IDAR 2022

FINAL Program

July 25 – 27th

Urbana IL 61801

Dear Colleagues and Friends,

Welcome you to IDAR 2022. Given the year-long delay in this meeting because of the pandemic, I am excited to see you at the in-person IDAR2022 at the University of Illinois.

IDAR2022 builds on successful past meetings, including IDAR2017 held in Varese Italy and IDAR2019 in Tokyo, Japan. We continue the tradition of presenting the most exciting research and developments in fields related to the study of D-amino acids. For IDAR2022, we especially thank Bruker, *Analytical Methods*, *Analytical Chemistry*, and the University of Illinois at Urbana-Champaign for their financial support for IDAR2022.

We have an exciting program highlighting the latest advances in the measurement of D-amino acids, the presence of D-amino acids in peptides and proteins, their impact in nutrition and diet, pharma and biotechnology, and their roles and effects in the central nervous system and peripheral organ diseases, in plants and microbes, and much more. The meeting has three cutting edge keynote talks, 39 exciting invited and contributed talks, a dynamic poster session, and plenty of opportunities for networking and discussions.

During the meeting, lunches, coffee breaks and snacks are provided. In addition, we have a Monday evening gathering at Riggs Brewery that includes dinner, in-house beer and live music. On Wednesday, the conference banquet will be held two blocks from the Beckman at the historic Illini Union.

I am looking forward to reconnecting with the D-AA community in July 2022 at the Beckman Institute.

Thanks,

Jonathan Sweedler, Chair IDAR2022

Scientific Committee

North America:

Thanh Do, University of Tennessee Knoxville, USA

Ryan Julian, UC Riverside, USA

Robert Kennedy, University of Michigan, USA

Lingjun Li, University of Wisconsin-Madison, USA

Janine Mauzeroll, McGill University, Canada

Michael Roper, Florida State University, USA

Tian 'Autumn' Qiu, Michigan State University, USA

Japan:

Yasuhisa Asano, Toyama Prefectural University, Japan

Kiyoshi Fukui, Tokushima University, Japan

Kenji Hamase, Kyushu University, Japan

Toru Nishikawa, Showa University School of Medicine, Japan

Tohru Yoshimura, Nagoya University, Japan

Europe:

Maria Di Fiore, University Campania, Caserta Italy

Jean-Pierre Mothet, CNRS - ENS Paris Saclay, France

Loredano Pollegioni, University of dell'Insubria, Varese, Italy

Alessandro Usiello, University of Campania, Italy

Herman Wolosker, Israel Inst. of Technology, Israel

COVID-19 Health & Safety Policies

IDAR2022 is an in-person event (with a virtual option) organized so that all individuals (attendees, speakers, staff and venue personnel) remain safe and comfortable throughout the event. We will follow all CDC, Illinois, Champaign country and University of Illinois campus guidelines, and will keep the conference web page with up to date with our current requirements.

UIUC Campus guidelines now allow individuals to be inside without masks and we cannot change this campus rule. However, we encourage attendees to be masked while at the conference.

For those needing to have a Covid test before flying internationally, there are several testing options. The closest is Campustown Urgent Care, which offers a variety of Covid test options (**covidtest.center**) including PCR tests. It is not a walk-in clinic: you need to sign up for an appointment (M-F between 9:00 am and 4:00 pm). When you go to their website, make sure to select the Champaign Campustown location (631 E Green St), which is only a short walk from the Beckman Institute. One of their screens asks for insurance details; if you are paying by cash, just enter anything and continue to the next screen.

Please do not attend IDAR2022 if you have a fever, COVID-19, COVID-19 symptoms, or are sick within 7 days of IDAR2022.

Masks & hand sanitizer will be made available.

Conference Questions?

Michelle Marquart
Lead Program Coordinator
Conference & Event Services
University of Illinois Urbana-Champaign
Phone: +1-217-244-8174

The Beckman Institute

Location: the IDAR 2022 sessions will be held at the Beckman Institute, 405 N. Mathews Ave., Urbana IL. The Beckman is a short walk from all three suggested hotels. If parking is needed, metered parking spaces are available near the Beckman:

- On Wright Street to the West
- In the circle drive to Beckman's East
- In the campus parking deck across Mathews Avenue, which requires paying via app or a payment machine near University and Mathews.

Meters are about $1 per hour. For more information on campus parking, please see the campus parking website.

Location of Sessions:

Oral Sessions: 5602 Beckman Institute (Fifth floor North side)
Coffee Breaks: 5269 Beckman Institute (Fifth floor, tower room)
Lunch and Poster Session: 1005 Beckman Institute (Ground floor)

Signs will be posted to these locations: there are building maps in the lobby

Evening Events

Monday evening:

5:15 pm. A bus will leave from the Beckman to go to Riggs Brewery (we have the entire place for the evening) with dinner and music, with several options to return to campus. There will be a buffet dinner and a cash bar with two free drinks included.

Our Beers – Riggs Beer Company

If you want to drive, their address is:

Riggs Brewery (Phone: +1 (217) 718-5345)
1901 S High Cross Rd,
Urbana, IL 61802

Tuesday evening:

On your own. Check on the virtual concierge included in your conference bag for dining and other suggestions (or ask a local Illini for ideas).

Wednesday dinner:

Conference banquet at the Illini Union, 1401 West Green Street

Location: Illini Room C and the South Lounge. The event starts at 5:30 pm with appetizers and a cash bar (with two free drink tickets). The sit down dinner starts at 6:30 pm.

IDAR Final Program

Location of Sessions:

Oral Sessions: 5602 Beckman Institute (Fifth floor)
Coffee Breaks: 5269 Beckman Institute (Fifth floor, tower room)
Lunch and Poster Session: 1005 Beckman Institute (Ground floor)
Please have the posters set up by Monday lunch

Monday July 25

9:00 am **Welcome**
Jonathan Sweedler (15 min)

9:15: am **Remembering Noriko Fujii (the 2019 IDAR conference chair)**
Kiyoshi Fukui and Takumi Takata (15 min)

9:30 am **Keynote 1** (Presider: Robert Kennedy)
K01. Dan Armstrong: Analytical approaches for analyzing D-amino acids and epimeric peptides in human samples (40 min)

10:10 am **Session 1: Analytical methods for D-amino acid characterization**
(Presider: Jean-Pierre Mothet and Michael Roper)

10:10 am L01. Kenji Hamase: Enantioselective analysis of amino acids and related compounds using multi-dimensional HPLC and its medical applications (25 min)

10:35 am L02. Janine Mauzeroll: Electrochemical sensor development for robust amino acid biosensing (25 min)

11:00 am **Coffee Break** (20 min)

11:20 am L03. Robert Kennedy: Deep chemical analyses of microdialysates by LC-MS: *in vivo* metabolomics (25 min)

11:45 am L04. Zhijun Zhu: Chiral pair isobaric DiLeu labeling strategy enabled separation and absolute quantitation of enantiomeric amino acids (10 min)

11:55 am L05. Gaoyuan Lu: Exploring crown ether as shift reagent for the differentiation of amino acid enantiomers and peptide epimers via ion mobility spectrometry (10 min)

12:05 pm **Lunch**

1:30 pm **Session 2: Physiology and metabolism of D-AAs**
(Presider: Kenji Hamase and Alessandro Usiello)

1:30 pm L06. Herman Wolosker: Import of L-serine across the BBB is critical for D-serine synthesis and neurodevelopment (25 min)

1:55 pm L07. Hisashi Mori: Physiological and pathological roles of serine racemase in the brain (25 min)

2:20 pm L08. Tian (Autumn) Qiu: D-Alanine in microbiome-gut-brain axis: sources, biodistribution, and functional roles (25 min)

2:45 pm **Coffee Break** (20 min)

3:05 am L09. Jean-Pierre Mothet: A right-handed excitatory amino acid tunes cortical neuronal inhibition (25 min)

3:30 pm L10. I-An Wei: Effect of D-amino acids on calcium dynamics in pancreatic islets (10 min)

3:40 pm L11. Giulia Murtas: Role of human D-3-phosphoglycerate dehydrogenase in amino acids and cellular metabolism (10 min)

3:50 pm **Session 3: Central Nervous System diseases related to D-amino acids metabolism**
(Presider: Janine Mauzeroll)

3:50 pm L12. Loredano Pollegioni: Omics analyses of serine metabolism in Alzheimer's disease (25 min)

4:15 pm L13. Daniel Liebl: Detrimental effects of reactive glial D-serine release on brain health following CNS injury (25 min)

4:40 pm L14. Dena Arizanovska: Microglial D-serine mediates synaptic damage following traumatic brain injury (10 min)

5:15 pm **Informal gathering** at RIGGS brewery (bus leaves from the BI): includes local beer, food and music Riggs Beer Company – On our farm, we grow beer.

Tuesday July 26

9:00 am **Keynote 2** (Presider: Jumpei Sasabe)

K02. Üner Kolukisaoglu: D-Amino acids in plants: How much do we really know? (40 min)

9:40 am **Session 4: D-Amino acids in mammals -- beyond the brain**
(Presider: Lingjun Li and Tian Autumn Qiu)

9:40 am L15. Ryan Julian: Isomerized residues in age-related disease (25 min)

10:05 am L16. Jumpei Sasabe: Life-long regulation of microbial D-amino acids in mammals (25 min)

10:30 am L17. Kenta Arisumi: Pathophysiological significance of D-amino acid metabolism in the experimental autoimmune encephalomyelitis. (10 min)

10:40 am **Coffee Break** (20 min)

11:00 am L18. Michael Roper: Effects of glucose stimulation on D-amino acid levels in rodent pancreatic islets of Langerhans (25 min)

11:25 am L19. Kiyoshi Fukui: Spatiotemporal distribution of D-amino acid oxidase in central neurons system and kidney: implication for aging, neurodegeneration and renal pathophysiology (25 min)

11:50 am L20. Stanislav Rubakhin: D-Amino acids as biomarkers in diabetes (10 min)

12:00 pm **Lunch and poster Session**

2:00 pm **Session 5:**
Central Nervous System diseases related to D-amino acids metabolism (2)
(Presider: James Checco)

2:00 pm L21. Uwe Rudolph: Glycine decarboxylase (GLDC): A novel indirect modulator of NMDA receptor activity and its potential role in the pathophysiology of psychosis (25 min)

2:25 pm L22. Alessandro Usiello: Duplication of the D-aspartate oxidase gene induces cortical abnormalities, social recognition deficits in mice and intellectual disabilities in human (25 min)

2:50 pm L23. Karen Zito: Regulation of spiny synapse plasticity by D-serine in health and disease (25 min)

3:15 pm **Coffee Break** (20 min)

3:35 pm **Session 6: Physiology and metabolism of D-AAs (2)**
(Presider: Silvia Sacchi)

3:35 pm L24. Lorenzo Chiariotti: Neuroepigenetics of D-Ser and D-Asp metabolism (25 min)

4:00 pm L25. Stefano Bruno: The role of structural flexibility and protein interactors in modulating the activity of serine racemase (25 min)

4:25 pm L26. John Gray: Privileged role for postsynaptic D-serine in synaptic plasticity (25 min)

Dinner on your own

Wednesday July 27

9:00 am **Keynote 3** (Presider: Herman Wolosker)
K03. Robin Roychaudhuri: Mammalian D-cysteine is a regulator of neural progenitor cell proliferation in the brain (40 min)

9:40 am **Virtual Session 1** (Presider: Elena Romanova)

9:40 am L27. Vibin Ramakrishnan: Tacticity directed functional programming of hetero-chiral peptide constructs for drug delivery (15 min)

9:55 am **Session 7: D-Amino acids in pharma and food** (Presider: John Gray)

9:55 am L28. Thanh Do: Cyclosporines: new stories from an old molecule (25 min)

10:20 am L29. Ann Bech Roskjær: D-Amino acid in processed foods on gut microbiota, health and disease (10 min)

10:30 am L30. Akina Matsuda: Gastric microbes actively produce D-amino acids in breast milk during infancy (10 min)

10:40 am **Coffee Break** (20 min)

11:00 am **Session 8: Physiology and metabolism of D-AAs (3)** (Presider: Kiyoshi Fukui)

11:00 am L31. Silvia Sacchi: So similar, so different: investigating human D-amino acid oxidase and D-aspartate oxidase regulation (25 min)

11:25 am L32. Mariella Cuomo: DNA methylation reshaping at DAO gene during brain development (10 min)

11:35 am L33. Akina Osaki: Physiological roles of catabolism of microbial D-amino acids in the energy metabolism in mammals (10 min)

11:45 am **Lunch**

1:15 pm **Session 9: D-Amino acid containing peptides** (Presider: Ryan Julian)

1:15 pm L34. James Checco: Understanding the roles of D-amino acid residues in cell-cell signaling peptides (25 min)

1:40 pm L35. Takumi Takata: Effect of C-terminal adjacent amino acid residues on deamidation/isomerization of asparagine residues in lens component proteins (25 min)

2:05 pm **Coffee Break** (20 min)

2:25 pm L36. Lingjun Li: Advancing neuropeptide research via novel application of ion mobility mass spectrometry (IM-MS) (25 min)

2:50 pm L37. Harvey Andersen: Exploratory recombinant expression and fractionation of eukaryotic L/D peptide isomerases (10 min)

3:00 pm: **Late Breaking and Virtual Session 2** (Presider: Elena Romanova)

3:00 pm L38. Toru Nishikawa: D-Serine and schizophrenia (25 min)

3:25 pm L39. Masakazu Umino: D-Serine-AMPA receptor interaction to development of a novel treatment for schizophrenia (15 min)

3:40 pm. TBD

4:00: pm **Conference wrap-up and closing**
Jonathan Sweedler (10 min)

5:30 pm **Banquet Dinner at the Illini Union**

Posters (in alphabetical order of presenting author last names)

P01. Harvey Andersen, University of Illinois Urbana-Champaign
Exploratory recombinant expression and fractionation of eukaryotic L/D peptide isomerases

P02. Kenta Arisumi, Department of Pharmacology, Keio University School of Medicine
Pathophysiological significance of D-amino acid metabolism in the experimental autoimmune encephalomyelitis.

