INRC 2025
International Narcotics Research Conference

July 8th – 11th, 2025 | Bologna, Italy

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Conference Handbook

VENUES

Belmeloro District, University of Bologna, Via B. Andreatta 8, Bologna
Palazzo Re Enzo, Piazza del Nettuno 1/C, Bologna
Palazzo De' Toschi, Piazza Minghetti 4/D, Bologna

WEBSITE

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DEPARTMENT OF CHEMISTRY "GIACOMO CIAMICIAN"

Activity funded with the contribution of the Department of Excellence program financed by the Minister of University and Research 2023-2027 (L. 232, 01/12/2016)

NIH National Institute on Drug Abuse

ORGANIZING SECRETARIAT

FONDAZIONE ALMA MATER

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INRC2025 Speakers

INRC2025 Plenary Speaker

Prof. Marzia Malcangio

Wolfson Sensory, Pain and Regeneration Centre,
King's College London

Marzia Malcangio is Professor of Neuropharmacology at King's College London where she has established an internationally renowned laboratory devoted to the study of the positive and negative modulation of pain transmission with particular emphasis on chronic pain. She has published more than 100 papers on pain and edited a book on Synaptic plasticity in Pain. Her current work explores novel approaches to target neuropathic and arthritic pain unveiling the involvement of microglia in the CNS and monocyte/ macrophages in the periphery and the mechanisms governing immune- neuronal cell communication.

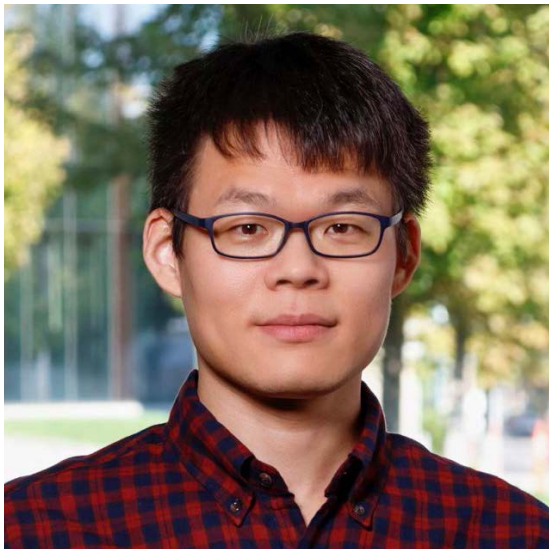
INRC2025 Founder's Lecture Awardee

Prof. Lee-Yuan Liu-Chen

Department of Neural Sciences, Lewis Katz School of Medicine,
Temple University, Philadelphia, PA (USA)

Lee-Yuan Liu-Chen obtained her BS degree in Pharmacy from National Taiwan University and her PhD degree in Neural and Endocrine Regulation from Massachusetts Institute of Technology. After postdoctoral training in Harvard Medical School, she worked for DuPont Pharmaceutical Co, where she started working on opioid receptors. She then established her own lab in Temple University School of Medicine. Her lab has been active in opioid receptor research for over 30 years. Her group was among the first to clone rat and human kappa opioid receptors (KORs). She and co-workers then elucidate the mechanisms of agonist-promoted KOR signaling, desensitization, internalization and downregulation as well as non-G protein interactions of the KOR, including GEC1 and NHERF, and the functional significance of the interactions. Her lab contributed to illustrate the structure of the MOR by demonstrating the covalent binding site of β-FNA bound to be Lys233(5.39) and the interaction of that the DRY motif in the TM3 with the i3 loop before X-ray crystal structure of the MOR was solved. In recent years, her lab has expanded into in vivo KOR pharmacological studies of agonists in rodents, including behavioral studies and investigations of KOR phosphorylation, and internalization and signaling and relationship between behavior and biochemical changes in brains. They demonstrated differences between nalfurafine, a clinically used KOR agonist, and U50,488, a prototypic KOR agonist, in rodent behaviors and brain signaling by phosphoproteomics study, contributing to understanding of biochemical basis of KOR agonist-induced side effects. In recent years, using two custom-generated KOR-tdTomato and KOR-iCre mouse lines, they are studying neuroanatomy and functional significance of KOR-related circuitries. The recent focus is on the paraventricular nucleus of thalamus (PVT), illustrating the importance of KOR in the PVT in affective behaviors.

INRC2025 Young Investigator Awardee



Prof. Tao Che

Center for Clinical Pharmacology, Department of Anesthesiology, Washington University in St. Louis, MO (USA)

Dr. Tao Che obtained his Bachelor's and Master's degrees in Science at Wuhan University in China between 2003 and 2010. He then pursued his Ph.D. under the mentorship of Dr. Paul Carey in Biochemistry at Case Western Reserve University from 2010 to 2014. Following this, Dr. Che conducted postdoctoral training in Dr. Bryan Roth's laboratory within the Department of Pharmacology at the University of North Carolina-Chapel Hill from 2015 to 2020. In March 2020, Dr. Che established his own laboratory in the Center for Clinical Pharmacology within the Department of Anesthesiology at Washington University in St. Louis. His research integrates structural and pharmacological methodologies to elucidate the molecular mechanisms governing opioid receptor signaling. The ultimate goal of his research is to dissect these mechanisms and explore their correlations with specific in vivo behavioral responses, paving the road for designing new generation of opioids

Program at a glance

July 9, 2025 | Belmeloro District		
8.30-9.30	**PLENARY LECTURE - Neuroimmune mechanisms in chronic pain** **Prof. Marzia Malcangio** (Wolfson Sensory, Pain and Regeneration Centre, King's College London)	
	ROOM A	**ROOM C**
9.30-11	**SYMPOSIUM 1 - Cortical opioid signaling regulates adaptive and maladaptive behaviors** (Chair: Max E Joffe. Speakers: Hugo Tejeda, Jacob Reeves, Rebecca Cole, Bruno Munoz)	**SYMPOSIUM 2 - AI-enabled drug discovery targeting opioid receptors** (Chair: Tao Che. Speakers: Tao Che, Marta Filizola, Michael Robertson, Christian Gruber W)
11-11.15	**COFFEE BREAK**	
11.15-12.30	**SYMPOSIUM 3 - From Neural Pathways to Molecular Insights: Advancing Treatments for Opioid Use Disorders** (Chair: Esi Domi. Speakers: Stephen Husbands, Leandro Vendruscolo, Roberto Ciccocioppo)	**SYMPOSIUM 4 - Uncovering GPR-Family G Protein Coupled Receptors as Novel Pain Regulators** (Chair: John Streicher. Speakers: Amanda Fakira, Erin Bobeck, John Streicher)
12.30-13.30	**CLINICAL SESSION ON THE USE OF OPIOID ANALGESICS - ROOM A** Chairs: Patrizia Romualdi, Daniele Caprioli. Talks & Speakers: "Treatment of fentanyl use disorder", Sebastiano Mercadante; "The five Ws of opioid prescribing in cancer pain management", Flaminia Coluzzi; "Taking care of chronic noncancer pain in Italy: how we manage opioids", Silvia Natoli.	
13.30-14.30	**LUNCH BREAK**	
	ROOM A	**ROOM C**
14.30-16	**SYMPOSIUM 5 - Exploring Roles of Habenula in Opioid Signaling and Behaviors** (Chair: Emmanuel Darcq. Speakers: Paul Kenny, Elyssa Margolis, Tom Hnasko, Dersu Odzemir. Hot Topic Speaker: Chiara Ebner)	**SYMPOSIUM 6 - Chemistry and Pharmacology of opioid receptor modulators** (Chairs: Meritxell Canals and Susruta Majumdar. Speakers: Rob Lane, Marthe Vandeputte, Amynah Pradhan, Bronwyn Kivell. Hot Topic Speaker: Mariana Spetea)
16-16.30	**DATA BLITZ SESSION A - ROOM A** (Chairs: Lucia Hipolito and Livio Luongo)	
16.30 - 16.45	**COFFEE BREAK**	
16.45-18.30	**POSTER SESSION A - First Floor**	

July 10, 2025 | Belmeloro District		
8.30-9.30	**FOUNDER'S LECTURE - My journey through the opioid field: Opioid research as a destiny?** **Prof. Lee-Yuan Liu-Chen** (Center for Substance Abuse Research & Department of Neural Sciences Temple University)	
	ROOM A	**ROOM C**
9.30-11	**SYMPOSIUM 7- Dynorphin-expressing accumbal neurons promote maladaptive behaviors and reduce reward** (Chairs: Ethan Anderson and Nicolas Massaly. Speakers: Catherine Marcinkiewicz, Ethan Anderson, Ream Al-Hasani, Nicolas Massaly)	**SYMPOSIUM 8 - N/OFQ receptor signaling at different scales: from molecular pathways to pain and stress-related behavior via brain networks** (Chairs: Lionel Mouledous and Chiara Ruzza. Speakers: Davide Malfacini, Lionel Mouledous, Chiara Ruzza. Hot Topic Speaker: Akanksha Mudgal)
11-11.30	**COFFEE BREAK**	
11.30-13	**SYMPOSIUM 9 - From Pain to Addiction and Back: Unraveling Psychological and Neural Connections in OUD and AUD** (Chair: Jesùs Lorente. Speakers: Jesùs Lorente, Jessica Cucinello-Ragland, Anushree Karkhanis, Hannah Harder. Hot Topic Speaker: Aya Osman)	**SYMPOSIUM 10 - The Opioid-Nociceptin Framework: Revealing Mechanisms of Neurotrauma** (Chairs: Kelly Standifer and Georgy Bakalkin. Speakers: Kelly Standifer, Brian Cox, Georgy Bakalkin. Hot Topic Speaker: Cathaline Robert)
13-14.30	**LUNCH BREAK**	
	ROOM A	**ROOM C**
14.30-16	**SYMPOSIUM 11 - Circuits, cell types, and neural dynamics underlying opioidergic neuromodulation** (Chairs: Gregory Scherrer and Michael Bruchas. Speakers: Catalina Zamorano, Hector Yarur, Gregory Scherrer. Hot Topic Speaker: Callie M Newson)	**SYMPOSIUM 12 - Opioids Beyond Pain-Roles in Mood and Motivation** (Chairs: Kathryn Braden and Anne Z Murphy. Speakers: Anne Z Murphy, Kathryn Braden, Blake Kimmey, Marie Eikemo)
16-16.30	**DATA BLITZ SESSION B - ROOM A** (Chairs Lucia Hipolito and Paola Sacerdote)	
16.30 - 16.45	**COFFEE BREAK**	
16.45-18.30	**POSTER SESSION B - First Floor**	

July 11, 2025 | Belmeloro District		
8.30-9.30	**YOUNG INVESTIGATOR AWARD LECTURE - Exploring the kappa opioid receptor signaling for safer analgesics** **Prof. Tao Che** (Center for Clinical Pharmacology Department of Anesthesiology Washington University in St. Louis)	
	ROOM 1	**ROOM 2**
9.30-11	**SYMPOSIUM 13 - The Endogenous Opioid System and Chronic Pain: Recent Advances and Therapeutic Potential of Opioid Peptides** (Chair: Bruehl Stephen. Speakers: Stephen Bruehl, Lin Tian, Jose Moron Concepcion, Azzurra Stefanucci)	**SYMPOSIUM 14 - Prenatal opioid exposure and its molecular, cellular, and behavioral outcomes** (Chairs: Julia Ferrante and Brady Atwood. Speakers: Julia Ferrante, Davian West, Anne Z Murphy, Teresa M Reyes)
11-11.30	**COFFEE BREAK**	
11.30-13	**SYMPOSIUM 15 - Novel insights into fentanyl's diverse effects across central and peripheral systems** (Chairs: Kasey Girven and Daniel Castro. Speakers: Krystal Flores-Felix, Megan Fox, Kasey Girven, Daniel Castro)	**SYMPOSIUM 16 - Sex-specific mechanisms underlying opioid reward, motivation, and pain-related outcomes** (Chair: Jessica Higginbotham. Speakers: Emilia Lefebre, Yanaira Alonso-Caraballo, Tiffany Wills, Jessica Higginbotham)
13-14.30	**LUNCH BREAK**	
	ROOM 1	**ROOM 2**
14.30-16	**SYMPOSIUM 17 - Novel Optical and Pharmacological Strategies to Probe the Opioid System In-vivo** (Chair: Luca Posa. Speakers: Luca Posa, Raaj Gowrishankar, Miriam Stoeber, Joshua Levitz)	**SYMPOSIUM 18 - Exploring the Interplay of Opioids, Cannabinoids, and Neuroimmune Mechanisms in Pain** (Chair: John K Neubert. Speakers: Robert M Caudle, Michael J Iadarola, Jay McLaughlin, Niall P Murphy)
16-16.30	**HOT TOPIC SESSION 1 - ROOM A** Chairs: Roberto Ciccocioppo, Laura Rullo Speakers : Evan S O'Brien, Michele Maritan, Bronwyn Kivell	**HOT TOPIC SESSION 2 - ROOM C** Chiars: Girolamo Calò, Monica Baiula Speakers: Sabrine Billel, Yusuke Hamada, Roozbe Bonsale
16.30-17.15	**BUSINESS MEETING - ROOM A**	
17.15-17.30	**END OF THE MEETING - ROOM A**	

Detailed program

July 8, 2025 • Palazzo Re Enzo

4.30 PM — PROFESSIONAL DEVELOPMENT SESSION
Thematic discussion groups where trainees may interact with a couple of more senior facilitators to ask questions and share experiences in a more direct and informal way.
Topics: challenges in starting your own lab; grant writing/paper writing; pros and cons of academic career; work/family balance.
Facilitators: Ream Al-Hasani, Monica Baiula, Andrea Bedini, Michael Bruchas, Lucia Hipolito, Nicolas Massaly, Jordan Mc Call, Amynah Pradhan, John Streicher

6 PM — **Historical perspective on "INRC and Opioid Research"**
Prof. Brian Cox, *Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, United States*

7 PM — Welcome Reception

July 9, 2025 • Belmeloro District

8.15 AM — Welcome and Opening Remarks • Room A
Prof. Susan Ingram, *INRC President*

8.30 AM — PLENARY LECTURE • Room A
Neuroimmune mechanisms in chronic pain
Prof. Marzia Malcangio, *Wolfson Sensory, Pain and Regeneration Centre, King's College London*

9.30 AM — MORNING PARALLEL SESSIONS
Symposium 1 • Room A
Cortical opioid signaling regulates adaptive and maladaptive behaviors
Chair: Max E Joffe
Speakers: Hugo Tejeda, Jacob Reeves, Rebecca Cole, Bruno Munoz

Symposium 2 • Room C
AI-enabled drug discovery targeting opioid receptors
Chair: Tao Che
Speakers: Tao Che, Marta Filizola, Ajith Karunarathne, Christian Gruber W

11 AM — Coffee break

11.15 AM — **Symposium 3** • Room A
From Neural Pathways to Molecular Insights: Advancing Treatments for Opioid Use Disorders
Chair: Esi Domi
Speakers: Stephen Husbands, Leandro Vendruscolo, Roberto Ciccocioppo

Symposium 4 • Room C
Uncovering GPR-Family G Protein Coupled Receptors as Novel Pain Regulators
Chair: John Streicher
Speakers: Amanda Fakira, Erin Bobeck, John Streicher

12.30 PM — CLINICAL SESSION ON THE USE OF OPIOID ANALGESICS
• Room A
Chairs: Patrizia Romualdi and Daniele Caprioli
Talks & Speakers:
Treatment of fentanyl use disorder, Sebastiano Mercadante
The five Ws of opioid prescribing in cancer pain management, Flaminia Coluzzi
Taking care of chronic noncancer pain in Italy: how we manage opioids, Silvia Natoli

1.30 PM — Lunch break

2.30 PM AFTERNOON PARALLEL SESSIONS

Symposium 5 • Room A

Exploring Roles of Habenula in Opioid Signaling and Behaviors

Chair: Emmanuel Darcq

Speakers: Paul Kenny, Elyssa Margolis, Tom Hnasko, Dersu Odzemir

Hot Topic Speaker: Chiara Ebner

Symposium 6 • Room C

Chemistry and Pharmacology of opioid receptor modulators

Chairs: Meritxell Canals and Susruta Majumdar

Speakers: Rob Lane, Marthe Vandeputte, Amynah Pradhan, Bronwyn Kivell

Hot Topic Speaker: Mariana Spetea

4 PM DATA BLITZ SESSION A • Room A

Chairs: Lucia Hipolito and Livio Luongo

4.30 PM Coffee break

4.45 PM POSTER SESSION A • First Floor

July 10, 2025 · Belmeloro District

8.30 AM FOUNDER'S LECTURE • Room A

My journey through the opioid field: Opioid research as a destiny?

Prof. Lee-Yuan Liu-Chen, *Center for Substance Abuse Research & Department of Neural Sciences, Temple University*

9.30 AM MORNING PARALLEL SESSIONS

Symposium 7 • Room A

Dynorphin-expressing accumbal neurons promote maladaptive behaviors and reduce reward

Chairs: Ethan Anderson and Nicolas Massaly

Speakers: Catherine Marcinkiewicz, Ethan Anderson, Ream Al-Hasani, Nicolas Massaly

Symposium 8 • Room C

N/OFQ receptor signaling at different scales: from molecular pathways to pain and stress-related behavior via brain networks

Chairs: Lionel Mouledous and Chiara Ruzza

Speakers: Davide Malfacini, Lionel Mouledous, Chiara Ruzza

Hot Topic Speaker: Akanksha Mudgal

11 AM Coffee break

11.30 AM **Symposium 9** • Room A

From Pain to Addiction and Back: Unraveling Psychological and Neural Connections in OUD and AUD

Chair: Jesùs Lorente

Speakers: Jesùs Lorente, Jessica Cucinello-Ragland, Anushree Karkhanis, Hannah Harder

Hot Topic Speaker: Aya Osman

Symposium 10 • Room C

The Opioid-Nociceptin Framework: Revealing Mechanisms of Neurotrauma

Chairs: Kelly Standifer and Georgy Bakalkin

Speakers: Kelly Standifer, Brian Cox, Georgy Bakalkin

Hot Topic Speaker: Cathaline Robert

1 PM Lunch break

2.30 PM AFTERNOON PARALLEL SESSIONS

Symposium 11 • Room A

Circuits, cell types, and neural dynamics underlying opioidergic neuromodulation

Chairs: Gregory Scherrer and Michael Bruchas

Speakers: Catalina Zamorano, Hector Yarur, Gregory Scherrer

Hot Topic Speaker: Callie M Newson

Symposium 12 • Room C

Opioids Beyond Pain- Roles in Mood and Motivation

Chairs: Kathryn Braden and Anne Z Murphy

Speakers: Anne Z Murphy, Kathryn Braden, Blake Kimmey, Marie Eikemo

4 PM DATA BLITZ SESSION B • Room A

Chairs: Lucia Hipolito and Paola Sacerdote

4.30 PM Coffee break

4.45 PM POSTER SESSION B • First Floor

July 11, 2025 • Belmeloro District

8.30 AM YOUNG INVESTIGATOR AWARD LECTURE • Room A

Exploring the kappa opioid receptor signaling for safer analgesics

Prof. Tao Che, *Center for Clinical Pharmacology Department of Anesthesiology Washington University in St. Louis*

MORNING PARALLEL SESSIONS

9.30 AM **Symposium 13** • Room A

The Endogenous Opioid System and Chronic Pain: Recent Advances and Therapeutic Potential of Opioid Peptides

Chair: Bruehl Stephen

Speakers: Stephen Bruehl, Lin Tian, Jose Moron Concepcion, Azzurra Stefanucci

Symposium 14 • Room C

Prenatal opioid exposure and its molecular, cellular, and behavioral outcomes

Chairs: Julia Ferrante and Brady Atwood

Speakers: Julia Ferrante, Devian West, Anne Z Murphy, Teresa Reyes

11 AM Coffee break

11.30 AM **Symposium 15** • Room A

Novel insights into fentanyl's diverse effects across central and peripheral systems

Chairs: Kasey Girven and Daniel Castro

Speakers: Krystal Flores-Felix, Megan Fox, Kasey Girven, Daniel Castro

Symposium 16 • Room C

Sex-specific mechanisms underlying opioid reward, motivation, and pain-related outcomes

Chair: Jessica Higginbotham

Speakers: Emilia Lefebre, Yanaira Alonso-Caraballo, Tiffany Wills, Jessica Higginbotham

1 PM Lunch break

2.30 AFTERNOON PARALLEL SESSIONS

Symposium 17 • Room A

Novel Optical and Pharmacological Strategies to Probe the Opioid System In-vivo

Chair: Luca Posa

Speakers: Luca Posa, Raaj Gowrishankar, Miriam Stoeber, Joshua Levitz

Symposium 18 • Room C
Exploring the Interplay of Opioids, Cannabinoids, and Neuroimmune Mechanisms in Pain
Chair: John K Neubert
Speakers: Robert M Caudle, Michael J Iadarola, Jay McLaughlin, Niall P Murphy

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4 PM HOT TOPIC PARALLEL SESSIONS

Hot Topic Session 1 • Room A
Chair: Roberto Ciccocioppo, Laura Rullo
Speakers: Evan S O'Brien, Michele Maritan, Bronwyn Kivell

Hot Topic Session 2 • Room C
Chairs: Girolamo Calò, Monica Baiula
Speakers: Sabrine Billel, Yusuke Hamada, Roozbe Bonsale

4.30 PM Business meeting • Room A

5.15 PM End of the meeting • Room A

8 PM Gala dinner • Palazzo de' Toschi

List of Posters – Session A

A1 – The Efficacy of PPL-138 to Reduce AUD-like and PTSD-like Symptoms in Female and Male Rats
Kylie Kealoha, Ali Idriss, Lawrence Toll, Andrea Cippitelli Yong Zhang, Panini Patankar, Kelly Standifer, Benjamin Carper

A2 – Adolescent social isolation increases stress and alcohol vulnerability via opioid gene espression changes in a sex-dependent manner
Loredana Maria Losapio, Adana Keshishian, Laura Rullo, Sofia Vellere, Massimo Ubaldi, Laura Soverchia, Sanzio Candeletti, Roberto Ciccocioppo, Patrizia Romualdi, Esi Domi.

A3 – Exploring the opioid system role on sex- and age-dependent effects of social isolation on binge-like alcohol drinking and decision-making in rats.
María Ros-Ramírez, Jesús David Lorente, Miguel Ángel Serrano Rosa, Ana Polache, Lucía Hipólito

A4 – Antidotal Action of Metal-Organic Frameworks (MOFs) on Opioid and Synthetic Cathinone Toxicity in Zebrafish: Toward Innovative Strategies for Substance Use Disorders
Olga Wronikowska-Denysiuk, Barbara Budzyńska, Anna Boguszewska-Czubara, Weronika Mrozek, Kornelia Hyjek, Grzegorz Kurowski, Przemysław J. Jodłowski

A5 – Pre-clinical characterization of heroin and oxycodone virus like particle nanovaccines in Sprague-Dawley rats
Davide Tronconi, Courtney Marecki, Bryan Hannon, Caroline M Kim, Fatima A Hamid, and Marco Pravetoni

A6 – Dopamine Dynamics And Genomic Correlates Of Addictive-Like Behaviors In An Opto-Intracranial Self-Stimulation Mouse Model
Esther Colantonio, Sarah Kada, Yahia Hadj-Arab, Victor Mathis, Judith Meyer, Mathieu Bruggeman, Emmanuel Darcq, Pierre-Eric Lutz

A7 – Dissecting Neural Mechanisms of Volitional Drug Seeking in Mice
Hansol Lim

A8 – Effects of Maternal Pain and Perinatal Opioid Exposure on Intravenous Oxycodone Self-Administration in Male and Female Rats
Hannah Harder, Jose Moron-Concepcion

A9 – Cebranopadol: A Novel Nop/Mop Dual Agonist To Treat Opioid Use Disorder
Manthoula Olga Kyratzi, Sofia Christina Gkolfinopoulou, Caitlin Crook, Laura Soverchia Chiara Cappelletti, Esi Domi, Massimo Ubaldi, Roberto Ciccocioppo.

A10 – Exploring the role of AMPAR auxiliary protein CNIH3 in opioid-seeking behavior and contributing risk factors using mouse models
Tania Lintz, Alex Liu, Talal Abdel Aal, Ashley Park, Jose A. Moron

A11 – Enhanced naloxone-induced conditioned place aversion following high-dose oxycodone in Zhx2 knockout mice
Sophia A. Miracle, Ava Glavine, Sophia Pavlidis, Kaylie R. Kaneshiro, Isabella C. Conti, Morgan, L. Hofmeyer, William B. Lynch, Camron D. Bryant

A12 – Regulation of the Opioid System by Ketamine and its Therapeutic Potential in Opioid Use Disorder
Aya Osman, Adam Dawoud, Achla Gupta, Ivone Gomes, Yasmin L. Hurd and Lakshmi A. Devi

A13 – Heroin preference over social interaction: the role of GABAergic transmission in the medial amygdala
Maria Chiara Ruano, Ginevra D'Ottavio, Sara Pezza, Soami Filippo Zenoni, Jacopo Modoni, Sofia Taddini, Fabio Fumagalli, Lucia Caffino, Francesca Mottarlini, Daniele Caprioli

A14 – Biased kappa opioid receptor agonist nalfurafine attenuates oxycodone seeking and craving in rats.
Wojciech Solecki, Barbara Jędrzejewska, Anna Ciejka, Anna Nalepa, Aleksandra Kaczmarska, Jakub Bilnicki, Ryszard Przewłocki

A15 – Preoperative Opioid Misuse Associations with Pain, Opioid Use, and Negative Affect after Abdominal Surgery
Jennifer M. Hah, Shana C. Levine, Saneel Khairnar, Luke Pirrotta, Gabrielle Hettie, Tina Hernandez-Boussard

A16 – Nicotine exposure via electronic cigarettes enhances Bdnf/TrkB transcription, dynorphin and OLIG2 levels specifically in the rat VTA
Camilla Morosini, Laura Rullo, Loredana Maria Losapio, Fabio Vivarelli, Moreno Paolini, Lucy C Fairclough, Donatella Canistro, Sanzio Candeletti, Patrizia Romualdi

A17 – Fentanyl and xylazine interactions result in the altered action potential firing rates in dopamine type 2 receptor-expressing striatal medium spiny neurons
Viktor Yarotskyy, Pamela E. Knapp, Kurt F. Hauser

A18 – Xylazine amplifies behavioral and cardio-respiratory impairments induced by fentanyl in mice
Bilel Sabrine, Corli Giorgia, Nicole Cocita, Bassi Marta, Marti Matteo.

A19 – Structural elucidation of a new nitazene-type synthetic opioid
Gyles Cozier, Alexander Simmons, Matthew Gardner, Peter Sunderland, Christopher R Pudney, Stephen M Husbands

A20 – Reversing poly-drug overdoses in a rat model
Aniah V. Matthews, Adriana Gregory-Flores, Leandro F. Vendruscolo, Nora D. Volkow, George F. Koob, Renata C.N. Marchette

A21 – Investigating the Respiratory Consequences of Fentanyl and Xylazine Poly-Drug Administration and the Rescue by Antagonists in CD1 Mice Using Whole-Body Plethysmography
Okubadejo R, Hart E, Cavallo D, Kelly E, Henderson G, Abdala Sheikh AP

A22 – Xylazine exhibits time-dependent conditioned-rewarding effects in a conditioned-place assay in Swiss Webster mice
Brandon Shepherd, Kerim Cakir, Ellen Walker

A23 – Nitazenes of the past, present, and future: Insights from in vitro μ-opioid receptor assays and in vivo behavioral studies in male C57BL/6J mice
Marthe M. Vandeputte, Grant C. Glatfelter, Donna Walther, Nathan K. Layle, Danielle M. St. Germaine, István Ujváry, Donna M. Iula, Michael H. Baumann, and Christophe P. Stove

A24 – Structure guided efficacy modulation to design opioid sparing analgesics
Barnali Paul, Peng Huang, Nokomis Ramos-Gonzalez, Junzheng Wang, Emily Wells, Haylee R. Hammond, Bo W Sortman, Parthasaradhireddy Tanguturi, Arghya Polley, Balazs R. Varga, Kevin Appourchaux, Soumen Chakraborty, Jakob Parker, Tori Taylor, Matthew Welch, Antonio Alves De Souza, Qianru Jiang, Andras Varadi, Rajendra Uprety, Shainnel O'Eans, Ryosuke Shinouchi, Sabrina Masood, Caleb A Seekins, Chao-Cheng Kuo, Jordan G McCall, Michael D. Cameron, Tao Che, Brandon L Warren, John M Streicher, Brian K Kobilka, Chunlai Chen, Jay P. McLaughlin, Evan O'Brien, Susruta Majumdar

A25 – Looking for NOP receptor biased agonists: a SAR study on N/OFQ(1-13)-NH2
Erika Morrone, Alessandra Rizzo, Erika Marzola, Giulio Meneguzzo, Davide Malfacini, Remo Guerrini, and Girolamo Calo'.

A26 – In vitro pharmacological characterization of novel NOP partial agonists
Michele Maritan, Alessia Frezza, Pietro Pola, Chiara Ruzza, Davide Malfacini, Arpit Doshi, Dennis Yasuda, Nurulain T. Zaveri, Girolamo Calo

A27 – In vitro pharmacological characterization of innovative opioid ligands with distinct binding profiles and improved pharmacological activity
Andrea Bedini, Marco Francescato, Luca Gentilucci

A28 – The Positive Allosteric Modulator BMS-122 Enhances the Anti-Allodynic Action of Opioid and Non-Opioid Analgesics in Rats with Nerve Injury
Ben M. Clements, Katherine E. Kerrigan, Che Hsuing-Lee, Ipek Berberoglu, Stephen W.P. Kemp, John R. Traynor

A29 – Squaramide Derivatives as μOR/κOR Ligands: Synthesis, Functional Evaluation, and Receptor-Ligand Interaction Analysis
Ryszard Bugno, Magdalena Tertil, Grzegorz Satała, Sabina Podlewska, Krystyna Nędza, Aneta Kozioł, Andrzej Bojarski, Ryszard Przewłocki

A30 – In vitro pharmacological characterization of novel derivatives of the NOP receptor ligand [6ACH2] N/OFQ(1-13)NH2
Riccardo Camilotto, Davide Malfacini, Matteo Gozzi, Antonella Ciancetta, Alessandra Rizzo, Erika Marzola,Remo Guerrini, Girolamo Calò

A31 – Replacement of Proline by Triazole in Endomorphin-1: Design and Identification of a Potent K- Opioid Receptor-Selective Agonist
Marco Francescato, Federica Santino, Piotr F.J. Lipìnski, Andrea Bedini, Luca Gentilucci.

A32 – Effects of Biased Analogues of The Kappa Opioid Receptor Agonist, U50,488, Using In Vivo Mouse Models of Pain And Side Effects
Loan Vu, Ross van de Wetering , Lindsay Kornberger, Dan Luo, Brittany Scouller, Sheein Hong, Kelly Paton, Thomas Prisinzano, Bronwyn Kivell

A33 – Determination of the antinociceptive effect of linalool, the main component of lavender essential oil, against thermal pain and its effect on anxiety-related or obsessive-compulsive disorder-related behaviors.
Wakako Fujita, Kiho Minagawa, Sho Fukubeppu, Hayato Matsunaga, Minoru Hatayama, Jun Aruga

A34 – A Novel In Vivo Protocol for Evaluating Evoked and Non-Evoked Migraine-Like Signs in Mice
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A35 – Kappa opioid receptor signaling in the mouse claustrum modulates pain-evoked behavioral states
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A36 – Set-up of a murine model of endometriosis and migraine comorbidity
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A37 – Enhancing Recovery Quality After Lumbar Fusion Surgery Through ERAS Implementation: A Retrospective Study
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A38 – Lack of contralateral morphine analgesia in the acid-induced fibromyalgia model and its reversal by therapeutic mirtazapine, but not pregabalin
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A39 – Delta Opioid Receptors Inhibit PACAP-PAC1 signaling following Opioid Induced Hyperalgesia in the vlPAG
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A40 – Kappa Opioid Receptor Antagonist Properties of Psychotropic Medications in a Mouse Model of Antinociception
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A41 – Fentanyl-Type Antagonist of the μ-Opioid Receptor: Important Role of Axial Chirality in the Active Conformation
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A42 – Title: GPR63 and GPR153 enhance pathological pain relief through the suppression of microglial activation
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A43 – In Vivo Chemogenetic Activation and Retrograde Mapping of Enkephalinergic Pain Circuits in Mice
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A46 – Enkephalin in the Dorsal Raphe Nucleus Modulates Aversive Processing
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A47 – Single early injection of a NOP receptor antagonist modulates emotional and social behaviors following traumatic stress in mice
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Chiara Ebner, Valentine Gilbart, Gabriele Giua, Judith Meyer, Hugues Jacobs, Olivia Wendling, Gilles Laverny, Emmanuel Darcq

A49 – Extended amygdala dynorphin regulates nociception and alcohol-induced analgesia in mice
Jessica A. Cucinello-Ragland, Yolanda Campos-Jurado, Jessica Higginbotham, Léa Becker, Jordan McCall, Jose A. Moron

A50 – Identification of Proteins Controlling mu Opioid Receptor Trafficking in Cultured Cells Using a Novel Chemical Biology Platform
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A51 – PACAP-PAC1 Signaling Enhances GABAergic Transmission in the vlPAG but Does Not Mediate CFA-Induced Hyperalgesia
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A52 – B-Caryophyllene Reduces Morphine Reward via the Adenosine A2a Receptor: Evidence from In Vivo CRISPR Knockdown and Conditioned Place Preference in Mice
Emily Camposeo, Abby Schwarz, Hager Shogaaeldein, John M. Streicher

A53 – The neuropeptide receptor GPR83 regulates anxiety-like behavior
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A54 – In vivo study reveals bidirectional contribution of lateral hypothalamic opioid-responsive neurons to affective states in mice
Giua G, Meyer J, Darcq E

A55 – Opioidergic activation of the descending pain inhibitory system underlies placebo analgesia in neuropathic pain model rat
Hiroyuki Neyama,, Yilong Cui

A56 – Kappa Opioid Receptor-Induced Cognitive and Emotional Dysfunction is Associated with Dysregulated Autophagic Signaling in the Brain
Christos Karoussiotis, Alexandra Symeonof, Angeliki Nomikou, Chrysanthi Charalampous, Ioannis Serafimidis, Ioannis Sotiropoulos, Zafiroula-Iro Georgoussi

A57 – Optical tools for probing and controlling nociceptive GPCR and G protein signaling dynamics in living cells
Waruna Thotamune, Dhanushan Wijayaratna, Sithurandi Ubeysinghe, Chathuri Rajarathna, Senuri Piyawardena, Marvin Meyers, Nokomis Ramos-Gonzalez, Susrutha Majumdar, and Ajith Karunarathne

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B1 – From Trauma to Tranquility: How Myrcene Helps the Brain Bounce Back
Bonsale R, Teweldemedhin M, Perrone M, Limongelli R, Piscitelli F, Belardo C, Guida F, Maione S, Luongo L.

