoto, W. E., and Bunch, L. (2015) Excitatory amino acid transporters: recent insights into molecular mechanisms, novel modes of modulation and new therapeutic possibilities. *Current opinion in pharmacology* 20, 116-123

Dr. Jensen was the main author, but with input about subject and structure, corrections and re-writes of the manuscript by all other authors, including myself.

2010

8. Eriksen, J., Bjørn-Yoshimoto, W. E., Jorgensen, T. N., Newman, A. H., and Gether, U. (2010) Postendocytic sorting of constitutively internalized dopamine transporter in cell lines and dopaminergic neurons. *J Biol Chem* 285, 27289-27301

My main contributions were ELISAs and the HA-DAT and Rab protein co-localization confocal microscopy, including the quantification of co-localization, as well as comments and ideas for extra experiments needed (the β 2-AR controls were only

included because I insisted).

Patents

2015

Submitted: Heim, J., Jensen, S., Klein, J., and Bjørn-Yoshimoto, W.E. (2015).
Methods and materials for identifying compounds promoting translational read-through of nonsense mutations (WO application number: WO2015082658A1). From my time at Evolva S/A.

I was research scientist, responsible for developing the assay in question (in collaboration with Jensen, S.). I was working under a project manager, but as only research scientist on the project, and I managed 1/3 full time lab technician and 1/2 full time student helper on the project. My project manager (Jensen, S.) was headhunted for another job, so I finished the remainder of this project on my own, and the results I obtained led to this patent application.

Own research results

After having studied molecular pharmacology aspects of the dopamine transporter in Professor Ulrik Gether's group during my bachelor and master thesis projects, I have studied medicinal chemistry and molecular pharmacology aspects of the excitatory amino acid transporters (EAATs) since I began my PhD study in the lab of Dr. Anders A. Jensen in 2013.

I have collaborated with the Bunch group on several structure-activity relationship studies of the first two EAAT1-selective inhibitors developed by the Jensen and Bunch groups [25,32,33]. Moreover, I have been working closely with the medicinal chemists on the development of a series of aspartate analogs, several of which displaying substantial subtype-selectivity profiles at the transporters [1]. Finally, I have identified a novel EAAT3-inhibitor in a 50,000 compound-library screening. Several of these compounds form the basis for some of the objectives in the present project.

I am currently finishing up a study delineating the importance of specific loops in the EAAT involved in the substance translocation process. In another study, I spent seven months in the lab of Dr. Susan Amara at the NIH. Here I worked on a project trying to identify interaction partners of EAAT3 that are important for an internalization of EAAT3, which is mediated by amphetamine uptake through dopamine transporters in the same cell. This project is still ongoing and has not yet been published.

Brief description of the research environment

The Anders A. Jensen (AAJ) group is part of the Experimental Pharmacology section headed by Professor Hans Bräuner-Osborne at Department of Drug Design and Pharmacology, SUND, University of Copenhagen, and the group presently consists of four PhD students (including me), one post doc and one master student. The AAJ group is fully equipped for the research activities outlined in the present project application.

The research of the AAJ group is focused medicinal chemistry and molecular pharmacology studies of ligand-gated ion channels and excitatory amino acid transporters (EAATs). The group has a long track-record of developing novel modulators for these targets, and several of these ligands have become commercially available pharmacological tools. Moreover, the group has over the years successfully applied several of these tools to delineate structure-function aspects of their respective receptors/transporters as well as to probe the physiological functions of the targets in their native environment.

For more than a decade the AAJ group has collaborated with the Lennart Bunch group on the development and characterization of EAAT ligands, and this collaboration has yielded several important tool compounds to the field. AAJ and I hope to be able to continue this work in the coming years, with me being the main person on this project in the group. We also aim to add at least one master student on the project during the three-year period, whom I will train.

Project description

In the present proposal, I aim to develop and characterize novel tools for the subtype-selective modulation of excitatory amino acid transporters (EAATs) by pursuing three objectives.

Objective 1 is to pursue a promising EAAT3-preferring inhibitor I recently. Judging from the structure of this lead, it is most likely an allosteric inhibitor, and we will perform a structure-activity relationship study to develop more potent and/or selective EAAT3 inhibitors. In objective 2 I will use mutational studies to map the molecular determinants for the subtype-selectivity of recently identified aspartate analogs targeting the orthosteric sites in EAAT1 and EAAT2 [1]. Objective 3 will be the screening of a compound library for activity at EAAT3 and EAAT4. During the last year, I will stay six months in the lab of Dr. Christoph Fahlke in Jülich, Germany, where I will learn sophisticated electrophysiological methods. Here I will characterize the binding and kinetics of select EAAT modulators.

Background

(S)-Glutamate (Glu) is the major excitatory neurotransmitter in the CNS, where it innervates ~40% of all neurons and is involved in all complex neuronal processes. Thus,

pharmacological intervention in glutamatergic mechanisms holds an immense potential for the future treatment of a wide range of psychiatric, cognitive and neurodegenerative disorders including schizophrenia, epilepsy, amyotrophic lateral sclerosis, Huntington's disease, Alzheimer's disease, and bipolar disorder [2-15]. The reuptake of Glu following its release into the synaptic cleft during neuronal firing is a key part of glutamatergic transmission, and this is mediated by the excitatory amino acid transporters (EAATs) (**Fig. 1A**) [16]. Five human EAAT subtypes have been identified, EAAT1-5, and the fact that they exhibit distinct cellular and regional expression patterns as well as different substrate specificities suggest that they govern different physiological functions (**Fig. 1A**) [17-19]. The EAAT-mediated translocation of Glu is driven by co-transport of Na^+ and H^+ and counter-transport of K^+ (**Fig. 1B**). In addition to its transport activity, the EAAT also has an anion conductance that is uncoupled from its transport, but increased by Glu binding [20]. This characteristic is particularly pronounced for EAAT4, but the physiological importance of this conductance is poorly understood.

Despite the widely accepted potential in EAATs as putative therapeutic targets, drug development attempts targeting the EAATs over the years have been sparse [21], most likely due to the ubiquity of glutamatergic signaling and the inherent risks of off-target effects. The resulting pronounced lack of truly selective pharmacological tools for the five transporters has severely hampered explorations of the physiological roles and therapeutic potential of the respective transporters. During the last decade, the medicinal chemistry and molecular pharmacology groups headed by Dr. Bunch and Dr. Jensen, respectively, have collaborated on the development and characterization of novel EAAT modulators. This has resulted in the discovery of several very interesting EAAT ligands, most notably the UCPH-100 series, the first class of EAAT1-selective inhibitors [22-24], and more recently another class of selective EAAT1-inhibitors [25] and 3-substituted aspartate (Asp) analogs displaying a wide spectrum of subtype-selectivities at the transporters [1]. In this project we will continue this development of novel pharmacological tools and also investigate the mechanisms of action underlying the modulation exerted by selected modulators at the transporters.