P03. Dena Arizanovska, The Miami Project to Cure Paralysis, Department of Neurosurgery, University of Miami Miller School of Medicine
Microglial D-serine mediates synaptic damage following traumatic brain injury (TBI)

P04. Shuangshuang Chen, Department of Chemistry, University of Illinois Urbana-Champaign
D-Amino acids in type 1 diabetes-affected human sera

P05. Mariella Cuomo, University of Naples "Federico II"
DNA methylation reshaping at DAO gene during brain development

P06. Oluwarotimi Folorunso, Harvard University
D-serine availability is important for proper prefrontal cortical interneuron development and adolescent social behavior

P07. Chen Huang, University of Illinois Urbana-Champaign
D-Alanine in the microbiome-gut-brain axis: utilizing germ free mice to decipher the origin and biodistribution of D-alanine in rodents

P08. Ran Inoue, University of Toyama
Blockade of D-serine signaling and adult hippocampal neurogenesis attenuates remote contextual fear memory following multiple memory retrievals

P09. Chiharu Ishii, Graduate School of Pharmaceutical Sciences, Kyushu University
Simultaneous determination of chiral amino acids in the plasma and brain of DAO deficient rats using a highly selective two-dimensional LC-MS/MS system

P10. Maltesh Kambali, Department of Comparative Biosciences, University of Illinois Urbana-Champaign
Evidence of a potential role of an increased copy number of the gene encoding glycine-decarboxylase (GLDC) in the pathophysiology of psychosis

P11. Haoqian Liang, Department of Biochemistry, University of Illinois Urbana-Champaign
Incorporation of heterologous reductase to generate D-alanine on class V lanthipeptide cacaoidin

P12. Gaoyuan Lu, University of Wisconsin-Madison
Exploring crown ether as shift reagent for the differentiation of amino acid enantiomers and peptide epimers via ion mobility spectrometry

P13. Akina Matsuda, Keio University School of Medicine
Gastric microbes actively produce D-amino acids in breast milk during infancy

P14. Giulia Murtas, Department of Biotechnology and Life Sciences, University of Insubria
Role of human D-3-phosphoglycerate dehydrogenase in amino acids and cellular metabolism

P15. Emmanuel Ogunkunle, Florida State University
Chiral liquid chromatography-mass spectrometry method for measuring D-amino acids in biological samples

P16. Samuel Okyem, University of Illinois Urbana-Champaign
Unusual neuropeptide modification in the nervous system of mammals: investigating potential isomers of galanin

P17. Akina Osaki, Department of Pharmacology, Keio University School of Medicine
Physiological roles of catabolism of microbial D-amino acids in the energy metabolism in mammals

P18. Tian (Autumn) Qiu, University of Illinois at Urbana-Champaign
Identifying sources of D-serine in *Caenorhabditis elegans* and their impact on behavior

P19. Ann Bech Roskjær, University of Copenhagen
D-Amino acids in processed foods on gut microbiota, health and disease

P20. I-An Wei, Florida State University
Effect of D-amino acids on calcium dynamics in pancreatic islets

P21. Elena Zerbini, Department of Biotechnology and Life Sciences, University of Insubria
Effects of pathological mutations in human PHGDH

P22. Zhijun Zhu, University of Wisconsin-Madison
Chiral pair isobaric DiLeu labeling strategy enabled separation and absolute quantitation of enantiomeric amino acids

K01.

Analytical Approaches for Analyzing D-Amino Acids and Epimeric Peptides in Human Samples

Daniel W. Armstrong, University of Texas at Arlington, Department of Chemistry & Biochemistry Arlington

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Studies on two classes of D-amino acids (i.e., proteinic and nonproteinic) in humans have increased tremendously over the last decade. Samples of various human fluids and tissues can be limited in amounts, involve complex matrices, and often have only trace levels of D-amino acids (D-AAs) or epimeric peptides. Since enantiomers and epimeric peptides have the same exact mass, separations play the dominant role in any/all analyses. Mass spectrometry, however, can be a highly useful detector given its sensitivity. Fluorescence detection is equally or more sensitive but does not provide m/z analyte corroboration which can be useful in complex samples with overlapping peaks. Multidimensional separations can be highly useful to minimize matrix effects for complex biological samples where multiple amino acids and/or peptides are to be determined in a single analysis. Since D-amino acids in physiological samples almost always exist in the presence of much higher levels of L-amino acid enantiomers, good chiral separations are necessary. Analysis of D-AA containing peptides generally require various enzymatic treatments that will be discussed.

Questions as to the origins and relevance of D-AAs in biological samples have been asked continually and the search for answers is escalating today. In mammals there are at least three important sources of D-AAs which are: 1) food intake 2) gut bacteria 3) intrinsic biosynthesis. The latter of these may be the most intriguing in terms of signaling molecules, biomarkers for disease, etc. The approaches we developed for human samples since 1990 - and the results of these studies will be discussed up to and including current studies involving cancer and Alzheimer's disease.

The essential analytical methodologies which have been developed for this field, will continue to improve and be a central part of the discovery process. They will be essential tools that allow a fundamental understanding of the mechanism of action and biological role of the often "misunderstood" D-amino acids. Hight throughput screening will required fast separations if such analyses are to move to the clinical setting.

K02.

D-Amino Acids in Plants: How Much Do We Really Know?

Üner Kolukisaoglu, University of Tuebingen/ZMBP

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D-Amino acids as enantiomers of the proteinogenic L-amino acids are known to be essential molecules, for instance as elements of peptidoglycan in the cell wall of bacteria or as co-agonists of NMDA receptors in animals. Interestingly, in plants their presence and functions have been neglected for a long time, although the uptake of D-amino acids has been shown before and there are a number of genes in plant genomes encoding for D-amino acid synthesizing and metabolizing enzymes. In the last years we focused on physiological functions of these molecules in higher plants. In the course of these works, we found growing evidence to explain the phenomenon of D-amino acid stimulated ethylene synthesis in plants. Thereby, the D-amino acid specific transaminase DAT1 plays a central metabolic function for this hormonal effect. We elucidated the metabolic pathway leading to ethylene in this case and found the favorite substrate of this enzyme, D-methionine, to cause different ethylene-dependent physiological responses in plants. Although, D-methionine is found rarely in nature, its excretion by several bacteria points to a widespread role of D-methionine in plant-microbe interaction. In another line of studies, we gathered several lines of evidence that seed plants harbor a peptidoglycan layer in their plastidic envelope with canonical elements like D-alanine in their peptide chain. It will be a future task to analyze and understand the evolution and functions of this subcellular structure in eukaryotic organelles. Altogether, our studies revealed a multitude of functions of D-amino acids in plants, which await to be further investigated.

K03.

Mammalian D-Cysteine is a Regulator of Neural Progenitor Cell Proliferation in the Brain

Robin Roychaudhur, Department of Neuroscience, Johns Hopkins School of Medicine

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D-Amino acids are being recognized as functionally important molecules in mammalian biology. The D-stereoisomer of the amino acid with the fastest in vitro spontaneous racemization rate, cysteine, has not been examined in mammals. Using chiral high-performance liquid chromatography and a stereospecific luciferase assay, we identify endogenous D-cysteine in mammalian brain. Serine Racemase (SR), which generates the N-methyl-D-aspartate glutamate receptor coagonist D-serine, is the candidate biosynthetic enzyme for D-cysteine. D-cysteine is enriched in the mouse brain at E9.5 with a concentration of approximately 4.5 mM, progressively decreasing with age. D-cysteine reduces the proliferation of cultured mouse embryonic neural progenitor cells (NPCs) by ~50 %, an effect not shared with D-serine or L-cysteine. The antiproliferative effects of D-cysteine is mediated by the Foxo family of transcription factors, Foxo1 and Foxo3a. The selective influence of D-cysteine on NPC proliferation is reflected in aberrant lamination of the cerebral cortex in neonatal SR knockout mice. Our unbiased screen for D-cysteine binding proteins in NPCs by immunoprecipitation with a conjugated D-cysteine antibody followed by mass spectrometry identified myristoylated alanine rich C-kinase substrate (MARCKS) and MARCKS like1 as putative D-cysteine–binding proteins. Together, these results establish endogenous mammalian D-cysteine and implicate it as a physiologic regulator of NPC homeostasis in the developing brain. Other D-stereoisomers may play novel roles in mammalian biology.

L01.

Enantioselective Analysis of Amino Acids and Related Compounds Using Multi-Dimensional HPLC and its Medical Applications

Kenji Hamase, Kyushu University, Graduate School of Pharmaceutical Sciences

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Most of the chiral compounds, including amino acids and hydroxy acids, have the homochirality in living beings, and one of the enantiomers are predominant. Concerning amino acids, L-forms are major and the D-enantiomers are minor (considered not present), especially in higher animals including humans. Along with the progress of analytical technologies, several D-amino acids were found in mammals and were reported to have biological functions and/or diagnostic values. However, the precise determination of the minor enantiomers in real world samples is practically difficult and the development/utilization of a highly sensitive and selective analytical method is essential. In the present study, we have developed selective multi-dimensional HPLC systems in combination with the sensitive pre-column fluorescence derivatization techniques. For the fluorescence derivatization, 4-fluoro-7-nitro-2,1,3-benzoxadiazole (NBD-F) was used for amino acids, 4-nitro-7-piperazino-2,1,3-benzoxadiazole (NBD-PZ) was used for hydroxy acids. For the simultaneous analysis of amino acids and hydroxy acids, 4-(N-chloroformylmethyl-N-methylamino)-7-nitro-2,1,3-benzoxadiazole (NBD-COCl) was used. For the multi-dimensional HPLC analysis, a microbore reversed-phase column was used in the first dimension to separate the analytes by their hydrophobicity. The target fractions were collected and introduced into the next dimension. To determine extremely trace levels of the enantiomers in complicated matrices, a mixed-mode separation or an anion-exchange separation was performed in the second dimension. These reversed-phase, mixed-mode and anion-exchange separations are always non-enantioselective modes, and the analytes are collected as their D plus L mixtures. The final dimension is the enantioselective mode and the chiral discrimination of the target compounds were performed. By using the whole fraction transfer technique for the multi-dimensional HPLC, the analytes could be determined selectively without losing the sensitivity. Concerning the neutral D-amino acids, relatively high levels of D-Ser were present in the frontal brain tissues including cerebrum and hippocampus. In the human plasma, small amounts of several D-amino acids including D-Ala, D-Asn, D-Pro and D-Ser were found and their concentrations were clearly related to the progress of kidney disfunction. Tissue distributions of acidic D-amino acids (D-Asp and D-Glu) were also studied, and high levels of D-Asp were observed in neuroendocrine tissues (testis, adrenal etc.). Concerning Glu, small but significant amount of the D-form was found in the testis. Enantioselective analysis of amino acids and related compounds are effective for the screening of new bio-functional/biomarker molecules, and further studies are ongoing.

L02.

Electrochemical Sensor Development for Robust Amino Acid Biosensing

Janine Mauzeroll, McGill University

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This talk focuses on the design improvements of D-amino acid oxidase-based electrochemical biosensors. Limitations in biosensor performance attributed to the bioreceptor component are discussed. The methodology used to tackle these challenges, which includes generating D-amino acid oxidase variants through single point mutations for use in biosensor development is presented. It is possible to alter enzyme selectivity and sensitivity to develop biosensors with desired performance properties. Moreover, the application of DAAO biosensors, focusing on D-serine detection through ex vivo and in vivo measurements from Xenopus, is discussed. The application of D-serine detecting biosensors allowed to measure neurochemical release in model animal systems. Finally, biosensor development is extended to other oxidase enzymes, such as glycine oxidase. This extension makes the application of biosensors highly attractive for multiple real-time chemical detection, not necessarily limited to biological applications.

L03.

Deep Chemical Analysis of Microdialysate by LC-MS: *in vivo* Metabolomics

Robert Kennedy, Brady Anderson, University of Michigan

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Brain extracellular space contains a wide range of molecules including neurotransmitters, neuromodulators, and metabolites. The chemical milieu in this space is indicative of cellular activity and is involved in regulation of that activity. Most work to date using microdialysis has focussed on measuring a small number of neurotransmitters or metabolites at a time. Recent advances in LC-MS technology for identifying and quantifying chemicals in complex mixtures have opened the possibility of tracking the concentrations of many more compounds. These trends are exemplified by metabolomics, the field of analysing the full complement of metabolites present in an organism or biofluid. In this work, we describe our efforts to improve and apply the tools of metabolomics to monitoring the brain extracellular space. The goal is to both identify the chemicals present and begin to understand their relationship to phenotype, behaviour, drug effects, or disease state. We also illustrate how these tools can highlight specific pools of glutamate within the brain.

Metabolomics can be performed as a directed or undirected analysis. In directed analysis, a select group of known compounds is determined. In undirected analysis, as many compounds as possible are detected using a general technique like LC-MS. Compounds of interest can be identified by matching the mass spectra to a database. Often only a small fraction of the signals detected can be attributed to specific compound. For example, it is not uncommon to detect 104 “features” (signal at a given retention time and mass) but only identify a few hundred compounds.

In this work, we illustrate how directed metabolomics methods can be used to uncover chemical differences of phenotypes, in this case the HR/LR behavioural model. We also describe strategies to identify over 300 compounds in dialysate and illustrate preliminary results using this method uncover unexpected chemical differences in phenotypes. Finally, we describe a LC-MS based method to using stable-isotope tracing to track specific pools of glutamate as a neurotransmitter. Potential of the methods for elucidating roles of d-amino acids in the brain will be discussed.

L04/P22.