B2 – In Vitro Validation of MOR and CB1R Residues Involved in Heteromerization and Crosstalk at Baseline and After Morphine Treatment
Chiara Cimetti, Elisabetta Cuna, Monica Baiula, Pedro Renault, Jesùs Giraldo, Andrea Bedini

B3 – Morphine-THC drug combination modulates MOR-mediated intracellular signaling and MOR- CB1 heteromerization in human and rodent neuronal cell models in vitro
Monica Baiula, Elisabetta Cuna, Chiara Cimetti, Pedro Renault, Jesus Giraldo, Andrea Bedini

B4 – Behavioral and Neuro-Immune Impact of Mμ-Opioid Receptor and CB2 Cannabinoid Receptor.
Emmanuel S. Onaivi; Berhanu Kibret, Zhicheng Lin; Venkatanarayanan Sharma

B5 – Exploring the effects of microglial KOR activation on inflammatory responses within the murine mesocorticolimbic system
Víctor Ferrís-Vilar, Javier Cuitavi, Marina Murillo-Martínez, Ana Polache, and Lucía Hipólito

B6 – Salvinorin A analogues activating the kappa opioid receptor modulate OPC differentiation, myelination and neuroinflammation in preclinical rodent models.
Rabia Bibi, Ross van de Wetering, Bria Pengelly, Kelly Paton, Dan Luo, Sam Williamson, Thomas Prisinzano, Anne La Flamme, Bronwyn Kivell

B7 – Central κ-Opioid Receptor-Mediated Suppression of Atopic Dermatitis-Associated Pruritus and Its Impact on Immune Modulation
Yu Okamoto, Michiko Narita, Yuta Sekiguchi, Haruaki Yamaguchi, Yukari Suda, Yusuke Hamada, Yasuyuki Nagumo, Tsuyoshi Saito, Hiroshi Nagase, Yozo Ishiuji, Naoko Kuzumaki, Minoru Narita

B8 – Prokineticin-2 Signaling and Glial Activation as Therapeutic Targets for Fabry Disease neuropathic pain
Giulia Galimberti, Silvia Franchi, Giada Amodeo, Laura Rullo, Loredana Losapio, Patrizia Romualdi, Sanzio Candeletti, Benedetta Riboldi, Stefania Ceruti, Paola Sacerdote

B9 – A possible mechanism for suppressing tumor progression and alleviating tumor-related symptoms via regulation of the peripheral μ-opioid system
Yusuke Hamada, Minori Tabata, Mutsumi Kamemoto, Natsumi Iwakiri, Yukari Suda, Michiko Narita, Naoko Kuzumaki, Minoru Narita

B10 – Evaluating the pharmacokinetics of prolonged release injectable buprenorphine in mice
Joseph Nelson, Christopher P. Bailey, Stephen M. Husbands, and Sarah J. Bailey

B11
Using ultra-large DNA-encoded chemical libraries to discover novel opioid modulators
Evan S. O'Brien, Vipin Ashok Rangari, Shainnel O. Eans, Haylee R. Hammond, Elizabeth White, Haoqing Wang, Yuki Shiimura, Kaavya Krishna Kumar, Kevin Appourchaux, Jay P. McLaughlin, Barry Morgan, Susruta Majumdar, Brian K. Kobilka

B12 – In vitro and in vivo pharmacological characterization of a new N/OFQ dimeric derivative
Pietro Pola, Alessia Frezza, Chiara Sturaro, Erika Morrone, Alessandra Rizzo, Giulio Meneguzzo, Davide Malfacini, Girolamo Calo', Remo Guerrini, and Chiara Ruzza

B13 – Peripherally Acting Mu Opioid Receptor Antagonists (PAMORAs): Investigating a Novel Strategy for Opioid Overdose Reversal
Brian C. Ruyle, Caroline G. Roth, Jose A. Moron-Concepcion

B14 – Chronic administration of NC-2800, a novel δ-opioid receptor agonist, exhibited antidepressant-like effects in mice without inducing tolerance
Akiyoshi Saitoh, Toshinori Yoshioka, Pengfei liu, Daisuke Yamada, Eriko Nakata, Tomio Yamakawa

B15 – Novel oxymorphone analogues, as bifunctional mu/delta opioid receptor agonists, produce antinociception without the risks of analgesic tolerance and physical dependence in mice
Mariana Spetea, Veronika Ernst, Maria Guastadisegni, Dominik Pircher, Barbara Brunner, Helmut Schmidhammer

B16 – Discovery of Phenyltriazole-Based Dual KOR Antagonists and MOR Agonists via Structure- Based Virtual Screening
Magdalena Tertil, Ryszard Bugno, Rafał Kurczab, Sabina Podlewska, Grzegorz Satała, Krystyna Nędza, Andrzej Bojarski, Ryszard Przewłocki

B17 – Design and Synthesis of Pyrazolomorphinan Derivatives as Novel Delta Opioid Receptor Agonists.
Hideaki Fujii, Chiharu Iwamatsu, Saki Ishizaki, Hideki Nakamura, Daisuke Yamada, Shuma Yamada, Junichi Niwa, Toshinori Yoshioka, Pengfei Liu, Shintaro Shirakura, Shigeto Hirayama, Akiyoshi Saitoh

B18 – Structure-Activity Exploration of Delta Opioid Receptor Positive Allosteric Modulators
Alexander J. Powell, Mengchu Li, Ruiyang Ling, Hannah Stewart, Sherrice Zhang, Andrew D. White, and John R. Traynor

B19 – Novel Hydroxynorketamine Analogs for Chronic Neuropathic Pain Management
Stefania Volpe, Gilles Zribi, Lakshmi Devi, Ivone Gomes, Lloyd Fricker, Irving Wainer, Lawrence Toll

B20 – TRPing receptors – modulatory effect of TRP channels on MOR
Julie Sanchez, J. Robert Lane, Meritxell Canals

B21 – Mapping Brain-Wide Neuronal Activation in Chronic Neuropathic Pain and Opioid-Mediated Analgesia
Madeline Martinez, Akihiko Ozawa, Lawrence Toll

B22 – Potentiation of opioid analgesia and delay of tolerance by ultramicronized palmitoylethanolamide: involved cells, underlying mechanisms, and proteomics insights
Laura Micheli, Alessandra Toti, Samuele Trisolini, Lorenzo Di Cesare Mannelli, Carla Ghelardini

B23 – Morphine antinociception restored by methadone in the morphine-resistant inflammatory pain state
Hirokazu Mizoguchi, Chizuko Watanabe, Asami Komiyama, Masaru Yoshizumi, Shinobu Sakurada

B24 – Sex-dependent neuronal activation and behavioural dysfunction caused by NTG-induced migraine is reversed by NOP receptor agonist
Akanksha Mudgal, Diana Pietrzak-Mitura, Olga Wronikowska-Denysiuk, Madeline Martinez, Lawrence Toll, Akihiko Ozawa, Katarzyna Targowska-Duda

B25 – The Effects of Mixed Mu/NOP Agonist on Mechanical Allodynia in NTG-Induced Acute and Chronic Migraine in Mice
Diana Pietrzak-Mitura, Akanksha Mudgal, Lawrence Toll, Akihiko Ozawa, Katarzyna Targowska-Duda

B26 – Implications of NOP and classical opioid receptor systems in treatment of migraine-like symptoms in mice
Katarzyna Targowska-Duda, Diana Pietrzak-Mitura, Akanksha Mudgal, Olga Wronikowska- Denysiuk, Lawrence Toll, Akihiko Ozawa

B27 – Decoding pain mediated shifts in social behavior following neuropathic injury in mice.
Carlee Toddes, Kevin Bai, Isabel Halperin, David Ottenheimer, Mitra Heshmati, Sam Golden

B28 – Spinal Lipocalin-2 Contributes to the Development of Central Post-Stroke Pain
Shogo Tokuyama and Kazuo Nakamoto

B29 – Understanding the Impact of Inflammatory Pain on Alcohol Use: A Study in Rats with a Focus on Sex and Dose
Yolanda Campos-Jurado, Youssef M. Saad, Alexandre Neptune, Bilal Zahoor, Haziq Latif- Jangda , Jose A. Moron

B30 – Convergent state-control of endogenous opioid analgesia
Blake A. Kimmey, Lindsay Ejoh, Lily Shangloo, Jessica A. Wojick, Samar Nasser Chehimi, Nora M. McCall, Corinna S. Oswell, Malaika Mahmood, Lite Yang, Vijay K. Samineni, Charu Ramakrishnan, Karl Deisseroth, Richard C. Crist, Benjamin C. Reiner, Lin Tian, Gregory Corder

B31 – The kappa opioid receptor (KOR) in paraventricular nucleus of the thalamus (PVT)
Chongguang Chen, Peng Huang, Kathryn Bland, Kevin Beier, and Lee-Yuan Liu-Chen

B32 – Hypothalamic Vasopressin - Dynorphin Neural Circuit in Endocrine Control of Left-Right Balance
Mengliang Zhang, Hiroyuki Watanabe, Yaromir Kobikov, Guifa Li, Olga Nosova, Emma Lindström, Karen Rich, Yoichi Ueta, Takashi Maruyama, Mathias Hallberg, and Georgy Bakalkin

B33 – Ventral tegmental area neurons expressing μ-opioid receptor make distal connections
Lucie Oriol, Melody Chao, Sarthak Singal, Thomas S. Hnasko

B34 – Medullary Raphe Serotonin Neurons are Influenced by Opioids and Improve Breathing
Ryan C. Pauly, Jessica R. Whitaker-Fornek, Keiko Arakawa, Erica S. Levitt

B35 – Functions of zona incerta MOR neurons in the control of hedonic balance in mice
Solène Poivey, Geoffrey Stuart-Lopez, Judith Meyer, Emmanuel Darcq

B36 – Excitatory synaptic transmission is differentially modulated by opioid receptors along the claustro-cingulate pathway
Jacob M. Reeves, Erwin Arias-Hervert, Gracianne E. Kmiec, William T. Birdsong

B37 – Autophagy in Reversing Neuronal Damage from HIV and Morphine
Myosotys Rodriguez, Florida Owens, Candy Carbajal, Dileepkumar Veeragoni, and Nazira El- Hage

B38 – A novel form of spatiotemporal bias – using mathematical models and electrophysiology in C57 mice to compare pre- and post-synaptic MOPrs
Sambrook, M.O., Bridge, L., Kelly, E., Williams, R.J. and Bailey, C.P.

B39 – Immunohistochemical and mRNA detection of opioid receptors and their endogenous ligands in human dorsal root ganglion neurons
Michael Schaefer, Mohammed Shaqura, Özgür Celik, Sascha Treskatsch, Shaaban Mousa

B40 – Role of central amygdala protein kinase C-delta, corticotropin-releasing factor, and somatostatin neurons in opioid-related mice behavior
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B41 – Dynamic Resilience: Stress Phenotypes and Their Impact on Opioid-Taking Behavior and Brain Activity
Emma Tyner, Kyle A. Windisch, Shiv Raman, and Julie A. Blendy

B42 – Characterization of SNC80 Behaviors in a DORfl/fl Parvalbumin Cre Mouse Line
Marie Walicki

B43 – Neurological deficits induced by brain injury: bipartite ipsilateral opioid signaling via humoral pathway
Hiroyuki Watanabe, Yaromir Kobikov, Olga Nosova, Daniil Sarkisyan, Vladimir Galatenko, Nikolay Lukoyanov, Mathias Hallberg, Mengliang Zhang, and Georgy Bakalkin

B44 – Critical Windows of Endogenous Opioid Influence on Breathing Development
Jessica R. Whitaker-Fornek and Erica S. Levitt

B45 – Isolating the role of endogenous μ-opioid activity in the Ventral Tegmental Area during natural reward
Catalina A. Zamorano, Brianna L. Smith, Ashritha B. Cheeyandira, Michael R. Bruchas

B46 – Inhibitors of Glycine Transporter 1 Reduce the Development of Morphine Antinociceptive Tolerance in Rats.
Sarah Kadhim Abbood, Anna Rita Galambos, Nariman Essmat, Imre Boldizsár, Jr., Tamás Tábi, Pál Riba, Laszlo G. Harsing, Jr., Ferenc Zádorand Mahmoud Al-Khrasani

B47 – The Role of AT1 Receptor and PPARγ in Opioid Antinociceptive Tolerance
Imre Boldizsár Jr, Dávid Árpád Karádi, Nariman Essmat, Zoltán Varga, Gábor Mórotz, Pál Riba, Mahmoud Al-Khrasani, Kornél Király

B48 – Inhibition of histone demethylase LSD1 alleviates opioid-induced tolerance and hyperalgesia
Ying He and Zaijie Wang

B49 – Presynaptic modulation of transmitter release in striatopallidal afferents by the μ Opioid receptor
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B50 – Investigation of Pregabalin, Tolperisone, and Naloxone in the Context of Morphine-Induced Tolerance and Constipation in Rats
Nariman Essmat, Kornél Király, Zoltán S Zádori, and Mahmoud Al-Khrasani

B51 – Morphine-induced mechanical hypersensitivity in mice requires prokineticin receptors
Giorgio Prosperi, Daniela Maftei, Antonia Manduca, Rossella Miele, Roberta Lattanzi

B52 – Sex-specific molecular alterations in mesolimbic brain region following methadone administration in morphine tolerant rats
Laura Rullo, Antonio Lacorte, Loredana M. Losapio, Paolo Mangione, Camilla Morosini, Marco Cristani, Sanzio Candeletti and Patrizia Romualdi.

B53 – CaMKIIβ in opioid tolerance, dependence, opioid-induced hyperalgesia, and neuropathic pain
Xuebi Tian, Giokdjen Ilktach, Xiao Guo, Meghna Gill, Michael A. Wang, Yavnika Kashyap, and Zaijie Jim Wang

B54 – Pharmacological characterization and evaluation of health risks of bovine BCM-7
Gerdes, E.K., Biechl, K., Gard, F., Deeg, C.A., Ammer, H.

B55 – Selective inhibition of hemokinin-1-induced pruritic behavior by non-opioid sendide derivatives.
Takafumi Hayashi, Kazuhiro Watanabe, Tsutomu Fujimura, Yasuyuki Agatsuma, Shinobu Sakurada

B56 – Aticaprant to the rescue: Targeting the κ-opioid receptor system to shield mood and memory from stress impairments
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B57 – A cellulose-rich diet disrupts gut homeostasis and leads to anxiety through opioid-mediated gut-brain axis in mice
Chihiro Nozaki, Haruka Hosoki, Kaede Ito, Hiroyuki Sasaki, Shigenobu Shibata

Symposium

Symposium 1 - Cortical opioid signaling regulates adaptive and maladaptive behaviors

Chair: Max E. Joffe

Abstract

The prefrontal cortex (PFC) plays a critical role in regulating decision-making, emotional regulation, and goal-directed behaviors. These processes rely on the precise coordination of excitatory and inhibitory circuits, which are tightly modulated by endogenous opioid receptors. Dysregulation of these systems has implications for a wide range of neuropsychiatric conditions and behavioral states. This symposium explores the fundamental roles of opioid receptor systems in shaping cortical circuits, presenting cutting- edge research from three accomplished scientists whose work spans preclinical and translational domains.

Hugo Tejeda, Senior Investigator at the National Institute of Mental Health (NIMH), leads groundbreaking research on opioid receptor systems in the prefrontal cortex (PFC). His studies employ advanced computational analyses and optogenetic approaches across species, including humans, non-human primates, and rodents, to elucidate how endogenous opioid peptides modulate inhibitory and excitatory microcircuits. Dr. Tejeda's exceptional work has been recognized with the prestigious 2025 Presidential Early Career Award for Scientists and Engineers (PECASE), highlighting his transformative contributions to addiction neuroscience and neuropsychiatric research. His findings offer a framework for combining opioid-based therapeutics with cognitive and neuromodulation therapies to treat addiction and its psychiatric comorbidities.

Jacob Reeves is a Ph.D. candidate in the Neuroscience Graduate Program at the University of Michigan, under the mentorship of Dr. William Birdsong. Jacob performed undergraduate research in biology at Wingate University and post-baccalaureate training at the University of Alabama at Birmingham. Jabob's work has been supported by prestigious NIH T32 training grants, and he has presented his findings at major national conferences, including INRC and the Winter Conference on Brain Research. His contributions to the field have been recognized through publications in eNeuro and forthcoming work on circuit-specific opioid modulation. His doctoral work, presented in this panel, focuses on the role of opioid modulation in somatostatin interneurons within the PFC.

Rebecca Cole is a Ph.D. candidate at the Center for Neuroscience of the University of Pittsburgh. Building on her undergraduate research at Boston University, where she co-authored articles in PNAS and The Journal of Neuroscience, Rebecca has received numerous accolades, including the Outstanding Poster Award at Pitt Psychiatry Research Day. Her current work in Dr. Max Joffe's lab explores opioid receptor modulation of GABAergic interneurons in the PFC. Rebecca's innovative research provides critical insights into the mechanisms underlying motivational and affective behaviors in opioid use disorder (OUD), advancing the development of targeted therapeutic interventions.

Braulio Muñoz, Assistant Professor at Indiana University and Investigator at the Stark Neurosciences Research Institute, investigates the role of corticostriatal circuits in addiction, with a focus on parvalbumin- expressing neurons in the anterior insular cortex and their impact on alcohol use disorder (AUD). Dr. Muñoz's exceptional contributions to addiction neuroscience have earned him multiple International Narcotic Research Conference (INRC) Travel Awards and recognition for his impactful publications in Nature Communications and eLife. His research highlights sex-specific mechanisms of opioid receptor- mediated plasticity and offers therapeutic insights into treating AUD.

Together, these distinguished speakers bring a wealth of expertise and achievements to the symposium, offering transformative insights into opioid receptor regulation in brain circuits. The panel of speakers are representative of a diverse range of career stages and backgrounds. The related but complimentary talks will form a collective effort to improve our understanding of cortical opioid signaling and to pave the way for innovative opioid-based treatments to address psychiatric diseases.

Prefrontal cortical opioid receptor regulation of recurrent and inhibitory microcircuits: from mice to man

Huikun Wang[1]*, Hector Yarur[1]*, Sofia Shirley[1]*, Chloe Noh[1], Valerie Tsai[1], Greg Corder[2], Karl Deisseroth[3], Charu Ramakrishnan[3], Stefano Marenco[1], Veronica Alvarez[1], Bruno Averbeck[1], Kareem Zaghloul[4], Hugo Tejeda[1]

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3. Stanford University School of Medicine
4. National Institute of Neurological Disorders and Stroke

The prefrontal cortex exerts top-down control over motivated, goal-directed behavior through long- range inputs, local recurrent excitatory, and inhibitory microcircuits. Endogenous opioid neuropeptides and their receptors are expressed in the prefrontal cortex, but their principles underlying their anatomical organization and modulation of "hard-wired"

synaptic circuits are just beginning to be uncovered. We and others have demonstrated that prefrontal cortical opioid receptors regulate excitatory and inhibitory synapses innervating the prefrontal cortex with specificity, but how endogenous opioid neuropeptide neurons communicate with opioid sensitive synapses was unclear. Further it is unclear whether functional regulation of inhibitory microcircuits is conserved in human and non-human primates (NHPs). Here, we will describe organizing principles of the endogenous dynorphin (Dyn) / kappa-opioid receptor (KOR) system and enkephalin (Enk) signaling through mu- (MOR) and delta-opioid receptors (DOR) from slice electrophysiology and anatomical studies performed in cortical slices from mice, NHPs, and human cortex. Our laboratory has demonstrated that in addition to directly inhibiting dopaminergic and recurrent local circuits and afferent excitatory synapses in a pathway specific manner, Dyn / KOR signaling also potently suppresses inhibitory microcircuits. Furthermore, Enk regulates inhibitory SST neurons through joint actions on MOR/DOR while inhibiting PV microcircuits solely through DOR. Through the NIH Comparative Brain Physiology Consortium and leveraging interneuron-specific enhancer driven optogenetic constructs our laboratory has been able to demonstrate that Dyn and Enk differentially regulate GABA release from distinct interneurons in NHPs. Moreover, using MOR promoter viruses we gained genetic access to MOR-positive neurons in NHPs and functionally demonstrated robust Enk inhibition of GABA release from MOR-positive interneurons. Further, we demonstrated that Dyn inhibits GABA release onto pyramidal neurons via KORs, while Enk inhibits GABA release through both MOR and DOR in human cortex. We also found the existence of functional Dynergic and Enkergic neuropeptidergic transmission that operates within prefrontal cortical circuits to inhibit glutamate and GABA release from opioid receptor positive neurons or hyperpolarize interneurons in mice. In collaboration with the NIMH Human Brain Collection Core, we have employed computational analyses (NeuronChat) that have revealed existence of intra-cortical Enkergic interactions within disinhibitory microcircuits similarly established in mice. Together, these findings demonstrate that endogenous opioid signaling shapes the activity of defined inhibitory microcircuits, a function that is conserved in primates. Future work is aimed at understanding how endogenous opioid systems regulate defined connectivity in primate cortex, specifically with novel tools implemented across species. These studies provide a framework for understanding opioid receptors in regulating prefrontal cortical circuits in health and neuropsychiatric disorder and elucidate multi- modal treatments consisting of existing and novel opioid therapeutics in tandem with cognitive therapy and/or neuromodulation (e.g. TMS) of cortical circuits.

Investigating the opioid sensitivity of anterior cingulate cortex somatostatin interneurons

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The anterior cingulate cortex (ACC) plays a role in pain processing. This pain processing is carried out by an interaction between excitatory principal neurons and inhibitory interneurons. Principal cells and inhibitory interneurons in the ACC receive afferent inputs from various cortical and thalamic regions. The endogenous opioid system can control this pain processing by activation of opioid receptors on both afferent inputs and inhibitory interneurons in ACC circuits. Activation of opioid receptors causes a decrease in neuronal firing and neurotransmitter release resulting in excitatory:inhibitory circuit changes. Moreover, activation of opioid receptors on inhibitory interneurons results in disinhibition of nearby pyramidal cells. In the ACC, there are several subtypes of inhibitory interneurons: including parvalbumin (PV) and somatostatin (SST) -expressing interneurons. We have previously found that PV interneurons are well characterized expressing the delta opioid receptor (DOR) but not the mu opioid receptor (MOR) or kappa opioid receptor (KOR).

Furthermore, PV interneurons play a functional role in thalamo-cortical feed-foward inhibitory circuits. While much is known about the role ACC PV interneurons play in the endogenous opioid system, little is understood about SST neurons and their role in the ACC endogenous opioid system. The aim of this study is to understand the expression of opioid receptors on somatostatin neurons in ACC and how it regulates the physiology of local circuits. We used a combination of brain slice electrophysiology and pharmacology in mice to characterize the effects of opioid receptors on SST neurons. We found that Met-Enkephalin activated a GIRK conductance on SST neurons. Opioid receptor specific agonists activated layer specific GIRK conductances in SST neurons with most layer 2/3 neurons functionally expressing DOR while most layer 5/6 neurons functionally expressed both MOR and DOR. These data suggest that SST neurons in the ACC exhibit differential expression of opioid receptors in a layer specific manner proposing different circuit functions across layers.

Oxycodone dependence alters Mu and Delta opioid receptor regulation of prefrontal cortex inhibitory transmission in a cell type-specific manner

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Opioid use disorder (OUD) is a chronic and relapsing brain disorder that is characterized by an inability to control drug use and an intense withdrawal syndrome upon cessation. While Mu opioid receptor (MOR)-based therapies alleviate somatic discomfort and attenuate craving in OUD, these treatments are less effective at addressing long-lasting psychological symptoms that maintain drug use, necessitating a more complete understanding of changes to opioid signaling that occur during dependence. The delta opioid receptor (DOR) system has been consistently linked to hedonic deficits in rodent models of affective and reward behavior, supporting a role for dysregulated DOR signaling in motivational and affective components of OUD. The prefrontal cortex (PFC) is heavily implicated in OUD, and a substantial preclinical literature has linked PFC function with affective and motivational behavior. Though existing studies have focused mainly on opioid actions at excitatory synapses, the PFC endogenous opioid system is strongly localized to GABAergic interneurons (INs) which tightly regulate PFC circuitry and affective behavior. Using whole-cell patch-clamp electrophysiology and slice pharmacology in male and female mice, we show that show that PFC MOR and DOR signaling suppresses spontaneous and evoked PFC inhibitory transmission through dissociable mechanisms. Furthermore, cell type-specific optogenetics revealed that PFC MOR and DOR signaling is synapse-specific and differentially expressed by PV, SST, and CCK-INs. An escalating dose regimen of oxycodone that produces physical dependence and motivational and
affective behavior alters SST- and PV-IN MOR and DOR signaling in a cell type-specific manner, suggesting that cell- and receptor-specific adaptations to PFC GABAergic transmission may promote different facets of opioid-related behavior following dependence. Ongoing work uses cell type- specific calcium imaging and chemogenetics to monitor PFC IN activity during the development of opioid dependence and withdrawal and probe their role in behavior.

Role of Mu opioid receptor and parvalbumin-expressing neurons in the anterior insular cortex-dorsolateral striatum circuitry in alcohol use disorder

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Alcohol use disorder (AUD) affects approximately one-third of the U.S. population at some point in their lives, underscoring the urgent need for effective therapeutic interventions. Current pharmacological treatments for AUD exhibit limited and varied efficacy. The dorsal striatum is a critical brain region implicated in the development and progression of AUD. Within this region, medium spiny neurons (MSNs) are the principal neurons influenced by excitatory inputs from extra- striatal glutamatergic synapses and GABAergic inputs. Importantly, recent findings highlight that long-range GABA inputs from the cortex also exert direct inhibitory control over the dorsal striatum, yet the role of these corticostriatal GABAergic synapses in regulating alcohol consumption remains largely unexplored. Our previous studies have demonstrated that alcohol selectively disrupts anterior insular cortex (AIC) glutamatergic inputs to the dorsolateral striatum (DLS), an essential cortical region in the addiction circuitry. We also identified that AIC parvalbumin-expressing (PV+) neurons provide direct GABAergic input to DLS MSNs, which are regulated by mu-opioid receptors (MOR), indicating a complex regulatory mechanism involving opioid signaling. Notably, in vivo activation of AIC-DLS glutamate synapses was found to reduce binge alcohol consumption in male subjects. Here, we found that acute alcohol exposure reduces the GABA release from AIC PV+ synapses, while chronic exposure differentially affects MSN subtypes in the DLS. Also, we found that the in vivo activation of PV+ AIC-DLS circuitry increases alcohol consumption only in females. These findings suggest that direct inhibitory projection from PV+ AIC neurons to MSN in the DLS plays a role in alcohol drinking.

Symposium 2 - AI-enabled drug discovery targeting opioid receptors

Chair: Tao Che

Abstract

Advances in artificial intelligence and machine learning (AI/ML) algorithms have made it possible to integrate and analyze large, unstructured datasets from various sources, accelerating any stage of the medication development process. This data-driven approach is particularly promising and have been adopted for developing new treatments for substance use disorders (SUDs). In this symposium, discovery of novel tool compounds for opioid receptors using AI tools and computational modeling will be discussed. This symposium aims to unite the fields of medicinal chemistry, structural biology, and computational biology with the expertise of neuroscientists and in vivo pharmacologists. The goal is to create a collaborative effort that harnesses the strengths of these disciplines to find effective solutions for combating the opioid crisis.

Tao Che

Assistant Professor of Structural Biology and Pharmacology Center for Clinical Pharmacology
Department of Anesthesiology, Washington University School of Medicine, St. Louis, USA

Structure-guided design of partial agonists at an opioid receptor

The delta opioid receptor (DOR) is a potential alternative target for non-addictive analgesics to alleviate chronic pain, made more attractive by its lack of the respiratory depression associated with mu opioid receptor agonists. Partial DOR agonists may offer more controlled activation of the receptor compared to full agonists, but the development of such ligands has been hindered by uncertainty over the molecular mechanism mediating partial agonism. Using a structure-based approach, we explored the engagement of the sodium binding pocket in DOR and developed a full range of DOR ligands from full agonists to partial agonists and antagonists.

Marta Filizola, Ph.D.

Dean, Graduate School of Biomedical Sciences
Sharon & Frederick A. Klingenstein-Nathan G. Kase, MD Professor Department of Pharmacological Sciences, Department of Neuroscience, and Department of AI & Human Health, Icahn School of Medicine at Mount Sinai (ISMMS), New York City, USA

Accelerating the Quest for Safer Opioids with Fine-Tuned Deep Transfer Learning Models

A longstanding question in opioid pharmacology is whether an enhanced therapeutic window arises from low intrinsic efficacy across all signaling pathways or from ligand bias, wherein certain transducer subtypes are selectively activated in a specific cellular context. Despite the promise of AI for such predictions, building deep learning models that reliably identify opioid receptor ligands with predefined bioactivities remains challenging, primarily due to limited high-quality data. To address this challenge, we have recently explored a range of ligand-based and structure-based drug discovery methods in combination with transfer learning, deep learning architectures, and transformer-based protein language models. By leveraging carefully curated data on known G protein-coupled receptor (GPCR) ligands, receptor sequences, and mutations that affect GPCR signaling, we have achieved improved opioid bioactivity predictions. Our models exhibit robust predictive performance, highlighting their potential to efficiently screen ultra-large chemical libraries and accelerate the discovery of safer, more effective opioid therapeutics.

Michael Robertson, Ph.D.

Assistant Professor of Structural Biology and Computational Biology Department of Biochemistry and Molecular Pharmacology, Baylor College of Medicine, Houston, USA

Targeting GPCRs with AI-Enabled Methods

G-protein coupled receptors play several key roles in substance use disorder. However, imaging these receptors bound to their ligands can prove incredibly challenging for both x-ray crystallography and cryoEM. Here I discuss how novel computational methods are greatly facilitating understanding the structures of these receptors and how they interact with their ligands.

Christian W. Gruber, Ph.D.

Associate Professor of Drug Discovery and Peptidomics Center for Physiology and Pharmacology
Medical University of Vienna, Vienna, Austria

Design of nature-inspired stabilized peptides as next generation opioid drugs

Peptides are critical signaling molecules that often act via GPCRs, including the κ- opioid receptor, which is a key target for pain management. To overcome the limitations of traditional peptide therapeutics, we leverage diverse bioresources, advanced chemical tools, and computational design to develop novel κ-opioid receptor ligands with enhanced stability, subtype selectivity, and functional bias.
These innovative peptides show potential as drug candidates for treating chronic abdominal pain and inflammatory conditions.

Symposium 3 - From Neural Pathways to Molecular Insights: Advancing Treatments for Opioid Use Disorders

Chair: Esi Domi

Abstract

This symposium will delve into the neurobiological and molecular underpinnings of opioid use disorders (OUD). By addressing opioid-induced changes in neural circuits and introducing innovative therapeutic strategies, the presentations will provide insights into potential advancements in the treatment of OUD.

Buprenorphine – the long and short of it

Nairong Liu, Joseph Nelson, Chris Bailey, Sarah Bailey, Stephen M Husbands
Department of Life Sciences, University of Bath, Bath, BA2 7AY

Since lockdown, long-acting injectable buprenorphine (LAIB) has become routine in Wales, Scotland and is being piloted in England as an alternative form of opioid substitution therapy (OST). There are currently over two thousand people using the treatment in Wales, representing around half of those in treatment for opioid use disorder. The new OST has been very popular with service users, with >85% of people remaining in treatment. People have reported that they are surprised to be spending their money on food rather than "gear," they appear to feel less anxious and can engage with treatment services.

We have been interested in whether kappa antagonism displayed by buprenorphine is more effective in providing anxiolytic effects when given by prolonged release – thus converting buprenorphine's short-acting kappa antagonism into constant blockade of the receptor.

We are investigating the pharmacodynamics and pharmacokinetics of LAIB compared to daily buprenorphine in various rodent models, with particular interest in the mu partial agonist and kappa antagonist activities, and whether the change in delivery affects brain and plasma levels of buprenorphine and key metabolites.

In both male and female rats, LAIB, at both 1 and 3 days post-administration, significantly blocked the antinociceptive effects of a kappa agonist (spiradoline) compared with vehicle treated controls whereas the antinociceptive response to spiradoline was not attenuated in animals treated with repeated daily acute buprenorphine (20h pretreatment). C57BL/6 mice showed an initial hyper locomotor and analgesic response to LAIB, with loss of these activities at later time points. This contrasts with our findings that after repeated daily administration of buprenorphine these activities were maintained. No norbuprenorphine was observed in the brain at any timepoint (LLOQ of 1.25 ng/mL) with either route of administration.

In summary, clinical findings indicate that LAIB is differentiated from daily dosing with buprenorphine and data from a variety of experiments in rodents confirm differences in observed pharmacology.

Funding: The work is supported by Camurus.

Opioid Addiction-Related Behaviors Are Controlled by Distinct Amygdala Circuitries

Leandro Vendruscolo, Stress and Addiction Neuroscience Unit, National Institute on Drug Abuse, Baltimore, MD, USA

Research in opioid-dependent mice reveals that inhibition of CeA-CRF neurons reduces irritability and somatic withdrawal signs, activation of CeA-SST reduces somatic withdrawal signs, and inhibition of CeA-PKC-delta alleviates opioid withdrawal-induced hyperalgesia and potentially reduces fentanyl intake. These findings underscore the diverse roles of CeA microcircuitries in opioid withdrawal-related behaviors and their potential as therapeutic targets for OUD.

Dual NOP/MOP Receptor Modulation: From neurocircuitries to Novel Mechanisms for Treating Opioid Use Disorders

Roberto Ciccocioppo, School of Pharmacy, Center for Neuroscience, University of Camerino, IT

Activation of Nociceptin/Orphanin FQ (N/OFQ) NOP receptors reduces the reinforcing properties of MOP agonists. This presentation will discuss preclinical data on compounds acting as dual NOP/MOP agonists, showcasing how concurrent activation of NOP and classical opioid receptors at both mechanistic and circuitry levels could lead to new therapeutic approaches for OUD.

Symposium 4 - Uncovering GPR-Family G Protein Coupled Receptors as Novel Pain Regulators

Chair: John Streicher

Abstract
The GPR family of G Protein Coupled Receptors was initially described as a large family of orphan receptors without known ligands or biological functions. Over time, many have been de-orphanized and their roles explored for different physiological states, including pain and analgesia. However, many remain orphaned, with little to nothing known of their ligands and mechanisms. In this symposium, we will explore recent advances in uncovering novel GPRs in pain, along with progress towards developing their ligands and uncovering their mechanisms of action. These findings represent new targets in pain physiology, with much to be explored in terms of their mechanisms, signaling, and pharmacology. Some may become new non-opioid targets for pain therapy.

Targeting the PEN-GPR83 pathway to enhance the efficacy of MOR agonists
Amanda Fakira, Rowan University

There is a need to identify targets that limit opioid abuse potential while maintaining the pain-relieving effects of opioids. Here we identify small molecule GPR83 ligands that block the rewarding effects of opioids while enhancing their pain-relieving effect.

GPR171 agonist relieves chronic pain and enhances morphine antinociception without enhancing adverse effects
Erin Bobeck, Utah State University

Chronic pain affects about one-third of the U.S. population, with opioids remaining the most prescribed treatment despite their side effects, including addiction. Novel therapeutics with reduced abuse liability are needed to treat these patients. We have been investigating the BigLEN- GPR171 neuropeptide-receptor system as a potential pain target. GPR171 is highly expressed in the periaqueductal gray, which is a key brain region involved in pain modulation. Despite not having any effects on acute pain, a GPR171 agonist reduces inflammatory and Paclitaxel-induced neuropathic pain in male mice, but has no effect in females. Interestingly, a GPR171 antagonist reduces morphine-induced antinociception, while an agonist enhances them, suggesting GPR171 can modulate opioid receptor signaling. Our findings also show that repeated administration of a GPR171 agonist does not enhance morphine tolerance, withdrawal, or reward suggesting it could enhance pain relief while minimizing opioid-related adverse effects.

GPR63 and GPR153 enhance analgesia in pathological but not acute pain by suppressing microglial activation in spinal cord
John Streicher, University of Arizona

We performed a phenotypic screen to identify novel GPRs in pain. We found that GPR63 and GPR153 knockdown in spinal cord blocks pain relief in pathological (neuropathic, paw incision) but not acute/nociceptive (tail flick) pain. We further found that both receptors work through microglia to regulate pathological pain relief by suppressing microglial activation. Both receptors are true orphans with no known ligands and little known of their function, so we will further detail our efforts to find GPR63/153 ligands using a novel microfluidic screening platform.

Symposium 5 - Exploring Roles of Habenula in Opioid Signaling and Behaviors

Chair: Emmanuel Darcq

Abstract

This symposium will explore the role of opioids in the habenula, a critical brain region in emotional regulation, addiction, and psychiatric disorders. Speakers will present insights into how habenular and opioid signaling influence mood, stress, and substance use, with potential implications for novel therapeutic strategies.

Paul Kenny will show that opioids inhibit the activity of medial habenula (mHb) neurons that project to the interpeduncular nucleus (IPn). This inhibitory action is mediated by mu opioid receptors (MORs) expressed by mHb neurons that project to the interpeduncular nucleus (IPn) and contributes to the positive reinforcing properties of opioids. The orphan GPR151 is co- expressed with MORs in the mHb and regulates their inhibitory actions on the mHb-IPn circuit. High-throughput screening identified small-molecule regulators of GPR151. One of these compounds modified mHb-derived excitatory transition in the IPn of wild-type but not GPR151 knockout (KO) mice. Direct infusion of this compound into the IPn modified intravenous opioid self-administration in wild-type but not GPR151 KO mice. These findings identify GPR151 is a novel regulator of MOR signaling in the mHb-IPn circuit and suggest that targeting GPR151 could offer new therapeutic strategies for opioid use disorder (OUD).

Elyssa Margolis will present her unpublished studies mapping MOR expression throughout the habenula. She will also describe ex vivo whole cell recordings where Elyssa Margolis's team measured responses to DAMGO in the mHb and lateral habenula (LHb). Overall, she observed larger inhibitory responses to MOR activation in the LHb, especially in LHb neurons that project to the parabrachial nucleus.