Project Activities

1. Development of an EAAT3-selective inhibitor and investigation of its mechanism of action.

In a recent screening of a 50,000 compound-library at EAAT3 at Harvard Medical School I did as part of my PhD, I have identified a hit compound displaying 10-fold more potent inhibition of EAAT3 ($\sim 8 \mu\text{M}$) than of EAAT1 and EAAT2, termed "Compound X" (**Fig. 2A**). Compound X shares no structural resemblance with the amino acid substrates for EAATs (due to intellectual property concerns, the structure is not shown), which suggests an allosteric mechanism of action at EAAT3. The medicinal chemistry optimization of Compound X will be an iterative process: In the first phase, commercially available analogs of the compound will be characterized at the transporters, and the structure-activity relationship (SAR) gained from this will in the subsequent phases be used to

guide the design of novel analogs that will be synthesized by the Bunch group. This approach is similar to what we have previously utilized to develop the first EAAT1-selective inhibitors [22,25], and the Jensen group has also developed several interesting modulators of other transporters and receptors using this strategy [25-28]. Hence, we find it plausible that it will be possible to develop potent and selective EAAT3 inhibitors from Compound X.

Concomitantly with these activities, we will delineate the binding site for Compound X (or an improved analog) in EAAT3 through characterization of the compound at chimeric EAATs (**Fig. 2B-D**) followed by studies at mutant EAATs where residues in regions found to be important for inhibitor binding have been point-mutated. Once the approximate location of its binding site in EAAT3 has been determined, Compound X will be docked into it in a homology model of the transporter, and other residues potentially involved in its binding according to this model will be probed in a final round of mutagenesis and testing. In a previous study, the binding site of UCPH-101 in EAAT1 has been described in great detail using this strategy [24].

2. Delineation of molecular determinants in EAATs for the subtype-selectivities exhibited by orthosteric 3-substituted aspartate analogs.

In a recent study, we have developed a series of 3-substituted Asp analogs as potent EAAT inhibitors, including several EAAT1-preferring and EAAT2-selective inhibitors (**Fig. 3A**) [1]. The Bunch and Jensen groups are currently developing additional analogs in this series, which will be characterized functionally at the EAATs throughout the project. In this project, we will study the molecular basis of the subtype-selectivity displayed by a selection of these inhibitors. Since the Asp analogs target the orthosteric (Glu-binding) site in the EAATs, we have docked the inhibitors into these sites in constructed homology models of EAAT1 and EAAT2 based on a crystal structure of a bacterial homolog with another Asp analog inhibitor, DL-TBOA, bound (**Fig. 3B**) [29]. In this way, we have identified four residues in proximity of the 3-substituent in the Asp analogs that differ between the two subtypes and thus could be responsible for or contribute to their respective subtype-selectivities (**Figs. 3C**). Initially, we will point-mutate and 'swap' the identity of these four residues between EAAT1 and EAAT2 (Q93 in TM2, S364 in HP1 and G443 and Q446 in HP2, using the EAAT1 numbers, **Fig. 3C**). Since the molecular basis for the selectivities could be more complex, we will also 'swap' bigger regions between the two transporters: the entire TM2, HP1 or HP2 and a part of TM4 (**Fig. 3D-E**).

Through this study, we will gain information about the binding modes of the 3-substituted Asp analogs in the orthosteric site of the EAAT and of residues/regions of importance for their respective subtype-selectivities. This insight will also be applied in our medicinal chemistry development of future 3-substituted Asp analogs, and thus it will hopefully aid in the design of even more potent and/or subtype-selective analogs in this series. Since we already have potent compounds with subtype selectivities, and have used this approach previously for similar projects, this is expected to yield a detailed map of residues involved in the selectivity, and this knowledge is likely to assist in designing novel compounds.

3. Screening for novel EAAT3 and EAAT4 modulators.

This part of the project consists of a screening of a small compound library at EAAT3 and EAAT4 in the search for novel ligands for these two subtypes. Subtype-selective modulators of these EAAT subtypes have not yet been reported, and the physiological roles and therapeutic potential of these two transporters are poorly elucidated. Although we already have identified "compound X", we believe it is worth to include EAAT3 in this screen, since the availability of additional EAAT3-preferring leads would be highly valuable for our development of high-potency and selective EAAT3-inhibitors. The screening will be performed using a 4500-compound library using a [³H]-D-Asp uptake assay implemented in our lab, analogously to the strategy used in previous screenings [22,25]. The follow-up to this screening will be conducted utilizing the same basic

approach as with the EAAT3-preferring inhibitor described above (objective 1). Initially, we will test commercially available analogs of the identified hits, and subsequently the Bunch group will synthesize non-commercial analogs. Any hits found in the screening will be developed and characterized as described for the EAAT3 inhibitors in project 1. Given our previous successes identifying EAAT1-selective inhibitors in this type of small screenings [22,25], as well as modulators of other molecular targets [26-28,30], we find it plausible that this screening could identify compounds with pharmacological activity at these two transporters. Furthermore, since much of the testing of such compounds could be done in conjunction with characterization in other objectives, I can perform a reasonable amount of characterization without much additional time dedicated to this objective.

4. Characterization of kinetic properties and the modes of modulation of anion conductance by selected EAAT modulators.

I will spend a 6-month period in the lab of Professor Christoph Fahlke (Forschungszentrum Jülich, Germany). Professor Fahlke is a world-renowned expert in sophisticated electrophysiological techniques, and for several years his lab has studied the electrophysiological characteristics of EAATs [22-24,31-33]. During this stay, I will study the modulation exerted by a selection of modulators developed in projects 1-3 at EAATs in patch-clamp recordings. The kinetic parameters of the modulators, including their binding on-/off-rates and the induced changes in transport and anion channel kinetics, as well as the modes of actions of their modulation of both EAAT-mediated Glu uptake and anion conductance will be investigated. The stay is scheduled for the last year of the project, and we do not know which specific modulators we have developed by then. However, I will study the more promising candidates from the different modulator series in these studies, which will include an analog from the EAAT3-preferring inhibitor series (objective 1), an Asp analog (objective 2) and identified EAAT3 or EAAT4 modulators (objective 3).

Significance of the project

Both from a basic scientific and medical point of view, the development of potent and selective EAAT modulators applicable for studies of the physiological functions and therapeutic potential of these transporters is of great interest. Moreover, a detailed delineation of the mechanisms of action of these modulators at the transporters will be an important contribution to the understanding of molecular structure/function aspects of these transporters. We aim to produce at least one publication per objective in high-impact scientific journals in the field such as Journal of Medicinal Chemistry, Journal of Biological Chemistry or Journal of Neuroscience, as well as one to two popular scientific articles in "Lægemedelforskning". Besides these direct scientific results, the project will also further educate and train me as a post doc with expertise in molecular neuropharmacology and electrophysiology. This project will also further my career by allowing me to follow through on some interesting results from my PhD, that are not far enough along to allow for high-impact publications at this time. Developing these leads

for publications and/or patents will be a great asset for my further academic career, as compared to leaving this project for someone else to finish, or publishing preliminary results in a low-impact journal.