Chiral Pair Isobaric DiLeu Labeling Strategy Enabled Separation and Absolute Quantitation of Enantiomeric Amino Acids

Zhijun Zhu, Shuling Xu, Zicong Wang, Daniel G. Delafield, Ting-Jia Gu, Gaoyuan Lu, and Lingjun Li

Department of Chemistry, School of Pharmacy, University of Wisconsin-Madison

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Introduction. It remains challenging to differentiate D-amino acids (D-AAs) from their enantiomeric L-forms by either gas-phase/liquid-phase separation or mass spectrometry (MS), let alone enantiomer-resolved quantitation. Here, we developed a high-throughput 4-plex isobaric N, N-dimethyl-L-leucine (L-DiLeu) and N, N-dimethyl-D-leucine (D-DiLeu) paired labeling strategy to qualitatively and quantitatively analyze L-AAs and D-AAs on LC-ion mobility (IM)-QTOF platform. The DiLeu tag serves as both a novel chiral reagent to generate diastereomers of D/L-AAs to facilitate both LC separation and IM identification and an isobaric tag for quantification. Utilizing D-DiLeu-L-AAs as surrogates to L-DiLeu-D-AAs, L-AA standards labeled by the last isobaric channel of both L-DiLeu and D-DiLeu could largely boost the signal of labeled D-/L-AAs in MS1 and serve as internal standards for accurate and confident absolute quantitation without extra D-AA standards. Brain tissues of male APP/PS1 Alzheimer's disease (AD) mouse model and wild type (WT) mouse were investigated.

Methods. A 4D database (m/z, CCS, RT, MS/MS) was built using L-DiLeu and D-DiLeu labeled L-AA standards. AAs extracted from different brain regions of both WT and APP/PS1 mice were labeled by L-DiLeu tags of channel 115, 116, and 117. L-AA standards were labeled by both L-DiLeu 118 and D-DiLeu 118, then mixed with L-DiLeu labeled AAs from brain samples for LC-IM-MS/MS analysis.

Preliminary data. Baseline RPLC separation of 20 D/L-AA counterparts after chiral pair DiLeu labeling was successfully achieved on a C18 column. Moreover, the distinguishable IM drift time differences between labeled D/L-AAs provided additional structural information, effectively reducing the interferences from co-fragmented isomers/isobars. Pooled brain cortex of male APP/PS1 mice (n=10) and WT mice (n=9) were tested in a pilot experiment. Taking the quantitation of L-Ile as an example, there were 2 groups of DiLeu reporter ions of different ratios at dt = 21.91 ms and at dt = 19.76 ms. Based on 4D database matching, the reporter ions at dt = 21.91 ms were from L-DiLeu-L-Ile while the other group of reporter ions were fragmented from an unknown co-eluted isomer/isobar. A significantly higher abundance of L-Ser, L-Lys, D-Ser, and D-Asp were found in the cortex of male APP/PS1 mice than in male WT mice, indicating that these AAs may be potential biomarkers of AD. D/L-AA levels in different brain regions and age-related D/L-AA level changes in WT and APP/PS1 mice are under investigation.

Novel Aspect. Chiral pair isobaric DiLeu labeling for enantiomer-resolved quantitation of D/L-AAs via LC-IM-QTOF.

L05/P12.

Exploring Crown Ether as Shift Reagent for the Differentiation of Amino Acid Enantiomers and Peptide Epimers via Ion Mobility Spectrometry

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Introduction. The differentiation and quantification of chiral biomolecules such as amino acid enantiomers and peptide epimers are necessary to elucidate their physiological roles, especially in food, the environment, and human health. Meanwhile, ion mobility spectrometry (IMS) is becoming an effective tool to study stereochemistry. However, it is extremely challenging to separate enantiomers by IMS. Peptide epimers such as D-amino acid-containing peptides (DAACPs) and their L-analogs also share small collision cross-section (CCS) differences (~1%) in most cases. These factors hinder the application of IMS in chiral analysis. Here we introduced a chiral crown ether, (−)-(18-Crown-6)-2,3,11,12-tetracarboxylic acid (18C6TA), as the shift reagent in IMS to differentiate chiral biomolecules. Amino acids can form a non-covalent ion complex diastereomer with crown ether to enable enantiomeric separation via IMS. The D/L structural differences (DLSDs) in peptide epimers can also be enhanced with the complexation. Not limited to electrospray ionization (ESI), the crown ether-amino acid/peptide ion complexes could also be preserved in matrix-assisted laser desorption/ionization (MALDI), allowing application in mass spectrometry imaging.

Methods. Chiral molecules (amino acids or peptides) and 18C6TA were dissolved in 50:50 methanol: water to reach a final concentration of 10 µM and 50 µM, respectively. For ESI experiments, samples were analyzed by a Waters Synapt G2 instrument via direct infusion. For MALDI experiments, samples were mixed with CHCA matrix and analyzed by a Bruker timsTOF fleX MALDI-2 instrument.

Preliminary Data. [Amino Acid+18C6TA+H]+ and [Peptide+18C6TA+H]+ ion complexes can be observed in both ESI and MALDI experiments. With limited IM resolving power of around 20, the enantiomeric differentiation of the D/L-phenylalanine enantiomer can be well achieved with 0.58 peak-to-peak resolution. Neuropeptides including achatin-1 (GDFAD), dermorphin 1–4 (YDRFG), and a series of other tetrapeptides with a D-residue at the second position from the N-terminus were examined as peptide epimer models. The DLSDs of the above DAACPs and their L-analogs can be enhanced by incorporating CCS coordinates of [Peptide+18C6TA+H]+ in addition to [Peptide+H]+. The stereoisomeric proportion of DAACPs or D-amino acids in a mixture solution can be further determined by measuring the drift time shift of the ion complexes from their L-analogs. Through computational modeling, the energy optimization structures of [D/L-Phe+18C6TA+H]+ indicated that the hydrogen bonds between 18C6TA and chiral molecules may play an important role in chiral recognition.

Novel Aspect. The chiral crown ether as the shift reagent in IMS for rapid differentiation of chiral biomolecules has been demonstrated.

L06.

Import of L-Serine Across the BBB is Critical for D-Serine Synthesis and Neurodevelopment

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D-Serine is a gatekeeper of N-methyl-D-aspartate receptors (NMDARs), which play an essential role in synaptic plasticity, neurodegeneration, and psychiatric disorders. We have shown that D-serine signaling is regulated by several Slc38 transporters, including system A (Slc38a1 and Slc38a2), which removes D-serine from the synapse and affects synaptic plasticity. D-serine synthesis and NMDAR activity are also controlled by a metabolic interplay termed the "serine shuttle", in which L-serine is exported from astrocytes to fuel the neuronal production of D-serine. We now show that D-serine production in the brain in the early postnatal period also requires the import of L-serine across the blood-brain barrier (BBB). We identified Slc38a5 as a serine transporter enriched at the BBB that mediates the import of serine during early postnatal development. Slc38a5 knockout mice (KO) exhibit lower brain serine content, behavioral and motor abnormalities, reduced brain volume, and impaired neurogenesis and synaptogenesis. Although Slc38a5 is widely known as a glutamine transporter, glutamine levels were unchanged in Slc38a5-KO, suggesting that this transporter is not required to maintain glutamine in the brain. Our observations indicate that Slc38 transporter family members, including Slc38a5, are essential regulators of L- and D-serine metabolism and are critical for optimal NMDAR function and neurodevelopment.

L07.

Physiological and Pathological Roles of Serine Racemase in the Brain

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Serine racemase (SRR) produces D-serine from L-serine. D-Serine regulates the activity of the N-methyl-D-aspartate-type glutamate receptor (NMDAR) as an endogenous co-agonist. The NMDARs involve in neurotransmission, neural circuit formation, synaptic plasticity, and higher brain functions. Hypoactivation and hyperactivation of the NMDARs relate to many neurodegenerative and psychiatric disorders. Thus, the manipulation of the activity or gene expression of SRR will contribute the development of new treatments to these disorders. To examine the roles of SRR and D-serine *in vivo*, we generated SRR gene knockout (SRR-KO) mouse strain with pure C57BL/6 genetic background and analyzed its phenotypes. Using contextual- and tone-dependent fear conditioning paradigms, we found that SRR-KO mice showed some specific impairment of extinction in the contextual fear conditioning. Furthermore, SRR-KO mice were resistant to neurotoxic states induced with acute injection of NMDA or A-beta oligomer in the brain, and diabetic retinopathy models. The change of SRR expression in the brain of a genetic Alzheimer's disease (AD) model mouse suggests the protective role of reduced expression of SRR in gliosis and neuronal damage in the AD model. SRR-KO mice will be valuable for the analyses of physiological and pathological roles of serine racemase in the brain.

L08.

D-Alanine in the Microbiome-Gut-Brain Axis: Sources, Biodistribution, and Functional Roles

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Discovery of microbial metabolites affecting function of the host's nervous and endocrine systems has become an important area in investigations of the microbiota-gut-brain axis, with one goal being discovery of new treatments for neurological and endocrine diseases. D-Alanine (D-Ala), the D-enantiomer of the proteinogenic L-alanine, is a microbial metabolite and a potential neuromodulator. D-Ala is an essential component of bacterial cell walls. In tissue and organs of higher animals such as rodents and humans, D-Ala is detected in low amounts compared to the L-alanine. Like D-serine, the known endogenous neuromodulator, D-Ala is a potent agonist of the glycine site of N-methyl-D-aspartate (NMDA) receptors and has been shown to help improving symptoms of schizophrenia. Interestingly, finding of D-Ala immunoreactivity in specific cells of endocrine tissues points to its involvement in glucose metabolism. However, neither the sources nor the potential physiological functions of D-Ala *in vivo* were sufficiently understood.

The two main points are studies to elucidate the sources and biodistribution of D-Ala in rodents and exploration of the functional roles of gut microbial D-Ala synthesis and metabolism in model animal *Caenorhabditis elegans* (*C. elegans*). Previous evidence suggested that microbiota may be a major contributor for D-Ala in rodents, complicating delineation of sources of D-Ala and biodistribution of gut-absorbed D-Ala *in vivo*. Using germ-free mice and stable isotopes of alanine, followed by quantitative mass spectrometry, we showed the biodistribution of gut-absorbed D-Ala without interference from microbiota and demonstrated the sources of D-Ala in mouse being diet and microbiota. No endogenous synthesis of D-Ala during experimental time frame was reliably observed. To explore whether the D-Ala synthesis and metabolism of gut microbe affect animal physiology and behavior, we evaluated the phenotype changes of the nematode *C. elegans* fed with microbial mutants lacking D-Ala synthesis or metabolism enzymes under normal or high glucose conditions. Results showed *C. elegans* locomotive behaviors and food preferences were affected by specific microbial mutant, as well as a mutant-mediated lifespan reduction under high glucose concentration, indicating the functional roles of gut microbial D-Ala synthesis and metabolism on host animal's phenotype and biological functions.

L09.

A Right-Handed Excitatory Amino Acid Tunes Cortical Neuronal Inhibition

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N-methyl-D-aspartate receptors (NMDARs) populate GABAergic interneurons, where they play a critical role in shaping circuit motifs and memory. However, we are largely ignoring whether and how NMDARs at GABAergic interneurons are gated by signals released in their surrounding microenvironment. Here we explore the dynamics of the co-agonist site occupancy by D-serine and glycine at glutamatergic synapses onto parvalbumin positive (PV+) GABAergic interneurons in the adolescent prefrontal cortex, an area central to complex cognitive operation. By combining cellular electrophysiology with the use of unique pharmacological interventions and genetic manipulations, we report that the firing activity of layer 5 fast-spiking-PV+ interneurons and their excitatory synaptic coupling with principal neurons is under the control of NMDA receptors which are gated by D-serine but not glycine and that the identity of the co-agonist is not determined by the synaptic regime of the excitatory input. We further show that D-serine-deficient mice, a model of NMDAR hypofunction that exhibits schizophrenia-like phenotypes display attenuated firing pattern of the interneurons and no long-term potentiation, and then explored some pharmacological interventions to restore these functional outcomes. Our study extends the physiological implications of right-handed amino acids and in notably D-serine in brain physiopathology by uncovering for the first time its control of inhibitory synaptic networks through NMDARs.

L10/P20.

Effect of D-Amino Acis on Calcium Dynamics in Pancreatic Islets

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D-Ser, D-Ala, and D-Asp are known as important signaling molecules in the central nervous system, playing critical roles in synaptic plasticity, the mechanism for learning, and memory. Recently, studies have shown their contributions to glucose regulation, characterized as a potential drug target for diabetes treatment. The presumptive targets for these D-amino acids are N-methyl-D-aspartate receptors (NMDA receptors), activated by binding its agonist (L-Glu) and co-agonist (Gly) to allow Ca2+ influx into the cells. D-Ser and D-Ala have been found to co-agonize at the Gly binding site, while D-Asp agonizes at the L-Glu binding site. Although evidence regarding the involvement of D-amino acids affecting insulin secretion has been obtained, divergent findings among the studies make the relationship unclear. In this report, we investigated the effect of D-amino acids on murine islet physiology. A microfluidic platform was integrated with a gravity-driven perfusion system to deliver 20 mM glucose and exogenous amino acids to islets.1 Fluorescence imaging was employed to monitor intracellular [Ca^{2+}] ([Ca^{2+}]$_i$), a marker of insulin secretion, to assess the impact of L- and D-amino acids on [Ca^{2+}]$_i$ oscillation patterns of isolated mouse islets under varied concentrations. Our findings show that the period of [Ca^{2+}]$_i$ oscillations significantly increased during exposure to 100 µM D-Ser and D-Ala but was not affected by the treatments of their enantiomers. For L- and D-Asp, prolonged oscillation periods existed only at concentrations of 500 µM or more. The results demonstrate the modulation of [Ca^{2+}]$_i$ dynamics, potentially through activation of NMDA receptors, indicating modulation of GSIS regulation.

L11/P14.