Tom Hnasko will present work on the habenulo-peduncular (HP) which is a hotspot for MOR expression and has emerged as a key substrate for aversion, anxiety, and addiction. He will describe recent work that reveals compartment-specific canonical inhibitory and non-canonical facilitatory effects of MOR in the HP circuit.

Dersu Ozdemir will discuss opioid-responsive neurons in the habenula (HbMOR) and their role in negative affect during opioid withdrawal. Using optogenetics and behavioral testing, Dersu and Darcq's team has shown that these neurons encode aversive states, with heightened activity during withdrawal. This research may inform new approaches to reducing withdrawal's emotional responses.

Mu-opioid receptor activation potentiates excitatory transmission at the habenulo-peduncular synapse

Sarthak M. Singhal, Agata Szlaga, Yen-Chu Chen, William S. Conrad, and Thomas S. Hnasko

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The continuing opioid epidemic poses a huge burden on public health. Identifying the neurocircuitry involved and how opioids modulate their signaling is essential for developing new therapeutic strategies. The medial habenula (MHb) is a small epithalamic structure that projects predominantly to the interpeduncular nucleus (IPN) and represents a mu-opioid receptor (MOR) hotspot. This habenulo-peduncular (HP) circuit can regulate nicotine and opioid withdrawal; however, little is known about the physiological impact of MOR on its function. Using MOR-reporter mice, we observed that MORs are expressed in a subset of MHb and IPN cells. Patch-clamp recordings revealed that MOR activation inhibited action potential firing in MOR+ MHb neurons and induced an inhibitory outward current in IPN neurons, consistent with canonical inhibitory effects of MOR. We next used optogenetics to stimulate MOR+ MHb axons to investigate the effects of MOR activation on excitatory transmission at the HP synapse. In contrast to its inhibitory effects elsewhere, MOR activation significantly potentiated evoked glutamatergic transmission to IPN. The facilitatory effects of MOR activation on glutamate co-release was also observed from cholinergic-defined HP synapses. The potentiation of excitatory transmission mediated by MOR activation persisted in the presence of blockers of GABA receptors or voltage-gated sodium channels, suggesting a monosynaptic mechanism. Finally, disruption of MOR in the MHb abolished the faciliatory action of DAMGO, indicating that this non-canonical effect of MOR activation on excitatory neurotransmission at the HP synapse is dependent on pre-synaptic MOR expression. Our study demonstrates canonical inhibitory effects of MOR activation in somatodendritic compartments, but non-canonical faciliatory effects on evoked glutamate transmission at the HP synapse, establishing a new mode by which MOR can modulate neuronal function.

Mu opioid receptor neurons of the habenula mediate opioid withdrawal

Dersu Ozdemir, Judith Meyer, Florian Pons, Cedric Champagnol-Di Liberti, Isabella Guimaraes-Olmo, Brigitte Lina Kieffer, Emmanuel Darcq

Université de Strasbourg (UNISTRA), INSERM UMR-S 1329, Strasbourg Translational Neuroscience and Psychiatry, Centre de Recherche en Biomédecine de Strasbourg, France.

Opioid use disorder is a chronic and relapsing disorder rising worldwide. Naloxone (Narcan®), a life-saving opioid antagonist, reverses overdose but induces severe negative affect. Neurons which mediate naloxone-induced aversion, remain unclear. Previously, we identified the habenula (Hb), an aversion center, as a site where mu opioid receptor-expressing (Hb-MOR) neurons encode negative emotional states. Here, we hypothesized that these neurons also drive naloxone aversion. In opioid-naïve mice, we found that high-dose naloxone produced conditioned place aversion (CPA) and increased activity of Hb-MOR neurons. Further, chemogenetic-inhibition of these neurons prevented high-dose naloxone CPA, indicating that blocking endogenous opioids at Hb-MOR neurons is aversive. In opioid-dependent animals, low-dose naloxone was sufficient to increase Hb-MOR neuron activity and produce CPA. Also, chemogenetic-silencing of Hb-MOR neurons prevented low-dose naloxone CPA, and alleviated somatic withdrawal symptoms. Altogether, under chronic morphine exposure, Hb- MOR neurons become highly sensitive to naloxone and are key to mediate naloxone-related adverse effects.

Symposium 6 - Chemistry and Pharmacology of opioid receptor modulators

Chairs: Meritxell Canals & Susruta Majumdar

NaloxoDARTs: development and characterization of tethered antagonists for blockade of Mu opioid receptors in discrete neuronal populations

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Opioid receptors are expressed in virtually all neural loci contributing to the experience of pain. Due to this widespread expression the contribution of specific cell types to the analgesic properties and deleterious side effects of opioids remains incompletely understood. Linking the activity of specific receptors in defined cells to behavioral or physiological processes remains a major challenge of translational pharmacology. In this study we describe the development of Drugs Acutely Restricted by membrane Tethering (DART) antagonists that contain an antagonist naloxone moiety linked to a Halo-tag reactive group. The optimized Naloxo-DART displayed robust blockade of a MOR agonist only when cells co-expressed a Halo-tagged membrane tether. We use the Naloxo-DART to selectively block MORs in locus coeruleus neurons. We propose the Naloxo-DART as a powerful approach to elucidating the physiological role of MORs expressed in specific neuronal populations with acute spatiotemporal control.

MOR bioassays in forensic toxicology: activity-based detection of new synthetic opioids

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In the past decade, new synthetic opioids (NSOs) have increasingly emerged on recreational drug markets worldwide. Their structural diversity and the limited pharmacological data available pose significant challenges for toxicologists in both forensic and clinical settings. While high-resolution mass spectrometry (HRMS) is the method of choice for broad NSO screening, its implementation is costly and untargeted data processing is time-consuming and labor-intensive. Furthermore, interpretation of medico-legal casework involving NSOs remains complex. In response, recent research has explored the application of in vitro bioassays for the untargeted and universal detection of NSOs, as well as for the interpretation of forensic cases involving novel opioids. Here, we discuss the successful application of a cell-based μ-opioid receptor (MOR) activation (β-arrestin 2 recruitment) assay for the detection of the novel synthetic opioid N-piperidinyl etonitazene (etonitazepipne) in a patient sample. In addition, the determination of 'fentanyl activity equivalents' facilitated the interpretation of etonitazepipne concentrations in this case. A modified protocol of this MOR bioassay can be applied to screen for both opioid agonists and antagonists in biological samples. In forensic cases where opioid involvement is suspected but conventional bioanalytical methods fail to detect any opioids, the MOR bioassay has the potential to offer a conclusive answer related to the (non-)relevant presence of opioids. Thus, the application of MOR bioassays extends beyond pharmacological characterization alone, with a promising role in forensic toxicology.

Development of PN6047, a novel G-protein biased delta opioid receptor agonist, for the treatment of opioid use disorder

Amynah A Pradhan

Background/Objectives

While opioids may provide acute pain relief, regular use of μ opioid receptor (MOR) agonists results in the development of physical dependence, tolerance, and opioid use disorder (OUD). Withdrawal and abstinence from chronic opioids produces a negative affective state, which includes hypersensitivity to emotional and painful stimuli. Delta opioid receptor (DOR) agonists offer a promising therapeutic target for treating negative affective states associated with opioid withdrawal and abstinence. However, the development of DOR agonists has been limited by seizurogenic activity and tolerance. G protein biased DOR agonists show reduced propensity for analgesic tolerance and pro-convulsant effects. PN6047 is a novel G protein biased DOR agonist developed by PharmNovo. The objective of this study was to evaluate PN6047 in pre-clinical models of opioid withdrawal-induced negative affect that may contribute to relapse.

Methods

The Pradhan lab tested PN6047 in animal models of opioid withdrawal-induced hyperalgesia, tolerance, and conditioned place preference. The Jutkiewicz lab tested PN6047 for adverse effects, specifically seizurogenic activity and abuse liability.

Results

In peripheral and cephalic models of opioid withdrawal induced hyperalgesia, PN6047 significantly inhibited established mechanical allodynia. This effect was dose-dependent and blocked by the DOR antagonist naltrindole. Co-administration of PN6047 also blocked the development of opioid induced hyperalgesia. Importantly, tolerance to PN6047 was limited, unlike the rapid tolerance induced by chronic treatment with the balanced DOR agonist, SNC80. In a self-administration paradigm, PN6047 did not maintain responding in rats trained to administer remifentanil. This lack of effect was also observed in animals that were morphine withdrawn, thus supporting the low abuse liability of PN6047. This compound also did not produce convulsions or changes in EEG activity reflective of seizurogenic activity.

Conclusions

Our studies indicate that PN6047 could be a promising therapy for negative affect associated with opioid withdrawal. PN6047 is well-prepared to enter future clinical trial for opioid use disorder.

Salvinorin A analogues activating the kappa opioid receptor modulate OPC differentiation, myelination and neuroinflammation in preclinical rodent models.

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There are no effective therapies currently available to repair damaged myelin or enable functional recovery in demyelinating diseases such as multiple sclerosis (MS). In MS, the protective myelin sheath is damaged, which impairs saltatory conduction and, over time, leads to neuronal cell death and loss of various motor and cognitive functions. The kappa opioid receptor (KOR) has been recognized as a potential therapeutic target for enhancing remyelination. In the present study, we utilized mouse in vitro and in vivo screens to identify salvinorin A analogues that were effective at promoting oligodendrocyte progenitor cell (OPC) differentiation into myelinating oligodendrocytes (OL) and quantified their ability to myelinate rhodamine stained nanofibers using primary mouse mixed glial cultures and high-throughput confocal microscopy. We then evaluated their effectiveness at promoting recovery using an experimental autoimmune encephalomyelitis (EAE) mouse (C57BL/6) model of demyelination in vivo. We found that all salvinorin A analogues enhanced OPC differentiation, with 16-ethynyl salvinorin A demonstrating the highest potency. It was also the most effective at increasing OL morphological complexity and promoting myelination in vitro. In the EAE model, treatment with mesyl salvinorin B, βTHP salvinorin B, and 16-ethynyl salvinorin A significantly decreased paralysis disease scores and increased the percentage of recovered mice. Furthermore, 16-ethynyl salvinorin A treatment reduced both microgliosis and astrogliosis in the white and grey matter of EAE mouse spinal cord. This identified KOR agonism acts via dual mechanisms to modulate neuroinflammation and OPC differentiation, to create an environment that promotes remyelination. Overall, these data highlight the potential for salvinorin A analogues to be developed into remyelinating therapies.

Symposium 7 - Dynorphin-expressing accumbal neurons promote maladaptive behaviors and reduce reward

Chairs: Ethan M. Anderson & Nicolas Massaly

Abstract

The nucleus accumbens (NAc) is a central structure within the mesolimbic circuit integrating both rewarding and aversive stimuli. The NAc is composed of 95% of medium spiny neurons expressing either enkephalin or dynorphin (Dyn) endogenous opioid peptides. While Dyn-expressing NAc neurons (NAc^{Dyn}) regulate both rewarding and aversive behaviors, the precise mechanisms through which they shape appropriate behavioral responses are still not fully understood. This symposium will provide unpublished data from four independent investigators to discuss the afferent/efferent circuit-based, activity-dependent, and epigenetic mechanisms that shape NAc^{Dyn} neuronal function in behaviors. First, Dr. **Catherine Marcinkiewcz** will characterize the serotoninergic (5-HT) monosynaptic inputs from the dorsal raphe nucleus onto NAc^{Dyn} neurons and discuss how alcohol consumption alters 5-HT-NAc^{Dyn} interactions to inhibit social rewards. Second, Dr. **Ethan Anderson** will demonstrate how epigenetic signaling decreases potassium channel expression in NAc^{Dyn} neurons, leading to increases in NAc^{Dyn} intrinsic excitability, and ultimately reduces alcohol drinking during times of stress in rodents. Next, Dr. **Ream Al-Hasani** will examine the sufficiency for dorsal NAc^{Dyn} neurons to block sucrose and fentanyl consummatory behaviors, using both optogenetic and chemogenetic approaches. Finally, Dr. **Nicolas Massaly** will investigate alterations in NAc^{Dyn} projections to the lateral hypothalamus induced by inflammatory pain to promote negative affective states. Overall, the proposed symposium will provide novel insights into the cellular and molecular mechanisms of NAc^{Dyn} neurons that impact a broad range of behavioral outcomes when rodents are exposed to rewarding (alcohol, social interactions, or consummatory rewards) or aversive (pain or stress) stimuli.

Epigenetic regulation of dynorphin-positive accumbal neurons reduces potentiated ethanol drinking in mice

Ethan M. Anderson, Ph.D. Louisiana State University, Department of Comparative Biomedical Sciences, Baton Rouge, LA, 70803

Stress perpetuates the cycle of excessive alcohol drinking and contributes to the transition to an alcohol-use disorder (AUD). A common mechanism that regulates both stress-sensitivity and alcohol use is epigenetic regulation of gene transcription. One epigenetic modifier implicated in AUD is G9a, a histone methyltransferase that dimethylates lysine 9 on histone 3 (H3K9me2). We recently showed that alcohol decreases G9a in the nucleus accumbens (NAc). Mimicking this reduction of NAc G9a with an AAV-mediated shRNA knockdown decreases stress-potentiated alcohol drinking; however, the mechanism is not fully understood. Since the dynorphin (Dyn) system plays a prominent role in stress/ethanol-related behaviors, and dynorphin is present in a major subset of NAc neurons (NAc^{Dyn+}), we hypothesized that G9a acts selectively through (NAc^{Dyn+}) neurons to alter stress- potentiated drinking. We tested this by injecting a novel cre-dependent AAV virus (AAV-DIO-shG9a) into the NAc of both dynorphin-cre and enkephalin-cre mice. These mice underwent 4 weeks of two- bottle choice drinking and then were injected with the kappa opioid receptor agonist U50,488 (5mg/kg, i.p.) before drinking. Control Dyn-cre mice exhibited stress-potentiated drinking, but the AAV-DIO-shG9a experimental mice did not, demonstrating an effect of G9a in NAc^{Dyn+} neurons. In contrast, experimental Enk-cre mice did exhibit stress-potentiated drinking, suggesting no effect of G9a in NAc^{Enk+}. A transcriptomic analysis revealed that NAc G9a alters potassium subunits associated with excitability, so we next used slice physiology to measure changes in current-evoked spiking activity. We found that a G9a knockdown led to an increase in NAc intrinsic excitability.
Combined, these results suggest that the effects of NAc G9a on stress-potentiated drinking are mediated by altering the excitability of NAc^{Dyn+} neurons. Thus, targeting G9a and/or NAc^{Dyn+} neurons could reduce escalated alcohol drinking in people with AUD.

Symposium 8 - N/OFQ receptor signaling at different scales: from molecular pathways to pain and stress-related behavior via brain networks

Chairs: Lionel Moulédous & Chiara Ruzza

Abstract

The nociceptin/orphanin FQ (N/OFQ) neuropeptide, discovered 30 years ago, acts through the NOP receptor, influencing a wide range of physiological processes, including pain perception, anxiety, depression, and addiction. Therapeutic research has highlighted its potential in developing novel analgesics that avoid the side effects associated with traditional opioids. Additionally, NOP receptor ligands show promise in treating mood disorders and substance abuse. However, the widespread expression of the N/OFQ system and its complex modulatory roles present challenges in achieving selective therapeutic outcomes. Targeting this system without affecting multiple pathways remains a key limitation, necessitating a significant refinement in our understanding of NOP receptor signaling at the molecular and brain circuit level, as well as a better characterization of the pathological conditions in which a pharmacological modulation of the receptor would be useful. The objective of this symposium is to address some of these pending questions and to discuss the opportunities and limitations of NOP receptor targeting for tempering stress-related neurobehavioral deficits. We have thus gathered presentations that analyze the consequences of NOP receptor modulation at various levels. At the intracellular signaling level, the first talk will test the hypothesis of differential coupling to G protein subtypes by different NOP ligands. At the neuronal circuit level, the second presentation will describe the impact of NOP signaling inhibition on the functional connectivity of regions involved in behaviors associated with the stress response. The final two talks will focus on the preclinical behavioral level in models of migraine and other stress-related pathologies. By bringing together molecular, circuit and behavioral levels of analysis, this symposium aims to better define the therapeutic opportunities linked to the N/OFQ system with a special focus on stress-related diseases.

Comprehensive transduceromic profiling of NOP receptor ligands reveals no essential G protein response differences

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The nociceptin/orphanin FQ (N/OFQ) receptor (NOP) is a GPCR implicated in the control of pain, anxiety, stress responses, and sleep regulation, making it an attractive target for new therapeutics. We performed a comprehensive transducerome analysis of NOP receptor signaling using the recently developed TRUPATH bioluminescence resonance energy transfer (BRET) biosensor platform1. A panel of 20 NOP receptor agonists – including the endogenous peptide N/OFQ, peptide analogues, and diverse non-peptide agonists was screened for coupling across multiple G protein subtypes in living cells. N/OFQ promoted NOP coupling with Gi1, Gi2, Gi3, GoA, GoB, Gz showing values of potency (pEC50) in the range of 8.96-10.46 and Emax ~ 20-30% inhibition of basal values. Of note, the signal to noise ratio of the assay was low. Our findings indicate that all tested ligands activate NOP receptor signaling with broadly similar G protein coupling profiles with no agonist producing a robust biased preference for any specific Gα subtype. This uniform signaling behavior across the ligand panel raises intriguing questions about NOP receptor biology: it may reflect an intrinsic limitation in the receptor's ability to differentially engage G proteins, or a lack of structural features in the evaluated agonists to drive G protein bias, or a low sensitivity of this BRET assay, or a combination of these factors. Nonetheless, these results provide a rigorous reference point for NOP pharmacology and an important foundation for future studies in the field of NOP functional selectivity.

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Pharmacological Modulation of NOP Receptors: Impact on Behavioral Stress Response and Brain Functional Connectivity

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Nociceptin, also known as orphanin FQ (N/OFQ), is an endogenous neuropeptide that signals through the nociceptin opioid peptide (NOP) receptor, a non-classical member of the opioid receptor family. Prior studies have shown that

the N/OFQ system plays a key role in modulating stress responses and adaptation to stressful stimuli. To investigate the contribution of endogenous N/OFQ to these processes, we subjected mice to an acute footshock stressor and pharmacologically blocked the NOP receptor using SB-612,111, a selective antagonist.
We then assessed (1) behavioral responses, (2) brain-wide neuronal activity and functional connectivity, and (3) delayed maladaptive behavioral outcomes.

NOP receptor blockade significantly increased freezing behavior in response to the aversive stimulus. Whole-brain activity mapping using c-Fos, an immediate early gene marker of neuronal activation, revealed that 7 of the 113 analyzed brain regions exhibited significantly increased activity in SB-treated mice, notably including the central amygdala (CeA). Furthermore, c-Fos expression in the CeA positively correlated with the intensity of freezing behavior. Functional connectivity analysis based on inter-regional c-Fos co-activation patterns revealed that SB-612,111 treatment markedly increased network density by doubling the number of significant positive correlations. It also led to a reorganization of network topology, with altered identity and centrality of key hub regions.

To examine the long-term impact of acute NOP receptor blockade, we assessed social behavior nine days after footshock exposure. In the three-chamber sociability test, SB-treated mice displayed significantly higher social preference compared to vehicle-treated stressed mice, suggesting a protective effect against stress-induced social deficits.

These findings demonstrate that endogenous N/OFQ signaling during acute stress has a broad impact on brain activity and network-level dynamics. Consistent with its established role in fear and anxiety, the CeA emerges as a central node mediating N/OFQ's behavioral effects.
Moreover, early NOP receptor antagonism may mitigate the delayed social impairments often associated with severe stress. Collectively, our data support the therapeutic potential of targeting the N/OFQ system to modulate acute stress responses and prevent long-term maladaptive outcomes.

Effects of NOP receptor modulation in pre-clinical migraine models

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Migraine is a prevalent neurological disorder, with stress among its key triggers. This study explores the role of NOP receptor modulation, via gene knockout and pharmacological activation, in murine migraine models.
Male and female wild-type and NOP knockout (NOP(-/-)) mice were used. Two selective NOP agonists were tested: AT-403 (brain-penetrant) and UFP-112 (peripherally restricted). Migraine- like signs were induced by a single injection of calcitonin gene-related peptide (CGRP, 0.1 mg/kg, i.p.) or by combining 4-day restraint stress (2 h/day) with a subthreshold dose of nitroglycerin (GTN, 0.1 mg/kg, i.p.). Periorbital mechanical allodynia (PMA), a marker of migraine-like pain, was assessed using von Frey filaments. To investigate the cellular mechanism, N/OFQ signaling was examined in NOP-expressing HEK293 cells and in human Schwann cells (hSCs), focusing on receptor internalization and cAMP modulation.
CGRP induced PMA in both genotypes, with no significant differences. Both AT-403 (1–30 μg/kg) and UFP-112 (0.1–10 pmol) fully prevented CGRP-induced PMA. Repeated restraint stress triggered PMA in wild-type mice, resolving within 21 days. Similar responses were observed in NOP(-/-) mice. However, GTN injection 21 days after stress reinstated PMA in previously stressed, but not in naïve, mice, indicating a priming effect. Both NOP agonists reversed PMA induced by stress alone and by stress combined with GTN.
In NOP-expressing cells, N/OFQ induced receptor internalization and Gαi recruitment at both the plasma membrane and endosomes. In hSCs, N/OFQ attenuated CGRP-induced cAMP elevation.
In summary, although genetic deletion of NOP had no major effect on PMA, pharmacological activation of NOP receptors markedly reduced migraine-like pain triggered by CGRP, stress, and stress plus GTN. The comparable efficacy of UFP-112 and AT-403 underscores the importance of peripheral NOP receptors. These findings support the therapeutic potential of NOP agonists as anti-migraine agents.

Symposium 9 - From Pain to Addiction and Back: Unraveling Psychological and Neural Connections in OUD and AUD

Chair: Jesús David Lorente Erenas

Abstract

This symposium will discuss the central role of pain as a factor contributing to drug use disorders, focusing on alcohol and opioids because their shared neurobiological mechanisms implicating mu opioid receptor activation in the mesocorticolimbic system. This bidirectional relationship, that has been overlooked in the past, is now gaining attention in the field because of previously unknown common neural mechanisms implicating endogenous opioid system, that can finally create a positive feedback loop between the suffering of pain and opioid/alcohol use disorders.

To this end, we will present data analyzing this relationship, and its neurobiological substrates involving endogenous opioid system, from different perspectives: from pain to the development of opioid/alcohol use disorder (OUD/AUD) and from alcohol/opioid consumption to the development of pain-related states. In addition, and to complete the global overview of pain-OUD/AUD relationship this symposium presents clinical and preclinical data including sex as main studied factor and different live-stages: adolescence, adulthood and the effect of maternal exposure to the offspring. This comprehensive overview of this problem will definitively impulse the discussion on this topic and future connections and collaboration between INRC members.

Chronic Pain, Negative Affect, and Vulnerability to Substance Use Disorders: Clinical and Preclinical Evidence with a Gender Perspective

Jesús David Lorente Erenas, María Ros, Francisco Molins, Javier Cuitavi, Paula Andres Herrera, Ana Minguez, Vicente Monsalve, José De Andrés, Miguel Ángel Serrano, Lucía Hipólito, Faculty of Psychology, European University of Valencia

Negative affect and stress are fundamental factors involved in substance use disorders (SUDs). Thus, situations that increases stress and/or anxiety, such is the presence of pain, can potentially act as a risk factor to promote drug seeking and relapse. Pain and motivated behavior share neurobiological substrates and because of pain, mu and kappa signaling in the mesocorticolimbic system is altered leading to negative affective states and altered alcohol and opioid reward processing. Indeed, clinical and epidemiological data have highlighted the connection between chronic pain and alcohol/opioid use disorders (AUD/OUD). This study aims to analyze the relationship between the presence of chronic pain, the development of negative affective states and how these states can increase vulnerability to opioid analgesics and alcohol misuse in a clinical and preclinal set up. Data from a Spanish clinical sample of chronic pain revealed a positive and significant correlation between negative affective states (anxiety, depression and anhedonia) and the level of pain and higher scoring in the current opioid misuse measurement (COMM), especially in women. Interestingly, in our population, the scores of the alcohol use disorders identification test (AUDIT) were very low and non-correlated with the self-reported levels of pain. Additionally, utilizing a reverse-translational strategy, we also observed that previous exposure to a short-access and controlled dose of morphine self-administration procedure impairs the pain-induced alcohol drinking behavior in female rats. Ongoing studies are investigating possible involvement of kappa opioid receptor signalling in the mesocorticolimbic system in these effects of pain. All together our findings suggest that pain serves as a risk factor for increased opioid or alcohol consumption that is mediated by the presence of negative affective states, especially in female subjects. Nonetheless the management of the pain condition with a controlled access to morphine impairs such effect of pain on alcohol consumption. These results highlight the complex interplay between pain and opioid and alcohol consumption. Further investigation into the neurobiological mechanisms underlying these interactions is warranted, with potential implications for developing gender-specific interventions targeting SUDs in pain-afflicted populations.

Sex-specific effects of inflammatory pain on hyperalgesia during protracted morphine withdrawal

Jessica A. Cucinello-Ragland (PhD Postdoctoral Fellow, Washington University in St. Louis, School of Medicine), Lila Hirshfelt, Tania Lintz, Jose Moron-Concepcion

Chronic pain is a global epidemic, a leading cause of disability world-wide, and a major driver for the onset, progression, and maintenance of opioid use disorder (OUD). Indeed, chronic pain is a common factor for the initiation of prescription opioid use, and people with chronic pain are at increased risk for OUD. Further, cessation of use often produces withdrawal-induced hyperalgesia, or increased pain sensitivity. Although pain during abstinence increases risk for return to use, preclinical research has most commonly investigated withdrawal-induced hyperalgesia during acute opioid withdrawal. To address this gap, the current study investigated the effects of persistent inflammatory pain, induced by complete Freund's adjuvant (CFA), on morphine withdrawal-induced mechanical hyperalgesia across three weeks of withdrawal. Mixed-sex cohorts of adult C57B6J mice received subcutaneous hind paw injection of either saline or CFA one week before the onset of 6 daily intraperitoneal injections of either saline or morphine (10, 20, 30, 40, 50, 50 mg/kg). Mechanical nociception was measured using the electronic von Frey test at baseline, one-week post-hind paw injection, 24 hours following the last morphine injection, and once per week for three weeks following the last morphine injection.

All animals regardless of sex displayed mechanical hyperalgesia during acute (24 hours) withdrawal. However, female mice, regardless of CFA treatment, did not recover from this hyperalgesic state across three weeks of withdrawal. Interestingly, male mice not treated with CFA recovered from morphine withdrawal-induced hyperalgesia by 1 week of withdrawal, while CFA-treated males remained hyperalgesic throughout protracted withdrawal. These findings suggest that the presence of persistent inflammatory pain prolongs withdrawal- induced hyperalgesia in a sex-specific manner. Ongoing studies are investigating differential activation of both mu and kappa opioid receptors throughout the brain following CFA treatment and protracted morphine withdrawal with the goal of identifying disruption endogenous opioid receptor signaling as a mechanism for CFA-induced potentiation of withdrawal-induced hyperalgesia.

Adolescent alcohol exposure induced pain and associated neurobiology

Abigail M. Kelley, Madison C. Heitkamp, Anushree N. Karkhanis (Associate Professor, Developmental Exposure of Alcohol Research Center, Department of Psychology, Binghamton University – SUNY, Binghamton, NY, USA)

Risky drinking behaviors during adolescence, a neurodevelopmentally critical period, exacerbates pain and lengthens recovery. Similarly, rats exposed to ethanol during adolescence exhibit heightened pain sensitivity in a nucleus accumbens (NAc) shell- dependent manner. The kappa opioid receptor (KOR) in the NAc shell is also involved in regulating pain sensitivity and dopamine transmission. Thus, we sought to elucidate whether the dopamine-dependent changes in pain sensitivity observed following adolescent chronic intermittent ethanol vapor (aCIE) exposure functions via a KOR- mediated mechanism. We exposed rats to ethanol vapor throughout adolescence (PD 28- 68) to achieve blood ethanol concentrations of 180 mg/dL. After a 21-day protracted abstinence period, we measured tactile sensitivity with and without administration of KOR agonist, U50,488 (0, 1.25, 2.50 mg/kg). In a separate group of rats, we used ex vivo fast scan cyclic voltammetry to assess KOR-mediated changes in dopamine transmission during protracted abstinence. As shown before, tactile sensitivity was greater in aCIE compared to air-exposed male and female rats. KOR activation reduced this sensitivity in all rats, albeit the lower dose was sufficient to reduced it in aCIE-exposed male and female rats compared to their air-exposed counterparts. Baseline levels of dopamine were significantly higher in aCIE-exposed compared to air-exposed male and female rats. Interestingly, while KOR- mediated inhibition of dopamine release was augmented in female aCIE- compared to air- exposed rats, no difference was observed between air- and aCIE-exposed male rats. It is possible that while the KOR on dopamine terminals is sufficient in altering pain stated in females, involvement of KOR in other brain areas or cell-types is necessary to alter pain in males. Together, these data highlight sex-differences in overlapping limbic addiction and pain circuitry.

Effects of maternal pain and perinatal opioid exposure on future drug seeking

Hannah Harder (PhD Postdoctoral Fellow, Washington University in St. Louis, School of Medicine), Jose Moron-Concepcion

Opioid use by women of reproductive age often leads to a secondary population of drug- exposed infants, who suffer from neonatal opioid withdrawal syndrome (NOWS) at birth. Relatively little is known about how opioid exposure during critical periods of early development affects future behavior, particularly drug use. Although there is increasing interest in studying perinatal opioid exposure via preclinical rodent models, these models are often not clinically translatable due to a lack of real-world complexities. Especially understudied is the role of maternal pain: although the primary reason reported by pregnant women for opioid use is for pain relief, few studies have been completed to investigate the intersection of maternal pain and opioid use on offspring's future behavior. Preliminary clinical studies indicate divergent outcomes for children exposed to opioids used for pain relief vs. maintenance therapies or addiction, making the combined study of maternal pain and opioid use critical. We have developed a novel rat model of maternal pain and perinatal opioid exposure wherein offspring are exposed to maternal neuropathic pain via chronic constriction injury (CCI), intermittent chronic oxycodone exposure, or both. Male and female adult offspring were then implanted with jugular vein catheters to acquire oxycodone self-administration behavior. Male offspring exposed to oxycodone during development made fewer lever presses for oxycodone; this effect was largely normalized in male rats exposed to both maternal pain and oxycodone. In addition, oxycodone-exposed male offspring had significantly higher body weights and decreased mechanical sensitivity threshold, which was again normalized in oxycodone- and pain-exposed rats. These results suggest an interaction between maternal pain, perinatal drug exposure, and sex on future drug use and abuse.

Symposium 10 - The Opioid-Nociceptin Framework: Revealing Mechanisms of Neurotrauma

Chairs: Kelly Standifer & Georgy Bakalkin

NOP receptor modulation of functional and cellular outcomes following TBI

KM Standifer[1], R Ullah[1], Y Zhang[1], M Stiles[1,2] and DM Sherry[1,2]

1. University of Oklahoma Colleges of Pharmacy, Oklahoma City, OK USA
2. University of Oklahoma Colleges of Medicine, Oklahoma City, OK USA

Traumatic brain injury (TBI) is a major cause of disability and mortality. Closed head injuries are the most common, resulting from sports injuries, falls and motor vehicle accidents, and affecting people of all ages. Previous studies by us and others demonstrated that loss of N/OFQ peptide (NOP) receptor or treatment with antagonists or partial agonists improved co-morbid nociceptive hypersensitivity and anxiety-like behaviors noted in preclinical models of PTSD. They also improved axonal recovery, dendritic outgrowth, and cerebral blood flow following TBI or spinal injury. We hypothesized that treatment with a NOP partial agonist (AT-035) following TBI would mitigate the actions of acutely released N/OFQ and improve functional and cellular responses following repeated closed head TBI. TBI worsened neurological score, rotarod latency and anxiety-like behaviors, and increased sensitivity to thermal and mechanical stimuli. Behavioral changes corresponded with reduced neuronal labeling intensity and glial cell activation. Daily injections of AT-035 (1 mg/kg, ip) for 7 days improved neurological score, rotarod latency to fall, tactile sensitivity, and neuronal labeling intensity and reduced glial activation markers compared to vehicle-treated TBI rats. These results support the hypothesis and further exploration of NOP modulator therapy for neurotrauma.

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Kappa opioid receptor neuromodulation of lateral habenula in mild traumatic brain injury

Brain M. Cox, Dept Pharmacology USUHS, Bethesda, US

The newly identified dynorphin-KOR synaptic inputs to the LHb will be highlighted and it will be discussed how the endogenous dynorphin/KOR system may modulate the excitatory/inhibitory transmission ratio of neurons in the latera habenula following mild traumatic brain injury in subsets of mice, and its contribution to hyperexcitability in that brain region, potentially increasing vulnerability to the development of depression following mTBI.

Left–Right Side-Specific Endocrine Signaling in Brain Injury: The Opioid Mechanism

Georgy Bakalkin[1], Nikolay Lukoyanov[2], Mengliang Zhang[3] and Hiroyuki Watanabe[1]

1. Uppsala University, Sweden
2. Departamento de Biomedicina da Faculdade de Medicina da Universidade do Porto, Portugal
3. Department of Molecular Medicine, University of Southern Denmark, Odense, Denmark

Each cerebral hemisphere is functionally connected to the contralateral side of the body through decussating neural tracts. These crossed pathways underlie the well- known contralateral effects of brain injury, such as hemiparesis and hemiplegia. Our studies demonstrate that, in addition to neural mechanisms, contralateral injury effects are also mediated by the humoral pathway, through neurohormones —including opioid peptides— that elicit side-specific physiological responses (1-3).

Specifically, this left–right-specific humoral signaling determines whether unilateral brain injury leads to effects on the left or right limbs. The hormonal signals originate from the hypothalamus and pituitary gland and act via receptors that are lateralized in the spinal cord, influencing side-specific control of symmetrical spinal motor circuits innervating the limbs.

Identifying the relative contributions of neurohormonal vs. neural mechanisms in post- injury outcomes is essential for understanding brain trauma and stroke—and for guiding the development of new therapeutic strategies. More broadly, lateralized neuroendocrine signaling may represent a fundamental biological principle for the regulation of left–right processes in bilaterally symmetric animals.

Supported by the Swedish Research Council.

References

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Symposium 11 - Circuits, cell types, and neural dynamics underlying opioidergic neuromodulation

Chairs: Michael Bruchas & Grégory Scherrer

Abstract

Endogenous opioids such as enkephalin modulate nociception and reward. However, the precise circuits and cell types that release and respond to these neuropeptides remain poorly characterized. Further, how endogenous opioids influence neural dynamics during pain perception and motivated behaviors is unclear. This symposium will provide novel information on the mechanisms of function of endogenous opioids in neural circuits, while describing cutting-edge techniques to studying opioidergic circuits. Ms. Zamorano uses a combination of genetic, fiber photometric, optogenetic, and behavioral approaches to determine the role of μ-opioid receptor activity on downstream dopamine release in the ventral tegmental area (VTA) and how lateral hypothalamic enkephalinergic projections to the VTA modulate natural reward. Dr. Yarur will describe novel findings on the mechanisms by which enkephalinergic activation of μ- and δ- opioid receptors in prefrontal cortex microcircuitry regulates valence processing, using slice physiology, in vivo Ca^{2+} imaging, enkephalinergic sensors and cell-type-specific pharmacological tools such as drugs acutely restricted by tethering (DART). Prof. Scherrer will presults results from viral tracing, transcriptomic, multiplexed error-robust fluorescent in situ hybridization (MERFISH) studies as well as single cell Ca^{2+} activity recordings in behaving mice that elucidate the distinct architecture of the endogenous opioid system in cortical and subcortical circuits. Additionally, this symposium will include one to two shorter talk(s) selected from the poster abstracts that were submitted.