Literature references

1. Hansen, J. C., Bjorn-Yoshimoto, W. E., Bisballe, N., Nielsen, B., Jensen, A. A., and Bunch, L. (2016) beta-Sulfonamido Functionalized Aspartate Analogues as Excitatory Amino Acid Transporter Inhibitors: Distinct Subtype Selectivity Profiles Arising from Subtle Structural Differences. *Journal of medicinal chemistry* 59, 8771-8786
2. Sigitova, E., Fisar, Z., Hroudova, J., Cikankova, T., and Raboch, J. (2016) Biological hypotheses and biomarkers of bipolar disorder. *Psychiatry and clinical neurosciences*
3. Javitt, D. C. (2004) Glutamate as a therapeutic target in psychiatric disorders. *Molecular psychiatry* 9, 984-997, 979
4. Niswender, C. M., Jones, C. K., and Conn, P. J. (2005) New therapeutic frontiers for metabotropic glutamate receptors. *Current topics in medicinal chemistry* 5, 847-857
5. Corona, J. C., Tovar-y-Romo, L. B., and Tapia, R. (2007) Glutamate excitotoxicity and therapeutic targets for amyotrophic lateral sclerosis. *Expert opinion on therapeutic targets* 11, 1415-1428
6. Bowie, D. (2008) Ionotropic glutamate receptors & CNS disorders. *CNS & neurological disorders drug targets* 7, 129-143
7. Javitt, D. C. (2015) Neurophysiological models for new treatment development in schizophrenia: early sensory approaches. *Annals of the New York Academy of Sciences* 1344, 92-104
8. Wesseling, J. F., and Perez-Otano, I. (2015) Modulation of GluN3A expression in Huntington disease: a new n-methyl-D-aspartate receptor-based therapeutic approach? *JAMA neurology* 72, 468-473
9. Plaitakis, A., and Shashidharan, P. (2000) Glutamate transport and metabolism in dopaminergic neurons of substantia nigra: implications for the pathogenesis of Parkinson's disease. *Journal of neurology* 247 Suppl 2, Ii25-35
10. Chang, P. K., Verbich, D., and McKinney, R. A. (2012) AMPA receptors as drug targets in neurological disease--advantages, caveats, and future outlook. *The European journal of neuroscience* 35, 1908-1916
11. Lerma, J., and Marques, J. M. (2013) Kainate receptors in health and disease. *Neuron* 80, 292-311
12. Bianchi, M. G., Bardelli, D., Chiu, M., and Bussolati, O. (2014) Changes in the expression of the glutamate transporter EAAT3/EAAC1 in health and disease. *Cellular and molecular life sciences : CMLS* 71, 2001-2015
13. Prentice, H., Modi, J. P., and Wu, J. Y. (2015) Mechanisms of Neuronal Protection against Excitotoxicity, Endoplasmic Reticulum Stress, and Mitochondrial Dysfunction in Stroke and Neurodegenerative Diseases. *Oxidative medicine and cellular longevity* 2015, 964518

14. Zhang, Y., Li, P., Feng, J., and Wu, M. (2016) Dysfunction of NMDA receptors in Alzheimer's disease. *Neurological sciences : official journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology* 37, 1039-1047
15. King, A. E., Woodhouse, A., Kirkcaldie, M. T., and Vickers, J. C. (2016) Excitotoxicity in ALS: Overstimulation, or overreaction? *Experimental neurology* 275 Pt 1, 162-171
16. Jensen, A. A., Fahlke, C., Bjorn-Yoshimoto, W. E., and Bunch, L. (2015) Excitatory amino acid transporters: recent insights into molecular mechanisms, novel modes of modulation and new therapeutic possibilities. *Current opinion in pharmacology* 20, 116-123
17. Danbolt, N. C., Lehre, K. P., Dehnes, Y., Chaudhry, F. A., and Levy, L. M. (1998) Localization of transporters using transporter-specific antibodies. *Methods in enzymology* 296, 388-407
18. Amara, S. G., and Fontana, A. C. (2002) Excitatory amino acid transporters: keeping up with glutamate. *Neurochemistry international* 41, 313-318
19. Bjørn-Yoshimoto, W. E., and Underhill, S. M. (2016) The importance of the excitatory amino acid transporter 3 (EAAT3). *Neurochemistry international* 98, 4-18
20. Machtens, J. P., Kortzak, D., Lansche, C., Leinenweber, A., Kilian, P., Begemann, B., Zachariae, U., Ewers, D., de Groot, B. L., Briones, R., and Fahlke, C. (2015) Mechanisms of anion conduction by coupled glutamate transporters. *Cell* 160, 542-553
21. Bunch, L., Erichsen, M. N., and Jensen, A. A. (2009) Excitatory amino acid transporters as potential drug targets. *Expert opinion on therapeutic targets* 13, 719-731
22. Jensen, A. A., Erichsen, M. N., Nielsen, C. W., Stensbol, T. B., Kehler, J., and Bunch, L. (2009) Discovery of the first selective inhibitor of excitatory amino acid transporter subtype 1. *Journal of medicinal chemistry* 52, 912-915
23. Erichsen, M. N., Huynh, T. H., Abrahamsen, B., Bastlund, J. F., Bundgaard, C., Monrad, O., Bekker-Jensen, A., Nielsen, C. W., Frydenvang, K., Jensen, A. A., and Bunch, L. (2010) Structure-activity relationship study of first selective inhibitor of excitatory amino acid transporter subtype 1: 2-Amino-4-(4-methoxyphenyl)-7-(naphthalen-1-yl)-5-oxo-5,6,7,8-tetrahydro-4H-chrom ene-3-carbonitrile (UCPH-101). *Journal of medicinal chemistry* 53, 7180-7191
24. Abrahamsen, B., Schneider, N., Erichsen, M. N., Huynh, T. H., Fahlke, C., Bunch, L., and Jensen, A. A. (2013) Allosteric modulation of an excitatory amino acid transporter: the subtype-selective inhibitor UCPH-101 exerts sustained inhibition of EAAT1 through an intramonomeric site in the trimerization domain. *J Neurosci* 33, 1068-1087
25. Hansen, S. W., Erichsen, M. N., Fu, B., Bjorn-Yoshimoto, W. E., Abrahamsen, B., Hansen, J. C., Jensen, A. A., and Bunch, L. (2016) Identification of a New Class of Selective Excitatory Amino Acid Transporter Subtype 1 (EAAT1) Inhibitors Followed by a Structure-Activity Relationship Study. *Journal of medicinal chemistry* 59, 8757-8770
26. Jørgensen, C. G., Frølund, B., Kehler, J., and Jensen, A. A. (2011) Discovery of Benzamide Analogues as a Novel Class of 5-HT₃ Receptor Agonists. *ChemMedChem* 6, 725-736
27. Trattig, S. M., Harpsøe, K., Thygesen, S. B., Rahr, L. M., Ahring, P. K., Balle,