Role of Human D-3-Phosphoglycerate Dehydrogenase in Amino Acids and Cellular Metabolism

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Human D-3-phosphoglycerate dehydrogenase (hPHGDH, EC 1.1.1.95) is responsible for the first and rate-determining step in the "phosphorylated pathway", which regulates the *de novo* biosynthesis of L-serine [1], by catalyzing the reversible transformation of D-3-phosphoglycerate (3PG, generated by glycolysis) into 3-phosphohydroxypyruvate (PHP) using NAD+ as cofactor. L-Serine is a nonessential amino acid that plays a major role in the metabolism of eukaryotic cells and is involved in several physiological activities, in particular in the development and function of the central nervous system. It is used for protein synthesis and as building block to produce phosphoglycerides, glycerides, sphingolipids, phosphatidylserine, and methylenetetrahydrofolate. Moreover, L-serine is the precursor of glycine and D-serine, two neuroactive signaling molecules modulating the activation of NMDA receptors [1]. hPHGDH is involved in nucleotide synthesis by supporting central one-carbon metabolism: the overexpression of the gene encoding PHGDH has been reported in human cancers and its inhibition reduce cell proliferation. Recently, hPHGDH levels have been related to Alzheimer's disease progression [2], and several mutations have been correlated to L-serine deficiency and neurodegenerative diseases [1].

hPHGDH belongs to the structurally most complex type I class of PHGDH: these dehydrogenases contain two common domains (the substrate-binding domain and the cofactor-binding domain) and two additional regulatory domains at the C-terminus: the 3D structure of the full-length hPHGDH has not been solved yet. With the aim to clarify the molecular mechanisms related to L-serine synthesis under physiological and pathological conditions (on the way to modulate D-serine levels), the biochemical properties of recombinant hPHGDH were investigated [3]. We clarified the substrate specificity by establishing the kinetic parameters in the forward and in the reverse direction, as well as the presence in solution of different conformations and/or oligomeric states of hPHGDH. Moreover, the effect of different ligands related to several metabolic pathways (such as α-ketoglutaric acid, acetyl-CoA, ATP, D- and L-amino acids, fumaric acid, glucose-6-phosphate, malic acid, succinic acid, UMP...) and ions on hPHGDH activity and structural properties was investigated to shed light on a putative allosteric regulation of its functionality. Furthermore, selected hPHGDH variants (namely V261M, V425M and V490M) corresponding to known SNPs related to pathologies were also characterized. This work was supported by PRIN 2017 2017H4J3AS "Dissecting serine metabolism in the brain".

References: (1) Murtas G et al., (2020). *Cell Mol Life Sci*; 77(24):5131-5148. (2) Chen et al., (2022). *Cell Metab*; 34(5):651-653. (3) Murtas G et al., (2021). *Int J Mol Sci*; 22(8):4231.

L12.

Omics Analyses of Serine Metabolism in Alzheimer's Disease

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The diagnosis of Alzheimer's disease (AD) may be delayed because early biomarkers are not available. Hyperactivation of N-methyl-D-aspartate receptors (NMDAR) have been associated with synapse dysfunction in AD. In the past years several studies reported that D-serine (D-Ser) is a main co-agonist at NMDAR in frontal brain area and AD patients show an increased levels of D-Ser in the CSF and in specific brain regions. Indeed, a correlation between cerebral glucose metabolism and synaptic activity has been described in AD patients. Glycolysis in astrocytes supports the production of L-serine (L-Ser), which is needed for the synthesis of a plethora of relevant biomolecules, among them glycine and D-Ser. In the brain, de novo L-Ser synthesis proceeds via the phosphorylated pathway (PP) consisting of three reactions involving 3-phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase (PSAT) and phosphoserine phosphatase (PSP).
We have demonstrated by chiral HPLC that the serum D-Ser level and the D-/total serine ratio significantly increase with the progression of AD [2]. We proposed these two parameters as novel and valuable biomarkers for the progression of AD, allowing to discriminate patients with moderate (CDR 2) and mild (CDR 1) symptoms from healthy (CDR 0) individuals.

Next, we have evaluated the alterations observed in hippocampal regions of AD patients compared to healthy individuals by using a multi-omics approach. The transcriptomic profile of AD patients did not show any variation in the expression levels of the three PP enzymes as well as in levels of the transcripts identified for each gene. On the contrary, PHGDH and PSAT protein levels were significantly increased in AD: these changes were associated with a significant decrease of L-Ser levels and an increase of D-/(D+L)-serine ratio in AD patients. Our data indicate that altered L-Ser level may contribute to damaged neurotransmission and synaptic plasticity in AD patients, likely by triggering an increase in brain D-Ser availability. This work was supported by PRIN 2017 2017H4J3AS "Dissecting serine metabolism in the brain".

L13.

Detrimental Effects of Reactive Glial D-Serine Release on Brain Health Following CNS Injury

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Synaptic damage caused by acute brain injury is a major contributor to the cognitive dysfunction observed in both animal models and human patients. The concept that synaptic loss may not be solely a passive event of CNS trauma is novel, where mechanisms that underlie the targeting and elimination of synapses are only beginning to be elucidated. Here, we demonstrate that reactive astrocytes and microglia produce and release D-serine following controlled cortical impact (CCI) in mice via upregulation of the enzyme serine racemase (SR) and the Slc1a4 transporter, respectively. Blocking D-serine production or release selectively from reactive glia via genetic and pharmacologic means prior to CCI preserves synaptic stability and cognitive function. We also show that prolonged hyperactivation of GluN2B containing extrasynaptic NMDA receptors by chronic glial D-serine release plays an important role in targeting synapses for elimination, where microglia are implicated in synaptic elimination. Importantly, the molecular perturbations that are downstream of reactive glial D-serine and cause synaptic loss in our mouse CCI model, are also conserved in brain tissue from human TBI patients. Conversely, analysis of sex differences has revealed key biological differences between male and female mice, where females are more resilient to the effects of brain trauma on synapse loss and memory. We anticipate our studies will provide a foundation for developing novel therapeutic strategies to protect the brain following CNS trauma.

L14/P03.

Microglial D-Serine Mediates Synaptic Damage Following Traumatic Brain Injury (TBI)

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Traumatic brain injury (TBI) is a major worldwide health concern, affecting nearly 70 million people each year. The molecular mechanisms underlying TBI are diverse, consisting of various cellular cascades that exert progressive damage well beyond the initial impact. A primary component conserved across all TBI pathology is diffuse synaptic damage, which is observed in mild to severe TBI and can result in long-term cognitive impairments. However, the mechanisms that regulate synaptic damage within the injured CNS remain to be elucidated, where extrasynaptic N-Methyl-D-aspartate receptors (NMDARs) are thought to play a critical role. We have previously shown that the enhanced synthesis and release of D-serine, the primary co-agonist for synaptic NMDARs, by reactive hippocampal astrocytes following TBI in mice causes impairments in synaptic plasticity and memory. Here, we identify microglia as a novel cellular source of D-serine in the injured brain and employ genetically modified mice to examine the role of microglial D-serine in synaptic damage. We demonstrate that genetic deletion of microglial serine racemase (SRR), the enzyme that converts L-serine to D-serine, abolishes injury-induced cognitive deficits as well as preserves dendritic spine density and morphology in the hippocampus following controlled cortical impact (CCI). This is likely due to reduced NMDAR activation, as microglial SRR ablation attenuates NMDAR subunit upregulation and consequent synaptic pruning. Furthermore, RNA-sequencing of isolated hippocampal microglial and astrocyte cells suggests a global effect of microglial D-serine on glial reactivity as well as highlights sex differences that contribute to neuroprotection from CCI injury. Together, these findings establish a novel, microglial specific mechanism of D-serine mediated damage and implicate a key pathological pathway that may be therapeutically targeted to preserve synaptic stability following TBI.

L15.

Isomerized Residues in Age-Related Disease

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Abnormal aggregation of proteins is a hallmark of aging, particularly in post-mitotic cells such as neurons that are not subject to frequent turnover. Although protein aggregation has drawn significant attention, more subtle changes such as isomerization of certain residues are also common but less explored or understood. As methods capable of identifying isomerization in complex samples have become available, the potential importance of isomerization in relation to disease is beginning to be realized. For example, in Alzheimer's it has been shown that isomerization is a better predictor for dementia than aggregation. Isomerization also offers a unique type of information because it is a spontaneous chemical modification that lies largely outside the realm of biological control. Within the context of long-lived proteins, isomerization is related to proteostasis by both exerting influence on and reporting the status of autophagic flux. Recent results obtained by novel proteomics data acquisition and processing related to such isomers will be discussed.

L16.

Life-Long Regulation of Microbial D-Amino Acids in Mammals

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Symbiotic microbes are associated with the host physiology and pathology, but metabolic interplays between microbes and their host are less understood. We previously found that mouse intestine is rich in free D-amino acids that are derived from the microbiota. We also have reported that oxidation of such microbial D-amino acids by a host enzyme, D-amino acid oxidase (DAO), modifies mucosal immune response to the gut microbiota. In my presentation, I would like to introduce our recent findings of how such microbial D-amino acids are metabolized in mammals through different life stages. In the embryonic stage, while embryos express DAO in the kidney, D-amino acid levels in their body fluid are not influenced by the presence of embryonic DAO, but modulated by maternal DAO. After the delivery, intestinal D-amino acids are significantly increased in parallel with colonization of intestinal microbes. The amounts of fecal D-amino acids in mammals are associated with total amount of gut microbes but not abundance of specific class of microbes. Such microbial D-amino acids are partly absorbed in the intestine, actively degraded by DAO, and, for the rest, excreted into urine. Therefore, D-amino acids in the blood are maintained at low levels without being affected by the fluctuating production of D-amino acids by gut microbes.

L17/P02.

Pathophysiological Significance of D-amino acid Metabolism in the Experimental Autoimmune Encephalomyelitis

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Pathogenesis of autoimmune diseases involves both genetic and environmental factors such as intestinal microbes. Gut microbial community impacts host immune responses through microbial fragment and/or metabolites and influences mucosal and systemic immunity, which modulates pathophysiology of autoimmune diseases.

Gut bacteria produce D-amino acids as essential components of their cell walls and as regulators of diverse cellular processes. Recently, we have shown that mammals regulate immune responses to microbes through metabolizing microbial D-amino acids by intestinal D-amino acid oxidase (DAO). DAO inhibits excessive mammalian immune responses to gut microbes to regulate symbiosis with them. However, pathological roles of DAO in the autoimmune diseases remain unclear. In this study, we investigated whether D-amino acid metabolism by DAO influences pathology of autoimmune diseases using a drug-induced mouse model of multiple sclerosis (experimental autoimmune encephalomyelitis, EAE). We induced EAE on female DAO-null mice (DAOG181R) and control mice at 9-13 weeks of age. Unexpectedly, lack of DAO improved clinical scores for EAE. The mRNA expressions of the proinflammatory cytokines, TNF-alpha, IFN-gamma, and IL-17, were increased in the thoracic spinal cord of wild-type animals after EAE induction, and further increased in DAO-null, in contrast to their symptoms. Levels of both L- and D-serine in the spinal cord were increased after EAE was induced even in the wild-type controls. Furthermore, the D/L-serine ratio in the spinal cord was further increased in the EAE-induced DAO-null mice. Considering that DAO is expressed in the hindbrain as well as in the small intestine, D-serine increase in the central nervous system due to loss of DAO might rescue the EAE pathology.

L18.

Effects of Glucose Stimulation on D-Amino Acid Levels in Rodent Pancreatic Islets of Langerhans

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Islets of Langerhans are clusters of distinct cell types that produce different hormones to regulate glucose metabolism. The evidence for D-amino acids (D-AAs) playing a role in islet physiology is increasing, with serine racemase being found in islets, D-Ala colocalizing within insulin-secreting β-cells, and D-Asp colocalizing within glucagon-secreting α-cells. The role that these small molecules play in islet physiology is unknown, but both D-Ser and D-Ala are co-agonists of GluN1 subunits of the N-methyl-D-aspartate receptor (NMDAR), a glutamate-gated ion channel. D-Asp acts as an agonist of the GluN2 subunit of the NMDAR. It has been shown that NMDARs can modulate glucose-stimulated insulin secretion (GSIS). To explore the role of D-AAs in islet physiology, we set out to quantitatively measure the levels of D-Ser, D-Ala, and D-Asp in islets, and to examine how exogenously applied D-AAs may affect islet function.

Intracellular and released levels of D-Ser, D-Ala, and D-Asp were quantified using a chiral liquid chromatography-mass spectrometry method with a modified derivatization using Marfey's reagent. Isolated rodent islets were incubated for 30 min with 3-, 11-, or 20-mM glucose for basal, half-maximal, and maximal stimulation. While both enantiomers of the three amino acids were observed in the lysate, no significant changes were found between the different glucose concentrations tested. In contrast, the levels of D-Ser and D-Ala released at 20 mM glucose were significantly reduced compared to the secreted levels at 3 mM. To examine the role that the D-AAs may play in islet function, the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) within islets were monitored using Fura-2 fluorescence microscopy as a proxy for insulin secretion. Incubation in 20 mM glucose induced ~5 min oscillations of $[Ca^{2+}]_i$. Oscillations of this intracellular 2nd messenger are widely hypothesized to be due to the oscillatory nature of glycolysis and oscillations of insulin release are observed from islets that coincide with the $[Ca^{2+}]_i$ oscillations. Notably, insulin oscillations are essential for maintenance of euglycemia. During exposure to 100 µM D-Ser or D-Ala, the period of Ca^{2+} oscillations were significantly increased, but not during delivery of their L-AA counterparts. D-Asp, up to 500 µM, was not found to induce a change in Ca^{2+} oscillations that were different than L-Asp. Together, these results are consistent with a potential autocrine or paracrine role of secreted D-AAs, specifically D-Ser or D-Ala, in modulating GSIS via NMDAR.

L19.

Spatiotemporal Distribution of D-Amino Acid Oxidase in Central Nervous System and Kidney: Implication for Aging, Neurodegeneration and Renal Pathophysiology

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D-amino acid oxidase (DAO) is a flavoenzyme, catalyzing oxidative deamination of D-amino acids to produce corresponding α-keto acids, ammonia and hydrogen peroxide. In our search of DAO activity among various tissues, using the assay based on hydrogen peroxide production involving enzyme-coupled colorimetric assay with peroxidase, we revealed that DAO activity was detected in kidney, cerebellum, medulla oblongata, midbrain and spinal cord, but not in liver. In addition, we observed that DAO activity and its expression were decreased in thoracic and lumbar regions of spinal cord in aged mice when compared with young mice, indicating that decreased DAO is involved in motoneuron degeneration during senescence. We also found gender difference in DAO activity in the kidney, suggesting that DAO activity is influenced by sexual dimorphism.