Catalina Zamorano, University of Washington

Opioid use disorder and opioid overdose death rates in the United States reached unprecedented levels during the COVID-19 pandemic. Understanding the critical role of endogenous opioid activity in natural reward behaviors is essential for understanding opioid use disorder, yet the fundamental mechanisms by which opioids affect these behaviors in the brain remain elusive. Here, we used a combination of genetic and molecular tools to isolate the role of μ-opioid receptor (MOR) activity in the ventral tegmental area (VTA) and identified a source of endogenous opioid release onto these receptors. To investigate the role of endogenous opioid activity on VTA^{GABA} neurons, we first used ex vivo 2-photon slice imaging. Preliminary results reveal that endogenous opioid peptides exert heterogenous effects on VTA^{GABA} neurons. Then, using fiber photometry and $Oprm1^{fl/fl}$ mice, we found that VTA MOR knockout decreased dopamine release to the cue but increased dopamine release to reward consumption in the nucleus accumbens (NAc) during Pavlovian conditioning. Next, using in situ hybridization and viral tracing methods, we identified enkephalin-containing neurons in the lateral hypothalamus (LH) that project to the VTA. Optical stimulation of these projections ex vivo elicited both excitatory and inhibitory post-synaptic currents. In vivo optical stimulation of these enkephalinergic projections during real-time place preference and intracranial self-stimulation suggests these projections play a role in reward and reinforcement. These results provide insight into the role of endogenous opioids in the VTA in modulating dopamine activity and motivated behavior. Current work is focused on further elucidating the role of endogenous μ-opioid activity in the VTA on GABA interneurons and determining how enkephalinergic projections from the LH to the VTA modulate natural reward. Ultimately, these studies lay the groundwork for understanding how opioid drugs of abuse elicit maladaptive changes in endogenous opioid systems to drive opioid use disorder.

Hector Yarur, National Inst. Mental Health

The medial prefrontal cortex (mPFC) plays a critical role in coordinating goal- directed behavior and executive function, processes often disrupted in neuropsychiatric conditions. These functions include learning context-dependent rules and integrating past experiences and outcome valence into schema representations that guide future behavior. Both opioid transmission and dysregulated mPFC activity have been linked to inhibitory-control deficits, which are characteristic of addiction and binge-type eating disorders. While endogenous opioids and their receptors are abundantly expressed in the mPFC, it remains unclear whether endogenous opioid transmission within this region directly modulates inhibitory control. In this study, we employed cutting-edge techniques to investigate the opioid system in mPFC circuitry. We found that enkephalin (Enk), mu-opioid receptors (MOR), and delta-opioid receptors (DOR) are expressed in distinct mPFC neuronal subpopulations. Enk was found in both excitatory and inhibitory neurons, with high expression in VIP and SST-interneurons subpopulations. MOR was robustly expressed in SST-positive interneurons, while DOR was expressed in SST- and PV- positive interneurons. These organizing principles and functional regulation of inhibitory interneurons are conserved in non-human and human primates. In-vivo calcium imaging of Enk- expressing neurons in the mPFC during a head-fixed associative learning task revealed their activation in response to both rewarding and aversive stimuli. These neurons appear to be actively engaged in processing task-related rewards and punishments. Preliminary data from Enk release monitoring using the μMASS biosensor indicated potential Enk release associated with task- related rewards and punishments. Additionally, blocking opioid signaling in the mPFC was found to alter behavior at both reward and aversive outcomes. Preliminary findings suggest that activating MOR signaling in the mPFC reduces food intake by increasing speed and distance traveled in food-restricted mice, suggesting that MORs may be constraining reward engagement. Using cell-type-specific pharmacological tools (DART), we observed that pharmacological blockade of

opioidergic transmission in SST-positive inhibitory interneurons within the mPFC increased lick frequency in response to reward outcomes, while no such effect was observed in PV-positive inhibitory interneurons. These results suggest that Enk signaling in the mPFC modulates reward-driven behavior, specifically through SST interneurons. Collectively, these findings shed light on the microcircuit mechanisms by which opioids regulate mPFC circuitry, ultimately influencing behavior by modulating specific interneuron subpopulations. This indicates that mPFC MOR signaling may play a role in modulating reward-driven behavior. Collectively, these results aim to shed light on the microcircuit mechanisms by which opioids potentially influence the exploitation-exploration tradeoff by regulating sustained engagement with rewards through actions in specific interneuron subpopulations.

Grégory Scherrer, University of North Carolina at Chapel Hill
Opioid receptors and peptides are broadly expressed along nociceptive neural circuits. Yet the region-specific organization by which each element of the endogenous opioid system modulates the sensory-discriminative, affective-motivational and cognitive-evaluative dimensions of pain experience remains elusive. We used single cell RNA- sequencing (snRNA-seq) and MERFISH to generate cellular and molecular atlases that clarify which cell types express each opioid peptide and receptor in the mouse brain, spinal cord and dorsal root ganglion. We discovered that standard scRNA-seq and bioinformatic methods can generate massive errors (~20-fold difference) in the quantification of cells expressing the μ-opioid receptor, based on which Oprm1 genomic regions containing reads were analyzed. We developed innovative approaches, including a novel gene annotation method, that promote both the retention of false negative and the removal of false positive Oprm1 reads, producing a faithful
atlas of Oprm1+ cell types in the nervous system. To investigate the cognitive dimension of pain, we then created a behavioral assay that generates placebo-like, endogenous opioid dependent, anticipatory pain relief in mice. In vivo calcium imaging and electrophysiological recordings in brain slices showed that expectations of pain relief increase the activity of ACC neurons that project to the pontine nucleus (Pn), a pre-cerebellar nucleus, and potentiate neurotransmission in this pathway. Optogenetic inhibition of the ACC→Pn→cerebellum pathway disrupted placebo analgesia and decreased pain thresholds, whereas activation elicited analgesia in the absence of placebo conditioning. Finally, we used a skilled reach-to-grasp task to determine how opioidergic neuromodulation impacts cortical neural dynamics and performance in behavioral assays, focusing on enkephalinergic signaling and Penk-expressing neurons. We found that superficial Penk+ neurons show distinct calcium activity patterns at the end of the reach-to-grasp behavior and that optogenetic stimulation of these neurons slows down behavior termination. These findings advance our understanding of the circuit-specific organization of the endogenous system by which opioid peptides and receptors modify behaviors.

Endogenous opioid receptor-mediated regulation of prefrontal cortex microcircuitry and valence processing

Hector Yarur[1], Valerie Tsai[1], Chloe Noh[1], Huikun Wang[1], Alice Graham[1], Brenda Shields[3], André Berndt[2], Michael Tadross[3], and Hugo Tejeda[1]

1. National Institute of Mental Health
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3. Duke University

The medial prefrontal cortex (mPFC) plays a critical role in coordinating goal-directed behavior and executive function, processes often disrupted in neuropsychiatric conditions. These functions include learning context-dependent rules and integrating past experiences and outcome valence into schema representations that guide future behavior. Both opioid transmission and dysregulated mPFC activity have been linked to inhibitory-control deficits, which are characteristic of addiction and binge-type eating disorders. While endogenous opioids and their receptors are abundantly expressed in the mPFC, it remains unclear whether endogenous opioid transmission within this region directly modulates inhibitory control. In this study, we employed cutting-edge techniques to investigate the opioid system in mPFC circuitry. We found that enkephalin (Enk), mu-opioid receptors (MOR), and delta-opioid receptors (DOR) are expressed in distinct mPFC neuronal subpopulations. Enk was found in both excitatory and inhibitory neurons, with high expression in VIP and SST-interneurons subpopulations. MOR was robustly expressed in SST- positive interneurons, while DOR was expressed in SST- and PV-positive interneurons. In-vivo calcium imaging of Enk-expressing neurons in the mPFC during a head-fixed associative learning task revealed their activation in response to both rewarding and aversive stimuli. These neurons appear to be actively engaged in processing task-related rewards and punishments. Preliminary data from Enk release monitoring using the μMASS biosensor indicated potential Enk release associated with task-related rewards and punishments. Additionally, blocking opioid signaling in the mPFC was found to alter the perception of both rewards and aversive outcomes. Using cell- type-specific pharmacological tools (DART), we observed that pharmacological blockade of opioidergic transmission in SST-positive inhibitory interneurons within the mPFC increased lick frequency in response to rewards, while no such effect was observed in PV-positive inhibitory interneurons. These results suggest that Enk signaling in the mPFC modulates reward-driven behavior, specifically through SST interneurons. Collectively, these findings shed light on the microcircuit mechanisms by which opioids regulate mPFC circuitry, ultimately influencing behavior by modulating specific interneuron subpopulations. Preliminary findings suggest that activating

MOR signaling in the mPFC reduces food intake but increases speed and distance traveled in food-restricted mice. This indicates that mPFC MOR signaling may play a role in modulating reward-driven behavior. Collectively, these results aim to shed light on the microcircuit mechanisms by which opioids influence the “exploitation-exploration dilemma”, regulating mPFC circuitry and motivating behavior through the inhibition of specific interneuron subpopulations.

Symposium 12 - Opioids Beyond Pain- Roles in Mood and Motivation

Chairs: Kathryn Braden & Anne Z. Murphy

Abstract

Pain is the primary indication for opioid treatment clinically and decades of research have characterized the analgesic effects of both endogenous and exogenous opioids. But the experience of pain is more than nociception alone; and opioids have effects far broader than analgesia. In this panel we will highlight some of the efforts being made to understand how both the endogenous opioid peptides and exogenous agonists can modulate mood and motivation on their own and in the context of pain.

Dr. Murphy will share a project from her lab examining how early life opioid exposure modulates stress responses and motivational circuitry in later life. This work highlights how exposure to opioids during development can have long-term consequences. Dr. Braden will share her postdoc work investigating how enkephalin within the Dorsal Raphe Nucleus (DRN) acts to buffer aversive responses to various stimuli, including pain. These studies implicate a convergent mechanism between pain and aversion that may be relevant to motivational dysregulation during chronic pain. Dr. Corder will share a project from his lab exploring how a distinct neural population within the ventrolateral periaqueductal gray (vlPAG) integrates pain, fear, and placebo analgesia through opioid-mediated signaling. These studies reveal the dynamic interplay of cognitive and nociceptive states on midbrain circuits, shedding light on novel targets for non-addictive pain management strategies and the broader role of endogenous opioids in mood and motivation. Finally, Dr. Eikemo will share work from her lab considering subjective and behavioral effects of opioids in healthy human volunteers and surgery patients. This clinical perspective will give insight into how subjective responses in humans can be used to inform the design of preclinical studies to better capture the elusive emotional experience of pain.

Early Life Opioid Exposure Effects on Mood and Motivation

Anne Z. Murphy, Ph.D. Professor
Neuroscience Institute, Georgia State University

Dorsal Raphe Nucleus Enkephalin Modulates Pain and Aversion

Kathryn Braden, Ph.D.
Postdoctoral Research Fellow, Castro Lab
Mallinckrodt Institute of Radiology, Washington University in St. Louis

Converging Pain and Placebo Mechanisms in Midbrain Opioid Circuits

Blake Kimmey Department of Psychiatry and Neuroscience, Perelman School of Medicine, University of Pennsylvania

Subjective Experience and Motivation for Opioids in Humans: Experimental and Clinical Evidence

Marie Eikemo, Ph.D. Researcher
Department of Psychology, University of Oslo, Norway

Dorsal Raphe Nucleus Enkephalin Modulates Pain and Aversion

Kathryn Braden, Ph.D.
Postdoctoral Research Fellow, Castro Lab
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Chronic pain is a complex disease, commonly associated with affective comorbidities such as depression, anxiety, and increase risk of suicide. It is important to understand the basic neurobiology behind the motivational dysregulation of pain to effectively treat it. Endogenous opioids in dorsal midbrain nuclei such as periaqueductal grey (PAG) and dorsal raphe nucleus (DRN) can regulate pain perception as well as mood and motivation. The PAG is canonically associated with opioid-mediated descending pain inhibition and is desensitized during chronic pain states. The specific role of the DRN during chronic pain has not been characterized despite studies showing opioid activity here also modulating pain and motivated behaviors. The aim of this study was to investigate the functional significance of DRN enkephalin signaling in pain sensitivity and related affective and motivational behaviors.

Methods

We disrupted preproenkephalin (PENK) in DRN using a Cre-dependent CRISPR- Cas9 viral vector injected into the DRN of Penk-Cre+ mice or their Cre- littermate controls. We ran a battery of appetitive and aversive behavioral assays to assess how knockdown of DRN enkephalin altered behavioral readouts in Cre+ mice compared to Cre- mice.

Results

We found that intraplantar injection of low-dose carrageenan reduced paw withdrawal thresholds by 50% in

Cre- controls, whereas knockdown of enkephalin peptide in the DRN produced a greater reduction (70%). Similarly, in an odor avoidance assay Cre- mice spent twice as much time sniffing an aversive odor than Cre+ mice. In appetitive behaviors such as social interaction, Cre- mice spent about 30% more time with a mouse than with a novel object but this was reduced in Cre+ mice to only 20%. Additionally, Cre- mice had an 85% preference for sucrose over water but this was reduced by 15% when PENK was knocked down in the DRN.

Conclusions
These results suggest that DRN enkephalin peptide acts to buffer aversive responses and its absence results in a shift towards enhanced avoidance of both aversive and appetitive stimuli. This attenuated reward/enhanced aversion phenotype is similar to behavioral changes observed in chronic pain states. Future studies will further investigate how DRN enkephalin contributes to affective dysregulation during chronic pain.

Symposium 13 - The Endogenous Opioid System and Chronic Pain: Recent Advances and Therapeutic Potential of Opioid Peptides

Chair: Stephen Bruehl

Abstract

Opioid analgesic medications are effective for pain management but their adverse effects when used daily in the long-term setting are well-documented, including hyperalgesia, tolerance, opioid misuse, and opioid use disorder. These adverse effects contraindicate their use for chronic pain management. Peptides are short chains of amino acids that exhibit biological activity.

Endogenous opioid peptides that bind to the three main classes of opioid receptors (mu, kappa, and delta) modulate not only pain, but also reward and aversion, and as analgesics may be associated with fewer adverse effects than traditional opioid analgesics. However, clinical use of endogenous opioid peptides has been limited by poor metabolic stability and blood-brain barrier penetration, making them ill-suited as therapeutic agents. This symposium will overview a diverse range of recent research on endogenous opioid peptides suggesting enhanced potential for their use as therapeutic analgesics. Topics addressed will include: 1) the impact of endogenous opioids on human chronic pain and responses to analgesic medications, 2) a novel optogenetic approach to studying endogenous opioid system dynamics in vivo, 3) the role of the endogenous kappa opioid receptor agonist dynorphin in chronic pain-related negative affect and its potential as a therapeutic target, and 4) the development of both centrally- and peripherally- acting endogenous opioid peptide analogues that show biological stability and analgesic efficacy in preclinical acute and chronic pain models.

Recent findings on the role of endogenous opioids as modulators of human chronic pain and responsiveness to opioid analgesic medications

Stephen Bruehl, Department of Anesthesiology, Vanderbilt University Medical Center, U.S.A.

Unlocking opioid neuropeptide dynamics with genetically encoded biosensors

Akash Pal, Ph.D., Yihan Jin, Ph.D., and Lin Tian, Ph.D. Max Planck Florida Institute for Neuroscience, U.S.A.

Dynorphin and pain-related negative affect: an opportunity for intervention?

Lucia Hippolito, Ph.D.[1] and Jose Moron-Concepcion, Ph.D.[2]
1. Department of Pharmacy and Pharmaceutical Technology, University of Valencia, Spain
2. Department of Anesthesiology, Washington University School of Medicine, U.S.A.

Development and analgesic activity of both centrally- and peripherally-acting endogenous opioid peptide analogues for chronic pain management

Azzurra Stefanucci, Department of Pharmacy, University of Chieti-Pescara, Italy

In the first presentation, Dr. **Bruehl** will describe human research examining the impact of individual differences in endogenous opioid function on chronic pain experience and responses to opioid analgesic medications. In this work, endogenous opioid function was quantified as the change in evoked laboratory pain responses between placebo and opioid blockade (i.v. naloxone) conditions. Results of these studies indicate that: 1) endogenous opioids inhibit chronic back pain intensity in a real world setting, 2) endogenous opioid analgesia can be increased by aerobic exercise training, and 3) individuals with low endogenous opioid inhibition exhibit greater analgesic and subjective responsiveness to opioid analgesics, suggesting that opioid analgesic medications might serve to supplement low endogenous opioid activity.Next, researchers from Dr. Tian's lab will present work regarding the use of novel optogenetic methods to study endogenous opioid system dynamics in vivo. Neuropeptide research has been limited by a lack of experimental tools that allow for the precise dissection of their complex and diverse dynamics in a circuit-specific manner. Development of a class of genetically encoded fluorescence sensors based on kappa, delta and mu opioid receptors to illuminate the spatiotemporal dynamics of endogenous opioid signaling in the brain will be described. Findings using this methodology to be discussed include determining the spatiotemporal scale of dynorphin volume transmission in brain slices and in vivo work detecting optogenetically-driven opioid release and differential opioid release dynamics in response to fearful and rewarding conditions. Recent progress to further optimize and expand the color-spectrum of these sensors to enable multiplex imaging with neuromodulators will be described.

In the third presentation, work in Dr. **Moron-Concepcion**'s lab will be described regarding the role of dynorphin, the endogenous agonist for the kappa opioid receptor (KOR), in pain-related negative affect. Evidence that dynorphin expression is increased in chronic pain conditions will be presented. Results of micro PET studies in rodents showing how pain affects dynamic interactions between KOR and dynorphin in the brain will also be described. Next, chemogenetic data showing that silencing dynorphin-containing neurons in the mesolimbic brain pathway prevents pain-induced negative affect will be overviewed. Then, potential therapeutic approaches will be described including development of novel intranasal formulations for kappa opioid receptor (KOR) antagonists to prevent elevated chronic pain-induced negative affect that contributes to escalation of opioid intake. Finally, recent work will be presented regarding the development of nanoliposomes loaded with KOR antagonists to prevent brain penetration and to allow for micro dosing that will avoid unwanted opioid adverse effects.

In the final presentation, Dr. **Stefanucci** will discuss her lab's development of both centrally- and peripherally-acting endogenous opioid peptide analogues for chronic pain management with reduced adverse opioid effects. Although opioid peptides exhibit therapeutic profiles with advantages over traditional opioid analgesics, their clinical use has been limited by poor metabolic stability and blood-brain barrier penetration. Work in Dr. Stefanucci's lab seeks to address these limitations. Bivalent mu opioid receptor (MOR) and delta opioid receptor (DOR) ligands are viable analgesics with improved therapeutic profiles versus selective MOR agonists. This presentation will overview the development and evaluation of MACE2, a bivalent MOR/DOR cyclic peptide with high metabolic stability and blood-brain barrier (BBB) penetration that exhibits high analgesic efficacy in a neuropathic chronic pain model. An alternative approach to reducing adverse opioid effects including tolerance is the development of peripherally-restricted opioid peptides. The presentation will describe the design and development of novel endogenous opioid peptide analogues with enhanced antinociceptive effects in vivo after peripheral administration that exhibit limited BBB penetration and high plasma stability. Therapeutic potential of these endogenous opioid peptide analogues will be discussed.

Symposium 14 - Prenatal opioid exposure and its molecular, cellular, and behavioral outcomes

Chairs: Julia R. Ferrante & Brady K. Atwood

Abstract

Prenatal opioid exposure has been a growing public health concern in many countries around the world. Prenatal opioid exposure produces many negative outcomes in children that persist well past the neonatal opioid withdrawal stage. However, little is known about the mechanisms whereby prenatal opioid exposure produces these impairments. Animal models of prenatal opioid exposure allow for deciphering molecular and cellular changes that result from this exposure and how those changes may relate to cognitive and behavioral difficulties measured in the prenatally exposed offspring. This proposal features 4 investigators that use highly translational animal models of prenatal opioid exposure to elucidate how prenatal opioid exposure affects the brain and behavior. The speakers are diverse in their backgrounds and in their career stages which supports INRC's goals regarding the composition of their speakers at their annual meeting.

Neonatal Opioid Exposure And Withdrawal Alters Microglia Regulation

Julia Ferrante[1], Khaled Althobaiti[2], Michelle E. Ehrlich[2]* and Julie A. Blendy[1]*

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2. Department of Neurology, Icahn School of Medicine at Mount Sinai, USA
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Infants exposed to opioids in utero are at risk of developing Neonatal Opioid Withdrawal Syndrome (NOWS), a combination of acute somatic withdrawal symptoms. In adult rodent models, there is evidence that morphine induces a pro-inflammatory response within the central nervous system, primarily through activation of microglia. It is unknown how in utero morphine exposure impacts developing microglia, and whether microglia play a role in producing withdrawal symptoms following perinatal exposure. We developed a mouse model of prenatal opioid exposure that encompasses the developmental equivalent of all three trimesters of human pregnancy ("three-trimester mice") in which mice receive morphine throughout gestation and the first two post-natal weeks. Our model produces significant developmental delays and spontaneous opioid withdrawal 24 hours following the final morphine injection. Microglia levels were assayed with Iba1 to examine microglia levels at postnatal day (PND)14 and PND15, timepoints representing chronic morphine exposure and spontaneous withdrawal, respectively. In multiple brain regions, microglia were increased in morphine treated mice at PND14, but levels were normalized at PND15. In a separate cohort of mice, microglia were isolated at PND14 and PND15 and sequenced. Gene ontology analysis revealed enrichment of cell cycle processes. Having established that our model causes quantitative and transcriptional changes to microglia, we assessed microglia as a therapeutic target in treating opioid withdrawal symptoms. Three-trimester mice were treated with PLX3397 to deplete microglia from PND7-12, and spontaneous opioid withdrawal was assessed. MOR-PLX3397 mice showed a reduction in somatic signs of withdrawal and thermal hyperalgesia, but not ultrasonic vocalizations, indicating that microglia may play a role in the production of physical, but not affective, withdrawal symptoms. Overall, this data suggests that early-life microglial modulation has potential as a novel therapeutic intervention for neonatal opioid withdrawal.

Behavioral And Biochemical Consequences Of Prenatal Methadone Exposure: Motor Effects And Changes In The Motor Cortex (M1) In Mice

Davian West, and Brady K. Atwood

Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN, USA

Prenatal methadone exposure (PME) poses significant risks to neurodevelopment, potentially altering sensorimotor behavior and function. Understanding PME's effects on the motor cortex (M1) is essential for elucidating mechanisms underlying altered motor behavior in exposed offspring. Here we investigate PME's motor effects and associated biochemical changes in M1, focusing on the differential expression and function of ADRA2A and HCN channels, their impact on glutamate transmission, and implications for synaptic function and motor behavior in PME mice. To test this, our lab developed a translationally relevant model of mice prenatally exposed to methadone, compared to prenatal saline controls. Motor behaviors were assessed using open field, rotarod, surface righting, and forelimb grip strength tests. Biochemical changes in M1 were analyzed through proteomics, immunohistochemistry, and Western blot techniques to evaluate neuronal density and protein expression patterns. Our findings indicate PME mice exhibit increased locomotor hyperactivity compared to controls. Biochemical analyses revealed altered expression of ADRA2A and HCN channels in M1, linked to changes in glutamate transmission and synaptic function. These findings highlight the significant impact of PME on motor behavior and biochemical alterations in M1. These findings underscore the importance of early interventions and the development of therapeutic strategies to mitigate the long-term effects of prenatal methadone exposure. Our ongoing research is aimed at elucidating the precise mechanisms underlying

these alterations and their relevance to human health, with the goal of finding potential therapeutic targets, as well as informing clinical practices and policies regarding prenatal drug exposure.

Long-Term Consequences Of Perinatal Morphine Exposure On Immune Signaling In Male And Female Rats

Hannah Harder and Anne Z Murphy

Neuroscience Institute, Georgia State University, USA

As a result of the current opioid crisis, the rate of children born exposed to opioids has skyrocketed. Later in life, these children have an increased risk for hospitalization and infection, raising concerns about potential immunocompromise, as is common with chronic opioid use. Opioids can act directly on immune cells or indirectly via the central nervous system to decrease immune system activity, leading to increased susceptibility, morbidity, and mortality to infection. However, it is currently unknown how perinatal opioid exposure (POE) alters immune function. Using a clinically relevant and translatable model of POE, we have investigated how baseline immune function and the reaction to an immune stimulator, lipopolysaccharide, is influenced by in utero opioid exposure in adult male and female rats. We report here that POE potentiates the febrile and neuroinflammatory response to lipopolysaccharide, likely as a consequence of suppressed immune function at baseline (including reduced antibody production). This suggests that POE increases susceptibility to infection by manipulating immune system development, consistent with the clinical literature. Investigation of the mechanisms whereby perinatal morphine increases susceptibility to pathogens is critical for the development of potential interventions for immunosuppressed children exposed to opioids in utero.

Perinatal Opioid Exposure Affects Executive Function And Prefrontal Cortical Transcriptional Responses In A Sex-Dependent Manner

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Healthcare resources are improving the survival rates for babies exposed to opioids in utero, however children born to opioid-using mothers have cognitive deficits, attentional problems, and are frequently diagnosed with ADHD. We are interested in better understanding the underlying neurobiological changes that link perinatal opioid exposure to changes in the developing brain and behavior, with a focus on prefrontal cortex. To that end, mouse dams are exposed to morphine or saline, prior to and during pregnancy, and extending through lactation. Transcriptional profiling is used to test whether perinatal MO exposure would cause sex-specific transcriptional changes in microglia that would relate to offspring executive function outcomes. Executive function is evaluated using the 5-choice serial reaction time task. Male MO-exposed offspring had reduced accuracy and female MO- exposed offspring had increased inattentive behavior. There were a similar number of genes altered in female vs. male microglia, but only 3 differentially expressed genes were evident in both sexes. Further, hierarchical clustering analysis and WGCNA identified genes that related to behavioral deficits. Together, our data identify individual genes and pathways in microglia within each sex that may relate to executive function deficits observed after perinatal opioid exposure, even though the transcriptional profiles are highly divergent between the sexes.

Symposium 15 - Novel insights into fentanyl's diverse effects across central and peripheral systems

Chairs: Kasey Girven & Daniel Castro

Abstract

The opioid epidemic is a nationwide condition affecting more than 2.5 million people in the United States. The most recent wave in overdoses is driven by synthetic opioids like fentanyl, which has high potency and lethality. This symposium brings together research on the neurobiological and metabolic effects of opioids, with a particular focus on fentanyl, across different brain regions and peripheral systems. Each speaker will showcase a suite of high-resolution methods that have expanded our understanding of fentanyl's effects. First, we will have a talk exploring how fentanyl modulates reward learning in the ventral tegmental area (VTA), identifying sex-specific differences in opioid-associated learning and the preferential activation of Cck-expressing VTA neurons. The second study uses intersectional viral strategies to examine how fentanyl alters gene expression in VTA projection neurons to the prefrontal cortex, nucleus accumbens, and basal amygdala, revealing distinct molecular and circuit-level differences during fentanyl exposure versus memory retrieval. Next, we will focus on understanding a less well-studied neuropeptide called neuropeptide S and its modulation of orbitofrontal cortex-mediated drug-seeking in an oral fentanyl self-administration task. Finally, we will address the metabolic consequences of opioid use, specifically fentanyl, on glucose metabolism, showing that opioid receptor signaling in pancreatic islets significantly influences insulin and glucagon secretion, with potential implications for opioid-induced metabolic dysfunction in individuals with comorbid conditions like diabetes. Together, these studies provide novel insights into the neural circuits underlying opioid reward and learning, as well as their peripheral effects on metabolic health, offering a comprehensive view of fentanyl's impact on both the brain and body.

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A Peri-Ceorulear Neuropeptidergic Pathway for Modulating OFC-Mediated Opioid- Seeking Behavior

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A relatively unknown neuropeptide, neuropeptide S (NPS), drives reward-seeking through activation of its cognate Gq-coupled protein receptor NPSR1. However, these studies lack the tools necessary to understand the system's role in reward-seeking. We generated both NPS-Cre and NPSR1-Cre driver mouse lines and isolated a population of NPS-containing cells that reside partly within and medial to the locus coeruleus (LC), known as the periLC. We found that the periLC sends excitatory projections to the orbitofrontal cortex (OFC) that connect directly with NPSR1-expressing neurons. Therefore, we hypothesized that periLC-NPS enhances reward-seeking through increased activation of OFC-NPSR1 post reward consumption. NPSR1-Cre and NPS-Cre mice received unilateral infusions of AAVDJ-DIO-GCaMP6s to either the OFC or periLC, respectively. In addition, all mice received unilateral fiber photometry implants to either the ipsilateral OFC for OFC-NPSR1 cell body recordings, or the ipsilateral periLC for periLC-NPS cell body recordings. These mice underwent Pavlovian and operant conditioning for sucrose reward, and an oral fentanyl self-administration task where mice received 120min sessions where they could nosepoke for sipper delivery of fentanyl solution (10 ug/ml). In a separate cohort, NPSR1-Cre mice expressing AAVDJ-DIO- GCaMP6s in the OFC with a 1mm diameter GRIN lens positioned above underwent two- photon imaging during Pavlovian conditioning for both natural and fentanyl reward. We found that OFC-NPSR1 neuron activity increases to cue delivery, but reduces during consumption of reward showing a bidirectional response. Then post consumption, these neurons have a rebound increase in activity. We then determined that during this post consumption event, periLC-NPS neuron activity is aligned and activated, an effect that is sensitive to periLC-NPS CRISPR deletion. These results continue to establish a critical role for the NPS system in both natural and drug-seeking behaviors.

Symposium 16 - Sex-specific mechanisms underlying opioid reward, motivation, and pain-related outcomes

Chair: Jessica Higginbotham

Abstract

Sex differences in opioid-mediated effects may contribute to the gender disparities in opioid misuse liability in the US. However, our understanding of the neural mechanisms underlying these differences, effectors, and negative outcomes remain limited. Therefore, this session will highlight emerging preclinical research focused on sex differences in opioid reinforcement, opioid-evoked plasticity, and opioid interactions with pain processing. Sexual dimorphisms in synaptic, cellular, and circuit-based mechanisms will be detailed in the context of opioid addiction. First, Dr. **Emilia Lefevre** will present her research on sex differences observed in the role of interneurons in fentanyl-evoked behavior. Her studies show that inhibition of nucleus accumbens (NAc) fast-spiking interneurons blunts the development of fentanyl sensitization in female but not male mice. In contrast, motivation for fentanyl self-administration is augmented by the inhibition of these interneurons in male but not female mice. Dr. Lefevre's studies suggest that fast-spiking interneuron circuitry plays a sex-specific role in opioid addiction-like behaviors. Next, Dr. **Yanaira Alonso-Caraballo** will focus on sex differences in the role of the paraventricular nucleus of the thalamus (PVT) to NAc shell (NAcSh) pathway in opioid abstinence. Her studies demonstrate that female rats express similar oxycodone self-administration, but higher levels of relapse compared to males after prolonged periods of abstinence. After long periods of abstinence, glutamatergic transmission in the PVT-NAc pathway increases in both males and females, with females exhibiting a slight increase in glutamatergic transmission earlier than males. Adding to this, she will provide data testing the hypothesis that inhibition of the anterior PVT-NAcSh decreases relapsing behaviors after long periods of abstinence from opioid administration. Then, Dr. **Tiffany Wills** will present work investigating the interaction of adolescent oxycodone exposure (AOE) and adult alcohol use to produce long-term changes in hyperalgesia. Her research demonstrates that AOE and adult alcohol produces long-term mechanical and thermal hyperalgesia that does not dissipate. Interestingly, the emergence of this phenotype is dependent on both, AOE and adult alcohol use, and is only observed in females. Adding to this, Dr. Wills will also discuss how this phenomenon produces higher sensitivity to a pain challenge and how it may be driven by sex differences in extended amygdala circuits. Finally, Dr. **Jessica Higginbotham** will discuss interactions between inflammatory pain and ovarian hormones that lead to maladaptive patterns of fentanyl use and altered function of dopamine reward circuitry. Using wireless in vivo fiber photometry, she will show how pain increases self-administration of fentanyl and associated dopamine neuron responses, selectively in males. Her talk will provide evidence for pain- and sex-specific estradiol mediated suppression of opioid-evoked dopamine neuron activity and describe its role in sexually divergent trends in opioid use under conditions of pain.

Sex-specific role of interneuron circuits in fentanyl-evoked behavior

Emilia Lefevre, PhD (Speaker), Assistant Professor, Department of Biomedical Sciences, University of Minnesota Duluth

Mapping the contribution of the PVT-NAc pathway on opioid abstinence

*Yanaira Alonso-Caraballo, PhD (Co-chair and Speaker), Postdoctoral Fellow, Paul Mermelstein and Mark Thomas Labs, Department of Neuroscience & Medical Team on Addiction, University of Minnesota

Sex differences in hyperalgesia from adolescent oxycodone and alcohol exposures

*Tiffany Wills, PhD (Speaker), Associate Professor, Department of Cell Biology & Anatomy, Louisiana State University Health Sciences Center

Sex-specific mechanisms underlying pain-facilitated fentanyl use

*Jessica Higginbotham, PhD (Chair and Speaker), Postdoc, Moron-Concepcion Lab, Department of Anesthesiology, Washington University School of Medicine

Symposium 17 - Novel Optical and Pharmacological Strategies to Probe the Opioid System In-vivo

Chair: Luca Posa

Abstract

Recent years have led to an increased appreciation of the complexity of opioid receptor signaling at the molecular, cellular, and neural circuit levels. To dissect the underlying mechanisms that mediate opioid action and to better harness opioid receptors as therapeutics, new techniques are needed to mechanistically probe opioid receptors with increased spatial, temporal, and genetic precision. This symposium will present and discuss transformative advancements in the development and application of targeted optical sensors and pharmacological approaches, which are revolutionizing our ability to investigate the opioid system in a wide range of contexts. Unlike traditional methods for detecting G protein-coupled receptors (GPCRs), innovative optical tools such as genetically encoded opioid sensors, photoactivable ligands, optogenetic approaches, and advanced nanobodies enable precise, real-time monitoring and manipulation of receptor activity with spatial and temporal resolution. These tools provide unique advantages for dissecting GPCR signal transduction and advancing discovery and therapeutic exploration.

The symposium will include a mix of cutting-edge work at the level of molecular engineering, structural biology, cellular physiology, neural circuit dissection, and behavioral modulation in the therapeutic contexts of pain management, addiction, and mood regulation. Dr. **Luca Posa** will present advances in the engineering of photochromic peripherally-restricted "opto-opioids," which allow precise spatial and temporal control of opioid receptor-mediated analgesia. Dr. **Raaj Gowrishankar** will present the characterization and application of novel opioid sensors, demonstrating rapid timescale opioid release during reward-related behavior in vivo. Dr. **Miriam Stoeber** will present the development and application of novel nanobodies that sense and modulate opioid receptors by selectively binding to extracellular or intracellular receptor domains. Finally, Dr. **Joshua Levitz** will present his work harnessing photopharmacological approaches to pinpoint the contribution of opioid receptors to the antidepressant action of ketamine. By integrating these advanced methodologies, we aim to deepen our understanding of the fundamental neuromodulatory mechanisms of opioid receptors and facilitate the development of safer, more effective therapies for conditions ranging from chronic pain to opioid use disorder to depression. Attendees will gain valuable insights into these advanced methodologies, which collectively deepen our understanding of the opioid system and have the potential for widespread application.

Importantly, these innovations align with the INRC meeting's focus on novel areas of opioid-related research for more effective therapeutic approaches. The diverse panel, from a wide range of nationalities, featuring two faculties at different career stages with impactful contributions in the opioid receptor field, two postdoctoral associates, one woman, three men from diverse ethnical backgrounds, and one representative of the LGBTQ+ community, is designed to engage attendees and foster interaction.

A photoswitchable morphinan agonist for reversible optical control of peripheral mu-opioid receptors in vivo

Luca Posa, Department of Biochemistry, Weill Cornell Medicine, New York, New York, USA

Elucidating the role of rapid endogenous opioid release for goal-directed behavior

Raaj Gowrishankar, University of Washington, Seattle, Washington

Sensing and modulating opioid receptor function with nanobodies

Miriam Stoeber, Department of Cell Physiology and Metabolism, University of Geneva, Geneva, Switzerland

Photopharmacological and transcriptomic mapping of opioid contributions to ketamine action to identify next-generation antidepressant targets

Joshua Levitz, Department of Biochemistry, Weill Cornell Medicine, New York, New York, USA

Symposium 18 - Exploring the Interplay of Opioids, Cannabinoids, and Neuroimmune Mechanisms in Pain

Chair: John K. Neubert

Abstract

Pain, addiction, and the opioid crisis have recently come to the forefront of public awareness as significant societal and economic issues. Chronic pain and neuroinflammation are complex conditions that profoundly affect patients' quality of life, necessitating a deeper understanding of their underlying mechanisms to develop more effective therapies. Recent studies have highlighted the dual roles of opioids and cannabinoids in modulating pain and inflammation through interactions with the neuroimmune system. These substances offer analgesic effects while also impacting neuroinflammatory pathways, presenting both opportunities and challenges for treatment. This symposium aims to explore the intricate mechanisms driving pain and neuroinflammation, with a focus on the roles of opioids, cannabinoids, and their bidirectional interactions with the neuroimmune system. Presentations will cover a wide range of research topics, including patch-seq characterization of joint neurons (Caudle); transcriptomic interrogation of the spinal-opioid circuits (Iadarola); the modulation of opioid effects by inflammasome NLRP3 (McLaughlin); and behavioral consequences of opioid and cannabinoid use in rodent pain models (Murphy). By bringing together experts in pharmacology, neuroscience, and clinical practice, this symposium will provide a comprehensive overview of current advancements and future directions in pain treatment. Attendees will gain insight into the molecular and cellular mechanisms involved, as well as the effects of opioid and cannabinoid therapies on pain and behavior in rodent models. The discussion will also cover emerging therapeutic approaches that balance the potential benefits of opioids and cannabinoids while minimizing adverse effects. Ultimately, the goal is to develop novel treatment strategies that maximize therapeutic benefits and improve patients' quality of life.