- T., and Jensen, A. A. (2012) Discovery of a Novel Allosteric Modulator of 5-HT₃ Receptors INHIBITION AND POTENTIATION OF CYS-LOOP RECEPTOR SIGNALING THROUGH A CONSERVED TRANSMEMBRANE INTERSUBUNIT SITE. *Journal of Biological Chemistry* 287, 25241-25254
28. Kragholm, B., Kvist, T., Madsen, K. K., Jørgensen, L., Vogensen, S. B., Schousboe, A., Clausen, R. P., Jensen, A. A., and Bräuner-Osborne, H. (2013) Discovery of a subtype selective inhibitor of the human betaine/GABA transporter 1 (BGT-1) with a non-competitive pharmacological profile. *Biochemical pharmacology* 86, 521-528
29. Boudker, O., Ryan, R. M., Yernool, D., Shimamoto, K., and Gouaux, E. (2007) Coupling substrate and ion binding to extracellular gate of a sodium-dependent aspartate transporter. *Nature* 445, 387-393
30. Damgaard, M., Al-Khawaja, A., Vogensen, S. B., Jurik, A., Sijm, M., Lie, M. E., Baek, M. I., Rosenthal, E., Jensen, A. A., Ecker, G. F., Frolund, B., Wellendorph, P., and Clausen, R. P. (2015) Identification of the First Highly Subtype-Selective Inhibitor of Human GABA Transporter GAT3. *ACS chemical neuroscience* 6, 1591-1599
31. Huynh, T. H., Shim, I., Bohr, H., Abrahamsen, B., Nielsen, B., Jensen, A. A., and Bunch, L. (2012) Structure-activity relationship study of selective excitatory amino acid transporter subtype 1 (EAAT1) inhibitor 2-amino-4-(4-methoxyphenyl)-7-(naphthalen-1-yl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carbonitrile (UCPH-101) and absolute configurational assignment using infrared and vibrational circular dichroism spectroscopy in combination with ab initio Hartree-Fock calculations. *Journal of medicinal chemistry* 55, 5403-5412
32. Hansen, S. W., Erichsen, M. N., Huynh, T. H., Ruiz, J. A., Haym, I., Bjorn-Yoshimoto, W. E., Abrahamsen, B., Hansen, J., Storgaard, M., Eriksen, A. L., Jensen, A. A., and Bunch, L. (2016) New Insight into the Structure-Activity Relationships of the Selective Excitatory Amino Acid Transporter Subtype 1 (EAAT1) Inhibitors UCPH-101 and UCPH-102. *ChemMedChem* 11, 382-402
33. Haym, I., Huynh, T. H., Hansen, S. W., Pedersen, M. H., Ruiz, J. A., Erichsen, M. N., Gynther, M., Bjorn-Yoshimoto, W. E., Abrahamsen, B., Bastlund, J. F., Bundgaard, C., Eriksen, A. L., Jensen, A. A., and Bunch, L. (2016) Bioavailability Studies and in vitro Profiling of the Selective Excitatory Amino Acid Transporter Subtype 1 (EAAT1) Inhibitor UCPH-102. *ChemMedChem* 11, 403-419

Short CV for collaborators that are not co-applicants

Two significant collaborators:

Lennart Bunch

Education

1998–2002 Ph.D. in organic chemistry/medicinal chemistry, KU (April 22, 2002)
 1996–1998 M.Sc. in organic chemistry, Florida State University, USA (August 10, 1998)

Employment

2006 – present Associate Professor, Dept. of Drug Design and Pharmacology, SUND/KU
 Group Leader, Bunch Research Group
 2015 – 2015 Visiting Scientist, w/ Ed Anderson, Dept. of Chemistry, University of Oxford, UK
 2003 – 2006 Assistant Professor, Dept. of Medicinal Chemistry, FARMA/KU
 2002 – 2003 Postdoc, Department of Chemistry, DTU

Publications

55 publications in peer-reviewed journals, hereof 37 as corresponding author.

Funding

External funding, 2006-to-date: 22.4 mio DKK total obtained from Government foundations (DFF/FSS, DFF/FNU), and private foundations: Lundbeck Foundation, Carlsberg Foundation, Novo Nordisk Foundation
 Internal funding, 2013-to-date: Two PhD fellowships

Christoph Matthias Fahlke

Education

1990 MD (summa cum laude), Medical School, University of Ulm
 1991 Diplom in Physik (with honors), School of Physics, University of Ulm
 1995 Habilitation (Member of the Medical Faculty at the University of Ulm)

Employment (2000-present)

1999-2005 Professor of Physiology, RWTH Aachen, Germany
 2005-2012 Professor and Chairman of the Institute of Neurophysiology, Medizinische Hochschule Hannover
 2012- Director of the Institute of Complex Systems 4 – Zelluläre Biophysik and Professor for Biophysics of the Cell, Heinrich-Heine University Düsseldorf

Select Grants (2000-present)

2001-2004 Deutsche Forschungsgemeinschaft „Molecular basis of anion conduction by neuronal glutamate transporters” (PI)
 2009-2011 Muscular Dystrophy Association „Chloride channel dysfunction in

myotonia congenita“ (PI)

2009-2012 Deutsche Forschungsgemeinschaft „Molecular mechanisms and cellular functions of EAAT anion channels“ (PI)

2015-2018 Deutsche Forschungsgemeinschaft „New Light-Driven Channels and Transporters for Optogenetics“ (PI)

Project start

01. maj 2017

Project end

30. april 2020

Place where the project will be carried out

The bulk will be carried out in the group of Associate professor Anders A. Jensen at the University of Copenhagen, Faculty of Health and Medical Sciences, Department of Drug Design and Pharmacology. A part will be carried out by collaborators in the group of associate professor Lennart Bunch in the same department. Then six months I will stay and work in the lab of professor Christoph Fahlke, Forschungszentrum Jülich, Institute of Complex Systems in Germany.

Explanation of budget

The salary is set. I have used tables provided from HR at the university of Copenhagen, with salary expense projections. Over the three years, this is DKK 1,647,544.

The running costs are estimates from previous PhD students and post docs doing similar types of experiments. The experience here is that running costs amount to approximately DKK 125,000 per year.

I hope to train at least one master student as part of this project. As such, I ask for an additional 75,000 for running costs related to the master student, and we will provide an additional 50,000 to be able to have a one-year research project.

The funding requests have been unevenly distributed over the three years to keep within the DKK 700,000 per year, but in reality, the expenses will likely be spent according to the time of the different activities outlined in the time plan. The master's student would preferably start sometime during the second year.

Running cost specifications applied from Lundbeck Foundation (estimated as a total over three years, totaling DKK 450,000), given in DKK.

Molecular biology reagents: 100,000
(incl. enzymes, DNA kits, competent bacteria, medium and buffers, DNA oligos, sequencing, electrophoresis equipment, and disposables)

Cell culture reagents: 125,000
(incl. cell culture medium, FBS, trypsin, buffers, antibiotics, transfection reagents, and disposables)

Pharmacological assay reagents: 125,000
(incl. radiolabeled chemicals, buffers, scintillation fluids and commercial control chemicals)

Various consumables: 25,000
(incl. plastics of various types for other purposes than the above)

Master student running costs: 75,000
(depending on specific project)

Other applications from the research group submitted for the current application deadlines?