In the kidney, its expression is detected in proximal tubules, and DAO is considered to play a role in the conversion of D-form amino acids to α-keto acids. LLC-PK1 cells, a pig renal proximal tubule cell line, were used to elucidate the regulation of DAO protein synthesis and degradation. We found that trypsinization of LLC-PK1 cells in culture system rapidly reduced the intracellular DAO protein level to ~33.9% of that before treatment, even within 30 min. Furthermore, we observed that the DAO protein level was decreased when LLC-PK1 cells were subjected to amino acid starvation. To determine the degradation pathway, we treated the cells with chloroquine and MG132. DAO degradation was found to be inhibited by chloroquine, but not by MG132 treatment. We next examined whether or not DAO was degraded by autophagy. We found that amino acid starvation led to an increased accumulation of LC3-II, suggesting that DAO protein is degraded by autophagy due to amino acid starvation. Furthermore, treatment with cycloheximide inhibited DAO protein degradation. Taken together, DAO protein is considered to be degraded by autophagy under starvation conditions.

These studies suggested that age- and gender-dependent DAO activity in each organ and potential dynamics of DAO protein may underlie the human pathophysiology regulated by D-amino acid metabolism.

L20.

D-Amino Acids in Healthy and Type Two Diabetes Affected Human Islets of Langerhans

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The islets of Langerhans are clusters of cells that function as endocrine units synthesizing and releasing insulin and a range of additional peptide hormones. The structural and chemical characteristics of islets change during type 2 diabetes. Although a range of metabolites including neurotransmitters has been reported in rodent islets, the involvement of these cell-to-cell signaling molecules within human pancreatic islets in the pathophysiology of type 2 diabetes is not well known, despite studies suggesting that these molecules impact intra- and inter-islet signaling pathways. We characterize the enigmatic cell-to-cell signaling molecules, D-serine (D-Ser) and D-aspartate (D-Asp), along with several classical neurotransmitters and related molecules, in healthy versus type 2 diabetes-affected human islets. Significantly lower D-Ser percentages and gamma-aminobutyric acid (GABA) levels were found in type 2 diabetes-affected islets compared to healthy islets. In addition, we observed the negative correlations of hemoglobin A1c (HbA1c) levels with absolute or relative levels of a number of the signaling molecules, such as D-Ser, D-Asp, serotonin, and GABA. Therefore, the progression of type 2 diabetes appears associated with the disruption in intra- or inter-islet signaling pathways. The findings indicate that these cell-to-cell signaling molecules can be used as therapeutic targets. This work is supported by the American Diabetes Association grant #1-18-VSN-19.

L21.

Glycine Decarboxylase (GLDC): A Novel Indirect Modulator of NMDA Receptor Activity and its Potential Role in the Pathophysiology of Psychosis

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NMDA receptor dysfunction is thought to play an important role in the pathophysiology of schizophrenia. In addition to the agonist glutamate, NMDA receptors are modulated by the co-agonists glycine and D-serine. Two patients with psychosis have been found to have a duplication or triplication of multiple 9p24.1 genes including GLDC (the gene encoding the enzyme glycine decarboxylase) which was predicted to increase degradation of the NMDA receptor co-agonist glycine. In the brain, GLDC is expressed by astrocytes, but not by neurons. Glycine and D-cycloserine treatment as "add-ons" to the antipsychotic drug clozapine led to a significant improvement of symptoms (J.A. Bodkin et al., *Biological Psychiatry* **2019**;86:523-535). Using gene targeting, CRISPR-Cas9 and trans-allelic recombination, we generated mice with a "duplication" (3 copies) and a "triplication" (4 copies) of the 9p24.1 genes that displayed an increased copy number in the two patients, and observed, e.g., latent inhibition and working memory deficits. Genomic fine-mapping with mice with 4 copies of only Gldc, or only the other 9p24.1 genes revealed that an increased copy number of Gldc is necessary and sufficient for startle habituation deficits, latent inhibition deficits, working memory deficits, social interaction and sociability deficits. Mice with 4 copies of Gldc display increased levels of GLDC protein and increased activity of the glycine cleavage system in the hippocampus. Synaptoneurosomal fractions of hippocampus showed decreased BDNF levels and reduced activation of the synaptic plasticity-related AKT-mTOR-CREB pathway in mice with 4 copies of Gldc, similar to what has previously been reported for serine racemase knockout which have a 90% reduction of D-serine. In mice with 4 copies of Gldc, the expression of miR-132 and the density of dendritic mushroom spines were reduced, consistent with reports on similar observations in serine racemase knockout mice. In the mice with 4 copies of Gldc, trace fear conditioning, which is in part dependent on the hippocampal CA1 region, is increased, whereas it was reported to be reduced in serine racemase knockout mice, suggesting functional differences between these two lines of mice.

In summary, our data show that an increased copy number of Gldc with resulting increased GLDC expression and glycine cleavage system activity is sufficient to induce molecular and behavioral features consistent with a schizophrenia-like phenotype. Our results suggest that in the patients with the 9p24.1 duplication/triplication the increase in GLDC copy number may be an important contributing factor to pathophysiology.

L22.

Duplication of the D-Aspartate Oxidase Gene Induces Cortical Abnormalities, Social Recognition Deficits in Mice and Intellectual Disabilities in Humans

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The D-aspartate oxidase (DDO) gene encodes the enzyme responsible for the catabolism of D-aspartate, an amino acid that occurs at high levels in the mammalian prenatal cortex and acts as an endogenous agonist for NMDA and metabotropic Glu5 receptors. Considering the key role of these receptors in neurodevelopmental processes and the cerebral prenatal abundance of D-aspartate, recent post-mortem findings suggest a link between D-aspartate dysmetabolism and schizophrenia. To clarify the still elusive role of this D-amino acid on brain function modulation, we used a knockin mouse model with additional Ddo gene copies, characterized by constitutive Ddo overexpression and depletion of D-aspartate. In these mice, we found reduced number of BrdU-positive dorsal pallium neurons during corticogenesis, and decreased cortical and striatal grey matter volume, measured by high-resolution structural magnetic resonance imaging, at adulthood. In line with this, we observed that Ddo retroviral transduction in embryonic day 14 telencephalic progenitor cells caused a significant reduction in the average size of clones. In addition to cortical abnormalities, we found that early D-aspartate depletion in Ddo-overexpressing mice induced social recognition deficits at adulthood. In order to translate our preclinical observations to humans, we reported the first clinical case of a young patient with severe intellectual disability, thought disorders and other behavioural abnormalities, harbouring a duplication of a chromosome 6 region, including the entire DDO gene. Overall, the present findings bring support for the role of D-aspartate metabolism in regulating neurodevelopmental processes in mice and provide translational evidence for an involvement of DDO duplication in the pathophysiology of intellectual disabilities in humans.

L23.

Regulation of Spiny Synapse Plasticity by D-Serine in Health and Disease

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Experience-dependent refinement of neuronal connections is important for brain development and learning. Signaling through NMDA-type glutamate receptors (NMDARs) is a key driver of learning-associated synaptic plasticity. Historically, the fundamental role of NMDARs in synaptic plasticity has been attributed to their specialized properties of ion flux through the receptor pore, which occurs following binding of both agonist (glutamate) and co-agonist (glycine or D-serine). More recently, a growing number of studies support that NMDARs also signal in an ion flux-independent manner. We show that ion flux-independent NMDAR signaling is required for the dendritic spine plasticity that is a vital component of brain circuit plasticity. We further define the key signaling pathways downstream of non-ionotropic NMDAR signaling in spine plasticity, which suggest that ion flux-independent NMDAR signaling is upstream of the cytoskeletal changes that support bidirectional spine structural plasticity. Notably, schizophrenia is associated with decreased levels of D-serine and decreased density of dendritic spines. Using the serine racemase knockout (SRKO) mouse model, which lacks the enzyme for D-serine production, we show that lowered D-serine levels enhance ion flux-independent signaling by the NMDAR, driving destabilization and loss of dendritic spines. Our results support a model in which modulation of D-serine levels occurring naturally and in association with disease can regulate the magnitude and direction of synaptic plasticity.

L24.

Neuroepigenetics of D-Ser and D-Asp Metabolism

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DNA methylation, by cross-talking in concert with a wide array of epigenetic performers, constitutes an ideal interface between our genome and environment. DNA methylation through 5-methyl- and 5-hydroxymethylcytosine, provides an epigenetic mechanism of gene regulation defining the gene expression program in neural development, function, and disorders. Recent surprising realization of a previously unanticipated plasticity of methylation profiles may well explain environmental adaptations, transient changes, and long-term alterations of the cell's transcriptomic profiles. Indeed, alteration of DNA methylation in brain have been recently found to greatly impact behaviour and mental health. The influence of environment, including early life experiences, stress or psychoactive molecules on epigenetic profiles in brain is currently under deep investigation. Notably, several genes that undergo physiological perinatal changes in DNA methylation are associated with neuropsychiatric conditions. In this context, we envisaged that epigenetic modifications during the perinatal period may drive essential and well-orchestrated physiological changes in the genes regulating brain levels of D-serine and D-aspartate. Potentially, dysfunction of this fine regulation may have long-lasting effects on brain functions and may contribute to the genesis of schizophrenia or other mental disorders, in which altered levels of D-amino acids are found.

L25.

The Role of Structural Flexibility and Protein Interactors in Modulating the Activity of Serine Racemase

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Human serine racemase (hSR) (EC:5.1.1.18) is a homodimeric pyridoxal-5'- phosphate (PLP)-dependent enzyme responsible for the biosynthesis of D-serine in the brain. As D-serine is a co-agonist of the NMDAR receptors, SR plays a crucial role in the modulation of glutamatergic neurotransmission. In vivo experiments have demonstrated that enzyme activity is modulated by post-translational modifications, allosteric effectors and protein interactors (Raboni et al., 2019). Prompted by these works, we have investigated hSR reactivity with nitric oxide and its interactions with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and the third PDZ domain of PSD-95. We have shown that S-nitrosylation stabilizes an open, less-active conformation of hSR and we concluded that ATP binding, glycine binding, and S-nitrosylation constitute a three-way regulation mechanism for the tight control of the enzyme activity (Marchesani et al., 2021). For GAPDH, we showed that hSR is not directly inhibited through an interaction with GAPDH but it is inhibited by the GAPDH substrate glyceraldehyde 3-phosphate in a time- and concentration-dependent fashion, likely through a covalent reaction of the aldehyde (Michielon et al., 2021). For PSD-95, we gathered evidence for a weak interaction, confirming the binding but supporting the hypothesis that a third protein partner is required to stabilize the complex (Giaccari et al., 2022).

L26.

Privileged Role for Postsynaptic D-Serine in Synaptic Plasticity

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N-methyl-D-aspartate receptors (NMDARs) are glutamate receptors that are master regulators of the bidirectional synaptic plasticity underlying learning and memory. In addition, NMDARs are unique among perhaps all receptors in biology in that, along with binding glutamate, they have an absolute requirement for a co-agonist, which can be either glycine or D-serine, to induce channel opening. However, the fundamental role of this co-agonist requirement, as well as the evolutionary conservation of dual co-agonists, remains poorly understood. Indeed, the identity of the synaptic NMDAR co-agonist is developmentally regulated and spatially restricted in the brain, with most forebrain synapses using glycine early on and later switching to D-serine. This differential and independent regulation of synaptic glycine and D-serine levels suggests they serve unique functions. We have recently shown that D-serine is released postsynaptically where it regulates synaptic NMDARs in an autocrine-like manner. Interestingly, basal NMDAR currents were minimally affected by the loss of postsynaptic D-serine, though long-term potentiation (LTP) was greatly impaired. These results suggested a specific role for D-serine in the induction of LTP. In a second line of evidence, NMDAR-mediated long-term depression (LTD) can occur during competitive antagonism of the co-agonist binding site, a phenomenon termed non-ionotropic LTD. Thus, we hypothesized that the level of occupancy of the co-agonist site by D-serine might bias the direction of synaptic plasticity. To test this hypothesis, we manipulated the occupancy of the NMDAR co-agonist site during the induction of synaptic plasticity using pharmacological approaches and enzymatic scavenging. We now show that reducing the occupancy of the NMDAR co-agonist site results in an overall bias towards synaptic depression whereas increasing extracellular D-serine levels biases plasticity towards synaptic potentiation. Surprisingly, we also found that D-serine completely inhibits non-ionotropic LTD, while exogenous glycine seems to have no effect on this form of plasticity. These results support our hypothesis that a primary role of the NMDAR co-agonist site may be to influence the direction of synaptic plasticity, with D-serine playing a privileged role in promoting synaptic potentiation by directly inhibiting synaptic depression. These results could lead to the development of novel approaches to modify synaptic plasticity for the treatment of neuropsychiatric diseases.

L27.

Tacticity Directed Functional Programming of Hetero-Chiral Peptide Constructs for Drug Delivery

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This work explains the utility of a design-centric approach in generating hetero-chiral peptide constructs by mutating the stereochemistry of peptide backbone for targeted drug delivery.

The introduction of cell-penetrating and tumor homing peptides has opened up new avenues for drug delivery applications using peptides. Cell-penetrating peptides (CPPs) are short peptides that can pass through the cell membrane while maintaining low levels of toxicity. Since the discovery of the transducing capabilities of the Tat peptide, many peptides capable of cell penetration have been discovered and utilized for transporting various cargoes and thus, show promising application as drug delivery vehicles. In this research project, we rationally designed three series of peptides each encoded with distinct design ideas for targeted drug delivery. The systemic development of Series-1 to Series-3 peptides, underlines the story of a design evolution starting from the structural engineering of a peptide molecule in a geometrical space, culminating in the generation of a complete drug delivery vector.

We adopted a strategy of geometry encoded functional programming of a peptide sequence, by adopting both L and D-amino acids through an 'informed walk' across Ramachandran map. The designed peptides displayed differential cellular uptake in cancerous cell types, revealing their potential for selective targeting. This corroborates our hypothesis that the topological specifications coded in the peptide design and the resulting electrostatic fingerprint play a critical role while interacting with the cell surface. All members in the designed peptide series retained their functional activity in serum, indicating their biocompatible nature, and showed negligible toxicity to mammalian red blood corpuscles (RBCs). Improved cytotoxicity to MTX resistant breast cancer (MDA-MB-231) cells by peptide-MTX conjugates, compared to the free MTX or THPs, guarantees the enhanced drug accumulation in cells, leading to the effective delivery of the designed molecules.