Patch-Seq Analysis of Joint Innervating Primary Afferent Neurons: Identifying Potential CBD targets

Robert M. Caudle, Department of Oral & Maxillofacial Surgery College of Dentistry, University of Florida

- Patch-Seq of TG TMJ and DRG knee capsule innervating neurons demonstrates that they are primarily nociceptive and express several proteins known to bind CBD.
- The single neuron transcriptomic data indicates these neurons have very few opioid, CB1, CB2, or Trpv1 receptors.
- The TG transcriptomic data also demonstrates differences between males and females that suggest potential mechanisms for why females have a higher incidence of TMD than males.

Human Spatial Transcriptomic Analyses of Spinal Opioid Circuits

Michael J. Iadarola, Department of Perioperative Medicine Clinical Center, National Institutes of Health

- Spatial transcriptomics of human spinal cord identified opioid peptide-expressing neurons in dorsal spinal cord
- The mu opioid receptor is the major receptor in cord, followed by kappa, then delta
- A population of spino-thalamic projection neurons also express the mu-opioid receptor
- Proenkephalin and Mu-receptors are co-expressed in some neurons suggesting autoreceptors
- A broad network of opioid peptides and receptors characterize the superficial layers of human spinal cord.

NLRP3 Inflammasome Modulation of Opioids and Pain

Jay McLaughlin, Department of Pharmacodynamics College of Pharmacy, University of Florida

- Prolonged morphine use activates the NLRP3 inflammasome, but little is known about NLRP3 modulation of basal opioid activity.
- Inhibition of NLRP3 with MCC950 potentiated the antinociceptive effects of morphine and fentanyl, but also exacerbated morphine-induced respiratory depression and conditioned place preference.
- MCC950 also reduced morphine-induced antinociceptive tolerance and hyperalgesia, further suggesting a role for NPRP3 in the response to both acute and chronic opioids.

Oxycodone-Cannabidiol Interactions in Pain Modulation and Reward Processing

Niall P. Murphy, Department of Biomedical Sciences College of Dentistry, Texas A&M University

- Summary of our recent studies on cannabidiol as an opioid-sparing approach for pain management
- Focus on pain, reward, and reinforcement-related mechanisms
- Studies conducted in rat models

Patch-Seq analysis of joint innervating primary afferent neurons: Identifying potential CBD targets
Robert M. Caudle, Bruna Balbino De Paula, and Airam Vivanco University of Florida, Department of Oral and Maxillofacial Surgery

Cannabidiol (CBD) has been used to treat many pain conditions in humans, but the evidence on its effectiveness is more anecdotal than definitive. However, recent studies have demonstrated antinociceptive efficacy when administered alone or in combination with opioids in different rodent models. These findings offer promise for novel pain therapies, but the mechanisms for CBD's effects are not known. CBD binds to several proteins which may or may not mediate these antinociceptive effects. We have examined CBD's effects and its interactions with oxycodone on voltage gated ion channels in dissociated trigeminal ganglion neurons. These findings suggest that CBD has direct effects on peripheral sensory neurons and that CBD may be synergistic with opioids.

NLRP3 Inflammasome Modulation of MOR Agonist Efficacy and Activity
Jay P. McLaughlin[1], Shainnel O. Eans[1], Ryosuke Shinouchi[1], Myosotys Rodriguez[2], Dileepkumar Veeragoni[2], Candy Carbajal[2], Florida Owens[2] and Nazira El-Hage[2]

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Anti-inflammatory compounds are commonly co-administered with opioids to synergistically improve pain management. However, opioid-induced activation of cytosolic multiprotein inflammasomes paradoxically promotes neuroinflammation thought to contribute to opioid induced hyperalgesia and antinociceptive tolerance. The activity of the NOD-like receptor (NLR) protein 3 (NLRP3) inflammasome implicated in pain and neuroinflammation also increases under morphine exposure, but little is known about the interactive effects of NLRP3 and opioid systems. Investigating NLRP3 modulation of basal opioid activity, MCC950 was administered to selectively inhibit NLRP3, and the effects of opioid agonists morphine and fentanyl assessed in mice. Using the 55 °C warm water tail withdrawal test, a 30 min pretreatment with MCC950 (40 mg/kg, i.p.) significantly ($F(2,88)=47.4$, $p<0.0001$; nonlinear regression analysis) potentiated the antinociceptive effect of morphine and fentanyl, shifting leftward the ED50 values 3.6-fold (from 2.78 to 0.77 mg/kg, s.c.) and 1.8-fold (from 0.051 to 0.028 mg/kg, s.c.), respectively. Further testing of acute and prophylactic treatment with MCC950 on opioid-induced hyperalgesia, antinociceptive tolerance and naloxone-precipitated withdrawal will be discussed. However, MCC950 inhibition significantly exacerbated the magnitude and duration of respiratory depression and hyperlocomotion caused by morphine and fentanyl, and MCC950 pretreatment potentiated the effect of morphine in the conditioned place preference (CPP) assay, enhancing a dose ineffective in control mice (0.1 mg/kg) to produce significant CPP. Consistent with an interaction between opioids and this inflammasome, brains isolated 4 or 24 hours after a single treatment with morphine or fentanyl, alone or in combination, biochemically displayed significant increases in NLRP3 inflammasome-related cytokines and chemokines which correlated with increased production of NLRP3, the MAPKs JNK and p38 proteins, and the transcription factor NF-kB, but also decreased expression of PSD-95. Pretreatment with MCC950 reduced the production of IL-6, IL-18, TNF-α, and expression of NLRP3 in morphine-treated brains, while reversing morphine inhibition of PSD-95, suggestive of NLRP3 modulation of these opioid- induced effects. In summary, pharmacological inhibition of NLRP3 mitigated the inflammatory and neurochemical factors affecting the behavioral effects of morphine and fentanyl, expanding current insights into NLRP3 inflammasome pathway control over opioid-induced antinociception and adverse effects.
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Poster session A

A1 - The Efficacy of PPL-138 to Reduce AUD-like and PTSD-like Symptoms in Female and Male Rats

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In the United States, alcohol use disorder (AUD) effects 9% of the population annually and post-traumatic stress disorder (PTSD) effects 6% of Americans within their lifetime. Epidemiological studies have revealed a high comorbidity between substance abuse disorders, like AUD, and mood disorders, like PTSD. A person afflicted with comorbid AUD-PTSD simultaneously experiences key biological and behavioral changes associated with both disorders. Physiological processing of substance-use and trauma require similar neural pathways and highly interactive brain structures – like the prefrontal cortex (PFC), amygdala, and HPA axis. These interactions result in a cascade of effects which are still being identified and deciphered. For example, the upregulation of nociceptin opioid peptide (NOP) receptors and its endogenous ligand nociceptin/orphanin FQ (N/OFQ) have been observed in independent rodent experiments of excessive alcohol consumption and rodent experiments of trauma. Specifically, NOP upregulation has been observed in brain structures relevant to AUD and PTSD, like the amygdala. We developed an experimental paradigm to study comorbid AUD-PTSD by combining current rodent models of alcohol self-administration and models of prolonged stress. Rodent AUD-like and PTSD-like symptoms were characterized by increased alcohol consumption, alcohol motivation, and anxiety- like behavior after trauma exposure. In this study, we assessed the efficacy of the novel compound PPL-138 – a partial agonist at NOP and mu receptors, an antagonist at kappa receptors – to reduce comorbid AUD-like and PTSD-like symptoms in rats. Consistent with previous pharmacological studies, we observed significant sex differences in development of both disorders and drug efficacy. Although there was not a sex difference in baseline alcohol consumption, males significantly increased consumption after trauma, while females did not. PPL-138 did not have an effect on AUD-only nor PTSD-only rats but did significantly reduced alcohol consumption within comorbid subjects. The findings of these studies will contribute to the improvement of pharmacotherapies for the treatment of alcohol use disorder and post-traumatic stress disorder in women and men. Further studies will aim to investigate the role of estradiol within this context.

A2 - Adolescent social isolation increases stress and alcohol vulnerability via opioid gene espression changes in a sex-dependent manner

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Chronic stress during developmental periods leads to altered functional connectivity and increases vulnerability to anxiety disorders and alcohol addiction in adulthood. Early-life stress may disrupt the maturation and function of brain regions involved in emotion and reward processing. These alterations may, in turn, heighten susceptibility to substance use by affecting reward circuitry. Specifically, dysregulation of the the dynorphin (DYN)/κ-opioid receptor (KOR) pathway, has been proposed as a key mediator of these long-term effects. However, the sex-specific role of the endogenous opioid system in mediating these outcomes remains poorly understood.

To investigate the long-term neurobiological effects of adolescent social isolation, male and female Wistar rats underwent grouped housing (GH) or social isolation (SI) conditions from post-natal day (PND) 21 to PND60. In adulthood (PND60-90), animals were tested for anxiety-like and alcohol- related behaviors. At PND60, gene expression analysis of DYN, KOR, μ-opioid receptor (MOR), and glucocorticoid receptor (Nr3c1) was performed in the prelimbic cortex (PrL) and amygdala, brain areas involved in emotional processing, stress-coping behavior, and reward.

Results showed that in the light dark test SI rats exhibited increased anxiety-like behavior in both males and females compared to their GH controls ($p=0.02$). Alcohol self-administration and motivation to obtain alcohol was significantly higher in SI females compared to control ($p=0.001$). Morever, SI females displayed a higher aversion resistance to punished alcohol self-administration compared to GH controls ($p<0.03$). We found that SI females showed a significantly lower extinction to alcohol compared to the other groups ($p<0.021$) and the pharmacological stressor yohimbine (0.625) elicited a significantly higher relapse to alcohol seeking in SI animals compared to the GH controls ($p=0.02$). At a molecular level, preliminary data showed no significant changes in MOR expression in the PrL of either sex. However, DYN and KOR mRNA levels were significantly higher in adult SI females ($p = 0.035$ and $p = 0.024$, respectively), indicating sex-specific transcriptional adaptations in response to adolescent social isolation.

These findings highlight the long-lasting, sex-specific impact of adolescent social isolation on stress- and alcohol-related behaviors, with females showing a higher vulnerability. These effects might rely on the upregulation of DYN and KOR in the prelimbic cortex of SI females suggesting a potential sex-dependent molecular mechanism in response to adolescent social stress.

A3 - Exploring the opioid system role on sex- and age-dependent effects of social isolation on binge-like alcohol drinking and decision-making in rats

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Poor decision-making is related to vulnerability to substance use disorders, yet the influence of sex and environmental stressors such as social isolation on this relationship remains unclear. Our objective was to evaluate whether social isolation modulates alcohol- induced impairments in decision-making in a sex- and age-dependent manner, using a rodent version of the Iowa Gambling Task (IGT).

Adult and adolescent Sprague-Dawley rats of both sexes were housed either in isolated or grouped conditions. Animals underwent IGT testing before and after a binge-like alcohol consumption protocol (Drinking in the Dark). Decision-making performance was analyzed in relation to alcohol intake, sex, age, and housing condition. In addition, brain tissue was collected for immunohistochemical analysis of c-Fos expression and RNAscope in situ hybridization will also be performed to examine expression patterns of kappa opioid receptors and dynorphins.

Our preliminary findings indicate that social isolation modulates both alcohol consumption and decision-making behavior in a sex- and age-dependent manner. In non-isolated animals, alcohol intake differs by sex and age: younger animals consume more than adults, and females drink more than males. However, these differences disappear when animals are previously exposed to social isolation.

Regarding decision-making, alcohol consumption appears to impair performance in males and improve it in females. Notably, in isolated animals, alcohol no longer influences decision-making, suggesting that prior social isolation overrides, or saturates, alcohol's impact on this cognitive function, masking further impairment. Instead, isolation alone significantly alters decision-making behavior, again in a sex- and age-specific manner. Ongoing experiments will provide information on the alterations in the mesocorticolimbic level associated to these behaviors.

These findings suggest that social isolation is a critical modulator of both alcohol intake and cognitive vulnerability. The interactions between sex, age, and environmental context highlight the complexity of risk factors contributing to substance use disorders.

A4 - Antidotal Action of Metal-Organic Frameworks (MOFs) on Opioid and Synthetic Cathinone Toxicity in Zebrafish: Toward Innovative Strategies for Substance Use Disorders

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Knowledge gap and objective
Although metal-organic frameworks (MOFs) have been widely studied for drug delivery and detoxification, their use as pharmacological antidotes remains largely unexplored. In vivo data on their efficacy against psychoactive substance toxicity - especially opioids and cathinones - are scarce. This study evaluated the effects of three zirconium (Zr)-based MOFs in a zebrafish model, focusing on their ability to modulate behavioral and cardiovascular responses to morphine and mephedrone.

Methods
Newly fertilized zebrafish embryos were exposed for 96 hours to E3 (control), morphine (25 mM), mephedrone (200 μM), or their combinations with MOFs - NU-1000, UiO- 66, and UiO-67. After exposure, heart rate was assessed under a stereomicroscope, and locomotor activity was measured by distance moved over 10 minutes.

Results
Morphine exposure significantly reduced larval locomotion ($p = 0.0051$), indicating a depressant effect on motor behavior. UiO-67 alone also decreased locomotor activity ($p = 0.0471$), suggesting intrinsic bioactivity. Notably, co-treatment with NU-1000 or UiO-67 effectively reversed morphine-induced hypolocomotion ($p = 0.0213$ and $p = 0.0398$, respectively), demonstrating their potential as functional antidotes. In parallel, morphine ($p = 0.0044$), NU-1000 ($p = 0.0078$), and UiO-67 ($p < 0.0001$) each significantly reduced heart rate. However, NU-1000 was uniquely able to restore normal heart rate when co-administered with morphine ($p = 0.0277$).
Conversely, mephedrone produced stimulant-like effects, markedly increasing both locomotor activity and heart rate ($p < 0.0001$, $p = 0.0054$, respectively). Co-treatment with NU-1000 or UiO-67 successfully attenuated mephedrone-induced tachycardia ($p = 0.0003$, $p = 0.0023$, respectively), while all three MOFs - NU-1000, UiO-67, and UiO-66 - were effective in reversing mephedrone-induced hyperlocomotion ($p < 0.0001$). Statistical analysis was conducted using two-way ANOVA followed by post hoc Tukey's test.

Significance
This study demonstrates that Zr-based MOFs can effectively reduce behavioral and cardiovascular alterations induced by morphine and mephedrone. These findings support their potential as novel agents for detoxification and supervised withdrawal, offering an innovative pharmacological strategy to mitigate acute toxicity and enhance treatment safety in clinical and emergency settings.

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A5 - Pre-clinical characterization of heroin and oxycodone virus like particle nanovaccines in Sprague-Dawley rats

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Opioid use disorder (OUD) poses a significant threat and remains a global challenge with limited treatments. Conjugate vaccines offer a promising alternative by inducing drug- specific antibodies that reduce unbound drug in the brain, thereby mitigating its central effects. Virus-like particles (VLPs) have emerged as effective vaccine platforms without requiring adjuvants, and only a few reports tested VLPs in the context of OUD. This study evaluated VLP-based oxycodone and heroin vaccines (OXY-VLP, M-VLP) as alternatives to sKLH-conjugated vaccines (OXY-sKLH, M-sKLH) formulated with aluminum salts (alum), currently in or nearing clinical trials (NCT04458545).

OXY and M haptens were conjugated to VLP or sKLH using carbodiimide (EDAC) chemistry, conjugation was confirmed by MALDI-TOF and dynamic light scattering (DLS). 35 Sprague-Dawley rats were immunized with saline, OXY-sKLH + alum (60+90 μg), M- sKLH + alum (60+90 μg), OXY-VLP (60 μg), or M-VLP (60 μg) three times at 14-days interval, and drug-specific antibody titer were quantified by ELISA one week after the second dose. One week after the third dose rats were challenged with 2.25 mg/kg subcutaneous (SC) oxycodone or 1 mg/kg SC heroin, and 30 minutes after the challenge, cardiopulmonary (heart rate, breath rate, SpO2 via PulseOX collar) and antinociceptive effects (latency to respond on a 54°C hotplate, 30s cutoff) were measured. Brains and trunk serum were harvested to quantify oxycodone, oxymorphone, heroin, and 6-MAM by LC-MS. OXY-VLP vaccination induced a robust immune response compared to saline ($p<0.05$) but lower than OXY-sKLH + alum ($p<0.05$). This led to an effect of treatment on SpO2 [$(F(2,15)=6.19, p<0.05)$] and increased serum sequestration of oxycodone [$(F(2,15)=128.6, p<0.05)$] and oxymorphone [$(F(2,15)=32.42, p<0.05)$]. Although towards significancy, M-VLP didn't elicit strong antibody production when compared to saline ($p=0.055$), and this yielded a main effect of treatment on thermal nociception [$(F(2,30)= 3.98), p<0.05$)]. However, only M-sKLH vaccination significantly protected against thermal nociception and increased 6-MAM sequestration in the serum when compared to M-VLP or saline ($p<0.05$).

Overall, these results suggest that while VLP platforms are considered inherently adjuvanted, they may still benefit from additional adjuvants depending on the context. Further studies are needed to identify optimal hapten-adjuvant combinations for VLP- based opioid vaccines.

A6 - Dopamine dynamics and genomic correlates of addictive-like behaviors in an opto-intracranial self-stimulation mouse model

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Substance use disorders are chronic conditions with serious societal and medical consequences, defined by maladaptive behaviors oriented toward compulsive drug consumption. Few studies have been conducted to determine how genomic plasticity may underlie inter-individual variability in such behaviors.

To address this question, our project combines advanced sequencing methodologies with a mouse model of opto-intracranial self-stimulation (oICSS). In this paradigm, the mu opioid receptor (MOR)-Cre knock-in line allows targeting MOR expressing neurons in the ventral tegmental area (VTA), which modulate the mesolimbic pathway. We recently showed that voluntary nose poke-induced optogenetic inhibition of VTA-MOR neurons (using the GtACR2 opsin) had reinforcing effects in both male and female mice. We processed three mouse cohorts over six weeks to assess the severity of three addictive-like behaviors: 1) persistence of reward-seeking when light stimulation was not available; 2) motivation for reward, evaluated using a progressive ratio schedule; and 3) resistance to punishment. This revealed significant individual differences, with animals showing high or low severity of addictive-like behaviors.

Next, given the central roles of the nucleus accumbens (NAc) and prefrontal cortex (PFC) in reward-seeking, we are now focusing on the neurophysiological and genomic correlates of addictive-like behaviors in these regions.

First, we monitored dopamine dynamics over the course of repeated oICSS sessions using in vivo fiber photometry and the dLight1.3b biosensor. We observed increased dopamine signal in the NAc shortly after laser stimulation delivery, and to a lesser extent in the PFC, showing that opto-inhibition of VTA-MOR neurons triggered the disinhibition of dopaminergic neurons, as expected. Analyses of dopamine signal evolution across repeated sessions are ongoing.

Second, we are investigating genomic plasticity in the PFC that may underlie the severity of addictive-like behaviors. To do so, we established protocols for cell-type specific analyses of gene expression and DNA methylation in neuronal cells, combining flow cytometry with RNA-sequencing or Enzymatic Methylation Sequencing, respectively. These protocols are currently used to investigate transcriptomic and methylomic changes associated with severity of addictive-like behaviors in the aforementioned cohorts.

Altogether, we expect to identify addiction-related molecular adaptations at high cellular resolution, with the long-term goal of identifying new therapeutic targets.

A7 - Dissecting Neural Mechanisms of Volitional Drug Seeking in Mice

Hansol Lim

Recent studies, including Lim et al., 2024, suggest that neural circuits involved in reward and aversion can overlap, encoding opposing valences depending on temporal or contextual factors. Opioid addiction exemplifies this duality, with both positive reinforcement during use and negative reinforcement during withdrawal driving compulsive seeking. However, the neural mechanisms that distinguish volitional drug-seeking from passive drug exposure remain incompletely understood.

In this study, we use a self-administration paradigm in mice to contrast intentional morphine intake with yoked, non-contingent exposure. This design allows us to isolate neuronal populations selectively activated during goal-directed opioid seeking. These neurons are genetically tagged and functionally interrogated via in vivo calcium imaging and input-output circuit mapping. Behavioral dynamics are aligned with neural activity through computational behavior analysis, and molecular identity is characterized using transcriptomic tools.

Preliminary results reveal behavioral and physiological phenotypes consistent with addiction in self-administering animals, including enhanced motivational drive and withdrawal responses. Distinct patterns of connectivity across affective brain regions further differentiate volitional use from passive exposure.
Altogether, this work provides a framework for identifying neural and molecular substrates of craving, offering insights for the development of targeted treatments for opioid addiction.

A8 - Effects of Maternal Pain and Perinatal Opioid Exposure on Intravenous Oxycodone Self- Administration in Male and Female Rats

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Despite the ongoing opioid epidemic in the United States and the prevalence of neonatal opioid withdrawal syndrome (NOWS), there is minimal clinical or preclinical evidence on the effects of perinatal opioid exposure on future drug use. Even less information is available regarding how maternal factors mediate potential effects, including the presence of chronic maternal pain. By utilizing a clinically-relevant rodent model of maternal neuropathic pain and/or oxycodone exposure, we investigate the developmental and behavioral consequences for male and female offspring exposed to maternal pain or perinatal opioids, particularly focusing on intravenous oxycodone self- administration. Adult male and female offspring exposed to maternal pain, perinatal oxycodone, or both, were implanted with jugular vein catheters and trained to self- administer oxycodone for three weeks (1 week at 0.15 mg/kg, 2 weeks at 0.06 mg/kg). We hypothesized that in utero exposure to opioids would increase oxycodone self- administration in male and female offspring, and that this would be ameliorated in offspring exposed to both maternal pain and opioids. We observed that male offspring exposed to maternal pain or in utero oxycodone made fewer lever presses for oxycodone, particularly during week 3 [Drug*Pain*Day interaction, $F(14,280)=2.667$, $p=0.0011$]. This effect was not seen in male offspring exposed to both maternal pain and oxycodone or in any group of female offspring. When comparing lever presses for different doses of oxycodone (0.06 mg/kg, 0.1 mg/kg, or 0.15 mg/kg), we observed that male rats exposed to oxycodone or maternal pain made fewer lever presses specifically for low dose oxycodone (0.06 mg/kg), suggesting decreased sensitivity to low doses (Dose*Pain*Drug, $F(2,12)=3.387$, $p=0.0515$). This was not observed in male rats exposed to both oxycodone and maternal pain or in female rats. Together, our results suggest that male offspring exposed to maternal pain or in utero oxycodone are less sensitive to low doses of oxycodone, but not high doses. This may predispose those offspring to abuse-like drug intake. This study highlights the importance of investigating the impact of prenatal experiences on future behavior, including drug use.

A9 - Cebranopadol: a novel nop/mop dual agonist to treat opioid use disorder

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Opioid use disorder (OUD) is a chronic psychiatric condition characterized by compulsive drug intake, severe intoxication, and abstinence episodes generally followed by relapse. Fentanyl is a potent opioid agonist that rapidly induces dependence, and its illicit use is associated with a high risk of respiratory depression and death. Cebranopadol is a recently developed long-acting compound that co-activates the mu-opioid (MOP) and nociceptin/orphanin FQ peptide (NOP) receptors. Previous studies have shown that cebranopadol reduces the motivation for heroin in rats. Based on this premise, here we evaluated the effect of cebranopadol on fentanyl self-administration and fentanyl induced respiratory depression in the rat.

Male Wistar rats were trained for 6 hours daily on fentanyl (2,5 μg/inf) self-administration under a fixed ratio 3 (FR3) schedule. Once a stable baseline of responding was established, the rats were pretreated with oral (os) cebranopadol (0, 12,5, 25, 50 μg/kg).

Our results demonstrate that acute treatment with cebranopadol significantly reduced fentanyl self- administration. RM ANOVA showed a significant main effect of treatment [$F(3, 30) = 3,37$; $p<0.05$]. Dunnett's post hoc multiple comparison test showed that cebranopadol treatment at the doses (12,5 and 25 μg/kg) significantly reduced the number of fentanyl infusions ($p<0.05$).

A separate group of rats trained for fentanyl self-administration was used to assess the effects of intravenous co-administration of cebranopadol (0, 25, and 50 μg/kg) and fentanyl (50 μg/kg) on respiratory function using a whole-body plethysmography apparatus.

Findings revealed that fentanyl markedly reduced respiratory parameters, but this effect was not potentiated by cebranopadol.

Together, these data indicate that cebranopadol can effectively reduce fentanyl consumption without increasing the risk of respiratory depression when fentanyl is taken alongside cebranopadol. These findings support the potential clinical development of cebranopadol for the treatment of opioid use disorder.

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A10 - Exploring the role of AMPAR auxiliary protein CNIH3 in opioid-seeking behavior and contributing risk factors using mouse models

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Cornichons are a conserved class of AMPAR auxiliary proteins that control the export of receptors from the endoplasmic reticulum to regulate synaptic excitability. Cornichon homolog 3 (CNIH3) co-assembles with AMPARs in the brain to enhance AMPAR sensitivity, slow receptor deactivation, improve channel permeability, and slow decay of excitatory post-synaptic currents. A 2016 genome-wide association study (GWAS) found that single nucleotide polymorphisms in CNIH3 provided significant protection, particularly in women, against the development of opioid use disorder in people with previous opioid exposure. Given the role of CNIH3 in AMPAR function, which is important in opioid-seeking behavior, we aim to investigate sex differences in the role of CNIH3 in the mechanisms underlying fentanyl-seeking behavior. In this study, we assess how CNIH3 deletion affects an array of behaviors that may impact vulnerability to opioid intake, such as depression-like and anxiety-like behavior. We also assessed natural reward-seeking and cognitive flexibility using operant sucrose self- administration, and fentanyl consumption using operant intravenous self-administration (IVSA) in male and female C57, wild-type (WT), and CNIH3 knockout (KO) mice. Our results indicate that CNIH3 deletion does not appear to affect risk factors for opioid-seeking but impairs the acquisition of sucrose self-administration and fentanyl IVSA, and blunts fentanyl intake in both sexes. Furthermore, CNIH3 KO dampens drug-seeking during cue-induced reinstatement in male CNIH3 KO mice. Additionally, we use principal component analysis (PCA) as an unbiased approach to identify the main contributing factors to behavioral differences between genotypes. This study is the first to uncover the role of CNIH3, a gene identified by clinical data to play a role in OUD, in opioid-seeking as well as contributing risk factors.

A11 - Enhanced naloxone-induced conditioned place aversion following high-dose oxycodone in Zhx2 knockout mice

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The opioid epidemic remains a major public health concern. Overprescription of oxycodone (OXY; active ingredient of OxyContin®) played a historical role in the current crisis and OXY misuse continues to fuel it. We mapped zinc-fingers and homeobox 2 (Zhx2) as a candidate quantitative trait gene underlying increased brain oxymorphone (OMOR), in BALB/cJ females (Beierle et al., 2022; JPET). OMOR is a much more potent and efficacious metabolite that was associated with increased OXY locomotion and reward in BALB/cJ females vs. BALB/cByJ mice. We used CRISPR-gene editing in BALB/cByJ mice to knock out exon 3 (Zhx2 KO) which modeled the loss-of-function variant in BALB/cJ mice and validated Zhx2 in brain [OMOR] and OXY behavior (Lynch et al., 2025; JPET). Here, we tested the hypothesis that Zhx2 KO would increase OXY withdrawal in a conditioned place aversion (CPA) paradigm. Mice were assigned to OXY/naloxone (NLX) or saline (SAL) control groups. On Day(D)1, all mice received SAL at 8am in the home cage and again at 12pm and were assessed for initial preference/aversion for 30 min in a two-sided apparatus. On D2 and D4, mice received OXY (40 mg/kg; IP) at 8am and NLX (1 mg/kg; IP) at 12pm and were confined to the right side for 30 min. Controls received SAL at both time points. On D3 and D5, mice received SAL at both timepoints and were confined to the left side for 30 min. On D8, all mice received SAL at both timepoints and were assessed for drug drug-free NLX-CPA for 30 min. On D9, OXY/NLX mice received SAL at 8am and NLX as a reminder injection at 12pm, while controls received SAL at both timepoints and once again, mice were tested for NLX-CPA. As predicted, Zhx2 KO mice showed enhanced NLX-CPA as indicated by significantly less time spent on the NLX-paired side. We are currently assessing brain OMOR in Zhx2 KO mice following high-dose OXY and future studies will assess changes in brain reward threshold during spontaneous OXY withdrawal. Overall, our results support Zhx2 in mediating brain OMOR levels and multiple OXY addiction model behaviors.

A12 - Regulation of the Opioid System by Ketamine and its Therapeutic Potential in Opioid Use Disorder

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Background
A major challenge in the treatment of opioid use disorder (OUD) is relapse, even after protracted abstinence driven by negative affect states including depression. Recently, a single sub- anesthetic dose of ketamine was shown to produce long-term antidepressant effects, with emerging evidence implicating the endogenous opioid system. Here we further characterize interactions between ketamine and the opioid system at the mRNA, protein and receptor signaling level. Additionally, effects of ketamine exposure during protracted abstinence on measures of relapse are assessed using a heroin self-administration paradigm.

Methods
Adult male Long Evan rats received saline (control) 3mg/kg or 10mg/kg ketamine injections (intraperitoneal) and sacrificed 24h, 72h or 7 days later. Brain samples were collected for qPCR, ELISA, and GTPγS signaling. A separate cohort of rats were catheterized and trained on a 14- day heroin Fixed Ratio 1 schedule. Rats were subsequently subjected to 14-days of forced abstinence and divided into a control vehicle treated group or a group receiving infusions of ketamine on days 9, 11 and 13 of forced abstinence. Rats were then assessed for cue induced and heroin primed drug seeking.

Results
Acute ketamine exposure alters transcription of opioid related genes in the nucleus accumbens (NAc) shell, with 10mg/kg ketamine eliciting more robust and longer lasting effects compared to 3 mg/kg. Additionally, 10mg/kg ketamine increases μ opioid receptor protein and signaling in NAc 7 days post-acute exposure. Finally, ketamine significantly reduces heroin primed drug seeking.

Conclusions
Ketamine exposure is associated with brain region specific, long-term changes to the endogenous opioid system providing novel insight into its long-term mechanisms. Additionally, ketamine exposure during abstinence from heroin reduces measures of relapse, indicating ketamine has therapeutic potential in the treatment of OUD.

A13 - Heroin preference over social interaction: the role of GABAergic transmission in the medial amygdala

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Background

Although millions of individuals use opioids each year, only a subset develop opioid use disorder, highlighting significant interindividual variability in vulnerability to addiction. By employing a discrete-choice paradigm between heroin and social interaction, we identified "vulnerable" and "resilient" phenotypes. Vulnerable rats exhibited elevated heroin intake and drug-seeking behavior, an increased number of burst events, and a persistent preference for the drug over social interaction.

Aim

Our objective is to identify the molecular substrate underlying the vulnerable rat phenotype. We focused on the glutamatergic and GABAergic systems of medial amygdala, a hub for social behavior and decision-making in general.

Materials and Methods

We trained 20 male rats to lever press (FR1) for social self-administration (2h-d/6days) followed by heroin self-administration (FR1) under a continuous-access, no-timeout condition (6h- d/15days). Next, we tested the rats for drug-seeking (30 min) at early and late abstinence day. In between, we employed a discrete-choice procedure (FR5) where rats faced a choice between heroin and social interaction (6-h/11days). Following the behavioral experiments, we sacrificed the animals and collected the brains. We conducted a Western blot analysis on medial amygdala (along with other brain regions) tissue to assess the expression levels of key proteins involved in GABAergic (GAT-1, GAT-3, GAD67, GABA-A α1/α2 subunits, GABA-B R1) and glutamatergic (GluN2A and GluN2B) neurotransmission along with Neuroligin-2 and Gephyrin.

Results and Conclusions

Of the twenty trained rats, six, referred to as "vulnerable", exhibited a marked preference for heroin. Western blot analyses of medial amygdala tissue revealed a significant decreased expression of GAT3 specifically in the "vulnerable" phenotype.

A14 - Biased kappa opioid receptor agonist nalfurafine attenuates oxycodone seeking and craving in rats

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Opioid use disorder (OUD) is one of the most important crises of contemporary societies with limited effective pharmacological treatments. Opioid use leads to the development of OUD symptoms, including drug craving – a hallmark of OUD. Opioid craving has been associated with the activity of the mesocorticolimbic dopamine system. Recently, nalfurafine (a biased kappa opioid agonist) has been demonstrated to modulate several opioid-induced behaviors, thus raising potential interest as a new candidate for OUD treatment. Here we aim to evaluate effect of nalfurafine treatment on mesocorticolimbic dopamine activity as well as oxycodone seeking and craving.

To evaluate effect of nalfurafine on dopamine release, we used fast scan cyclic voltammetry (FSCV) as well as in vivo fiber photometry in male Sprague-Dawley rats. To evaluate effects of nalfurafine on oxycodone seeking we used oxycodone self-administration as well as several tests of oxycodone seeking and craving in male Sprague-Dawley rats. In control studies we evaluated nalfurafine effect on food seeking behaviors as well as locomotion and anxiety-like behaviors (open field test) and dysphoria (conditioned place aversion test).

Systemic and intra-VTA nalfurafine administration decreased phasic dopamine release in the NAc as well as in the mPFC and BLA of anesthetized rats. Nalfurafine administration decreased oxycodone seeking both during early (withdrawal day 1; WD1; $p<0.01$) and protracted (WD30) abstinence ($p<0,01$) as well as oxycodone-induced reinstatement of oxycodone seeking (but with no effects on stress-induced reinstatement). This effect was present in doses which did not induce sedation or anxiety-like behaviors nor dysphoria. Similarly, nalfurafine administered via osmotic pumps (WD1-14) decreased oxycodone seeking in novel context ($p<0.05$). Lastly, systemic nalfurafine administration decreased phasic dopamine release induced by oxycodone cues in freely moving rats.

These results demonstrate that nalfurafine effectively attenuates phasic dopamine release in the forebrain as well as decreases oxycodone seeking and craving. Such nalfurafine effects were not due to its potential sedative or dysphoric properties. We propose that nalfurafine administration leads to blunted dopaminergic responses to oxycodone conditional stimuli thus decreasing their incentive motivational effect on oxycodone seeking behavior.

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A15 - Preoperative Opioid Misuse Associations with Pain, Opioid Use, and Negative Affect after Abdominal Surgery

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Objective
Preoperative opioid misuse is associated with worse postoperative outcomes. This prospective longitudinal cohort study evaluated the association between preoperative opioid misuse and prolonged pain and opioid use after elective abdominal surgery; and examined postoperative trajectories of patient-reported outcomes over one year.

Methods
187 patients undergoing elective spine surgery completed pre-surgical and weekly postoperative longitudinal assessments of pain and opioid use and monthly assessments of depression and anxiety. Cox regression analyzed the effect of preoperative opioid misuse (defined as a score of 9 or higher on the Current Opioid Misuse Measure; COMM) on time to pain and opioid cessation while linear mixed-effects models examined longitudinal changes in postoperative outcomes.

Results
16% (n=30) reported a positive preoperative COMM score. Adjusting for age, sex, race, ethnicity, and any preoperative opioid use, preoperative opioid misuse (COMM-Positive) was associated with delayed pain cessation (HR 0.50; 95%CI 0.27-0.92; p = 0.03). There was no significant association between preoperative opioid misuse and either delayed return to baseline opioid dose or delayed opioid cessation. Patients with preoperative opioid misuse reported higher pain intensity at week 1 (p=0.01), and there was a significant interaction between preoperative opioid misuse and time (p<0.0001). Scores diverged from those without preoperative opioid misuse after week 28 for COMM-positive patients. At week 52, COMM-positive patients reported moderate pain (6.33, 95%CI 5.49-7.17) while those without preoperative opioid misuse reported minimal pain (0.22, 95%CI 0.08-0.37).
COMM-Positive patients reported significantly higher NIH PROMIS depression and anxiety scores at postoperative week 4 which remained stable over time.