Yes, Anders A. Jensen has sent two applications. One will, if provided, also be utilized in this project (compound library) to increase the number of compounds I will screen in objective (3). The number of compounds listed in this application (4500) is what we can do if the application by Anders A. Jensen for a new library is declined:

2016-2319

Allosteric Modulation of Alpha6* Nicotinic Acetylcholine Receptors

2016-2615

Search for novel modulators of neurotransmitter receptors and transporters

References / recommendations

Recommendation from Anders A. Jensen:

It is a great pleasure for me to write this recommendation of Walden Emil Bjørn-Yoshimoto in connection with his application “The molecular basis for subtype-selective modulation of excitatory amino acid transporter modulators” to the Lundbeck Foundation.

Walden is currently finishing up his PhD project focused on excitatory amino acid transporters (EAATs) in my research group at the Department of Drug Design and Pharmacology, SUND, University of Copenhagen, from where he is expected to obtain his PhD degree in March 2017. In the course of this project, Walden has been an important participant in the collaboration between my group and the medicinal chemistry group headed by Associate Professor Lennart Bunch on the development of various EAAT ligands, several of which displaying interesting selectivity profiles at the various EAAT subtypes. These projects have resulted in a total of five publications in international peer-reviewed scientific journals, and a couple of the ligands identified in these projects will form the basis for project activities proposed by Walden in his application. Furthermore, Walden has co-authored two review articles on the EAATs, one of these as first-author. Finally, during a stay in the lab of Susan Amara at the NIH (Bethesda, MD, USA) Walden has been working on the role of EAAT3 on the effects mediated by the psychostimulant amphetamine, and he is currently finishing up a study investigating the functional importance of a specific loop in EAAT2 for the substrate translocation of this transporter. We expect the results of both of these projects to be published in high-impact internal peer-reviewed scientific journals.

As outlined above, Walden has been very productive during his time in my group, where he has been a driven, dedicated and focused as PhD student that has worked very independently on his project. He has with a constructive attitude worked on solving the challenges that inevitably will arise in any research project, and he has shown great fighter spirit and stamina in the efforts to solve these challenges and to get the project back on track again. Throughout his time in my group Walden has displayed a high work ethic and responsibility, and he has taken great pride in producing scientific data of high quality. Finally, he has combined his practical lab work with a substantial theoretical insight into the EAAT field and thus contributed considerably to the direction of the research in his project.

I have been interested in EAATs and the important roles of these transporters in glutamatergic neurotransmission for close to a decade now, and I would be very pleased if Walden could continue as a post doc in my group and continue his work on these fascinating transporters. Since I consider Walden a highly dedicated, skilled and promising scientist and think the project described in his application is very interesting, I whole-heartedly recommend the Lundbeck Foundation to support his application and enable Walden to continue his pursuit of a career in science.

Anders A. Jensen, Ph.D.
Department of Drug Design and Pharmacology
Faculty of Health and Medical Sciences
University of Copenhagen
Universitetsparken 2
DK-2100 Copenhagen Ø
Denmark
Phone: +45 23962434
Email: aaj@sund.ku.dk

Abstract in Danish

Baggrund

Glutamat er medvirkende til stort set alle processer i hjernen, og er involveret i talrige sygdomme som Skizofreni, Alzheimers sygdom, epilepsi, bipolar affektiv sindslidelse, obsessiv-kompulsiv tilstand. En familie af eksitatoriske aminosyre transportører (excitatory amino acid transporters, **EAAT**'er) medierer genoptag af glutamat in hjernen. Der er fem subtyper af EAAT'er i mennesker, men kun specifikke farmakologiske redskaber mod EAAT1 (udviklet i vores lab) og EAAT2. Dette har begrænset forskning af EAAT'er, både i raske hjerner og sygdomstilstande.

Mål og objektiver

Jeg vil udvikle redskabsstoffer med subtype-selektivitet, og karakterisere deres molekylære basis for modulering og EAAT præference. Til det vil jeg forfølge tre objektiver:

1. Optimere og karakterisere en inhibitor jeg har identificeret, som foretrækker EAAT3
2. Karakterisere molekylære determinanter for subtype selektivitet i nyligt identificerede EAAT1- og EAAT2 selektive stoffer.
3. Lave et lille screen af stoffer med EAAT3 og EAAT4 modulering, udvikle disse til mere selektive/potente stoffer

Metoder

Projektet vil afhænge af etablerede metoder i laboratorierne. Dette vil f.eks. være molekylær biologi, radio-substrate assays, fluorescens baserede assays og elektrofysiologiske metoder, primært "two-electrode voltage clamp" og "patch clamp".

Forventede resultater og perspektiver

Jeg forventer at et eller flere nye redskabsstoffer vil blive udviklet og karakteriseret fra objektiv (1). Objektiv (2) vil give indsigt i mekanismen for subtype selektive EAAT modulatorer, som vil hjælpe i fremtidig design af nye stoffer. Jeg tror, baseret på tidligere erfaringer, at det er sandsynligt at objektiv (3) vil give os 1-3 nye stof grupper, som kunne lede til de første stoffer med EAAT3 eller EAAT4 selektivitet. Til sammen vil disse redskaber og ny viden muliggøre megen ny forskning i glutamaterg neurotransmission.

Budget

Year Type	Name	Amount of money	Details
1			
	Applied from the Lundbeck Foundation		
	Running costs	161.000,00 kr.	Running costs for consumables and smaller lab equipment needed (estimated from previous experience from the PI). See "explanation of budget" for details.
	Salary - post doc	538.344,00 kr.	Salary, as calculated from 8 months in 2017 and 4 months in 2018 from information provided by our HR department
		<u>699.344,00 kr.</u>	
	Applied from others		
	Running costs	0,00 kr.	
	Salary - post doc	0,00 kr.	
		<u>0,00 kr.</u>	
	Provided by workplace		
	Running costs	31.000,00 kr.	~25000 for Computer and ~6000 for conference participation
	Salary - post doc	0,00 kr.	
		<u>31.000,00 kr.</u>	
	Provided by others		
	Running costs	0,00 kr.	
	Salary - post doc	0,00 kr.	
		<u>0,00 kr.</u>	
2			
	Applied from the Lundbeck Foundation		
	Running costs	150.000,00 kr.	Running costs for consumables and smaller lab equipment needed (estimated from previous experience from the PI). See "explanation of budget" for details.
	Salary - post doc	549.108,00 kr.	Salary, as calculated from 8 months in 2018 and 4 months in 2019 from information provided by our HR department
		<u>699.108,00 kr.</u>	
	Applied from others		
	Running costs	0,00 kr.	