L28.

Cyclosporines: New Stories from An Old Molecule

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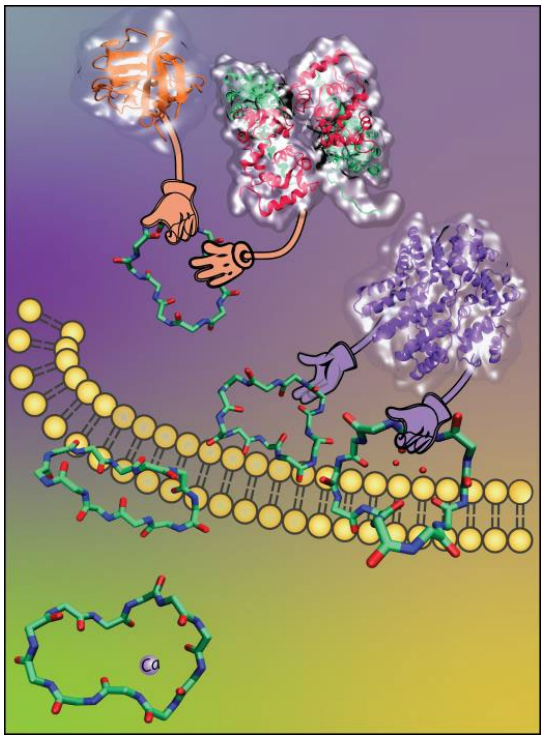
Cyclosporine A (CycA) has revolutionized the organ transplant medicine for its immunosuppressive property since 1980s. Despite of its lipophilic nature, CycA is orally available and can passively cross cellular membrane to reach its intracellular targets. The molecule acts via binding cyclophilin A and two units of calcineurin. The 11-residues long macrocyclic peptide contains seven N-methylated residues which can induce cis-trans isomerization of peptide bond sand one D-amino acid (D-Ala8). The conformational spaces of CycA and its analogues are highly complex; theoretically CycA can adopt up to 128 conformers with distinct cis/trans amides. Only a small number of cyclosporine crystal structures have been reported, leading to conflicting data on structure-function relationships. The classical view on cyclosporine binding to cyclophilin assumed that the bound state is among those that are highly populated in solution. However, our data suggest that while the main conformers in solution dictate membrane permeability, those that bind to protein targets are minor conformers that are difficult to resolve with traditional techniques. This presentation will highlight a novel strategy to capture aqueous and biologically active conformers of cyclosporine's using a combination of ion-mobility spectrometry mass spectrometry, X-ray/neutron crystallography, 2D nuclear magnetic resonance, and computational modeling. The subtle changes caused by salt additives and the importance of D-alanine(D-Ala8) will also be discussed.



L29/P19.

D-Amino Acid in Processed Foods on Gut Microbiota, Health, and Disease

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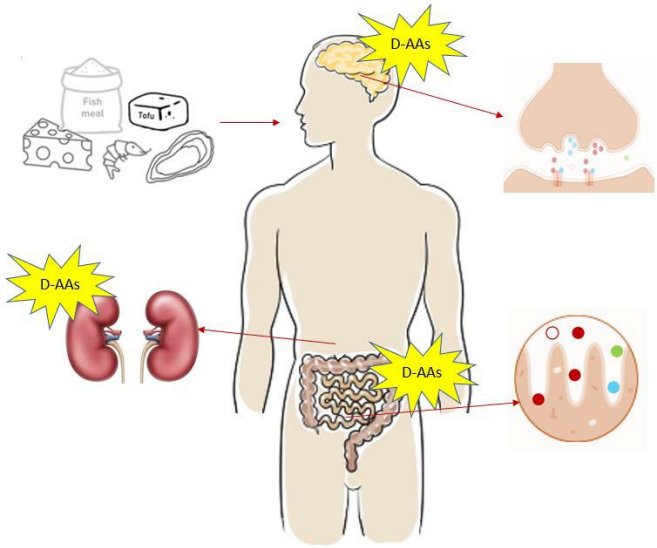
The drastic changes in food processing and eating habits worldwide have resulted in high exposures to D-amino acids (D-AAs) from ultra-processed foods. Research on D-AAs in human nutrition remains limited and it is unknown whether the increasing consumption has an impact on the D-A content in tissues and bio-fluids. Another major source of D-AAs are bacteria in the food chain, in food fermentation, and in our gastro-intestinal tract. In order to understand sources and physiological effects of D-AA exposures we have reviewed the nutritional literature on D-AAs. Human metabolism, transport, and localization of D-amino acids is reviewed as well. D-AAs have important functions in bacterial physiology, and considerable amounts of D-AAS are synthesized by intestinal bacteria. Preliminary data show that bacterially produced D-AAs and D-peptides have cross-reactive immune dominating effect with the host. In the intestine, kidney, and brain some polymorphic amino acid transporters absorb or reabsorb D-AAs well. When high amounts of D-AA are absorbed into the blood, the capillary D-AA concentration can increase up to three-fold in the proximal convoluted tubule. Highly stereoselective amino acid transporters are present in the tubules and secretion of D-AAs occur. Some D-AAs are crossing the blood brain barrier across both the luminal and abluminal membranes. D-amino acids are involved in multiple processes in the brain and a tight regulating is important to ensure a healthy brain. Overall, the current data indicate that regulation of D-AAs serve important functions but that high exposures may have pathophysiological effects; the current literature is insufficient to evaluate the relative importance of foods and gut microbial sources of D-AAs and health consequence of increased exposures from ultra-processed foods cannot therefore be assessed.



L30/P13.

Gastric Microbes Actively Produce D-Amino Acids in Breast Milk During Infancy

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Environmental factors during prenatal period and early childhood have influences on future health and susceptibility to certain diseases. This concept is known as Developmental Origins of Health and Diseases (DOHaD). Factors involved in the DOHaD theory include nutrition and gut microbiota, which may lead to the development of DOHaD-related diseases such as obesity and hypertension. While the gut microbiota and its metabolites in infancy have critical associations with the development of diseases in adulthood, it remains largely unknown whether maternal bacterial metabolites affect the immunity or energy metabolism of infants via breast milk. This study was performed to investigate whether mother-infant interaction mediates maternal D-amino acids. We found that P10 mouse infants had high levels of D-Ala and D-Leu in the breast milk filled in their stomach. In the germ-free condition, the gastric D-amino acids in P10 mice were at undetectable levels, suggesting that gastric D-amino acids in the infancy were from microbes. Since treatment with D-Ala in drinking water for dams resulted in significant increase of gastric D-Ala in the P10 pups, D-Ala can be transferred to breast milk. However, the levels of the gastric D-Ala in the P10 pups were much higher than maternal blood D-Ala. Also, we found that relative bacterial amounts in the stomach to other intestinal regions was significantly higher in the infancy compared to those in the adulthood. Therefore, we assume that gastric D-Ala in the infancy is not mainly from the breast milk, but from gastric microbes in situ. In support of this view, human breast milk showed only similar levels of D-amino acids to the blood. Thus, those results suggested that D-amino acids are not concentrated in the breast milk but are actively produced by microbes in the infant stomach. Our study warrants future studies to examine the significance of D-amino acids or their metabolism during infancy in the disease susceptibility developed in the adulthood.

L31.

So Similar, So Different: Investigating Human D-Amino Acid Oxidase And D-Aspartate Oxidase Regulation

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In the CNS, the FAD-dependent flavoenzymes D-amino acid oxidase (DAAO) and D-aspartate oxidase (DASPO) are involved in the modulation of N-methyl-D-aspartate (NMDA) receptor activity since they are responsible for the catabolism of the receptor's co-agonist D-serine (D-Ser) And the alternative agonist D-aspartate (D-Asp), respectively. DAAO and DASPO share the same chemical mechanism, a high sequence identity and have been proposed to originate from a common ancestor. However, the human enzymes (hDAAO and hDASPO) possess exclusive biochemical features. hDASPO shows a 10-fold higher specific activity on the physiological substrate compared to hDAAO. Moreover, the interaction of the two apoproteins with the cofactor is extremely different: FAD binding is weak in hDAAO (Kd = 8.0 μM) [1] and strong in hDASPO (Kd in the nanomolar range) [2]. These findings suggest that the two flavoenzymes evolved to fulfil their specific physiological roles in a different way. In the brain, the low activity of hDAAO maintains D-Ser in a physiological range, avoiding both its abnormal accumulation and an excessive degradation that would lead to NMDA receptor dysfunction. On the other hand, the highly efficient hDASPO keeps D-Asp at low and tightly controlled levels during post-natal development.

We recently studied the role of post-translational modifications on the modulation of hDAAO and hDASPO structural and functional properties. Biochemical and cellular studies indicated that, differently from hDAAO [3], both nitrosylation and sulfhydration of cysteine residues barely affect hDASPO properties. Indeed, both hDAAO and hDASPO interact with the primate specific regulatory protein pLG72, which affects the protein stability thus reducing the enzymatic activity. However, again the extent of the observed effect is different: contrary to what observed for hDAAO [4], pLG72 only partially inactivates hDASPO when present in a large molar excess.

These findings shed light on the activity modulation of the two related flavoenzymes hDAAO and hDASPO, showing how they are finely tuned in the brain to control the physiological levels of their substrates D-Ser D-Asp.

L32/P05.

DNA Methylation Reshaping at DAO Gene During Brain Development

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D-Amino acid Oxidase (DAO) gene is involved in the degradation of D-Ser and is strictly regulated during brain development to ensure correct modulation of D-Ser levels. We tested the hypothesis that the activity of DAO gene is finely controlled by DNA methylation through orchestrated methylation and demethylation events at their regulatory regions. We performed a comprehensive DNA methylation and mRNA expression analyses of DAO gene in mice during brain development in different brain areas and neural cell types. We evaluated DNA methylation state using amplicon bisulfite sequencing and performed an in-depth single molecule methylation approach in order to assess the cell-to-cell methylation heterogeneity. We found strong spatio-temporal changes in DNA methylation during development, especially in cerebellar astrocytes and at specific CpG sites. Some of these CpGs accumulated a high degree of 5-hydroxymethylcytosine at P1 suggesting an increase of the activity of TETs enzymes over the DNMTs activity at DAO gene promoters. Following these events, DAO gene dramatically underwent demethylation in later developmental stages. The data indicate that a programmed active erasure of DNA methylation signatures at DAO gene causes the post-natal increase od DAO enzyme. Furthermore, the here applied single-molecule methylation analysis (epiallele analysis) of DAO gene allowed us to identify different patterns of DNA methylation likely due to cell-type composition and function in different brain areas and developmental stages.

L33/P17.

Physiological Roles of Catabolism of Microbial D-Amino Acids in the Energy Metabolism in Mammals

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All proteinogenic amino acids expect for glycine have D-and L-enantiomers, but L-amino acids are selectively used for ribosomal protein synthesis and for most of biological processes. Despite predominant use of L-amino acids, accumulating evidence point to the distinctive roles of D-amino acids in mammalian physiology. Several D-amino acids, such as D-alanine, are originated from commensal gut bacteria1. Recent studies showed high amount of D-alanine is accumulated in two organs, ACTH-positive cells in the anterior pituitary gland and βcells in the pancreas, with the circadian rhythm in mammals2,3. In contrast to knowledge for intrinsic D-amino acids, physiological roles of bacterial D-amino acids in mammals have been poorly understood. In this study, we investigated whether metabolism of microbial D-amino acids influences mammalian energy metabolism using mice lacking the enzymatic activity of D-amino acid oxidase (DAO), a flavoenzyme catabolizing D-amino acids. With normal diet, loss of DAO activity increased mouse body weight, central obesity, with age. The increase in its body weight was accelerated by feeding with high fat diet. In addition, reducing gut bacteria by treatment with antibiotics minimize their weight gain. These results indicated involvement of microbial-amino acids in energy metabolism in mammals. As a result of overweight, DAO-null mice presented persistent insulin secretion under glucose challenge but without insulin resistance. On the other hand, gut microbial quantity and diversity did not show any characteristics to tend to obesity. Thus, our findings suggest that metabolism of microbial D-amino acids modulates hormone(s) orchestrating energy metabolism in mammals.

References

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L34.

Understanding the Roles of D-Amino Acid Residues in Cell-Cell Signaling Peptides

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Neuropeptides play critical roles in communication between cells, and the processing of neuropeptides after translation directly dictates their final physiological functions. Understanding the active structures and post-translational processing of neuropeptides is important both to understand normal cell-cell signaling and to design therapeutic compounds that modulate these signaling pathways for the treatment of disease. Neuropeptides can undergo an unusual post-translational modification: the isomerization of an amino acid residue from the L-stereoisomer to the D-stereoisomer. This change in stereochemistry has a significant impact on the functional properties of the resulting D-amino acid-containing peptide (DAACP). Despite the importance of stereochemistry for the biological function of many DAACPs, little is known about how D-residues influence interactions on the molecular level, particularly with their cognate receptors. Here, we utilize a diverse array of tools to identify DAACPs, characterize their properties, and understand their interactions with cell surface receptors. Ultimately, research in this area enables a better understanding of how post-translational isomerization influences cell-cell signaling and may give insight into the evolution of this elusive modification.

L35.

Effect of C-Terminal Adjacent Amino Acid Residues on Deamidation/Isomerization of Asparagine Residues in Lens Component Proteins

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[Purpose] Amino acids are chiral molecules that take two forms, L-form and D-form, but the amino acid residues in protein/peptide that constitute our body are exclusively L-form. On the other hand, there is an aspartic acid residue (Asp) that undergoes significant site-specific alteration to D-form (including isomerization) during aging. To screen for factors that affect site-specific Asp isomerization, we first generated a model protein that rapidly forms isomerization intermediates under mild conditions in vitro. In order to investigate the effect of C-terminal adjacent residues on the isomerization reaction, we replaced the C-terminal adjacent residues of the target Asp with various amino acids and compared the isomerization rates of model protein.