Conclusion
Preoperative opioid misuse is a significant risk factor for delayed pain cessation even after adjusting for preoperative opioid use and is associated with a significant increase in pain severity in the year following surgery. Targeted risk stratification and interventions to address preoperative opioid misuse may reduce the development of persistent post-surgical pain.

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A16 - Nicotine exposure via electronic cigarettes enhances Bdnf/TrkB transcription, dynorphin and OLIG2 levels specifically in the rat VTA

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Different drugs of abuse affect CNS neuronal networks and reshape the expression of neuroplasticity- related genes in key regions of the mesocorticolimbic reward circuitry, such as the ventral tegmental area (VTA) and the nucleus accumbens (NAc). Recent evidence suggests that neuronal activity and life experiences, including repeated drug exposure, can modulate oligodendrogenesis, thus altering neuronal myelination. This study aimed to investigate whether prolonged exposure to nicotine, via electronic cigarettes, affects oligodendrocyte differentiation.
To this end, male Sprague-Dawley rats were exposed to nicotine using a commercially available e- cigarette device for four weeks. Subsequently, the VTA and NAc were collected for gene and protein expression analyses to assess the Bdnf/TrkB and DYN/KOR systems, OLIG2 (a marker of oligodendrogenesis), its epigenetic regulator Kdm6b, as well as neurofilament protein levels, given their critical role in maintaining axonal integrity.
Results showed that mainstream nicotine exposure significantly increased the expression of OLIG2 ($p < 0.05$), a transcription factor essential for oligodendrocyte differentiation, in the rat VTA. This effect was associated with increased mRNA levels of the epigenetic enzyme Kdm6b ($p < 0.05$), which is involved in regulating OLIG2 expression and synaptic plasticity. In the same brain region, nicotine upregulated Bdnf ($p < 0.05$) and TrkB gene expression ($p < 0.01$), as well as dynorphin peptide levels ($p < 0.01$), without significant changes in KOR protein expression ($p > 0.05$). These data suggest adaptive/maladaptive changes in oligodendrocyte regulation, potentially contributing to addiction-related neurobiology. Notably, these molecular changes occurred alongside a reduction in neurofilament light levels ($p < 0.01$), suggesting potential axonal remodelling associated with enhanced oligodendrogenesis. No significant changes in investigated parameters were detected in the NAc ($p > 0.05$), thus suggesting that oligodendrogenesis and associated molecular alterations selectively localized in the VTA. Protein correlation analysis revealed that prolonged nicotine exposure primarily impacts neuroplasticity-related protein networks within this area.
Overall, these findings suggest that prolonged nicotine exposure, through electronic cigarettes, induces alterations of oligodendrogenesis-related parameters specifically within the VTA. These molecular changes may impact axonal conduction velocity and reward circuitry connectivity, promoting neuronal adaptations that could contribute to the development of addictive behaviour.

A17 - Fentanyl and xylazine interactions result in the altered action potential firing rates in dopamine type 2 receptor-expressing striatal medium spiny neurons

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The combined use of fentanyl and xylazine, μ-opioid receptor and pan-α2-adrenoceptor agonists, respectively, is a major public health threat in the USA. A recent study has shown fentanyl and xylazine interact to significantly increase the likelihood of fentanyl overdose. We find that fentanyl alone can significantly alter the activity of dopamine type 2 receptor (D2) expressing medium spiny neurons (MSN), and investigated whether xylazine co-exposure would exacerbate the effects of fentanyl on the activity of D2 MSNs in electrophysiological recordings of ex vivo striatal slices. Striatal slices were obtained from 2-3 months old male B6.Cg-Tg$^{(Drd2\text{-}EGFP)S118Gsat}$ eGFP-expressing mice to identify D2 MSNs. We found that 10 μM xylazine applied acutely caused a significant reduction in action potential firing rates at depolarizing current injections equal to or greater than 325 pA (3.1 Hz – 9.6 Hz mean change in range, *p<0.05, n=11, two-way repeated measures ANOVA). Additionally, 10 μM xylazine promoted action potential firing adaptations measured as an increase in the slope of normalized inter-action potential intervals vs. inter-action potential interval count curve from 0.0257 ± 0.0046 in controls vs 0.0571 ± 0.0071 in the presence of xylazine (*p<0.001, n=11, Student's t-test), which agreed with earlier reported effects of fentanyl on D2 MSN firing rate adaptations. In addition to the effects of each drug individually, combined acute application of 100 nM fentanyl and 10 μM xylazine resulted in novel interactions in D2 MSN function not seen when these drugs are applied separately.
Specifically, fentanyl and xylazine co-exposure caused a leftward shift in the firing frequency vs. injected current dependence increasing the sensitivity of D2 MSNs to 150 pA to 250 pA stimuli (n=10, *p<0.05, Student's paired t-test), reducing the rheobase from 205.0 pA ± 15.3 pA to 167.5 pA ± 21.4 pA (**p<0.01, n=10, Student's paired t-test), and increasing the initial momentary frequency response to injected currents > 175 pA (10.6 % - 69.4 % mean increase in range,
*p<0.05, n=10, two-way repeated measures ANOVA). We conclude that fentanyl and xylazine uniquely interact to increase the sensitivity of D2 MSNs to extra- and intrastriatal afferents.

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A18 - Xylazine amplifies behavioral and cardio-respiratory impairments induced by fentanyl in mice

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Gap of knowledge

Xylazine, commonly referred to as "Tranq-Dope" is a veterinary sedative that has been detected in some illicit drug supplies in USA and Europe. Recently, fentanyl samples have been found adulterated with xylazine, leading to reports of heightened effects among users. Moreover, this combination also significantly raises the risk of fatal overdoses. Despite the absence of an approved medication to counteract overdoses involving both fentanyl (FENT) and xylazine (XYL), naloxone is frequently administered. It remains unclear whether xylazine independently increases the risk of fatal overdose or the specific mechanisms through which its combination with fentanyl exacerbates toxicity. The aim of this study is to investigate the pharmaco-toxicological effects of Xylazine in combination with fentanyl.

Methods

We evaluated in vivo the behavioral (Visual placing, acoustic, spontaneous locomotion and tail pinch tests) and the cardio-respiratory effects of Xylazine (0.1-60 mg/kg i.p.) alone and in combination with fentanyl (FENT 1 mg/kg + XYL 10 mg/kg i.p.). Opioid receptor specificity was investigated using naloxone (NLX; 6 mg/kg i.p.).

Results

Our results revealed that xylazine dose-dependently impaired sensorimotor, motor and cardio-respiratory responses in mice. Co-administration of FENT and XYL worsened these effects compared to either substance alone. Interestingly some effects of XYL were also potentiated with NLX pre-treatment. Overall, NLX was not effective in blocking the behavioral and the cardio- respiratory impairments induced by the combination of FENT and XYL.

Conclusion

These data contribute to the understanding of the risks associated with the increasing spread of "Tranq-Dope" and highlight the need for further studies to explore the mechanisms by which these combinations can intensify the effects of new synthetic opioids

A19 - Structural elucidation of a new nitazene-type synthetic opioid

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We have developed a model for supporting local police forces with rapid testing of drugs for intelligence purposes. Current forensic testing can take many months and does not effectively support public health concerns from contaminated/strong batches. In our model, police forces identify substances of concern that are not destined for evidentiary analysis, e.g. at the site of an overdose. These are brought to our labs and within ~1 day we return the substance ID and some quantification information. We now support 5 police forces in SW England. With partners at other Universities we are expanding this model to the rest of the country, with 15 force areas in England and Wales now covered, via 5 institutions. We anticipate 5 new partner institutions to begin testing in 2025 to further expand information provision.
Recently a sample believed to contain a new synthetic opioid was brought to Bath from London. Within days a further sample, from Bath, was found with the same opioid present. Analysis indicated the London sample contained 55% paracetamol, 40% caffeine, 1.5% of the new nitazene, 1% medetomidine and <1% clozapine with the Bath sample being 48% paracetamol, 39% caffeine, 8% heroin, 1% the new nitazene, 0.5% medetomidine, <0.5% clozapine along with other street heroin constituents. The presence of medetomidine and clozapine, and at similar ratios, indicates that they are from the same starting batch but with the Bath sample being 'cut' with heroin.

Identification of the Nitazene.
Molecular weight (LCMS) of 390 indicating an even number of nitrogen atoms. 1H NMR indicated an analogue of isotonitazene but with a different benzimidazole ring substituent. 1H1H-COSY, 1H13C-HSQC and 1H13C-HMBC support a nitazene structure and identify a carbon at 118 ppm, consistent with a nitrile. DOSY and NOESY NMR confirmed final structure, including location of nitrile on the ring.
In summary, the sample is identified as isotocyanazine (5-cyano isotodesnitazene), a new synthetic opioid.

Funding
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A20 - Reversing poly-drug overdoses in a rat model

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Among synthetic opioid-involved overdose deaths, almost 80% involves another drug or alcohol. Opioid overdose deaths are caused by respiratory depression followed by cardiac arrest. We have developed a rat model of opioid overdoses in which we use whole-body plethysmography to assess the respiratory depression caused by opioids alone (DOI: 10.1124/jpet.122.001476) or in combination with other substances. Using whole body plethysmography, we analyzed ventilation parameters on a breath-by- breath basis. Female and male Long-Evans rats underwent intravenous catheter surgery. After habituation to plethysmography chambers, the rats were tested in within- subjects, Latin-square design with tests one week apart. The combination of ethanol and fentanyl led to a marked decrease on the amount of air inhaled per minute (minute ventilation, $F_{3,51} = 16.69$, $p < 0.0001$) and a supra-additive increase on the frequency of apneic events. The combination of fentanyl and xylazine led to dose-dependent additive effects ($F_{3,45} = 34.95$, $p < 0.0001$). Xylazine potentiated and prolonged the duration of fentanyl-induced reduction of minute ventilation ($F_{4,72} = 7.040$, $p < 0.00001$) and increase in apneic events ($F_{4,72}=15.377$, $p<0.00000$). We then compared the efficacy of naloxone, nalmefene, and buprenorphine to reverse polydrug overdoses. The depression of minute ventilation by combinations of fentanyl and ethanol and fentanyl and xylazine was transiently rescued by naloxone (<5 min), nalmefene (<5 min), and buprenorphine (<10 min). The increased frequency in apneic events was partially blunted by nalmefene only (20% reduction). These findings suggest that understanding the mechanisms underlying apnea are key for the treatment of polydrug overdoses.

Funding
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A21 - Investigating the Respiratory Consequences of Fentanyl and Xylazine Poly-Drug Administration and the Rescue by Antagonists in CD1 Mice Using Whole-Body Plethysmography

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The α2-adrenoceptor agonist and sedative xylazine has increasingly been used as a fentanyl adulterant over the past five years, becoming a growing public health concern. In this study, experiments were conducted to investigate the ventilatory depressive effects of xylazine, with and without fentanyl, and whether medium-dose combinations rescue the depression. We also examined the rescue potential of reversal agents (atipamezole for xylazine and naloxone for fentanyl) when co- administered. Ventilatory parameters were evaluated using unrestrained whole-body plethysmography in male and female CD-1 mice, with doses of 3 and 10mg/kg for xylazine, and 0.05, 0.15 and 1.35mg/kg for fentanyl, and 3mg/kg and 1mg/kg for naloxone and atipamezole respectively. Xylazine and fentanyl independently caused ventilatory depression: fentanyl via inspiration, with 1.35mg/kg increasing inspiratory time (Ti) by 167% of baseline, and xylazine by expiration, with 10mg/kg increasing expiratory time (Te) by 197% of baseline, both depressing ventilation rate ($p<0.05$). At these doses, fentanyl and xylazine induced changes in the ratio of inspiratory and expiratory times (Ti/Te) of +65% and -30.5% respectively, and the ratio of peak inspiratory flow and peak expiratory flow (PIF/PEF) of -42% and -23% respectively, potentially reflecting opposing mechanisms of ventilatory depression. When xylazine and fentanyl were co-administered, the effect size to Ti and Te was equal ($d=0.05$), and there was no change in the Ti/Te ratio ($p>0.05$). However, PIF was more depressed than PEF after co-administration of xylazine and fentanyl, with a decrease in PIF/PEF ratio ($p<0.01$), signifying a greater effect size on inspiration over expiration. Only combined naloxone and atipamezole treatment fully restored ventilation rate to baseline following co-fentanyl and xylazine administration, compared to that during saline infusion ($p<0.01$). All interventions mitigated the depression of Ti/Te and PIF/PEF, with naloxone and the combination of naloxone and atipamezole showing the most significant improvements ($p<0.001$). Our results highlight the increased clinical complexity of xylazine-fentanyl co-use. With the differing implications on inspiratory and expiratory components of breathing and the unknown importance of central regulation over airway patency. As such we now intend investigate the underlying mechanisms of these pattern alterations using an in situ model called the working heart-brain preparation.

A22 - Xylazine exhibits time-dependent conditioned-rewarding effects in a conditioned-place assay in Swiss Webster mice

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In recent years the veterinary anesthetic xylazine has become a common adulterant of illicit opiate supplies, particularly in the Northeastern United States. In a 2023 illicit drug checking program in Philadelphia, xylazine was present in 99% of samples where fentanyl was the primary drug (Philadelphia Department of Public Health, 2023). Self-reports from people who inject drugs (PWID) suggest that xylazine-adulterated fentanyl (XAF) may produce more severe withdrawal symptoms than fentanyl alone, and that xylazine may give fentanyl "legs," extending its perceived duration to give it a heroin-like effect (Friedman, 2022). To investigate these self-reports, we developed a conditioned-place aversion (CPA) assay using male Swiss Webster mice in which we paired a chamber to withdrawal from fentanyl and/or xylazine precipitated by naloxone and/or idazoxan. We first established a procedure that produced CPA from withdrawal fentanyl and used that paradigm to investigate xylazine alone and in combination with fentanyl. We found that xylazine withdrawal precipitated by idazoxan did not produce CPA, and that XAF did not alter fentanyl CPA. Unexpectedly, we found that mice conditioned to the drug-paired chamber 4hr after administration of 10 mg/kg xylazine exhibited a place-preference for the drug-paired chamber. This effect is not seen with when mice are conditioned 2hr after xylazine ($p=0.0319$). This data suggests that xylazine has a delayed conditioned-rewarding effect, which may explain the self-reports of extended time course of XAF compared to fentanyl alone. Future studies will investigate time course effects of XAF further, as well as respective dose ratios of xylazine to fentanyl. This work was supported by Peter F. McManus Charitable Trust, P30 DA013429, and NIDA Training Grant 5T32 DA7237-35.

A23 - Nitazenes of the past, present, and future: Insights from in vitro μ-opioid receptor assays and in vivo behavioral studies in male C57BL/6J mice

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Objectives
New synthetic opioids (NSOs) continue to contribute to drug-related fatalities worldwide. Since 2019, various NSOs with a 2-benzylbenzimidazole scaffold ('nitazenes') have been detected in the recreational drug market. In this work, we performed pharmacological characterization of 15 structurally diverse nitazene opioids that might be predicted to emerge or grow in popularity. The aim was to expand the existing knowledge about nitazene opioid structure-activity relationships (SAR), while also strengthening preparedness against current and future nitazenes that may cause harm to people who use drugs.

Methods
Mu-opioid receptor (MOR) affinity was determined via competition radioligand ([^{3}H]DAMGO) binding assays in rat brain tissue. MOR activation was studied by means of a cell-based β-arrestin 2 recruitment assay. Complementing in vitro findings, in vivo experiments were performed to investigate opioid-like effects (antinociception/hot plate latency, locomotor activity, body temperature changes) of seven etonitazene analogues after subcutaneous administration in male C57BL/6J mice.

Results
Binding assays revealed that all nitazenes bind to MOR with nanomolar affinities (Ki=8-431 nM). Functional potencies ranged from 0.588 nM (etonitazene) to 1266 nM (ethylene nitazene). Methionitazene (EC_{50}=5.28 nM) and α'-methyl etonitazene (EC_{50}=1.00 nM) exceeded the potency of fentanyl (EC_{50}=17.0 nM). Most newly studied nitazenes were less active than their corresponding comparator 2-benzylbenzimidazole; notable exceptions were iso-butonitazene (EC_{50}=10.3 nM) and sec-butonitazene (EC_{50}=7.62 nM), the potencies of which exceeded that of butonitazene (EC_{50}=34.2 nM). Furthermore, methylnitazene (EC_{50}=70.2 nM) and propylnitazene (EC_{50}=9.50 nM) were more potent than nitazene (EC_{50}=312 nM). Efficacies ranged from 82.8-254% (compared to hydromorphone). In vivo, dose-dependent effects were observed for antinociception, locomotor activity, and body temperature changes in mice. The most and least potent analogues were α'-methyl etonitazene (ED_{50}=0.060 mg/kg) and ethyleneoxynitazene (ED_{50}=11 mg/kg), respectively. Bell-shaped curves were obtained for locomotor activity. The maximum distance traveled by the animals was largely comparable (~320-410 m), and all analogues induced a comparable maximum decrease in body temperature compared to baseline (2.0-2.6°C).

Conclusion
By expanding our knowledge of nitazene SARs, this study allows to inform relevant stakeholders regarding nitazene opioids that have been actively contributing or might be anticipated to contribute to the nitazene as well as the larger opioid crisis.

A24 - Structure guided efficacy modulation to design opioid sparing analgesics

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Introduction

Mu opioid receptor (MOR) agonists with minimal efficacy- lower than partial agonist buprenorphine but higher than antagonist naloxone- have been proposed as alternatives to develop safer analgesics. To discover such agents, we used structure-based design to develop a new class of opioid sparing analgesics.

Methods

Novel ligands on the mitragynine pseudoindoxyl (MP) template were synthesized. The G-protein activity of analogs was characterized using biochemical C cell- based assays, assays in recombinant and heterologous systems and ex-vivo brain slice recordings. Two cryo-EM structures were solved in both active and inactive states.

Molecular dynamic simulations (MD) were run on both structures. To capture TM6 outward movement and subsequent Gi binding, we utilized a single molecule FRET (smFRET) assay. Antinociception was assessed using 55°C tail withdrawal, CCI, CFA and CIPN in mice.

Additionally, respiration using CLAMS and addiction potential using CPP and IVSA were measured in rodents.

Results

Screening of compounds led to the development of BP1-G4 (10-Cyclopropyl MP), which exhibits intermediate efficacy depending on receptor availability. In amplified assays like cAMP, Nb39 and GTPturnover it exhibited efficacy higher than naloxone and CTAP but lower than buprenorphine and the parent template, MP. In BRET Gi assays and [35S]-GTPγS assays, the ligand behaved as an antagonist.

Cryo-EM structures of BP1-G4 in active and inactive states, SAR on analogs and MD simulations, identified a novel 'Y1.39Y2.64H7.56' pocket- which was responsible for the intermediate efficacy observed with BP1-G4. smFRET demonstrated that BP1-G4's ability to induce TM6 outward movement and G-protein binding was weaker than its precursor, MP and stronger than the antagonist naloxone.

BP1-G4 showed MOR-dependent antinociception in thermal assays and anti-allodynia in neuropathic, chemotherapy induced neuropathy and inflammatory pain models while showing reduced respiratory depression, conditioned place preference in mice. BP1-G4 also showed no signs of IV self-administration in rats.

Conclusions

We identified an efficacy range required to achieve analgesia while minimizing adverse effects at MOR. We determined that engagement of a 'YYH' pocket in the receptor can modulate activation and lead to decreased efficacy, this can potentially be extrapolated to other GPCRs to fine tune efficacy. Supported by R01DA05SS78 and R01DA03C24C.

A25 - Looking for NOP receptor biased agonists: a SAR study on N/OFQ(1-13)-NH2

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Nociceptin/orphanin FQ (N/OFQ) controls several biological functions by selectively activating the Gi-coupled N/OFQ peptide (NOP) receptor . Here, we aimed to identify NOP receptor biased agonists by evaluating the ability of N/OFQ(1-13)-NH2 derivatives to promote NOP-Gβ1 and NOP-β-Arrestin 2 (β- Arr2) interaction in a bioluminescence-resonance energy transfer (BRET) based assay.
The first series of N/OFQ(1-13)-NH2 derivatives was obtained by swapping each amino acid from positions 5 to 13 with a Lys(Ac) residue. N/OFQ(1-13)NH2 showed superimposable potency (pEC50) in both pathways, with pEC50 values of 8.16 and 8.04 for G protein and β-Arr2, respectively. Substitutions from position 5 to 13 led to an overall decrease in agonist potency with the exception of the Lys(Ac)10 derivative that displayed similar potency as N/OFQ(1-13)-NH2. Then a panel of 10 different non natural amino acids were used for substituting Lys(Ac) in positions 5, 6, and 13 thus generating 30 novel peptides. None of these chemical modifications modified ligand efficacy. In terms of potency modifications at position 5 and 13 (<10 fold loss of potency) were better tolerated than at position 6 (> 10 fold loss of potency). However, all the changes in peptide potency were similar in NOP/G protein and NOP/β-Arr2 experiments. The only exception to this rule was [Lys(benzoyl)^{5}]N/OFQ(1-13)-NH2 that displayed high potency and efficacy in NOP/G protein and lower potency and efficacy in NOP/β-Arr2 experiments thus behaving as a G protein biased NOP agonist (bias factor calculated as $\Delta\Delta\log(\tau/KA) = 1.61$). Further studies are now needed to investigate the in vivo implications of NOP receptor biased agonism and eventually its therapeutic potential.

A26 - In vitro pharmacological characterization of novel NOP partial agonists

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Nociceptin/orphanin FQ (N/OFQ) is the endogenous ligand for the Gαi coupled N/OFQ peptide (NOP) receptor. NOP agonists are studied for their analgesic, anxiolytic, antitussive and hypnotic effects, while antagonists as possible treatments for depression and Parkinson's disease. The aim of the study was the investigation of the novel NOP receptor ligands AT-200, AT-336 and AT-523, comparing them to reference NOP ligands including the endogenous agonist N/OFQ, the full agonist AT-403 and the partial agonist AT-090.
Compounds were evaluated in vitro using BRET assays to monitor NOP–G protein and NOP–β-arrestin2 interactions, and calcium mobilization assays in cells expressing NOP or classical opioid receptors and chimeric G proteins.
In the NOP-G protein interaction assay N/OFQ potency was high (pEC_{50} 8.85), AT- 403 potency and maximal effect were close to that of N/OFQ, while AT-090, AT- 200, AT-336 and AT-523 behaved as moderate potency (pEC_{50} values ranging 7.40 – 7.67) partial agonists (alpha values ranging 0.23 – 0.48). In the NOP-β-arrestin2 interaction assay, N/OFQ showed a very similar profile as at G protein (pEC_{50} 8.31), while AT-403 showed slightly lower potency and Emax than in the G protein assay. AT-523 and AT-090 displayed similar effects as in the G protein interaction assay while AT-200 and AT-336 showed no agonist activity in the arrestin interaction assay, but were able to antagonize N/OFQ effects. In the calcium mobilization assay performed on cells expressing NOP receptor, N/OFQ and AT-403 displayed similar high potency and maximal effects. The other AT compounds displayed 4-20 fold lower potency and slightly lower maximal effects (alpha values 0.81 – 0.93). All compounds were inactive in cells expressing classical opioid receptors except for AT-336 which displayed limited NOP selectivity (35-fold over kappa receptors).
In conclusion, we identified novel NOP-selective partial agonists that were unbiased (AT-523) or G protein-biased (AT-200 and AT-336) NOP ligands. These compounds are valuable tools for future in vivo studies, helping to clarify how NOP receptor functional selectivity impacts therapeutic outcomes.

A27 - In vitro pharmacological characterization of innovative opioid ligands with distinct binding profiles and improved pharmacological activity

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Opioids targeting mu opioid receptor (MOR) are still the most widely used pain killers, despite their limited efficacy in different chronic pain conditions, their relevant adverse effects and abuse liability. To overcome these issues, innovative opioids with improved pharmacology have been sought by exploring multiple alternatives (e.g.: G protein-biased or peripherally restricted agonists at MOR, KOR, DOR). Ligands simultaneously modulating multiple opioid receptors (e.g.: MOR/NOPr dual agonists) have been recently attracting increasing interest for their potentially enhanced effectiveness, reduced side effects, uncomplicated PK/PD.
Here, we aimed to characterize the in vitro pharmacological profile of three EM-1 analogues containing modified urea (urea-EM-1) to identify innovative opioid ligands with distinct binding profile to one or more opioid receptors and possibly improved pharmacology.
To assess compounds affinity profile, competition binding assays were carried out in HEK-293 cells selectively overexpressing human MOR, DOR, KOR, or NOPr as previously described. Ligands activity at their target opioid receptor(s) was investigated by evaluating their ability to inhibit forskolin-induced cAMP accumulation in HEK-293 cells overexpressing the receptors and in PMA- differentiated SH-SY5Y human neuroblastoma cells (a human neuron-like model) endogenously expressing them. Ligands-mediated effects on expression of inflammatory mediators and opioid receptors were evaluated via qPCR in PMA-differentiated SH-SY5Y cells.
MF134 displayed high affinity to MOR (ki=3.5±0.5 nM) and some affinity to DOR (ki=6052±259 nM), and significantly inhibited adenylyl cyclase in PMA-differentiated SH-SY5Y cells (IC_{50}=0.25±0.08 nM; E_{max}=68±6 %).
MF195 displayed high affinity to MOR (ki=3.5±0.5 nM) and good affinity to NOPr (ki=214.3±15.2) nM), and significantly inhibited adenylyl cyclase in PMA-differentiated SH-SY5Y cells in a NOPr- dependent way (IC_{50}=0.63±0.05 nM; E_{max}=49.7±6.5 %).
MF192 displayed high affinity and selectively to NOPr (ki=4.0±0.3 nM).
Peptides effects on the expression of inflammatory mediators and opioid receptors are being investigated, and data will be presented at the conference.
Three novel EM-1 derivatives containing modified urea were identified and characterized: MF134, a MOR potent partial agonist (with some low affinity to DOR); MF195, MOR/NOPr dual ligand displaying potent partial agonism at NOPr; MF195, a high affinity NOPr-selective ligand. Thus, emerging as innovative opioid ligands with promising, potentially improved profile.

Supported by QSPainRelief (H2020 grant agreement n.848068).

A28 - The Positive Allosteric Modulator BMS-122 Enhances the Anti-Allodynic Action of Opioid and Non-Opioid Analgesics in Rats with Nerve Injury

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Opioid therapy for acute pain is effective but carries risk of abuse and overdose. Opioid therapy for chronic pain is even riskier, as efficacy decreases, and duration of treatment extends.

Adjunct medications to enhance opioid analgesia without enhancing adverse effects could provide a useful method for opioid sparing. Positive allosteric modulators (PAMs) interact with agonist-occupied receptors to increase agonist affinity and/or signaling. One PAM of the μ- opioid receptor, BMS-122, has been shown to enhance opioid activity in vivo without increasing negative effects. However, these studies were performed with opioids on acute antinociception. Our objective is to determine the capacity of BMS-122 to enhance opioid activity in chronic pain and to potentially enhance the activity of non-opioid analgesics.

Studies were conducted using the spared nerve injury model (SNI) of mononeuropathy in male and female Sprague-Dawley rats. All rats developed substantial tactile allodynia as measured with von Frey filaments. Animals then received a pretreatment of BMS-122 (10 mg/kg, s.c.) or vehicle, followed by (R)-methadone (0.1, 0.32, or 1 mg/kg, s.c.) or morphine sulfate (1, 3.2, or 10 mg/kg, s.c.). Tactile thresholds were measured for 2 hours. Doses of opioid with minimal effects on allodynia were enhanced with by pretreatment of with BMS-122. The combination increased anti-allodynia to a similar or greater extent than a fully efficacious dose of the opioids. To test more clinically relevant therapeutics, we repeated the study using the opioid partial agonist buprenorphine (0.01, 0.032, or 0.1 mg/kg, s.c.) or the non-opioid analgesic gabapentin (30 or 100 mg/kg). BMS-122 enhanced the action of even the highest dose of buprenorphine, supporting the capacity for PAMs improve the efficacy of partial agonists. In addition, BMS-122 enhanced the activity of gabapentin at a low dose, to that of a fully efficacious dose.

Gabapentinoids and opioid drugs synergize in nerve injury, and these data suggest a possible interaction between gabapentin and endogenous opioid peptides. Overall, our data suggest that opioid PAMs may be effective in treating neuropathic pain, either as opioid sparing agents or as adjuvants to non-opioid analgesics.

These studies were supported by R37 DA039997 (J.R. Traynor) and T32 TR004764 (B.M. Clements).

A29 - Squaramide Derivatives as μOR/κOR Ligands: Synthesis, Functional Evaluation, and Receptor-Ligand Interaction Analysis

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Since the 2016 publication of PZM21 as a potential analgesic with reduced side effects, its structure has inspired extensive structural modifications, especially with regard to its G protein-biased signaling over β-arrestin-2 recruitment. Structure–activity relationship (SAR) studies of new PZM21 derivatives, together with data on key interactions from the crystal structure of PZM21 bound to the μ-opioid receptor, have significantly advanced our understanding of the relationship between ligand structure and pharmacological activity.

PZM21 features four key pharmacophoric features: a positive ionizable group (PI), a hydrogen bond acceptor/donor (HBA/HBD), and two aromatic rings (AR), whose spatial arrangement is critical for modulating potency, selectivity, and overall pharmacological profile. In this study, we investigate the SAR of a partially rigidified series of PZM21-like compounds featuring a squaramide moiety, capable of acting as both a hydrogen bond donor and acceptor.

The synthesized squaramide derivatives exhibited high μOR affinity (3–271 nM), diversified κOR affinities (26–4024 nM), and low δOR binding (≥700 nM). In functional in vitro assays, three derivatives displayed partial agonist activity at μOR with a preference for G-protein- biased signaling over β-arrestin-2 recruitment. The remaining compounds in this series behaved as antagonists at both μOR and κOR. Additionally, in silico docking studies revealed distinct ligand–receptor contact patterns within μOR and κOR binding pockets, supporting the observed pharmacological profiles.

This work highlights a novel series of structurally related μOR or dual μOR/κOR ligands with divergent intrinsic activities and signaling preferences, offering insights into the structural determinants of opioid receptor modulation.

Acknowledgments

The study was funded by Grant 2018/31/B/NZ7/03954, financed by the National Science Centre, Poland (www.ncn.gov.pl).

A30 - In vitro pharmacological characterization of novel derivatives of the NOP receptor ligand [6ACH^2]N/OFQ(1-13)NH$_2$

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The nociceptin/orphanin FQ (N/OFQ) control different biological function by selectively activating the N/OFQ peptide (NOP) receptor, a naloxone-insensitive member of the opioid receptors family. Based on the active model of the NOP receptor predicted in silico we synthesized and tested a small library of peptides constrained at positions Gly^2 and Gly^3; this lead to the identification of [6ACH^2,desGly^3]N/OFQ(1–13)-NH$_2$] (3B) as a novel NOP ligand (Ciancetta et al. 2024). In the present study we designed novel [6ACH^2,desGly^3]N/OFQ(1–13)-NH$_2$ derivatives by substituting Phe^1 with Tyr, Nphe, Dmt, and Pheϕ), resulting in the generation of four pairs of enantiomers. The peptides were assayed in vitro by bioluminescence resonance energy transfer (BRET) for NOP-G protein and NOP-β- arrestin 2 interactions. In the NOP- G protein BRET assay, N/OFQ behaved as a potent (pEC$_{50}$ 8.22) agonist. All the novel molecules were inactive up to 10 μM with the exceptions of Phe^{1}ϕA which behaved as low potency (pEC$_{50}$ 6.40) partial agonist (α 0.32) and Phe^{1}ϕB which displayed low potency (pA$_2$ 6.19) antagonist properties. Additionally, all compounds were tested at β-arrestin 2 interaction, with N/OFQ being a potent (pEC$_{50}$ 8.37) agonist while none of the derivates showed agonist effects up to 10 μM. The two Phe^{1}ϕ enantiomers showed similar antagonist potency (pA$_2$ 6.63 and 6.66). Overall, these datasets highlight the enantiomers Phe1ϕA and Phe1ϕB as intriguing tools to finely tune NOP receptor efficacy and functional selectivity. To the best of our knowledge, this is the first example of enantio-specific triggering of functional selectivity at the NOP receptor.

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A31 - Replacement of Proline by Triazole in Endomorphin-1: Design and Identification of a Potent K-Opioid Receptor-Selective Agonist

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The κ-opioid receptor (OR)/dynorphin system modulates key physiological functions, including stress, mood, and emotional behaviours. Its dysregulation is linked to neuropsychiatric disorders such as depression, anxiety, and substance use disorders. In this context, in the last few years we became interested in developing κOR ligands as potential treatments for these conditions.
For instance, the cyclopeptide c[D-Trp-Phe-Gly-β-Ala], derived from [D-Trp]CJ-15,208, resulted to be aκ OR-selective biased agonist, displaying functional selectivity toward G-protein-dependent signalling[1], while another cyclopeptide, c[D-Trp-Phe-β-Ala-β-Ala] emerged as the first κOR-selective negative allosteric modulator reported to date[2]. Interestingly, the modification of the endogenous, potent and selective κOR agonist endomorphine-1 (H-Tyr-Pro-Trp-PheNH2, EM1), by substitution of Pro with a β[2]-pseudoPro, yielded H-Tyr-Amo-Trp-PheNH$_2$, a κOR-selective partial agonist[3]. In this work, we discuss other EM1 derivatives designed by replacement of Pro with a 1,2,3-triazole, obtained by Cu(I)-catalyzed alkyne-azide cycloaddition (click chemistry) (Figure). The dipeptide-azide N$_3$-(S or R)-Trp-(S)-PheNH$_2$ was indeed coupled to a (S)-Tyr derivative carrying the alkyne substituent in the form of amide (Y = O, X = NH), amine (Y = HH, X = NH), ester (Y = O, X = O), or ether (Y = HH, X = O).

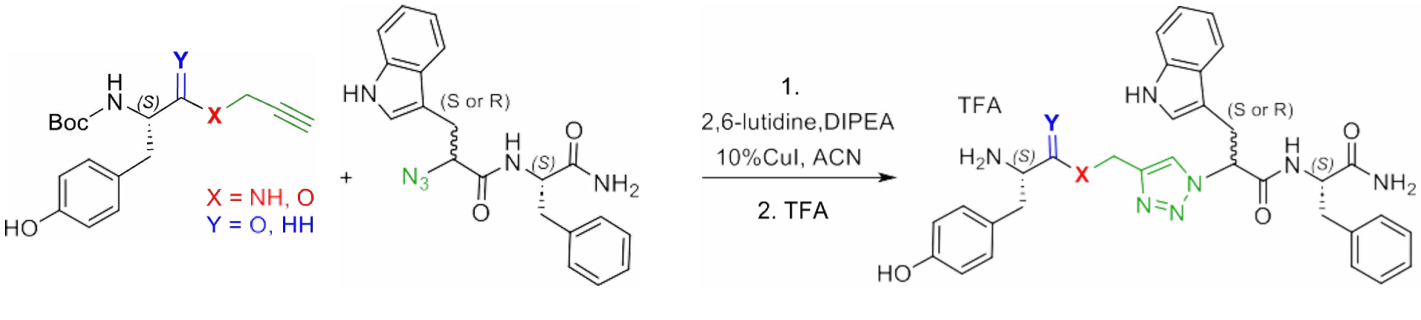

Figure. Synthesis by click chemistry of EM1 derivatives containing a triazole ring at the position 2.

The triazole scaffold shares several physicochemical similarities with peptide bond, including the distance between the substituent, the dipole moment, the planarity and hydrogen bond acceptor/donor behaviour. In addition, the modification is expected to significantly improve metabolic stability and bioavailability. Also, the triazole-peptides adopt an extended all-trans geometry. These modifications lead to the discovery of (S)- Tyrol-triazole-(S)-Trp-(S)-PheNH$_2$ (FS302), which showed good affinity towards κOR in a displacement binding assay (Ki = 84 nM), while the cAMP test in HEK-293 cells revealed a κOR agonist behaviour, since it significantly inhibited forskolin-induced cAMP accumulation (IC$_{50}$ = 2.4 ± 0,1 nM; Emax 88 ± 12%). From these first findings, FS302 came to the spotlight as a potent κOR selective agonist, confirming that the introduction of triazole represents an effective tool for generating effective peptidomimetics. Further investigations have been conducted to disclose compound's functional profile and metabolic stability.