Year	Type	Name	Amount of money	Details
2				
		Applied from others		
		Salary - post doc	0,00 kr.	
			<u>0,00 kr.</u>	
		Provided by workplace		
		Running costs	61.000,00 kr.	~6,000 for conference participation, ~ 5,000 for publication expenses and ~50,000 of the 125,000 for running cost of 12 months of master student
		Salary - post doc	0,00 kr.	
			<u>61.000,00 kr.</u>	
		Provided by others		
		Running costs	0,00 kr.	
		Salary - post doc	0,00 kr.	
			<u>0,00 kr.</u>	
3				
		Applied from the Lundbeck Foundation		
		Salary - post doc	560.092,00 kr.	Salary, as calculated from 8 months in 2019 and 4 months in 2020 from information provided by our HR department
		Running costs	139.000,00 kr.	Running costs for consumables and smaller lab equipment needed (estimated from previous experience from the PI). See "explanation of budget" for details.
			<u>699.092,00 kr.</u>	
		Applied from others		
		Salary - post doc	0,00 kr.	
		Running costs	0,00 kr.	
			<u>0,00 kr.</u>	
		Provided by workplace		
		Salary - post doc	0,00 kr.	
		Running costs	16.000,00 kr.	~6000 for conference participation and ~10000 in publication expenses.
			<u>16.000,00 kr.</u>	
		Provided by others		
		Salary - post doc	0,00 kr.	

Year	Type	Name	Amount of money	Details
3				
		Provided by others		
		Running costs	50.000,00 kr.	As seen in the letter of support from Professor Christoph Fahlke, his lab is equipped to supply running costs for the work performed in his lab.
			<u>50.000,00 kr.</u>	
			<u>2.097.544,00 kr.</u>	

Co-applicant(s) / Mentor

Name Anders Asbjørn Jensen
Email aaj@sund.ku.dk
CV

Personal Data

Name
Anders Asbjørn Jensen

Birth date
August 11, 1970

Birthplace
Glostrup, Denamrk

Home address
Griffenfeldsgade 37C 3. mf. th., DK-2200 Copenhagen, Denmark

Degrees

1995
M.Sc. (Pharm), The Royal Danish School of Pharmacy

2001
Ph.D., The Royal Danish School of Pharmacy

Positions

1995-1997
Academic Employee
Quality Assurance / Chemical Production
H. Lundbeck A/S
Copenhagen
Denmark

1998-present
Ph.D. student (1998-2001)
Assistant Professor (2001-2006)
Associate Professor (2006-)
Department of Drug Design and Pharmacology
Faculty of Health and Medical Sciences
University of Copenhagen
Denmark

Scientific Visits

1993-1994

Department of Molecular Pharmacology

Bioscience

Novo Nordisk A/S

Denmark

In the lab of Dr. Peter H. Andersen

1998

Acadia Pharmaceuticals Inc.

San Diego, CA

USA

In the lab of Dr. Mark R. Brann

2003-2004

Department of Physiology

Pharmazentrum

University of Basel

Switzerland

In the lab of Professor Bernhard Bettler

Awards

2001

Novo Nordisk Ph.D. Plus Prize.

2001

The Danish Academy of Natural Sciences Ph.D. Award.

2011

Scientist of The Year, Faculty of Pharmaceutical Sciences, University of Copenhagen.

Funding Since 2010

DKK 20,846,559 and two industrial PhD students.

Funding is from The Danish Research Counsel, The Lundbeck Foundation, the Novo Nordic Foundation, Augustinus Foundation, Innovation Fund Denmark, and the University of Copenhagen.

Mentoring Experience

Current post docs: 1

Former post docs: 2

Current PhD students: 6 (4 as Principal Supervisor)

Former PhD students: 15-20 (6 as Principal Supervisor)

Master Students: >25 (13 as Principal Supervisor)

Other Scientific, Academic and Innovation Activities

Member of the Editorial Advisory Board of the journal *Combinatorial Chemistry & High Throughput Screening*.

Regular reviewer: *Br. J. Pharmacol.*, *Neuropharmacology*, *J. Neurochem.*, *Eur. J. Pharmacol.*, *Comb. Chem. High Throughput Screen.*, *J. Med. Chem.*, and *ChemMedChem*.

Occasional reviewer: *Proc. Natl. Acad. Sci. USA*, *J. Neurosci.*, *Trends Pharmacol. Sci.*, *J. Biol. Chem* and *ACS Chem. Neurosci*.

Reviewer of grants for the Wellcome Trust, The German National Research Council and The Australian National Research Council.

Co-developer of the PhD Course “*Molecular Pharmacology*” and the Graduate Course “*Advances in Medicinal Chemical Research*” at the School of Pharmaceutical Sciences.

Chairman or external member of the evaluation committees for 11 PhD thesis’s.

Co-founder and Co-CEO of the company Hugin NeuroChemicals I/S.

Publications

Total peer reviewed publications: **114**

Total peer reviewed publications excluding book chapters: **109**

Patent applications: **2**

Select recent publications related to excitatory amino acid transporters.

1. Haym, I., et al. (2016) *ChemMedChem* **11**, 403-419
2. Hansen, S. W., et al. (2016) *ChemMedChem* **11**, 382-402
3. Hansen, S. W., et al. (2016) *J Med Chem* **59**, 8757-8770
4. Hansen, J. C., et al. (2016) *J Med Chem* **59**, 8771-8786
5. Pedraz-Cuesta, E., et al. (2015) *BMC Cancer* **15**, 411
6. Jensen, A. A., et al. (2015) *Curr Opin Pharmacol* **20**, 116-123
7. Erichsen, M. N., et al. (2014) *Neurochemical research* **39**, 1964-1979

8. Assaf, Z., et al. (2013) *J Med Chem* **56**, 1614-1628
9. Abrahamsen, B., et al. (2013) *J Neurosci* **33**, 1068-1087
10. Huynh, T. H., et al. (2013) *SpringerPlus* **2**, 1
11. Huynh, T. H., et al. (2012) *J Med Chem* **55**, 5403-5412
12. Huynh, T. H., et al. (2012) *Bioorg Med Chem* **20**, 6831-6839
13. Erichsen, M. N., et al. (2010) *J Med Chem* **53**, 7180-7191
14. Jensen, A. A., et al. (2009) *J Med Chem* **52**, 912-915
15. Bunch, L., et al. (2009) *Expert Opin Ther Targets* **13**, 719-731
16. Sagot, E., et al. (2008) *J Med Chem* **51**, 4085-4092
17. Colleoni, S., et al. (2008) *J Pharmacol Exp Ther* **326**, 646-656
18. Faure, S., et al. (2006) *J Med Chem* **49**, 6532-6538
19. Bunch, L., et al. (2006) *J Med Chem* **49**, 172-178
20. Brauner-Osborne, H., et al. (2005) *Org Biomol Chem* **3**, 3926-3936
21. Alaux, S., et al. (2005) *J Med Chem* **48**, 7980-7992
22. Jensen, A. A., et al. (2004) *Biochem Pharmacol* **67**, 2115-2127



Forschungszentrum Jülich GmbH · IC5-4 · 52425 Jülich · Germany

Institute of Complex Systems
IC5-4: Cellular Biophysics
Director of the Institute:
Prof. Dr. med. Christoph Fahlke

Tel: +49 2461 61-3016
Fax: +49 2461 61-4216
Email: c.fahlke@fz-juelich.de

Jülich, 8th Sept. 2016

To Whom It May Concern

I am very pleased about the possibility to continue my fruitful collaboration with Dr. Anders A. Jensen and his research group when it comes to the post doc project of Walden Bjørn-Yoshimoto entitled *"The molecular basis for subtype-selective modulation of excitatory amino acid transporters by novel modulators"*.