[Method] Recombinant human αA-crystallin Asp151Asn (D151N) was prepared in E. coli by targeting the 151st Asp of human αA-crystallin, which has been previously reported to be isomerized. Next, D151N/A152X (X: any amino acid), in which the 152nd alanine (A) in D151N was replaced with another amino acids, were produced and purified. The structure of these D151N/A152X were checked, then subjected to LC-MS analysis after heating, and the deamidation/isomerization ratio were compared to D151N.

[Results and Discussions] The structural analyses confirmed that the substitution of Ala152 with other amino acids did not affect the higher-order structure of αA-crystallin mimics. On the other hand, comparison of modification rates showed that deamidation/isomerization of Asn151 increased when the C-terminal amino acid residue of the D151N was small, while decreased when it was replaced by a larger amino acid residue. These results support the hypothesis that steric hindrance of the C-terminal amino acid residue affects the formation of succinimide intermediate of Asp. Among the αA-crystallin substitutions produced in this study, D151N/A152G produced intermediate in a very short time. We obtained a useful model protein for further screening of various isomerization-promoting and -suppressing substances.

L36.

Advancing Neuropeptide Research Via Novel Application of Ion Mobility Mass Spectrometry (IM-MS)

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The neurons in our brain are bathed in a complex suite of neuropeptides that modulate their response and affect the activities of neuronal networks. Development of highly sensitive and selective analytical tools for neuropeptide identification and quantitation is in great demand. Although considerable progress has been made in the past decade, remaining technical challenges exist for more complete neuropeptidomic analysis. Here, we explore novel use of ion mobility mass spectrometry (IM-MS) to address some of these unique challenges. For example, we developed a site-specific strategy to rapidly and precisely localize D-amino acids in peptides by IM-MS analysis of tandem MS-generated epimeric fragment ions. The development of this strategy is based on the concept that the epimeric fragment ions resulting from D/L-peptide epimers exhibit conformational differences, thus showing different mobility in IM-MS as measured by drift time and collision cross section. This difference is used as a criterion to localize the D-amino acid substitution. Additionally, the application of multidimensional ion mobility spectrometry to the study of metal-enhanced enantiomeric discrimination of D/L-amino acid containing Abeta monomers and chiral effect on oligomerization process will be presented. Finally, the use of D-/L-dimethylated amino acid to facilitate peptide epimer differentiation and quantitation on different IMS platforms and the exploration of chiral molecular shift reagents to enhance separation of D-amino acid containing peptides will be discussed.

L37/P01.

Exploratory Recombinant Expression and Fractionation of Eukaryotic L/D Peptide Isomerases

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A subtle post-translational modification is the enzymatic conversion of a ribosomally generated all L-amino acid containing peptide into a D-amino acid containing peptide (DAACP). Compared to their all L-amino acid epimers, DAACPs can exhibit differential chemical stability and receptor binding characteristics. They have also been shown to be prevalent across phylogenetically diverse species and occur in a wide array of functional contexts, from venom peptides to neuropeptides. As these modifications are largely carried about by peptide processing enzymes, studies to characterize and identify such enzymes are crucial to further investigation of the physiological actions and prevalence of their post-translationally modified peptide substrates. Recently DAACPs have been detected in the nervous system of a marine mollusk, Aplysia californica. The presence of such DAACPs had led to the hypothesis that a L/D peptide isomerase should exist in the nervous system of Aplysia, and an assay has been developed to partially purify and characterize the protein responsible for the isomerase activity. A list of isomerase candidate proteins has been obtained through proteomics studies of purified CNS extracts. These proteins were further analyzed through single cell transcriptomics, and a system was developed for recombinant expression of candidate proteins. Isomerase activity has also been detected in rodent tissue homogenates using non-endogenous synthetic peptide as well as on an endogenous peptide, Neuropeptide FF. However, while this activity suggests the presence of endogenous DAACPs, they have not yet been confirmed. An assay has also been developed to characterize the isomerase activity in rodent systems, and a system for activity guided purification was developed to obtain partially pure protein fractions, which were then subjected to proteomics analysis to obtain a list of candidate proteins. In addition to being critical for elucidating the biosynthetic pathway for DAACP production, the discovery of novel peptide isomerases will aid in a search for similar enzymes though sequence similarity and substrate specificity. In turn, investigations of tissue/cell type specific expression of these enzymes will help elucidate the function of the DAACPs.

L38.

D-Serine and Schizophrenia

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D-Serine is an endogenous coagonist for the N-methyl-D-aspartate type glutamate receptor (NMDAR) with high brain-predominant tissue and extracellular concentrations, NMDAR-like distribution, and metabolic processes. These features suggest that D-serine signaling plays a critical role in the expression and regulation of higher brain functions involving the NMDAR and in the pathophysiology of their disorders. The coagonist nature of D-serine allow us to postulate that its concentrations in the extracellular fluid of synapses acting on the NMDAR must be maintained in an appropriate constant range, rather than dropping rapidly after quantum release as in neurotransmitters. Therefore, we have investigated the regulatory molecular and cellular mechanisms of the extracellular concentrations of D-serine and their possible disturbances in schizophrenia, since schizophrenia is assumed to implicate NMDAR hypofunction based on observations of schizophrenia-like psychoses caused by NMDAR antagonists and autoantibodies. In animal experiments using an in vivo dialysis technique or in vitro gene expression system of xenopus oocyte, we have found that the prefrontal extracellular D-serine concentrations are markedly decreased in the prefrontal cortex lesioned by the neuronal cell body-selective neurotoxin quinolinate, and that they are downregulated by neuronal depolarization and glial activity attenuation and modulated by Asc-1 neutral amino acid transporter, 3'-phosphoadenosine 5'-phosphosulfate transporter (PAPST1), serine racemase, calcium-permeable AMPA type glutamate receptor, and GABAA receptor. In the studies on schizophrenia patients, mRNA expression of ASC-1 and three types of GABAA receptor subunits are observed to be significantly altered in the postmortem prefrontal cortical areas, and significant association of PAPST1 with schizophrenia is detected in blood DNA samples. However, there was no significant changes in the tissue levels of D-serine in the prefrontal cortical areas of postmortem brains from schizophrenia patients. Together with the results of other investigators indicating increased density of NMDAR glycine site and reduced radiolabeled ligand binding to the phencyclidine site within the NMDAR channel revealed by in vivo PET study, our data support the following views: (1) neuronal and glial cells participate in the extracellular release of D-serine, (2) aberrant expression of the extracellular D-serine signaling-related molecules might be implicated in the pathophysiology of schizophrenia, and (3) attenuation of extracellular D-serine signaling to the NMDAR could occur in the brain of schizophrenia patients.

L39.

D-Serine-AMPA Receptor Interaction to Development of a Novel Treatment For Schizophrenia

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NMDA-type glutamate (Glu) receptor (NMDA receptor) antagonists and autoantibodies have been observed to mimic positive and negative symptoms and cognitive dysfunction of schizophrenia, suggesting NMDA receptor hypofunction in the brain in schizophrenia. Since currently available antipsychotic drugs that primarily block D2-type dopamine receptors have not been successful in treating negative symptoms and cognitive dysfunction, facilitation of NMDA receptor function is expected to be a new therapeutic approach. To obtain an insight into a novel treatment for schizophrenia, we have studied the effect of the calcium-permeable (CP)AMPA-type Glu receptor (CP-AMPA receptor) antagonist, IEM1460, on pharmacological animal models of schizophrenia based on the following findings and suggestions: (1) NMDA receptor blockade has been reported in the rat frontal cortex to induce a compensatory increase in Glu release which stimulates extra-NMDA type Glu receptors such as AMPA, kainite and metabotropic Glu receptors, (2) we have revealed that AMPA receptor agonist S-AMPA reduces the extracellular concentrations of the NMDA receptor coagonist, D-serine, in the rat prefrontal cortex in a CP-AMPA receptor antagonist reversible manner, and (3) these observations indicate that CP-AMPA receptor blockade could prevents the exacerbation of NMDA receptor hypofunction in schizophrenia elicited by a decrease in D-serine signaling consequent upon AMPA receptor overactivation, and restore or enhance the extracellular D-serine levels and NMDA receptor activity. In support of the above idea, subcutaneous administration of IEM1460 in mice significantly reduced the augmented movements that occurs after acute (subcutaneous) administration of phencyclidine or dizocilpine (MK801), NMDA receptor blockers, widely used as animal models of schizophrenia IEM1460 also significantly suppressed hyperactivity in another model of mice treated with the dopamine agonist methamphetamine, which induces positive schizophrenia-like symptoms. These results suggest that excessive activation of CP-AMPA receptors might involved in impaired Glu transmission and Glu-dopamine interactions in schizophrenia and that CP-AMPA receptor blockers could be useful as novel therapeutic agents for this disorder.

P04.

D-Amino Acids in Type 1 Diabetes-Affected Human Sera

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A number of D-amino acids(D-AAs) are involved in novel cell-to-cell signaling functioning as neurotransmitters and neuromodulators and influencing behavior and physiology.[1] Intriguingly, the presence of these D-AAs, including D-serine and D-alanine,[2,3,4,5]have been suggested in rodent and human pancreatic islet beta-cells, of which the autoimmune-mediated destruction can cause C-peptide and insulin dysregulation and further lead to type 1 diabetes (T1D).[6,7,8] Since the alterations in serum metabolite profiles are correlated with islet autoimmunity, we ask whether D-AAs can be used as serum biomarkers to assess the diagnosis and prediction of T1D development. To answer this, we investigate correlations between human serum levels of D-AAs, C-peptide, and disease duration looking for chemical trends in T1D progression. Chiral liquid chromatography-tandem mass spectrometry (LC-MS/MS) is used in measurements of the D/L-alanine, serine, aspartate, and proline levels in T1D-affected human serum samples. The LC-MS/MS system has high sensitivity for these analytes in serum. Derivatized by modified Marfey's reagent, both D-and L-amino acid stereoisomers are detected and identified in a single measurement.[9] Results show positive correlations between the amounts of D-alanine, D-aspartate, and D-serine with C-peptide levels, suggesting dysregulation of D-AAs in T1D-affected serum. Additionally, our results demonstrate lower D-alanine percent ages and higher D-aspartate percentages in serum samples with no detectable C-peptide compared to samples exhibiting C-peptide levels >0.2 nmol/L. There is no significant difference in each D-AA amount as well as in D-AA percentage in relation to T1D duration. Though the correlation between disease duration and D-AA levels is not sufficient to allow D-AA levels alone to determine the progression of T1D, D-AAs, when referenced with C-peptide levels, correlate with T1D development. This work is supported by the American Diabetes Association grant #1-18-VSN-19.

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P06.

D-SERINE AVAILABILITY IS IMPORTANT FOR PROPER PREFRONTAL CORTICAL INTERNEURON DEVELOPMENT AND ADOLESCENT SOCIAL BEHAVIOR

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Alterations in the ratio of activity in the cortical excitatory and inhibitory (E/I) balance are thought to be central to the etiology of multiple neurodevelopmental disorders. Evidence suggests that disruptions in the prefrontal cortex (PFC) inhibitory neuron (CIN) activity play an essential role in the pathophysiology of psychiatric disorders with shared social deficits. For example, there is a reduction of a subset of GABAergic neurons that expresses the genetic marker, parvalbumin (PV+), in the PFC in post-mortem brains of subjects with schizophrenia. Any means to compensate or prevent the reduction in CIN activity may be a productive approach to relieve a deficit in social cognition. Although the social abnormalities in these orders are associated with altered cortical circuitry in adulthood, our understanding of the timing and mechanisms involved in normative CIN circuit maturation that subserve these behaviors are limited. Glutamate N-methyl-D-aspartate receptors (NMDARs) are unique compared to other glutamate receptors because, in addition to the binding of glutamate, NMDAR activation requires the binding of a co-agonist, either glycine or D-serine. D-serine is racemized from L-serine by the enzyme serine racemase (SR). We and others have shown that NMDAR hypofunction rodent models have social and cognitive deficits. However, an outstanding question is whether the onset of phenotypic changes has a developmental time course that reflects symptom onset. Furthermore, mice lacking D-serine due to genetic deletion of SR (SR knockout; SR-/-) display forebrain NMDAR hypofunction. We previously demonstrated that SR-/- mice had reduced GABA immunostaining embryonically and fewer PV+ CINs in the prelimbic cortex at postnatal day 16 (PND16). Here, we investigate how constitutive NMDAR hypofunction affects prelimbic cortex E/I balance at juvenile and adolescent timepoints. We further determine whether aberrant social behavioral phenotypes are present in adolescent SR-/- mice which could inform our understanding of maturational events in NMDAR signaling relevant to schizophrenia onset. At PND 16, there was a significant reduction in the inhibitory postsynaptic potentials (IPSP), but not the excitatory postsynaptic potentials (EPSP) component of the compound EPSP/IPSP, resulting in an increased E/I ratio in Layer 2/3 of the prelimbic cortex and enhanced pyramidal neuron's intrinsic excitability. At PND27-31, male SR-/- mice displayed a social novelty preference that differed from the deficit in adult mice in the three-chamber social interaction test, which engages the prefrontal circuitry. Adolescent female SR-/- mice showed a significant increase in social novelty preference compared to WT. These findings elucidate the neurodevelopment effect of NMDAR co-agonist D-serine required for channel opening on aspects of prefrontal cortex neural circuits development important for the social behaviors disturbed in psychiatric disorders.

P07.