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A32 - Effects of Biased Analogues of The Kappa Opioid Receptor Agonist, U50,488, Using In Vivo Mouse Models of Pain And Side Effects

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Kappa opioid receptor (KOR) agonists have well-established antinociceptive effects. However, many KOR agonists have negative side effects, which limit their therapeutic potential. Some researchers have suggested that the development of biased agonists that preferentially stimulate KOR G-protein pathways over β-arrestin pathways may yield drugs with fewer adverse side effects. This was investigated in the current study. We describe the synthesis and characterization of three U50,488 analogues, 1, 2, and 3. We evaluated the acute and chronic antinociceptive effects of these compounds in mice using the warm-water tail flick assay and in a paclitaxel-induced neuropathic pain model. Side effects were investigated using open-field, passive wire hang, rotarod, elevated zero maze, conditioned place aversion, and whole-body plethysmography, with some tests being conducted in KOR or β-arrestin2 knock out mice. All compounds were highly potent, full agonists of the KOR, with varying signaling biases in vitro. In the warm-water tail withdrawal assay, these agonists were ~10 times more potent than U50,488, but not more efficacious. All KOR agonists reversed paclitaxel-induced neuropathic pain, without tolerance. Compound 3 showed no significant side effects on any test. Signaling bias did not correlate with the antinociceptive or side effects of any compounds and knockout of β-arrestin2 had no effect on U50,488-induced sedation or motor incoordination. These findings highlight the therapeutic potential of 3, with its lack of side effects typically associated with KOR agonists, and also suggest that G-protein signaling bias is a poor predictor of KOR agonist-induced side effects.

A33 - Determination of the antinociceptive effect of linalool, the main component of lavender essential oil, against thermal pain and its effect on anxiety-related or obsessive-compulsive disorder-related behaviors

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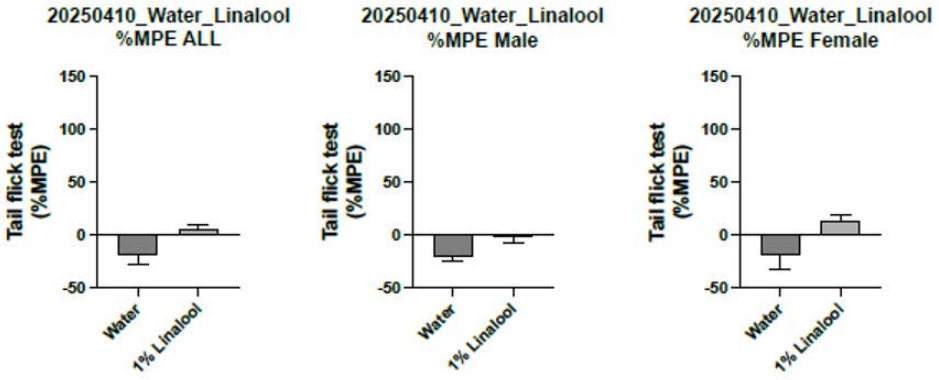
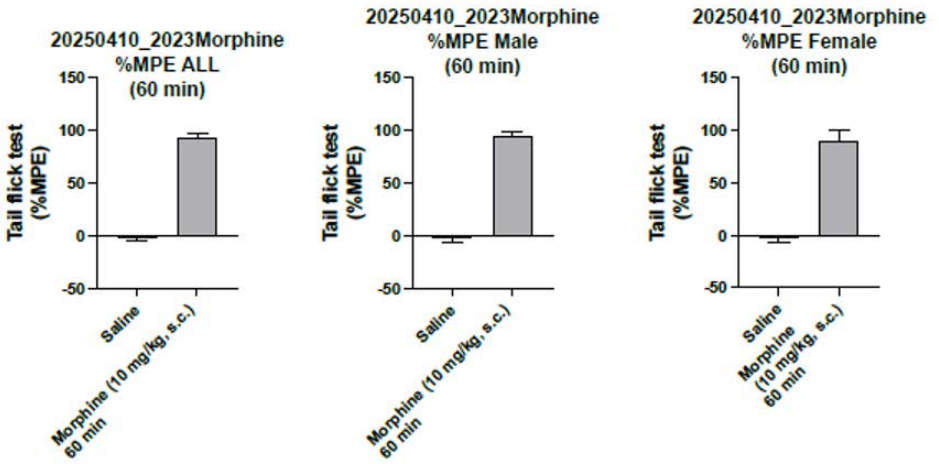
Lavender essential oil (LEO) is the most widely used aromatic plant, and has been shown to have favorable therapeutic effects on inflammation, cancer, and neuropathic pain in humans and rodents. Linalool, the main component of LEO, reduces inflammatory responses and relieves pain in both humans and rodents. In addition, clinical and preclinical studies have suggested that essential oils rich in linalool exhibit various pathophysiological effects such as antidepressant action. In this study, we examined the effects of lavender essential oil and linalool on pain responses to thermal stimulation (tail-flick test), anxiety-related behavior (marble-burying test), and obsessive-compulsive disorder (OCD)-related behavior (splash test) in adult male and female mice. The inhalation of 1% lavender for 5 min did not affect the tail-flick test. However, inhalation of 1% linalool modestly, but significantly, extended the response time to heat stimulation in the tail-flick test, suggesting the modest antinociceptive effects of 1% linalool inhalation. The effect of 1% linalool was 16.4-fold lower than that of morphine (i.e., the%MPE of 1% linalool was 5.6%, while that of morphine (10 mg/kg, s.c.) was 92.8%). In addition, the effect of 1% linalool was more significant in female mice than in male mice, whereas the effect of morphine (10 mg/kg, s.c.) did not differ between male and female mice. In contrast, there were no changes in the marble-burying or splash tests after treatment with 1% lavender or 1% linalool inhalation.
Collectively, these findings suggest that linalool inhalation has modest antinociceptive effects. However, linalool did not affect anxiety-related or OCD-related behaviors following inhalation treatment. Additional studies are required to understand the detailed mechanisms underlying its antinociceptive effects.

(参考，再解析データ、 %MPE)



p=0.0030 (ALL), p=0.0182 (Male), p=0.0312 (Male), vs. Water, unpaired t-test.

n= 46 (ALL), n= 22 (Male), n= 24 (Female)



P<0.0001 (ALL), P<0.0001 (ALL), P<0.0001 (ALL), vs. Water, unpaired t-test.

n= 12-14 (ALL), n= 6-7 (Male and Female)

A34 - A Novel In Vivo Protocol for Evaluating Evoked and Non-Evoked Migraine- Like Signs in Mice

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Background
Migraine is one of the most prevalent neurological disorders worldwide, especially among women (3:1 female-to-male ratio). Despite available therapies, many patients are still dissatisfied, highlighting the need for new therapeutic strategies. Preclinical models are essential for understanding migraine pathophysiology and testing new treatments. Traditional models rely on pharmacologically induced migraine-like states, with periorbital mechanical allodynia (PMA) as the primary measurable outcome. However, given the complexity of migraine symptoms in humans, a robust animal model should integrate multiple migraine-like signs. The mouse grimace score (MGS) and photophobia are non-evoked migraine-associated signs, but existing protocols to assess these endpoints lack standardization and often produce highly variable results.

Objective
This study aimed to develop a novel experimental protocol for evaluating multiple evoked (PMA) and non-evoked migraine-like signs (photophobia and MGS) in the same animal, in accordance with the 3R principle. The effects of nitroglycerin (NTG) and calcitonin gene-related peptide (CGRP) were assessed in parallel in male and female mice from two different strains (CD-1 and C57BL/6).

Methods
Male and female CD-1 and C57BL/6 mice received NTG (10 mg/kg, i.p.), CGRP (0.1 mg/kg, i.p.), or vehicle. PMA was assessed using von Frey filaments, following the up-down method. Photophobia was assessed with light/dark box test (10–40 min post-injection), and MGS was scored at baseline and 60 min post-injection.

Results
In traditional models, both NTG and CGRP significantly increased PMA in all groups, independent of sex or strain. However, our refined protocol revealed that CGRP induced photophobia, increased MGS, and PMA in male mice, whereas in females, CGRP increased only MGS and PMA. NTG exclusively induced PMA, without affecting photophobia or MGS.

Conclusion
This study establishes a novel, standardized protocol for assessing multiple migraine- like signs in mice, reducing animal use while improving translational relevance. The findings highlight differential effects of CGRP and NTG and suggest a sex-specific role of CGRP in photophobia. This model may enhance preclinical evaluation of anti-migraine therapies.

A35 - Kappa opioid receptor signaling in the mouse claustrum modulates pain-evoked behavioral states

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Pain is a multidimensional experience with both sensory and affective components. The claustrum has been implicated as a potential integrator of sensory and emotional states, but its role in pain and opioid signaling remains unclear. In this study, we tested the hypothesis that kappa opioid receptor (KOR) signaling in the claustrum contributes to pain-related affective behaviors.

We combined fluorescent in situ hybridization (FISH) and in vivo fiber photometry in mice to assess the role of KOR in the claustrum across different models of pain. Our FISH experiments revealed that Fos expression (a marker of neuronal activity) is upregulated in KOR-expressing claustrum neurons following acute noxious stimulation. Furthermore, real-time calcium imaging demonstrated that the claustrum is activated in response to noxious sensory stimuli.

We next explored how KOR activity in the claustrum affects pain-related changes in affective behavior. Using a complete Freund's adjuvant (CFA) model of inflammatory pain, we observed a significant increase in binge-like consumption of sucrose pellets, suggestive of an altered affective state. Systemic administration of the KOR antagonist norbinaltorphimine (norBNI) blocked this effect, implicating a role for KOR signaling in mediating pain-induced changes in appetitive behavior.

Together, our results reveal the importance of claustral KOR activity in modulating behavioral responses to acute and persistent pain. This study identifies the claustrum as a KOR-sensitive that links nociception to affective behavior, which may underlie the neural basis of pain-induced affective dysregulation.

A36 - Set-up of a murine model of endometriosis and migraine comorbidity

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Endometriosis (EM) is a painful condition characterized by the presence of ectopic endometrial tissue, mostly located in the peritoneal cavity. EM is frequently associated with comorbidities, including migraine, which occurs in EM patients at roughly twice the rate observed in healthy women. Additionally, migraine appears to be a risk factor for developing endometriosis.

The objective of this study was to set up a mouse model of endometriosis and migraine comorbidity.
First, donor mice received subcutaneous estradiol benzoate to stimulate endometrium growth. After 7 days, uterine fragments from donor mice were suspended in phosphate buffered saline (PBS) and injected intraperitoneally (i.p.) in recipient (EM) mice; sham mice received an injection of PBS. To mimic migraine, glyceryl trinitrate (GTN) (0.1, 1 or 10 mg/kg) was injected i.p. every two weeks, starting on day 14 after EM induction. EM-like and migraine-like behaviors were assessed every 2 weeks up to 57 days post- implantation. As EM signs, abdominal mechanical allodynia (AMA) and spontaneous pain-like behaviors were assessed. As a hallmark of migraine, periorbital mechanical allodynia (PMA) was measured.

Starting at 14 days post-implantation (DPI), EM mice exhibited significantly reduced abdominal mechanical thresholds and increased spontaneous pain-like behavior compared to sham controls. Only EM mice showed susceptibility to GTN 0.1 and 1 mg/kg, while sham mice displayed reduced periorbital mechanical thresholds only in response to GTN 10 mg/kg. In this model, EM significantly worsened migraine signs. On the other hand, EM mice displayed no GTN-dose dependent differences in EM signs, implying that the severity of the migraine trigger did not worsen EM in this model.

In summary, this model successfully replicates EM and migraine comorbidity, highlighting EM-induced susceptibility to subthreshold migraine triggers. This study has provided a valuable tool for investigating potential therapeutic targets and pathophysiological mechanisms underlying EM-migraine comorbidity.

A37 - Enhancing Recovery Quality After Lumbar Fusion Surgery Through ERAS Implementation: A Retrospective Study

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Purpose
This study explored the impact of Enhanced Recovery After Surgery (ERAS) implementation on postoperative recovery quality in patients undergoing 1–2 level lumbar fusion surgery.

Method
A retrospective quasi-experimental study was conducted at a medical center in southern Taiwan between January 2023 and February 2024. Forty-nine patients undergoing 1–2 level lumbar fusion were assigned to an ERAS group (n=24), which received ≥60% of a standardized 21-item ERAS protocol across pre-, intra-, and postoperative phases, or to a traditional care group (n=25), which received routine perioperative management without structured ERAS implementation. Outcomes included length of stay, time to first ambulation, pain scores on surgery day and at discharge, pain improvement from surgery day to discharge and from admission to discharge, complications, nausea/vomiting, and 30-day readmission rates.

Result
ERAS significantly reduced hospital stay (4.67±0.3 vs. 7.36±0.57 days; $p<0.001$), accelerated ambulation (1.63±0.15 vs. 2.52±0.21 days; $p=0.001$), and lowered pain scores on surgery day (2.38±0.16 vs. 3.56±0.20) and discharge (1.75±0.14 vs. 2.52±0.13), both $p<0.001$. Pain improvement from surgery day to discharge was greater (1.04±0.21 vs. 0.16±0.24; $p=0.008$), whereas improvement from admission to discharge did not differ significantly ($p=0.106$). No significant differences were observed in nausea/vomiting ($p=0.668$), complications ($p=0.349$), or readmissions ($p=0.490$).

Conclusion
ERAS significantly enhanced postoperative recovery and functional outcomes without increasing adverse events. Findings support its integration into routine lumbar fusion care. Future implementation should focus on outpatient engagement, protocol adherence, and long-term functional evaluation.

Keywords
ERAS, lumbar fusion, recovery quality, postoperative care, length of stay

A38 - Lack of contralateral morphine analgesia in the acid-induced fibromyalgia model and its reversal by therapeutic mirtazapine, but not pregabalin

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When acid saline (pH4.0) was unilaterally administered twice 5 days apart into the gastrocnemius muscle of mice, chronic and bilateral (generalized) hyperalgesia (AcGP) was observed. The AcGP model showed similar pathophysiological and pharmacotherapeutic features observed in fibromyalgia patients in clinic. For example, the hyperalgesia in the AcGP model showed the female-dominance under the condition of gonadectomy and was reversed by pregabalin or duloxetine, but not by anti-inflammatory drug diclofenac. Moreover repeated i.c.v. injections of pregabalin or alpha2-adrenergic antagonist-type antidepressant mirtazapine completely cured the established hyperalgesia in the AcGP model. However, we found unique brain analgesic actions of morphine in this model. When morphine (i.c.v.) was given in the AcGP model, analgesic actions were observed only on the ipsilateral, but not contralateral side of paw. The lack of contralateral morphine analgesia was significantly reversed by a single i.c.v. injection of MK801, an NMDA receptor antagonist in the AcGP model. When morphine was microinjected into the NRPG, a most sensitive brain site for morphine analgesia, the analgesia was detected only on the ipsilateral side of paw of naïve mice. Therefore, it is speculated that the intense pain signal following the second muscular acid injection activates anti-opioid NMDA receptor in the contralateral lower brain stem including NRPG and counterbalances the morphine actions. As the lack of contralateral morphine analgesia was also significantly reversed by repeated i.c.v. treatments with mirtazapine, but not by pregabalin, mirtazapine may have independent actions of the cure of chronic pain in the AcGP model and reversal of lack of morphine analgesia.
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Wakako Fujita

In this study, we focus on the mechanism of antinociceptive tolerance to morphine.
Recent study revealed that the RTP4, a receptor chaperone protein targeting GPCR including opioid receptors is involved in the antinociceptive tolerance to morphine.
Interestingly, RTP4 levels were significantly upregulated only in the hypothalamus and the brain region specific deletion of RTP4 leads to the partial suppression of the development of antinociceptive tolerance to morphine.
Although the decrease in antinociception (i.e., tolerance) was observed in both male and female animals but the increase of RTP4 in the hypothalamus was only observed in male but not in female animals.
Furthermore, chronic morphine known to induce hyperalgesia, but I found that only in female but not in male mice showed the trends to decrease of the withdrawal threshold.
Furthermore, I found that the inflammatory cytokines including TNFalpha and IL-1beta showed the trend to increase only in female but not in male animals.
These results suggest that there may be a sex differences in the mechanism of development of antinociceptive tolerance to morphine and in the mechanism of development of hyperalgesia after repeated morphine administration.

A39 - Delta Opioid Receptors Inhibit PACAP-PAC1 signaling following Opioid Induced Hyperalgesia in the vlPAG

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Opioid induced hyperalgesia (OIH) is a prevalent chronic pain condition that remains a challenge for millions globally. Pituitary adenylate cyclase-activating peptide (PACAP) promotes hyperalgesia via the PAC1 receptor which is densely expressed in the ventrolateral periaqueductal gray (vlPAG). The vlPAG also expresses opioid receptors, including the mu opioid receptor (MOR) and the delta opioid receptor (DOR), which inhibit pain. Although DOR's have minimal effects in naïve animals, they are upregulated in chronic pain. Using whole-cell patch-clamp recordings from neurons in ex vivo slices containing vlPAG from male and female C57/B6 mice, we examined the effects on PACAP and the DOR agonist Deltorphin II on synaptic transmission. Mice were treated with repeated injection of morphine (20mg/kg for 3 days, 40mg/kg on day 4, SC) to induce OIH. We hypothesized that PACAP would enhance GABA signaling via the PAC1 receptor within the vlPAG and that OIH treated mice would have increased endogenous PACAP. We also hypothesized that DOR agonists would be effective at reversing OIH changes in PACAP signaling.

In saline mice, PACAP (5nM) potentiated electrically evoked GABA release in the vlPAG. In mice treated with morphine, the effect of PACAP was potentiated. To determine whether chronic morphine treatment increased endogenous PACAP within the vlPAG, we applied M65 to slices. M65(100bM) inhibited evoked GABA release in OIH mice without an effect in saline treated mice suggesting that morphine treatment induces the release of endogenous PACAP. The DOR agonist deltorphin II (1mM) inhibited PACAP potentiation in both saline and morphine treated mice. There is a trend toward greater effects of deltorphin following morphine treatment. The effects of deltorphin II were reversed with the delta opioid antagonist ICI 174,864 (1mM). The results indicate that DOR agonists may be useful as therapeutics for OIH. Future experiments will examine the signaling pathways involved in DOR reversal of PACAP effects in the vlPAG.

A40 - Kappa Opioid Receptor Antagonist Properties of Psychotropic Medications in a Mouse Model of Antinociception

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Background/Objectives
Approximately one in five people experience an episode of major depressive disorder, with 30% of these individuals resistant to standard antidepressant treatments, resulting in treatment-resistant depression (TRD). While opioids were historically used to treat melancholia, addiction risks led to their disuse. Recently, kappa opioid receptor (KOR) antagonism has been proposed as a therapeutic strategy in TRD. This study investigates whether commonly used psychotropic medications possess KOR antagonist properties that contribute to their efficacy in TRD.

Methods
We evaluated the antinociceptive effects of seven psychotropic drugs—mianserin, mirtazapine, venlafaxine, reboxetine, risperidone, amisulpride, and zolpidem—in mouse models using hotplate and tail-flick assays. The effects were tested in combination with naloxone (non-selective opioid antagonist), Nor-BNI (kappa-selective antagonist), and U50,488H (kappa agonist).

Results
All drugs exhibited dose-dependent antinociceptive effects, reversed by naloxone and Nor- BNI, indicating opioid system involvement. Co-administration of the KOR agonist U50,488H with mianserin, venlafaxine, or risperidone produced significant leftward shifts in the dose- response curves (e.g., ED_{50} of U50,488H decreased from 4.8 to 0.5 mg/kg with mianserin; $p < 0.05$), suggesting KOR antagonism. Reboxetine, zolpidem, amisulpride, and mirtazapine were not significantly potentiated with U50,488H.

Conclusion
Based on these findings, we propose that certain psychotropic medications may exert their effects in part via kappa opioid receptor antagonism. These FDA-approved agents for depression or related conditions may be suitable for repurposing as monotherapy or augmentation in TRD. Given their availability, the feasibility of conducting future trials to assess this approach is evident.

A41 - Fentanyl-Type Antagonist of the μ-Opioid Receptor: Important Role of Axial Chirality in the Active Conformation

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In recent years, synthetic opioids have merged as a predominant cause of drug-overdose- related fatalities, causing the "opioid crisis." To design safer therapeutic agents, we accidentally discovered μ-opioid receptor (MOR) antagonists based on fentanyl, which is one of the strongest MOR agonists.[1]
We presumed that fentanyl analogues should exhibit E/Z-isomerism around the N–(C=O) bond and atropisomerism based on the Ar–N(C=O) bond. While fentanyl was known to exist predominantly in the E-form, the atropisomeric property has not been investigated. We successfully created atropisomers of fentanyl analogues by introducing substituents at the 2′ or 6′ position of the anilino moiety and separated the enantiomeric forms. Examination of the affinity at the MOR revealed that one of the atropisomers, the (–)-form, of the fentanyl analogues serves as an agonist and the other, the (+)-form, as an MOR antagonist. Subsequently, we conducted in vivo study by using mice. The intraperitoneal administration of the (+)-atropisomer antagonized the effects of morphine to a degree comparable to naloxone.
This pioneering discovery was achieved by introducing atropisomerism into the fentanyl framework. Although atropisomeric properties are often overlooked, many drugs exhibit latent chirality. Hence, this study provides a novel perspective and valuable insights for developing pharmaceuticals. Furthermore, these SAR studies offer a clue to the future drug design of fentanyl analogues with agonistic/antagonistic activity against MOR.

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A42 - GPR63 and GPR153 enhance pathological pain relief through the suppression of microglial activation

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Abstract

Chronic pain impacts approximately 20% of the global population. While opioids are effective at providing pain relief, they have a surplus of side effects including the risk of tolerance, addiction, and respiratory depression. This leads us to the search for novel pain treatments. Orphan receptors represent promising drug targets because, despite limited characterization, several have been implicated in pain modulation pathways. G-protein coupled receptors (GPCRs) make up approximately 35% of current drug targets on the market underscoring the value of investigating these less-understood receptors. Through a screening campaign, we show that the orphan receptors GPR63 and GPR153 play a key role in pathological pain relief. RNAscope revealed that GPR63 is present in 50-60% of microglia and GPR153 is present in near-100% of microglia. Using non-selective and microglia-selective CRISPR to perform knockdowns we have found that elimination of GPR153 blocks pain relief and recovery in paw-incision and both GPR63 and GPR153 block pain relief in chemotherapy-induced peripheral neuropathy (CIPN). Neither GPR63 nor GPR153 affect antinociception in acute pain assessed through the tail flick assay, suggesting the receptors act only in pathological pain states. We further performed microglial inhibitor and morphology assays and found that these receptors suppress microglial activation, suggesting a novel mechanism of action in pathological pain. Since these receptors have no known ligands, we are currently searching for agonists and antagonists of these receptors using a combination of in silico and in vitro assays. Our computational approach includes the use of molecular dynamics simulations and geometric deep learning models to perform virtual ligand screening. The top hit compounds will be assessed via PRESTO-Tango arrestin recruitment assays. Overall, we have described a completely novel role for these orphan receptors in regulating microglial activity during pathological pain. Through our basic science and drug discovery efforts, we will further determine whether these receptors could also be effective therapeutic targets for the management of pathological pain.

Acknowledgments

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A43 - In Vivo Chemogenetic Activation and Retrograde Mapping of Enkephalinergic Pain Circuits in Mice

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The endogenous opioid system is crucial for pain modulation, particularly within the descending pain pathway. While extensive research has elucidated the roles of opioid receptors and exogenous opioids, the specific contributions of endogenous opioid peptides, such as enkephalins, and their neural circuitry remain underexplored. This study seeks to delineate how enkephalinergic neurons modulate acute nociception via projections to the ventrolateral periaqueductal gray (vlPAG), and whether this circuitry exhibits sex-dependent differences. To investigate this, we utilized PENK-IRES2-Cre mice and conducted stereotaxic viral injections for retrograde tracing and chemogenetic manipulation using DREADDs. Quantitative PCR, immunohistochemistry, and pain behavior assays (plantar and formalin tests) were employed to assess behavior and molecular outcomes. Chemogenetic activation of enkephalinergic neurons projecting to the vlPAG using DCZ significantly increased antinociception relative to saline- treated controls (plantar: $p = 0.0076$; formalin: $p = 0.0091$). Retrograde tracing identified multiple brain regions containing enkephalin-positive neurons that project to the vlPAG, including the prefrontal cortex, amygdala, anterior cingulate cortex, insula, nucleus accumbens, and rostral ventromedial medulla. Furthermore, qPCR revealed significant sex differences in proenkephalin (PENK) gene expression across these regions, most notably the amygdala ($p = 0.0007$), suggesting a sexually dimorphic organization of enkephalinergic input to the vlPAG. Collectively, these findings offer novel insights into the previously uncharacterized enkephalinergic pain circuits and highlight the importance of examining peptide-based mechanisms alongside traditional receptor-targeted approaches. Furthermore, by identifying sex- specific differences within these circuits, this study lays important groundwork for the development of more personalized and sex-informed strategies for pain management.

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A44 - VGF in the nucleus accumbens: roles in synaptic plasticity and opioid-evoked behaviors

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The nucleus accumbens (NAc) relies on neuropeptides to tune reward-related behaviors, and exogenous opioid intake can topple the balance of neuropeptides within the NAc and increase vulnerability to addiction. Neuropeptides derived from VGF (non-acronymic) may be impacted, as prior work demonstrated that transcription of VGF increased in the NAc of mice following a week of opioid exposure. VGF-derived peptides are known to mediate plasticity, but their action on NAc physiology and opioid-evoked behaviors is unknown. Our objective is to characterize VGF expression in the NAc and determine its contribution to opioid-evoked behaviors.

We used RNA in situ hybridization to visualize VGF expression across the NAc and determine its colocalization with known NAc cell types. Using Cre-expressing viruses injected in the NAc of floxed VGF mice, we ablated VGF-derived peptides active in the NAc and treated mice with acute fentanyl injections. Locomotion was measured to determine psychomotor sensitization and tolerance, a behavioral readout of opioid-evoked plasticity.

We found that VGF is expressed ubiquitously across the NAc, and colocalizes with both somatostatin interneurons and dopamine receptor D1 and D2-positive medium spiny neurons. Viral disruption of VGF-derived peptides in the NAc had no effect on locomotion at low or high doses (0.063, 0.2, 0.4, or 0.63 mg/kg) of acute injections of fentanyl compared to control animals. However, when 0.4 mg/kg of fentanyl was administered daily for one week, animals with disrupted VGF in the NAc showed amplified psychomotor sensitization over time compared to control animals ($p = 0.028$, time x virus interaction, 2-way ANOVA).

These results indicate that VGF-derived peptides in the NAc do not impact the acute stimulatory effects of fentanyl, but may be necessary for normal opioid-evoked plasticity following chronic fentanyl exposure. Future experiments will assess the impact of VGF-derived peptides on excitatory postsynaptic currents in the NAc using whole-cell patch-clamp electrophysiology. This study implicates VGF as a mediator of opioid-evoked plasticity. Dissecting the mechanisms by which these neuropeptides are recruited to influence maladaptive synaptic plasticity and NAc- driven behaviors could therefore identify targets for addiction treatment.

A45 - Region-specific modulation of brain mu-opioid and oxytocin receptors by gut microbiota during early development in male rats

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The gut microbiota is critical in early brain development, influencing neural, immune, and metabolic pathways. Disruptions to microbial communities, as seen in germ-free (GF) rodents or following antibiotic exposure, have been linked to altered neurodevelopment, including changes in social, reward, and stress-related behaviours. However, the neurobiological mechanisms underlying these effects remain unclear. Since mu-opioid (MOPr) and oxytocin receptors (OTR) regulate social, stress-related and reward processing, we investigated how gut microbiota perturbation affects their brain distribution and density using quantitative receptor autoradiographic binding.

We measured OTR ([^{125}I]-OVTA) and MOPr ([^{3}H]DAMGO) binding, with [^{125}I]-OVTA and [^{3}H]DAMGO respectively, in the forebrains of conventional (CON) and GF male and female rats at postnatal days (PND) 8, 22 and 116–150 (adults). We also examined OTR and MOPr binding in male rats exposed to a clinically relevant antibiotic cocktail (2 mg/mL of Penicillin G and 4 mg/mL of Streptomycin), from PND 21–27.

In male but not female PND 22 GF rats, MOPr binding was significantly increased in the cingulate cortex ($P<0.05$), motor cortex ($P<0.05$) and nucleus accumbens shell ($P<0.05$), compared to CON. Interestingly, this effect was not observed in PND 8 and adult GF rats ($P>0.05$), thus restricting it to PND 22 rats. A similar increase in MOPr binding was detected in the accumbens shell ($P<0.01$) of antibiotic-treated male PND 27 rats compared to non-antibiotic control group.

For OTR, GF male but not female rats showed elevated binding in the cingulate cortex ($P<0.001$), prelimbic cortex ($P<0.05$), orbital olfactory cortex ($P<0.05$) and septum ($P<0.05$) across all age groups. Antibiotic-treated male PND27 rats also displayed increased levels of OTR binding in the ventral anterior olfactory nucleus ($P<0.01$) and ventral caudate putamen ($P<0.01$) compared to non-antibiotic control group.

These findings from two microbiota knockdown models indicate that gut microbiota modulates MOPr and OTR levels in a region- and sex-specific manner, with effects most prominent in early postnatal development and in males. This work provides mechanistic insight into how early-life microbiota disruption may influence neurodevelopmental trajectories and highlights potential neurochemical pathways through which antibiotics might affect infant brain development and behaviour.

A46 - Enkephalin in the Dorsal Raphe Nucleus Modulates Aversive Processing

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Chronic pain is a complex disease that is commonly associated with comorbidities such as depression, anxiety, and increased risk of suicide. It is important to understand the basic neurobiology behind the motivational dysregulation of pain to effectively treat it. Endogenous opioids in dorsal midbrain nuclei such as periaqueductal grey (PAG) and dorsal raphe nucleus (DRN) are important for modulating both analgesia and motivation. The PAG is canonically associated with opioid-mediated descending pain inhibition and is desensitized during chronic pain states. The specific role of the DRN during chronic pain has not been characterized despite studies showing opioid activity here also modulating pain and motivated behaviors. The aim of this study was to investigate the functional significance of DRN enkephalin signaling in pain sensitivity and related affective and motivational behaviors.

Methods
We disrupted preproenkephalin (PENK) in DRN using a Cre-dependent CRISPR- Cas9 viral vector injected into the DRN of Penk-Cre+ mice or their Cre- littermate controls. We ran a battery of appetitive and aversive behavioral assays to assess how knockdown of DRN enkephalin altered behavioral readouts in Cre+ mice compared to Cre- mice.

Results
We found that intraplantar injection of low-dose carrageenan reduced paw withdrawal thresholds by 50% in Cre- controls, whereas knockdown of enkephalin peptide in the DRN produced a greater reduction (70%). Similarly, in an odor avoidance assay Cre- mice spent twice as much time sniffing an aversive odor than Cre+ mice. In appetitive behaviors such as social interaction, Cre- mice spent about 30% more time with a mouse than with a novel object but this was reduced in Cre+ mice to only 20%. Additionally, Cre- mice had an 85% preference for sucrose over water but this was reduced by 15% when PENK was knocked down in the DRN.

Conclusions
These results suggest that DRN enkephalin peptide acts to buffer aversive responses and its absence results in a shift towards enhanced avoidance of both aversive and appetitive stimuli. This attenuated reward/enhanced aversion phenotype is similar to behavioral changes observed in chronic pain states. Future studies will further investigate how DRN enkephalin contributes to affective dysregulation during chronic pain.

A47 - Single early injection of a NOP receptor antagonist modulates emotional and social behaviors following traumatic stress in mice

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Post-traumatic stress disorder (PTSD) is characterized by persistent symptoms following trauma, including re-experiencing, avoidance, cognitive alterations, reactivity disturbances, and social impairments. Preclinical evidence suggests that the nociceptin (N/OFQ) system, composed of the neuropeptide nociceptin and its receptor NOP, contributes to the development of these symptoms. Indeed N/OFQ mediates some negative effects of stress in several acute stress models, through its action in brain regions involved in perception, anxiety, fear and emotional regulation.
To test this hypothesis, we blocked the N/OFQ system through a single early injection (either before or after stress) of a NOP antagonist — namely SB-612,111 (SB, i.p., 10 mg/kg) — in a murine model of PTSD based on strong contextual fear conditioning (CFC), in which mice received 5 electric shocks (1 mA). We then assessed the impact of stress and N/OFQ system blockade on fear learning, fear memory extinction, anxiety, and sociability.
SB injection 30 minutes prior to the conditioning session resulted in an overall decrease in freezing percentage (2-way ANOVA on repeated measures, group $p=0.0294$; time $p<0.001$; interaction $p=0.0521$) and, 4 days later, in a shorter latency to exit the dark compartment in the Light/Dark test (Mann-Whitney, $p=0.0387$). These results suggest that the reduction of the freezing behavior induced by N/OFQ system blockade during conditioning leads to an anxiolytic effect.
When SB was administered right after conditioning, mice showed the following day an increase in hypo-locomotion upon re-exposure to the aversive context (Student's t-test, $p=0.0113$). Moreover, when tested 9 days later, SB-treated mice displayed higher interaction time with the unknown mouse (2-way ANOVA followed by post-hoc test, $p=0.0134$) and social preference (One-way ANOVA followed by post-hoc test, $p=0.00280$) than vehicle-treated stressed mice in the 3-chamber sociability test. These results show that early blockade of the N/OFQ system after CFC can prevent the long-term deterioration of social behaviors induced by traumatic stress.
Our work suggests that therapies targeting the N/OFQ system could exert a prophylactic effect on some PTSD symptoms.

A48 - Unraveling of a cross-habenular neuronal population expressing the μ-opioid receptor in hedonic balance

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The habenular complex (Hb) exhibits one of the highest levels of mu-opioid receptor (MOR) expression in the brain and plays a crucial role in modulating reward processing, stress responses, and negative affect during opioid withdrawal. However, the contribution of MOR- expressing cells to the habenular regulation of hedonic balance remains unclear.
Using male and female MOR-Cre mice with unilateral viral vector injections of AAV-DIO- GtACR2, we performed optogenetic inhibition across the HbMOR population. This inhibition induced significant place preference in real-time place preference testing ($p=0.014$), suggesting that suppressing HbMOR neuronal activity produces reward-like effects. While rewarding, these effects were non-reinforcing in an optogenetic intracranial self-stimulation protocol. Long-term HbMOR inhibition also produced anxiolytic effects in the elevated plus maze ($p=0.008$) and open field test ($p=0.028$), therefore affecting exploratory and anxiety-like behavior. In parallel, we found that endogenous opioid tone in HbMOR populations decreases during stress events (z-score: -3.2±0.8) using the DeltaLight biosensor in MOR-Cre mice, reinforcing the HbMOR role in negative affect. Characterization of endogenous opioid tone at the level of the habenula during rewarding behaviors is in progress.
We demonstrated that HbMOR neurons control hedonic balance, however as HbMOR neurons are expressed in both the medial (MHb) and lateral (LHb) subdivision of the Hb, the exact contribution of these distinct populations is not known. To characterize the HbMOR subpopulations, we performed high-throughput in-situ hybridization (300 transcripts, Xenium, 10X Genomics) on 9 tissue sections. Spatial mapping identified four major MOR-expressing neuronal populations with distinct transcriptional profiles, suggesting diverse functional roles in reward and stress circuits. The identification of marker genes for these subpopulations is in progress to guide future targeting experiments and to unravel the distinct subpopulations of HbMOR neurons in hedonic balance.
Together, these complementary functional and molecular approaches provide novel insights into how HbMOR neurons regulate affective states. Ongoing studies will determine how these distinct subtypes differentially contribute to reward processing and negative affect, potentially offering new therapeutic targets for mood disorders and addiction.