I have recently collaborated with Anders on an investigation of the mechanism of action of the EAAT1-selective allosteric inhibitor UCPH-101 on this transporter. In that collaboration the respective expertise of the groups supplemented each other nicely and it resulted in publications in the two high-impact journals *Journal of Neuroscience* and *Current Opinion in Pharmacology*. My group has been interested in the EAATs and in the exploration of the modes of action of novel modulators for many years, and this new project is thus very much at the core of our research interest.

The 6-months stay for Walden in my lab is scheduled to take place in the last year of his three-year post doc project. Since the medical chemistry development of the ligands during the first two years of the project is expected to optimize the EAAT modulators currently available, the specific modulators in focus during his stay in my lab has not yet been planned. However, I am very excited about the EAAT3-prefering inhibitor, recently identified by our collaborators, and look forward to study of this compound or an optimized analog of it. During Walden's stay, we plan to study the mechanism(s) of action and the nature of modulation exerted by one or two selected EAAT modulators in detail in electrophysiological recordings at EAATs expressed in mammalian cell lines:

- 1) In addition to mediating Glu uptake the EAATs also an anion conductance. We will investigate the mode of action of the EAAT modulator(s) on both of these functions.
- 2) We will investigate the kinetic parameters characterizing the modulation exerted by the EAAT modulator(s), including determination of binding on-/off-rates, as well as changes in transport and anion channel kinetics upon addition of these compounds.

My lab is highly experienced in and fully equipped to conduct these types of studies, and I have the financial resources for the operational costs connected to these studies.

In conclusion, I hereby express my full support to the application for funding to this project, and I look forward to collaborate with Walden and Anders on it.

Yours sincerely,

Christoph Fahlke

Letter of Support from Professor Christoph Fahlke:

A letter of support from Dr. Christoph Fahlke, confirming his dedication to the project, and confirmation of his agreement of me coming to his lab, etc.

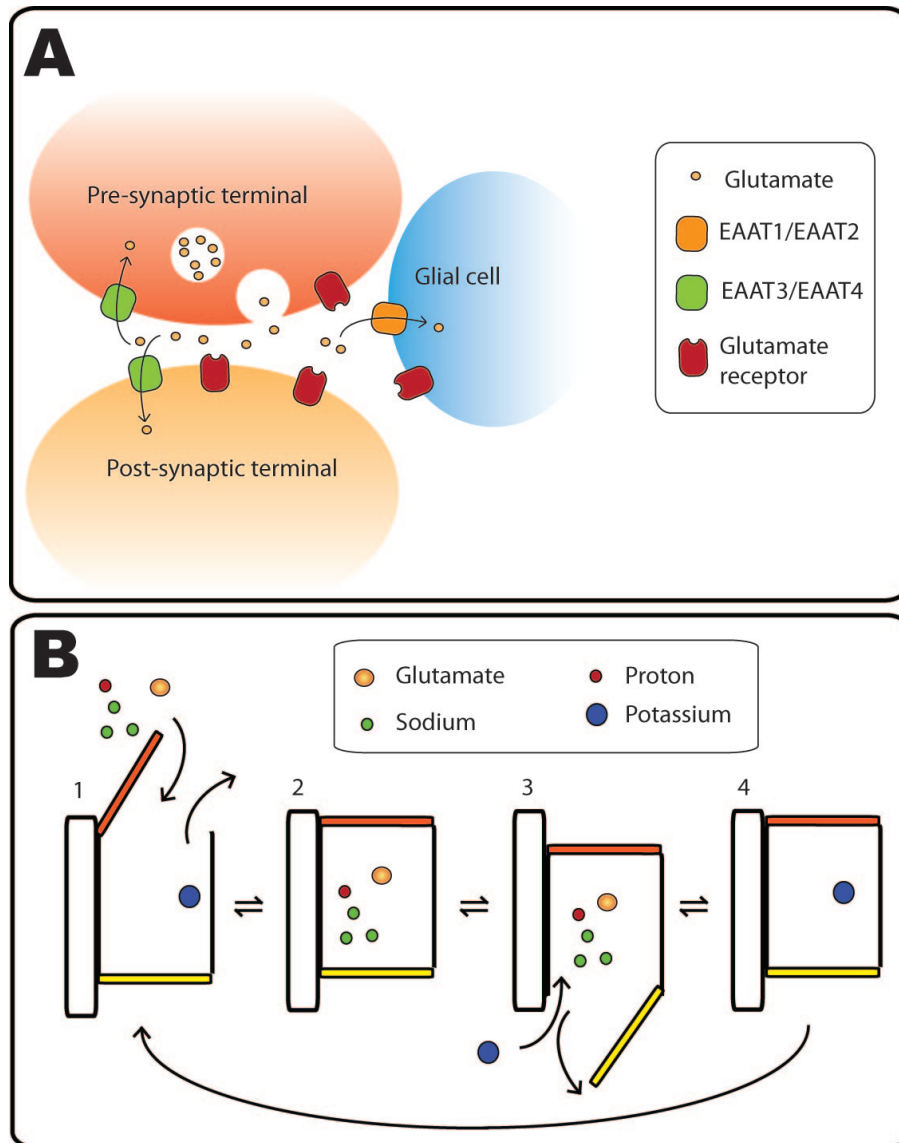


Figure 1 - Glutamate synapse and EAAT mechanism:

(A) The glutamatergic synapse. Glutamate is released by the presynaptic neuron and acts at a plethora of glutamate receptors. The EAATs mediate the uptake of glutamate from the synapse into either glial cells (mainly by EAAT1 and EAAT2) or neurons (mainly by EAAT3 and EAAT4). (B) The mechanism of Glu uptake. The EAATs function by alternating the access to the extracellular and intracellular space. From the top: Glu enters the EAAT together with 3 Na⁺ and one H⁺. The transporter blocks access by moving HP2 (orange), then rearranges to an inward open state by moving HP1 (yellow). The ions exit the EAAT into the cytoplasm, and a K⁺ binds. The transporter rearranges to the outward open state where K⁺ leaves, and the cycle can begin anew.