D-Alanine in the Microbiome-Gut-Brain Axis: Utilizing Germ Free Mice to Decipher the Origin and Biodistribution of D-Alanine in Rodents

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The gut-microbiota-brain axis is a novel area of research in understanding neurological diseases. One focus within this area is the neuromodulators involved in the bi-directional communication between the host central nervous system and the gut microbiome. Since d-alanine has been linked to the gut microbiome, and is found within the brain, it is a suitable target to study as a potential neuromodulator in the gut-microbiota-brain axis. Here, utilizing germ-free mice, we aim to explore the origin and the biodistribution of d-alanine in rodents. We orally administer C56BL/6 germ-free mice with isotopic alanine to look at (1) the short (1-hour) and long (2-week) term biodistribution of gut-absorbed d-alanine in tissues without the influence of the gut microbiome, and (2) the racemization of l- to d-alanine with or without the microbiota. A group of germ-free mice were fed with D-alanine-$^{13}C_3$,^{15}N (I-D-Ala) for aim 1. A separate group of germ-free and regular age-matched mice were fed with L-alanine-2,3,3,3-d4 (I-L-Ala) for aim 2. Once sacrificed, the plasma, brain, pituitary, islets, acinar tissue, small intestine, colon, and the colon contents were collected. These tissues were then extracted for amino acids and reacted with a modified Marfey's reagent. The reacted samples were then eluted with an ultra-high pressure liquid chromatography (UHPLC) equipped with a phenol-hexyl column and analyzed with triple quadruple mass spectrometer using multiple reaction monitoring (MRM). The resulted chromatograms were processed using the MS Data Analysis software from Bruker. Our results showed that in both short- and long-term feeding, the gut-absorbed I-D-Ala in germ-free mice were distributed throughout the body, with the highest level in the plasma, pancreatic islets, and acinar tissues. The brain and pituitary showed low levels of I-D-Ala in short-term treatment, but significantly higher levels of I-D-Ala in the long-term group, demonstrating that the gut-absorbed I-D-Ala can pass the blood-brain barrier and accumulate over time. In the germ-free mice samples, racemization of I-L-Ala to D-enantiomer were not reliably detected while it was readily observed in regular mice possessing a normal microbiota. This suggests the lack of observable endogenous l-alanine racemization in mice during the evaluated time span with our analysis method.

P08.

Blockade of D-Serine Signaling and Adult Hippocampal Neurogenesis Attenuates Remote Contextual Fear Memory Following Multiple Memory Retrievals

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The mechanistical basis of exposure-based therapy for patients with post-traumatic stress disorder (PTSD) is the characteristic manifestation of re-experiencing trauma event in PTSD. Memory retrieval by re-exposure to trauma-associated events can trigger destabilization followed by reconsolidation for maintaining or enhancing original fear memory. Therefore, prevention or blockade of reconsolidation by manipulating the neurobiological factors at the molecular and cellular levels could weaken the original fear memories. The N-methyl-D-aspartate (NMDA) receptor and hippocampal neurogenesis play crucial roles in hippocampus-dependent memory processes, including reconsolidation. Using contextual fear conditioning paradigm with multiple retrievals, we attempted to weaken the original contextual fear memory by repeatedly disrupting retrieval-induced reconsolidation via downregulation of NMDA receptor signaling and inhibition of neurogenesis. In the first experiment, prior to fear conditioning, NMDA receptor signaling was downregulated by the genetic reduction of its co-agonist, D-serine, and the neurogenesis was ablated by focal X-ray irradiation on the hippocampus. We found that simultaneous D-serine reduction and neurogenesis inhibition resulted in a progressive decrease in freezing following each retrieval, leading to an attenuation of remote contextual fear memory on day 28. In the second experiment using the same behavioral protocols, after conditioning, pharmacological approaches were conducted to simultaneously block D-serine signaling and inhibit neurogenesis, resulting in a similar suppressive effect on the remote fear memory. The present findings provide insights into the role of D-serine-mediated NMDA receptor signaling and neurogenesis in memory retrieval and the maintenance of remote fear memory and provide a new strategy to improve exposure-based therapy for PDST treatment.

P09.

Simultaneous Determination of Chiral Amino Acids in the Plasma and Brain of DAO Deficient Rats Using a Highly Selective Two-Dimensional LC-MS/MS System

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Owing to the discovery of several D-amino acids in the physiological fluids and tissues of mammals, analytical methods have been improved to perform more sensitive, precise and comprehensive determination. However, the simultaneous analysis of various chiral amino acids by widely used liquid chromatographic methods has still difficulty due to the co-elution of uncountable intrinsic substances with target compounds. Not only the sensitivity but the selectivity of the methods should be improved to overcome this problem, and the multi-dimensional analysis would be one of the effective solutions. Therefore, in this study, a highly selective two-dimensional (2D) LC-MS/MS system consisting of reversed-phase and enantioselective separations and the detection of specific precursor/product ion pairs was designed. The developed system was applied to the chiral amino acid analysis in the plasma and brain of rats with D-amino acid oxidase (DAO) deficiency focusing on the alteration of D-amino acid levels. The plasma, cerebellum and cerebrum samples were deproteinized using 20-fold volumes of methanol. The supernatants were dried and amino acids were derivatized using 4-fluoro-7-nitro-2,1,3-benzoxadiazole. Isotope-labeled L-amino acids were used as internal standards. After the reaction, aliquots (20 µL) were subjected to the 2D LC-MS/MS system. Ala, Asp, Glu, Leu, Lys, Met, Phe, Pro, Ser and Val were selected for the target analytes, and the analytical conditions were investigated to develop a 2D LC-MS/MS system.

For the first dimension, all targets were isolated by an ODS column (Singularity RP18, 1.0 x 500 mm) using the gradient elution of aqueous 10-30% acetonitrile containing 0.05% trifluoroacetic acid within 270 min. For the second dimension, enantiomers were separated by Pirkle-type columns (Singularity CSP-001S and 011S, 1.5 x 150 mm) using the mixture of methanol/acetonitrile containing formic acid within about 20 min. The detection was performed by an ESI-MS/MS. Integrating these conditions, an automated 2D LC-MS/MS system was developed, and D-amino acids in the plasma and brain of control and DAO deficient rats were determined. As a result, all target analytes were successfully determined without severe interferences from other biological substances. In the DAO deficient rats, the amounts of D-Ala, Leu, Met, Pro and Ser in the plasma were higher than those of the control rats. In both cerebellum and cerebrum, the amounts of D-Ala, Leu and Met increased in the DAO deficient rats, while D-Ser increased only in the cerebellum. Further applications to various tissues are expected to clarify the distribution/alteration of trace levels of intrinsic D-amino acids.

P10.

Evidence of a Potential Role of an Increased Copy Number of the Gene Encoding Glycine-Decarboxylase (Gldc) in the Pathophysiolgy of Psychosis

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Genomic copy-number-variants (CNVs) have been implicated in etiology of schizophrenia (SCZ) and bipolar disorder. To determine the neurobiological relevance of a genomic duplication/triplication involving multiple genes including GLDC on chromosome 9p24.1, which has been detected in two patients, we developed and studied mice with 4 copies of the 9p24.1 region and mice with 3 or 4 copies of Gldc only. We hypothesized that an increased copy number of Gldc would lead to increased degradation of glycine, an NMDA receptor co-agonist, and thus to NMDA receptor hypofunction, which may contribute to the manifestations of schizophrenia. Performing qRT-PCR, Western blot, immunofluorescence and behavioral studies, we found an increased expression of GLDC, which was detected in astrocytes but not in neurons, an increased activity of the glycine cleavage system, a decreased expression of BDNF and a reduced activation of BNDF-dependent synaptic-plasticity-related pathways, e.g., Akt-mTOR-CREB, and increased expression of miR-137, which has been linked to SCZ by GWAS. The mice displayed deficits in startle habituation, latent inhibition, working memory and social interaction and preference. Our results provide evidence that an increase in copy number of the Gldc gene alone induces molecular alterations and behavioral changes that may contribute to the pathophysiology of SCZ.

P11.

Incorporation of Heterologous Reductase to Generate D-Alanine on Class V Lanthipeptide Cacaoidin

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Lanthipeptides are ribosomally synthesized and post-translationally modified peptides characterized by lanthionine (Lan) and/or methyllanthionine (MeLan) residues1. Recently, a novel lanthipeptide cacaoidin that displays potent antimicrobial activity against Gram-positive bacteria was discovered2. Cacaoidin carries multiple unusual structural features, including four D-alanine (D-Ala) and one D-butyrine (D-Abu) that are believed to contribute to peptide stability and bioactivity3. These D-amino acids are formed through dehydration of original L-amino acids, followed by reduction. In this study, other than F420-dependent reductase CaoJC from cacaoidin biosynthetic gene cluster, the heterologous NADPH-dependent reductase NpnJA was introduced into cacaoidin biosynthetic pathway to generate D-amino acids in cooperation with dehydratases complex4. MALDI-TOF MS demonstrated that 4 reductions were catalyzed on the fully dehydrated peptide by NpnJA both in vitro and in vivo. Tandem MS revealed the existence of 4 D-Ala on the resultant product, and the reduction pattern was consistent with wild type cacaoidin.

P15.

Chiral Liquid Chromatography-Mass Spectrometry Method for Measuring D-Amino Acids in Biological Samples

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D-amino acids are important biomolecules that are involved in neurotransmission. They have been detected in various tissues, including the brain and the endocrine portion of the pancreas, the islets of Langerhans. Of particular interest are D-serine, D-alanine, and D-aspartate, since they are co-agonists for the ionotropic N-methyl-D-aspartate receptors (NMDARs) found in islets and there are strong suggestions that these D-amino acids may have autocrine or paracrine signaling functions. To understand the roles of these D-amino acids, we set out to examine the secretions from islets induced by glucose using a chiral liquid chromatography-tandem mass spectrometry (LC-MS/MS) method capable of resolving 13 common L- and D- amino acids. Analytes were derivatized with a chiral derivatizing agent, Marfey's reagent and the resulting diastereomers were separated on a reversed-phase 2.1 x 30 mm column with 2.7 µm superficially porous C18 particles. Through the optimization of separation conditions, all enantiomers were baseline resolved (Rs > 1.5) using a standard acidic mobile phase system consisting of 0.1% formic acid / 10mM ammonium formate in water and 0.1% formic acid in acetonitrile. Derivatization rates between amino acids varied, with some needing more than 6 hours for complete derivatization. With the optimized conditions, limits of detection (LODs) for most compounds were less than 100 nM. The applicability of the method was demonstrated by examining the D-amino acids released from human and murine islets of Langerhans upon glucose stimulation. The method was further applied to brain tissues and secretions to demonstrate the ability of the method to detect D-amino acids in complex matrices.

P16.

Unusual Neuropeptide Modification in The Nervous System of Mammals; Investigating Potential Isomers of Galanin

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The limited genetic sequences encode for twenty amino acids; this chemical repertoire is expanded by numerous post translational modifications, resulting in a highly complexed proteome used to carryout cellular activities. Isomerization of one or more amino acids from L to D and the conversion of aspartate to iso-aspartate residues can change the physiological properties of a peptide but do not result in a mass change. Current mass proteomic approaches cannot easily detect these post translational modifications. The incorporation of ion mobility into mass spectrometry have allowed isomeric peptides with similar chromatographic properties to be distinguished. Here, we employed a suite of enzymatic assays and a four-dimensional separation technique that combines ion mobility with liquid chromatography and quadrupole time of flight to discover D-amino acid and iso-aspartate containing peptides in the central nervous system of mammals. Specifically, we describe the occurrence of a possible isomeric form of galanin, an important neuroendocrine peptide that has been implicated in feeding, sleep, wakefulness, memory etc.

P18.

Identifying Sources of D-Serine in *Caenorhabditis elegans* and Their Impact on Behavior

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Free D-serine (D-Ser) is a potent co-agonist of the N-methyl-D-aspartate receptor (NMDAR) in glutamate neurotransmission and regulates NMDAR functions in the nervous system. Serine racemases convert L-serine to D-Ser and are believed to be the major source of D-Ser in animals. In *Caenorhabditis elegans*, a knockout of the serine racemase gene *serr-1* results in behavioral changes, but the level of D-Ser is unaffected. By growing *C. elegans* on peptone-free nematode growth medium (PF-NGM), we delineated the sources of D-Ser, both exogenous from peptone in the culturing media and endogenous from the serine racemase gene *serr-1*, and a potential serine/aspartate racemase, Y51H7C.9, identified by sequence similarity network (SSN) analysis. We also discovered a new serine dehydratase (*aka* serine ammonia-lyase), K01C8.1 (now the gene renamed as *srdh-1*), in *C. elegans*. We identified the *serr-1* knockout and PF-NGM culturing conditions as two independent factors that impact *C. elegans* locomotion behavior after off-food, both short-term and long-term, and no interactions were found between the two factors. The *serr-1* knockout showed impact on worm swimming speed, wave initiation rate, and curling in swimming mode and short-term and reversal time and distance in crawling mode and long term. Interestingly, supplement of exogenous D-Ser to the *serr-1* mutant can rescue the behavioral change in reversal time and distance in crawling, but not wave initiation rate and curling in swimming, indicating the different regulatory roles of endogenous and exogenous D-Ser in *C. elegans* locomotive behavior.

P21.

Effects of Pathological Mutations in Human PHGDH

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In the human brain, 3-phosphoglycerate dehydrogenase (hPHGDH, EC 1.1.1.95) catalyzes the first step of the "phosphorylated pathway" (PP), a short, cytosolic pathway that in astrocytes leads to the de novo synthesis of L-serine (L-Ser) by re-routing the metabolic fate of the glycolytic intermediate 3-phosphoglycerate [1]. L-Ser is a non-essential amino acid crucial for the cellular metabolism and for neurotransmission [2], being the primary source of D-serine (D-Ser) and glycine, the two co-agonists of N-methyl-D-aspartate (NMDA) receptors. Considering its relevance, a deeper understanding of pathological defects in hPHGDH function would allow to gain further insight into the mechanisms affecting L-Ser and, consequently, D-Ser synthesis. For this purpose, the effects of three missense mutations in the human PHGDH gene, discovered in the context of a rare neurometabolic disorder named 3-PHGDH deficiency [3], were investigated. The recombinant hPHGDH variants carrying the V261M substitution in the nucleotide binding domain and the V425M and V490M substitutions in the carboxy-terminal regulatory domain were characterized. For all hPHGDH variants, kinetic analyses revealed a lower catalytic efficiency in both the forward and the reverse reactions and a reduced affinity for NADH cofactor compared to the wild-type enzyme. Moreover, structural studies indicated differences in the tertiary and quaternary structure. The subcellular localization and the changes in the expression levels of the PP enzymes and in the L-Ser cellular content have been investigated in U251 human astrocytoma cells ectopically expressing the V261M, V425M and V490M hPHGDH variants. The properties of the pathological variants of PHGDH are providing relevant information to develop new approaches aimed to treat neurological disorders acting on the modulation of brain L-Ser and D-Ser levels.This project was founded by" PRIN-2017 - Dissecting serine metabolism in the brain".

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