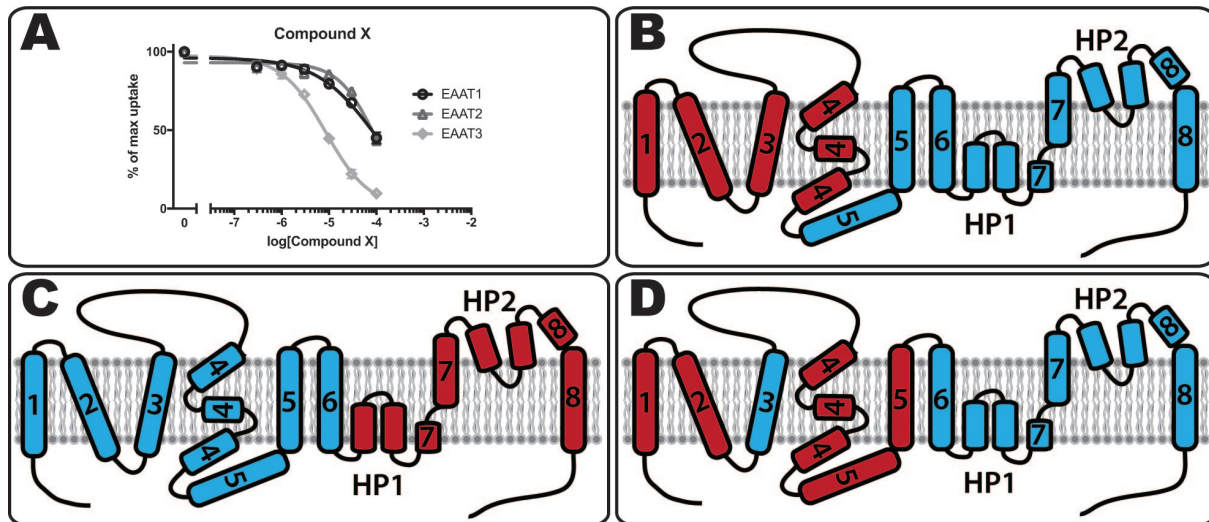


Figure 2 - Compound X:

(A) Compound X. Concentration-inhibition curve of compound X in [3H]-D-Asp uptake assay on EAAT1, -2 and -3 cell lines. (B-D) Search for the binding site of compound X in EAAT3. Schematic representation of different chimeric transporters planned for elucidating the binding site of the EAAT3 inhibitor (red: EAAT3, blue: EAAT2). After narrowing down the binding site region, we will investigate specific residues in these regions.

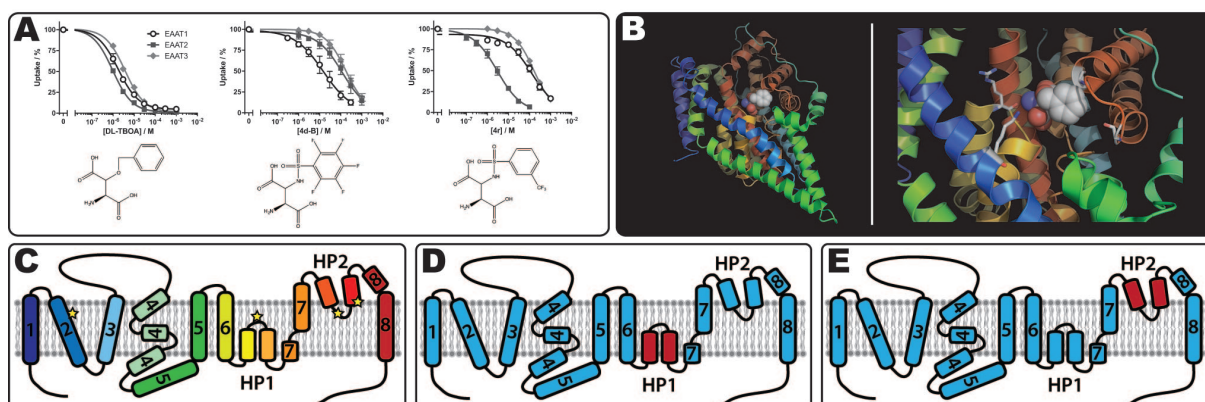


Figure 3 - Aspartate analogs:

(A) Chemical structures of TBOA and the two 3-substituted Asp analogs 4d-B and 4r and their functional at EAAT1, -2 and -3 in the $[^3\text{H}]$ -D-Asp uptake assay. (B) Crystal structure of GltPh. Left: a single EAAT protomer from a side view. Right: Close-up of the competitive inhibitor TBOA in the Glu binding site of the transporter with the four residues predicted to be putative determinants of the EAAT1- and EAAT2-selectivities of the 3-substituted Asp analogs (indicated by stars in B) shown as sticks colored by element. The transporter is colored in 'rainbow', according to the topology plot, and TBOA is colored by element. (C-E) Examples of different mutations and chimeras represented by different EAAT subtypes in either red or blue. (C) EAAT topology with stars indicating residues differing between EAAT1 and EAAT2 predicted to be in vicinity of 3-substituent of the Asp analogs. (D) switching HP1, (E) switching HP2. Additional point mutations will be created based on the initial results with chimeras.

Objective	Activity	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12
Objective 1 EAAT3 inhibitor (Approx. 20 months full time)	Molecular biology		Create chimeras				Create point mutations						Finish and publish
	Pharmacological characterization	Test commercial analogs		(if any good compounds are found from commercial analogs, test on chimeras)	Test synthesized compounds on wild type transporters	Test synthesized compounds on chimeric transporters to find binding site			Test novel synthesized compounds on wild type transporters	Test on mutant transporters to find binding site			Finish and publish
	Chemical synthesis		Synthesis (chemists)	Synthesis (chemists)	Synthesis (chemists)		Synthesis, round 2 (chemists)	Synthesis, round 2 (chemists)					
Objective 2 3-substituted aspartate analogs (Approx. 7 months full time)	Molecular biology			Create chimeras and point mutations				Create point mutations, round 2 (based on chimeras)					Finish and publish
	Pharmacological characterization				Test compounds at mutant transporters to find binding determinants				Test compounds at mutant transporters to find binding determinants, round 2				Finish and publish
Objective 3 EAAT3 and EAAT4 screen (Approx. 5 months full time)	Screening	Screen library											Finish and publish
	Molecular biology						Create chimeras for EAAT4 compound(s)	Create chimeras for EAAT4 compound(s)	Create point mutations for EAAT4 compounds				Finish and publish
	Pharmacological characterization		Verify hits	Test commercial analogs		Test synthesized compounds	Test synthesized compounds	Test best EAAT3 compounds on mutants created in "objective 1"	Test best EAAT4 compounds on chimeric transporters to find binding	Test best EAAT4 compounds on mutant transporters to find binding			Finish and publish
	Chemical synthesis				Synthesis (chemists)	Synthesis (chemists)							
Stay abroad, related to all objectives (6 months)	Electrophysiological characterization										Stay in Germany, electrophysiological characterization	Stay in Germany, electrophysiological characterization	

Figure 4 - Project timeline:

The 12 quarters of the project are listed at the top, and the different objectives and specific activities are listed on each line while denoting the expected time in which each part will be conducted. There will be some overlap in project progression, thus there is overlap where each project will be worked on part time during some durations. The grey parts are done by collaborators, and do not take my time. In summary, approximately 18 months will be spent on project (1), approximately 7 months on project 2, and approximately 5 months on project 3 (though some of this can be done in parallel with parts of objective 1, reducing the de facto extra time spent on this objective), while an additional 6 months will be spent in the lab of Professor Christoph Fahlke. I have left the last quarter open for tying up loose ends and preparing manuscripts.