A49 - Extended amygdala dynorphin regulates nociception and alcohol-induced analgesia in mice

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Chronic pain is a leading cause of disability worldwide, significantly reduces quality of life, and is highly co-morbid with various psychiatric conditions, including negative affective and substance use disorders (SUDs), including alcohol (AUD). The endogenous opioid system, including kappa opioid receptors (KORs) and their endogenous ligand dynorphin (DYN), is heavily implicated in pain chronification and negative affect. This is due, in part, to the concentrated expression of DYN and KOR in the extended amygdala, a cluster of regions including the central nucleus of the amygdala (CeA) and bed nucleus of the stria terminalis (BNST). However, the specific role of DYN extended amygdala circuitry in multidimensional aspects of persistent pain has not yet been investigated. Here, we use electrophysiological, photometric, and chemogenetic approaches to characterize a role for dynorphinergic CeA-BNST circuitry in regulating nociceptive behaviors. Our electrophysiological studies show that persistent inflammatory pain, induced by complete Freund's adjuvant (CFA), decreases excitability of prodynorphin (Pdyn) cells in the right CeA, and further anatomical tracing shows that CeA^{Pdyn} cells send dense projections to the BNST. Consistent with these findings, using wireless fiber photometry, we find that terminal activity of CeA^{Pdyn}-BNST neurons is attenuated during thermal nociception, and this effect is exacerbated by CFA. This effect, however, is blunted following intraperitoneal (IP) injection of alcohol (1.5g/kg). Finally, to characterize the functional contributions of CeA^{Pdyn}-BNST neurons, we chemogenetically manipulated the circuit prior to testing mechanical nociception. While chemogenetic activation of the circuit produces anti-nociception in both females and males regardless of pain status, inhibition of CeA^{Pdyn}-BNST neurons produces hyperalgesia in both sexes in the absence of CFA but attenuates hyperalgesia in males treated with CFA. Further, chemogenetic inhibition of the circuit prevents acute alcohol-induced analgesia (1.5g/kg, IP) in animals without persistent pain. Collectively, these preliminary data indicate that CeA^{Pdyn}-BNST neurons regulate nociception in a sex-dependent manner. Ongoing studies are further investigating terminal activity of this circuit in response to innocuous and noxious stimulation, cue-associated foot shock, and the light-dark box test to further characterize its contributions to nociceptive and affective components of persistent pain.

Support
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A50 - Identification of Proteins Controlling mu Opioid Receptor Trafficking in Cultured Cells Using a Novel Chemical Biology Platform

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Regulation of mu opioid receptor (MOR) signaling is critical for maintaining cellular homeostasis and responsiveness to opioids. One aspect of MOR regulation occurs through agonist-induced receptor internalization to endosomes followed by proteolytic downregulation in lysosomes – a process proposed to contribute to opioid tolerance. Interestingly, MORs can also be actively returned to the plasma membrane from endosomes, which protects them from lysosomal downregulation. The mechanism that selects MOR for this endosomal recycling pathway remains unresolved. Here, we used functional genomics paired with a novel sensor of receptor downregulation to identify cellular proteins which control MOR downregulation. We found that the central regulator of MOR post-endocytic trafficking is the Retromer complex, which rescues MOR from opioid-induced downregulation by promoting MOR recycling from endosomes to the plasma membrane. We show that MOR accesses Retromer-dependent recycling through its established non-canonical bi-leucine recycling motif. This Retromer/bi- leucine pathway is a necessary, sufficient, and conserved mechanism which protects MOR from agonist-induced downregulation in response to multiple clinically relevant opioids including fentanyl and methadone. This novel pathway for accessing Retromer-dependent recycling is also present in other classes of membrane proteins. Overall, this study revealed a novel mechanism by which MOR is regulated following chronic exposure to opioid drugs.

A51 - PACAP-PAC1 Signaling Enhances GABAergic Transmission in the vlPAG but Does Not Mediate CFA-Induced Hyperalgesia

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The ventrolateral periaqueductal gray (vlPAG) is a critical hub for descending modulation of pain. Pituitary adenylate cyclase-activating polypeptide (PACAP) is implicated in pain pathophysiology via its high-affinity PAC1 receptor that is highly expressed in the PAG. Yet, the role of PAC1 signaling in vlPAG neurons is unknown.
Whole-cell patch clamp recordings from vlPAG neurons from C57/B6 male and female mice assessed the effects of PACAP and the selective PAC1 receptor M65 on excitability and synaptic transmission. We hypothesized that PACAP would enhance GABAergic synaptic transmission in the vlPAG, resulting in hyperalgesia. In addition, we hypothesized that CFA induced inflammation would increase PACAP tone in the vlPAG.
exacerbates inflammatory pain mediated by vlPAG neuronal hyperexcitability and increased GABAergic transmission. Inflammation was induced with Complete Freund's adjuvant (CFA) (20µl) into the hindpaw of C57/B6 male and female mice. Recordings were done 5-7 days following inflammation. In current-clamp experiments, PACAP (5 nM) had minimal effect on excitability. In voltage-clamp experiments, PACAP (5 nM) increased both spontaneous and evoked GABAergic synaptic currents. The effect of PACAP on evoked inhibitory post-synaptic currents (eIPSCs) was increased in CFA-treated mice. M65 (100 nM) effectively reversed the effect of PACAP in all experiments. The effect of PACAP is not dependent on sex. In contrast to our hypothesis, there was no evidence of PACAP tone in the vlPAG of CFA-treated mice.
Consistent with this finding, M65 did not reverse CFA-induced hyperalgesia. We interpret these findings as PACAP activation of PAC1 can increase GABA release in the vlPAG that is consistent with hyperalgesia but PACAP does not mediate CFA-induced hyperalgesia.

A52 - B-Caryophyllene Reduces Morphine Reward via the Adenosine A2a Receptor: Evidence from In Vivo CRISPR Knockdown and Conditioned Place Preference in Mice

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Opioids are gold standard for pain management, but their therapeutic value is limited by severe side effects, such as tolerance, addiction, and respiratory depression. Current strategies lack effective options to mitigate opioid-induced reward. B-Caryophyllene (BCP), a terpene found in Cannabis sativa, has demonstrated anti-reward properties through an unknown pathway. We hypothesized that BCP attenuates morphine-induced reward behavior via the adenosine A2a receptor.

To test this, the Conditioned Place Preference (CPP) assay was used to assess reward association. Experiments in male and female CD-1 mice showed that administration of BCP with morphine significantly reduced the paired-box preference when compared to morphine-only mice. Additionally, BCP-only mice displayed aversion. These results suggested that the aversive properties of BCP may be responsible for mitigating the positive association of morphine.

To investigate the mechanism, adult male and female CD-1 mice underwent intracerebroventricular (ICV) cannulation followed by CRISPR-mediated knockdown of the adenosine A2a receptor. CPP assays were performed to assess reward association in knockdown or non-coding mice, which were paired to chambers in three groups: vehicle, BCP-only, and morphine-only. This experiment showed that in the A2a knockdown, the aversive effects of BCP were reversed, while the morphine-only group retained its preference. This confirmed the A2a pathway's role in mitigating the aversive preference of BCP, but not morphine.

Our current project is establishing A2a's role in mitigating reward when BCP is administered with morphine. This is achieved with the knockdown of A2a in the whole brain via ICV cannulation, followed by CPP in mice who receive both BCP and morphine. This research will confirm that the A2a is also responsible for BCP's lack of ability to reduce morphine reward.

This work highlights the potential for BCP as a promising therapy in minimizing the risk of opioids through an adenosine A2a receptor-mediated mechanism—addressing the critical need for preventing addiction during pain management.

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A53 - The neuropeptide receptor GPR83 regulates anxiety-like behavior

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In our society there is a growing feeling of isolation and loneliness despite our perceived connectivity via social media and smart phones. This isolation has resulted in sharp increases in anxiety amongst adolescents, especially among young girls. Loneliness and social isolation during the critical developmental period of adolescence is likely to have long-lasting impacts on brain chemistry and connectivity. In fact, there are reports, both preclinical and in patients, indicating that isolation in adolescence has significant effects on overall health, feelings of anxiety, nicotine and alcohol intake, as well as motivation for heroin. GPR83, whose endogenous ligand is a neuropeptide called PEN, was first identified as a protein regulated by the glucocorticoid dexamethasone, indicating that this receptor may play a role in stress- responses. In fact, when mice are treated with corticosterone or dexamethasone, GPR83 expression is reduced in the amygdala. Moreover, GPR83 knockdown in the BLA increases anxiety-related behaviors in female mice. We have recently identified small molecule ligands for GPR83 based on a homology model and in silico screening. Compounds were tested for binding and signaling through GPR83 identifying Cpd 1 as an agonist for the receptor. The goal of the current study is to determine whether our small molecule agonist could regulate anxiety- related behaviors in a model of adolescent social isolation in mice. In addition, we sought to determine whether GPR83 expression was regulated in the brain following this model. Overall, we find that treatment with Cpd1 alleviates anxiety-related behaviors in a dose-dependent manner in female mice. This may be linked to increased expression of GPR83 following adolescent social isolation. Future studies will investigate the functional consequences of social isolation on GPR83 function in neurons and the overall impact on opioid abuse liability.

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A54 - In vivo study reveals bidirectional contribution of lateral hypothalamic opioid-responsive neurons to affective states in mice

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The lateral hypothalamus (LH) is a critical hub for integrating metabolic, emotional, and environmental signals to guide motivated behaviors. Disruptions in LH function have been linked to conditions such as eating disorders, addiction, and mood disturbances. Within the LH, a distinct population of neurons expresses the mu-opioid receptor (MOR), a key receptor in the modulation of reward, stress, and emotional processing. Given the convergence between MOR signaling and LH function, LH-MOR neurons may represent a pivotal substrate for emotional states, motivated behaviors, and related pathologies. However, the specific contribution of these neurons has yet to be investigated.

To determine LH-MOR contribution in affective states, we conducted a functional characterization of LH-MOR neurons using the MOR-Cre mouse model in combination with in vivo fiber photometry and optogenetics. MOR-Cre mice were injected with a Cre-dependent GCaMP viral vector in the LH and implanted with an optical fiber to record LH-MOR Ca^{2+} activity during exposure to stimuli with varying affective valence. Preliminary fiber photometry results indicate that LH-MOR neurons encode affective stimuli, both rewarding and aversive, exhibiting bidirectional responses aligned with stimulus valence. Indeed while the activity of these neurons significantly decreased in response to natural rewards (e.g. palatable food consumption and social intetraction) the opposite was found in response to aversive stimuli (e.g., anxiogenic, stressful, or fear-inducing conditions). Then, to investigate the causal relationship between LH-MOR activity and motivational states, a second cohort of MOR-Cre mice were injected in the LH with Cre- dependent optogenetic constructs—GtACR2 for inhibition or ChR2 for activation—and implanted with optical fibers. Consistent with the photometry results, optogenetic inhibition of LH-MOR neurons induced approach behavior in a real-time place preference task, whereas their activation promoted avoidance.

Together, these findings reveal the bidirectional role of LH-MOR neurons in encoding and shaping affective states. Their dysfunction may contribute to the maladaptive emotional processing seen in conditions such as eating and substance use disorders.

A55 - Opioidergic activation of the descending pain inhibitory system underlies placebo analgesia in neuropathic pain model rat

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Placebo analgesia is caused by inactive treatment, implicating the involvement of endogenous brain functions. Neuroimaging studies have shown increased neuronal activity in the dorsolateral prefrontal cortex (dlPFC) and rostral anterior cingulate cortex (rACC) during placebo administration. Naloxone abolishes both the activation in these regions and placebo effects, suggesting that the endogenous opioidergic system in the frontal brain plays a crucial role in placebo analgesia. Recently, Chen et al. reported that the opioid circuit of the cortex-pons-cerebellum pathway is also involved in placebo analgesia for acute pain (Chen C et al., Nature, 2025). However, the neurobiological basis of placebo analgesia has not been fully elucidated, especially in the context of chronic pain. Previously, we established an animal model of placebo analgesia using pharmacological conditioning with four consecutive gabapentin injections (Zeng Y et al, 2018). In these placebo analgesia model rats, we observed increased neuronal activity in the medial prefrontal cortex (mPFC) following placebo administration, as analyzed by [18F] FDG-PET-based small-animal neuroimaging. Moreover, neuronal activity in the mPFC was positively correlated with that in the ventrolateral periaqueductal gray (vlPAG), a key brain region in the endogenous pain-modulating system. Importantly, these changes were abolished by naloxone administration. In the present study, we aimed to elucidate the detailed neural basis of how opioidergic signals in the frontal brain area are involved in placebo analgesia. To this end, we established MOR-Cre knock-in rats and found that MOR-positive neurons in the mPFC largely overlapped with GABAergic neurons. Chemogenetic activation of MOR-positive neurons in layer 5 of the mPFC, or selective inhibition of the mPFC-vlPAG pathway abolished placebo analgesia. Furthermore, the analgesic effect induced by selective inhibition of MOR-positive neurons in layer 5 of the mPFC in neuropathic pain rats was also blocked by immunotoxin-mediated ablation of the mPFC-vlPAGpathway. These results suggest that MOR activation in the mPFC, leading to the engagement of the mPFC-vlPAG pathway, plays a crucial role in the fundamental neurobiological basis of placebo analgesia. This study was supported by JSPS KAKENHI 20K16511 (H.N.), 24659574, 26112003, 15K14328, 16H06276 (Y.C).

A56 - Kappa Opioid Receptor-Induced Cognitive and Emotional Dysfunction is Associated with Dysregulated Autophagic Signaling in the Brain

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Introduction

Chronic psychological stress increases the release of endogenous dynorphin A, thereby activating κ-opioid receptor (κ-OR) signaling that modulates anxiety-related behaviors across various brain regions. Concurrently, chronic stress alters neuronal autophagy, a critical lysosomal degradation pathway responsible for maintaining protein homeostasis and regulating synaptic structure.

Results

We have previously demonstrated that κ-OR agonists induce macroautophagy through a novel Gαio/ERK1,2/CREB signaling pathway in Neuro-2a cells and primary neurons. To investigate the in vivo relevance, wild-type mice were administered with the κ-OR agonist U50,488H (5 mg/kg for 15 days) and subjected to behavioral, molecular, neurostructural and proteomic analyses on the hippocampus. Behavioral assessments, including the elevated plus maze and novel object recognition test, demonstrated that sustained κ-OR activation induces anxiogenic responses [~40% increase] and cognitive impairments [~60%; p=0.032, p=0.0118]. U50,488H administration showed increased expression of autophagic markers [~50%] and decreased pre- and post-synaptic protein levels [~40%; p=0.0121, p=0.042]. Moreover, Golgi-based 3D neuronal reconstruction analysis of dentate gyrus hippocampal neurons showed that U50,488H treatment reduced the dendritic length and number of branches [~35%], indicating compromised hippocampal neuroplasticity [p=0.0121]. Interestingly, proteomic analysis of hippocampal purified autophagosomes further showed altered autophagic cargo, implicating autophagy as a key mechanism in κ-OR-mediated synaptic modulation [t-Test FDR=0.05, S0=0.1]. Additionally, the selective κ-OR antagonist nor-BNI effectively prevented both autophagy induction [~40%] and synaptic protein loss [~30%] triggered by forced swim stressor [2-way ANOVA $p<0.05$], supporting the role of κ-OR mediated autophagy in stress-induced synaptic deficits.

Conclusion

These findings identify a novel signaling mechanism through which stress induced κ- OR activation contributes to synaptic and behavioral alterations. Modulating κ-OR induced autophagy using specific receptor antagonists may offer a new therapeutic approach for neuropsychiatric disorders.

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A57 - Optical tools for probing and controlling nociceptive GPCR and G protein signaling dynamics in living cells

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Pain perception is a complex process, primarily regulated by G protein Coupled Receptors (GPCRs), including opioid, cannabinoid, serotonergic, and glutamate receptors. The current understanding of these pain receptor pathways comes from the structural data that only show one or few possible conformations out of many, as well as the static biochemical information about the signaling involved. While these approaches are beneficial, the dynamic and ligand- dependent behavior of these pathways are crucial for therapeutic interventions. Addressing these issues, using structure-guided protein engineering, we develop probes to examine real-time signaling dynamics of pain receptor activities in living cells, such as GαGTP generation and Gβγ signaling with subcellular spatial and millisecond temporal resolution. We also engineer optogenetic tools to selectively control the signaling pathways downstream of pain receptors within spatially confined subcellular regions, such as selected regions of the plasma membrane or the Golgi apparatus. Our tools, together with advanced live cell imaging, allow for the detection of downstream effects of receptor and G protein signaling and their fate over time. Further, they enable the precise spatio-temporal optical control of receptor and receptor-independent G protein activity. By elucidating the dynamic regulation of pain receptor signaling, our work will enhance the current understanding of nociceptive pathways and the development of therapeutics tailored to manage pain without developing tolerance and addiction.

Poster session B

B1 - From Trauma to Tranquility: How Myrcene Helps the Brain Bounce Back

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Abstract

Mild traumatic brain injury (mTBI) is a major public health issue associated with persistent cognitive, emotional, and behavioral deficits. Despite its high prevalence, therapeutic interventions to mitigate mTBI-related impairments remain inadequate. While cannabinoids derived from cannabis herbs have been extensively studied for their neuroprotective potential, emerging evidence suggests that non-cannabinoid constituents, particularly terpenes, may also exert this effect.

This study is the first to investigate the neuroprotective effects of myrcene in alleviating mTBI- induced neuronal damage. Given the prominent sequelae of mTBI, including pain sensitivity, motor dysfunction, behavioral disturbances (e.g., aggression, and depression), and diminished quality of life, there is a critical need for novel therapeutic approaches. Using the C57 mice model (divided into 4 different groups: Sham/Veh, mTBI/Veh, Sham/Myrcene, mTBI/Myrcene), we evaluated the efficacy of myrcene in mitigating these mTBI-associated deficits. The results of the behavioral tests indicate that myrcene treatment ameliorates pain perception by increasing the mechanical withdrawal thresholds (mTBI/Veh: 0.07310 g vs. mTBI/Myrcene: 1.010 g), enhances general well-being evaluated by nesting score (mTBI/Veh: 2.800 vs. mTBI/Myrcene: 4.600), and improves mood-related behaviors such as aggression evaluated by the total fighting time (seconds) in the resident intruder test (mTBI/Veh: 50 s vs. mTBI/Myrcene: 0 s) and depression evaluated based on the immobility time in the tail suspension test (mTBI/Veh: 120 ± 16.77 seconds vs. mTBI/Myrcene: 72 seconds).

Furthermore, myrcene restored neurotransmitters level by decreasing Glu (mTBI/Veh: 6.9352 pmol/10µl vs. mTBI/Myrcene: 2.209 pmol/10µl), and increasing Gaba (mTBI/Veh: 2.4069 pmol/10µl vs. mTBI/Myrcene: 10.311 pmol/10µl) and morphological alterations, potentially through reducing the endocannabinoid level such as AEA (mTBI/Veh: 1.480 pmol/10µl vs. mTBI/Myrcene: 0.6250 pmol/10µl) and 2-AG (mTBI/Veh: 9.020 pmol/10µl vs. mTBI/Myrcene: 3.675 pmol/10µl). These findings highlight myrcene as a promising therapeutic candidate for mTBI recovery.

Keywords: Mild traumatic brain injury, Cannabis, Myrcene, Behaviors, Microdialysis, Morphology, Endocannabinoids.

B2 - In Vitro Validation of MOR and CB1R Residues Involved in Heteromerization and Crosstalk at Baseline and After Morphine Treatment

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Background and Objective

Chronic pain is a debilitating condition that remains insufficiently treated in many patients[1]. Co- activation of mu-opioid receptors (MOR) and cannabinoid receptor 1 (CB1R) has been proposed as a strategy to enhance analgesia[2]. Their close proximity suggests potential for heteromerization and functional crosstalk, but the molecular interfaces involved are not well understood. This study aimed to validate MOR and CB1R residues computationally predicted to mediate their interaction, both at baseline and following morphine exposure, to support the development of a Quantitative Systems Pharmacology platform for chronic pain therapy.

Methods

Site-directed mutagenesis was used to generate five MOR and four CB1R variants. HEK-293 cells were co-transfected with wild-type or mutant MOR and CB1R. Confocal microscopy was used to assess receptor colocalization, while morphine-induced inhibition (10^{-4}–10^{-12} M) of forskolin-stimulated cAMP accumulation was quantified.

Results

All mutants were successfully generated, and MOR-CB1R heterodimerization was confirmed in HEK- 293 cells. Mutations significantly altered receptor colocalization after morphine treatment. Most mutants showed increased colocalization ($p < 0.05$ or $p < 0.01$ vs. wild-type), while CB1R T125A led to decreased colocalization (Pearson's $r = 0.6171$ vs. 0.6880 in wild-type, $p < 0.05$). As expected, morphine concentration-dependently inhibited cAMP accumulation in wild-type MOR (EC_{50}: wt = 1.28 ± 0.29 nM) and MOR-mutans exhibited significant differences compared to wild-type (V82A = 0.58 ± 0.04 nM; F86A = 0.11 ± 0.05 nM; $p < 0.001$). Notably, CB1R mutations also impacted MOR-dependent inhibition of cAMP accumulation (EC_{50}: wt = 0.54 ± 0.04 nM; F180A = 0.032 ± 0.011 nM; T125A = 0.78 ± 0.13 nM; $p < 0.001$).

Conclusion

This study identified MOR and CB1R residues critical for heteromer formation and signaling modulation. Mutation of these residues altered colocalization and morphine-induced signaling, highlighting the distinct pharmacological profile of MOR-CB1R heteromers. These findings support the rational design of opioid/cannabinoid co-therapies and the refinement of Quantitative Systems Pharmacology models for chronic pain.

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B3 - Morphine-THC drug combination modulates MOR-mediated intracellular signaling and MOR-CB1 heteromerization in human and rodent neuronal cell models in vitro

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Chronic pain is a debilitating life-long condition, which severely reduces patients' quality of life and their socio-economic contribution to society, representing a significant global burden [PMID: 37111650]. The combination of mu-opioid receptor (MOR) and cannabinoid type-1 (CB1) receptor agonists has been proposed as an improved analgesic cotreatment with enhanced efficacy/safety. Nevertheless, molecular determinants that regulate MOR-CB1 crosstalk and heteromerization are largely unknown.

In this study, we aimed to evaluate the combinational effects of morphine and Δ9- tetrahydrocannabinol (THC) on MOR-mediated signaling in various human and rodent primary neuronal cells, as well as in HEK293 cells co-expressing Flag-MOR and HA-CB1. This evaluation was conducted following both acute and prolonged (morphine 1μM, 24h) MOR stimulation. Additionally, we sought to quantify MOR-CB1 heteromerization after acute morphine-THC co-treatment in HEK293 cells expressing either wild-type receptors or their mutants.

Morphine inhibition of forskolin-induced cAMP production was measured by ELISA in rat and mouse primary neurons, PMA (16nM, 5 days) differentiated SH-SY5Y cells, and HEK-293 cells, both under basal conditions and when co-treated with THC (10-100nM). cAMP production was also measured in HEK293 cells co-expressing wtMOR and wtCB1 or their mutants, which were obtained through site-directed mutagenesis. MOR-CB1 heteromerization in HEK-293 cells was quantified using confocal microscopy.

Under basal condition, morphine-mediated inhibition of adenylyl cyclase was significantly potentiated by THC coadministration in rat cortical primary neurons (morphine IC_{50}=2.26±0.29nM vs morphine + THC IC_{50}=1.46±0.62, $p<0.05$). Contrarily, in rat striatal, rat DRG, mouse cortical, and mouse striatal primary neurons, THC significantly reduced morphine's ability to inhibit adenylyl cyclase. Following prolonged MOR activation, in rat cortical, rat striatal primary neurons, and human PMA-differentiated SH-SY5Y cells, THC rescued the reduced response to morphine. Analyzing MOR-CB1 heteromerization in transfected HEK293 cells, morphine (Pearson's coefficient (PC)=0.3795) reduced wt-MOR-wt- CB1 colocalization, and THC reverted this effect (PC=0.5341, $p<0.001$). In V82A-MOR-wt-CB1-HEK293 cells, morphine did not modify heterodimerization (Pearson's coefficient=0.6066), while THC increased MOR-CB1 colocalization (PC=0.7011, $p<0.001$).

Our results demonstrate that THC co-administration improves morphine effects, both under basal conditions and following prolonged MOR stimulation; moreover, analyzing MOR-CB1 heterodimerization, some residues were shown to be important for receptor colocalization. Therefore, THC-morphine cotreatment may be considered a promising drug combination to improve chronic pain management. Supported by QSPainRelief (H2020 grant agreement n.848068).

B4 - Behavioral and Neuro-Immune Impact of Mμ-Opioid Receptor and CB2 Cannabinoid Receptor

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Marijuana (cannabis) and cannabinoids are getting global medical and recreational approvals in this era of the opioid epidemic. Opiates have been used for medical and recreational purposes for millennia. Both opiates and cannabis are often co-abused, and their use increase the risk of opioid and cannabis use disorders (OUDs and CUDs), and dependency. Opioid and cannabinoid systems are complex, have endogenous ligands, and share many neuromodulating and pharmacological effects by activating opioid and cannabinoid receptors, respectively. Inflammation is increasingly implicated in many diseases, and mu-opioid receptor (MOR) and CB2 cannabinoid receptor (CB2R) have been linked to neuroinflammation. The hypothesis that changes in MOR and CB2R mediated behavioral and cytokine alterations are associated with their roles in inflammation was tested here. Naïve male C57Bl/6J as wild type, MOR KO mice with deletion of MOR, DAT-Cnr2 cKO mice with deletion of CB2R from dopamine neurons and CX3Cr1-Cnr2 cKO mice with deletion of CB2R from microglia were used in the study. Behavioral analysis using wheel running activity (WRA) for five minutes, and tail-flick latencies for nociception with cut-off at twelve seconds were assessed in the animals. After the behavioral experiments animals were prepared for the proinflammatory cytokine assays. ELISA assay was used to investigate the level of cytokines and chemokines in the cerebellum and prefrontal cortical regions of the animals. MOR KO, DAT-Cnr2, and CX3Cr1-Cnr2 mice displayed differential phenotypes with genotypic and brain region alterations of TNFα, IL-1α, IL-1β, IL-6, IFNγ, MCP-1 and CXCL10 in the cerebellum and prefrontal cortical regions of the animals. DAT-Cnr2 cKO mice displayed hyper-psychomotor WRA responses while the CX3Cr1-Cnr2 cKO were less sensitive to the tail-flick latencies compared with MOR KO DAT-Cnr2 cKO and WT controls. The results indicated that deletion of MOR, and selective deletion of CB2Rs from either dopamine neurons or microglia had differentially modified motor activity and nociceptive responses, with genotypic, and brain region (cerebellum and prefrontal cortex) alterations of cytokines and chemokines. Modulation of components of the eCBome and opioid systems could thus offer immune regulation strategies in terms of therapeutic targets for disorders associated with inflammation in chronic pain conditions.

B5 - Exploring the effects of microglial KOR activation on inffammatory responses within the murine mesocorticolimbic system

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Inflammation in the central nervous system has been proven to be related to the development of neuropsychiatric disorders and negative affective states. Particularly, changes regarding microglia, as inflammatory responder cells in the mesocorticolimbic system (MCLS), are linked to the appearance of behavioural changes, such as anxiety-like behaviour. Despite being pivotal regulators in the MCLS, the role of opioid receptors and the dynorphinergic system on brain inflammatory responses is yet to be unravelled. Generally, Kappa Opioid Receptor (KOR) activation leads to anti-inflammatory responses upon pre-existent inflammation, but its influence on microglial pro-inflammatory pathways lacking a previous inflammatory insult is unclear. We hypothesized that KOR activation may still lead to rather anti-inflammatory outcomes. To study this, two parallel in vivo and in vitro experiments were carried out. The in vivo model consisted of a rat retrodialysis protocol using KOR agonist U69,593 targeted to the nucleus accumbens, while the in vitro approach corresponded to the culture of BV2 (mouse microglia-derived) cells with KOR agonist U50,488 and antagonist norbinaltorphimine (Nor-BNI). In both cases, the levels of extracellular inflammatory mediators (TNF-α, IL-1β, IL-6, IL-10, CCL2) were measured through flow cytometry or ELISA, while components of related intracellular pathways (AKT, NLRP3, CASP1, COX2, iNOS) were also studied through Western blotting with BV2 cells, 4 hours post-treatment. Preliminary results were able to prove that KOR blockage through Nor-BNI exposure does not affect inflammatory responses in microglia. Interestingly, pro-inflammatory intracellular mediators did not appear to be impacted by U50,488 administration in BV2 cells, either. Thus, our results regarding the effects of KOR activation could imply a selectivity of the receptor to drive pro- or anti-inflammatory responses only when a previous inflammatory context is present. Such interpretation represents a solid step forward for the study of the link between opioid receptors, inflammation in the MCLS, and negative affective states.

B6 - Salvinorin A analogues activating the kappa opioid receptor modulate OPC differentiation, myelination and neuroinflammation in preclinical rodent models

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There are no effective therapies currently available to repair damaged myelin or enable functional recovery in demyelinating diseases such as multiple sclerosis (MS). In MS, the protective myelin sheath is damaged, which impairs saltatory conduction and, over time, leads to neuronal cell death and loss of various motor and cognitive functions. The kappa opioid receptor (KOR) has been recognized as a potential therapeutic target for enhancing remyelination. In the present study, we utilized mouse in vitro and in vivo screens to identify salvinorin A analogues that were effective at promoting oligodendrocyte progenitor cell (OPC) differentiation into myelinating oligodendrocytes (OL) and quantified their ability to myelinate rhodamine stained nanofibers using primary mouse mixed glial cultures and high-throughput confocal microscopy. We then evaluated their effectiveness at promoting recovery using an experimental autoimmune encephalomyelitis (EAE) mouse (C57BL/6) model of demyelination in vivo. We found that all salvinorin A analogues enhanced OPC differentiation, with 16-ethynyl salvinorin A demonstrating the highest potency. It was also the most effective at increasing OL morphological complexity and promoting myelination in vitro. In the EAE model, treatment with mesyl salvinorin B, βTHP salvinorin B, and 16-ethynyl salvinorin A significantly decreased paralysis disease scores and increased the percentage of recovered mice. Furthermore, 16-ethynyl salvinorin A treatment reduced both microgliosis and astrogliosis in the white and grey matter of EAE mouse spinal cord. This identified KOR agonism acts via dual mechanisms to modulate neuroinflammation and OPC differentiation, to create an environment that promotes remyelination. Overall, these data highlight the potential for salvinorin A analogues to be developed into remyelinating therapies.

B7 - Central κ-Opioid Receptor-Mediated Suppression of Atopic Dermatitis-Associated Pruritus and Its Impact on Immune Modulation

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κ-Opioid receptors (KORs), expressed widely in the CNS including the hypothalamus and spinal cord, exhibit high affinity for the endogenous ligand dynorphin, rendering them compelling targets for the management of pain and pruritus. In this study, we investigated the effects of KOR agonists —specifically, the centrally active KOR agonists and its peripherally restricted analog on pruritus-associated behaviors in a murine model of allergic dermatitis. While a peripheral KOR agonist had no effect on spontaneous scratching behavior or alloknesis scores, systemic administration of KOR agonists led to a robust suppression of both parameters. These findings indicate that brain or spinal KOR activation is likely critical for the amelioration of chronic itch in this model. Further immunophenotypic analysis of splenic lymphocytes in the atopic dermatitis model revealed using fluorescence-activated cell sorting (FACS) that although the overall number of splenic T cells remained unaffected, the proportion of spleinc T cells expressing high levels of inhibitory immune checkpoint receptors was significantly reduced following nalfurafine treatment. These data suggest that, beyond its antipruritic effects, KOR agonists may exert immunomodulatory functions by attenuating the immunosuppressive phenotype often induced under chronic itch conditions. Thus, central KOR agonism emerges as a dual-action therapeutic strategy—simultaneously targeting sensory and immune dysregulation in atopic dermatitis-associated pruritus.

B8 - Prokineticin-2 Signaling and Glial Activation as Therapeutic Targets for Fabry Disease neuropathic pain

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Neuropathic pain is a hallmark symptom of Fabry-Anderson disease (FD), typically beginning in childhood, persisting throughout life, and remaining largely unresponsive to current therapies.
Therefore, identifying new and effective therapeutic strategies is urgently needed.
We hypothesized that modulating the neuroinflammatory response could alleviate FD-associated pain.
To address this, we tested two pharmacological approaches: inhibition of microglial activation with minocycline and blockade of prokineticin-2 signaling using the selective receptor antagonist PC1. Male GLA^-/- mice (a murine model of FD), aged 10 and 25 weeks, were used in this study. These animals exhibited key sensory alterations, including mechanical allodynia, thermal hyperalgesia, hyposensitivity to cold stimuli, and abdominal pain.
Mice were treated for two weeks with either minocycline or PC1, and their sensory responses were evaluated.
Inflammatory and neuroinflammatory profiles were assessed in the gut, peripheral nervous system (sciatic nerves and dorsal root ganglia, DRGs), and central nervous system (spinal cord). FD mice displayed significant intestinal inflammation, characterized by elevated levels of prokineticin-2 and pro-inflammatory cytokines. In the sciatic nerve, severe early neuroinflammation was observed, which diminished over time. This included early upregulation of prokineticin-2, pro-inflammatory cytokines, and GFAP, followed later by Iba1. In the DRGs, neuroinflammation was pronounced and sustained, with persistent overexpression of prokineticin-2, cytokines, Iba1, GFAP, and histone demethylases KDM6A and KDM6B. Spinal cord inflammation was also evident and progressed with age, marked by increased levels of IL-1β, TNF-α, IL-6, Iba1, and GFAP.
Treatment with minocycline or PC1 significantly reversed sensory deficits in both age groups. These treatments significantly reduced inflammatory and neuroinflammatory markers in the gut, sciatic nerve, and DRGs by lowering the expression of prokineticin-2 and pro-inflammatory cytokines. Both treatments also enhanced levels of the anti-inflammatory factor PPARγ.
Importantly, they prevented the development of spinal microgliosis and astrogliosis, thereby mitigating central sensitization.
In conclusion, our findings underscore the critical role of neuroinflammation in FD-related neuropathic pain and support the therapeutic potential of targeting the prokineticin system, glial activation, and cytokine production—while promoting anti-inflammatory mediators—as a promising strategy for pain management in FD.

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B9 - A possible mechanism for suppressing tumor progression and alleviating tumor-related symptoms via regulation of the peripheral μ-opioid system

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While central μ-opioid receptors (MORs) are well established as mediators of analgesia, peripheral MORs have been primarily implicated in adverse effects, including gastrointestinal dysfunction and immunosuppression. However, the role of peripheral MORs in cancer pathophysiology remains poorly understood. In this study, we investigated the functional contribution of peripheral MORs to tumor progression and cancer-associated symptoms using both pharmacological and advanced genetic approaches. Allogenic tumor-bearing mouse models of lung and pancreatic cancer were established for this purpose. Repeated administration of a peripheral MOR agonist significantly enlarged the tumor grafts and reduced survival, whereas treatment with peripheral MOR antagonists markedly suppressed tumor expansion, alleviated cancer- associated anorexia, and prolonged survival relative to controls. Mechanistically, conditional gene deletion of MORs in peripheral neurons, but not in immune cells, suppressed tumor growth and improved survival. Furthermore, in pancreatic tumor- bearing mice, peripheral neuron-specific MOR knockout significantly ameliorated cancer-induced anorexia, while muscle weakness remained unaffected. These findings suggest that the blockade of MORs expressed in peripheral nerves may represent a novel strategy for mitigating tumor progression and cancer cachexia. Ongoing investigations are focused on delineating the underlying cellular and molecular mechanisms mediating this anti-tumor effect.

B10 - Evaluating the pharmacokinetics of prolonged release injectable buprenorphine in mice

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Introduction

Buprenorphine is a partial mu-opioid receptor (MOPr) agonist and kappa-opioid receptor (KOPr) antagonist (Cowan and Lutfy, 2003, Curr. Neuropharmacol. 2 95) that is used clinically for the treatment of opioid use disorder. With more prolonged release formulations now being utilised, we investigated the pharmacokinetics and pharmacodynamics of a prolonged-release injectable formulation of buprenorphine (PRL-BUP) and its metabolites following subcutaneous administration.

Methods

Adult B6.129S2-Oprm1tm1Kff/J mice (JAX) (MOPr -/-) and wild-type C57BL/6 mice, both sexes, were anaesthetised before a subcutaneous injection (30 mL/kg) of vehicle or PRL-BUP (30mg/kg, n=3-5). At 1,3,6,10,14 or 21 days post-administration, the mice underwent a warm-water (52oC) tail withdrawal assay followed immediately by an open field locomotor test (30 min). 40 uL blood samples (Bailey and Sadler, 2013 Lab. Anim 47(4):316-9) and brains were collected before buprenorphine and its metabolites were analysed using cyclic mass spectrometry.

Results

In wild-type, but not $MOPr^{-/-}$, C57BL/6 mice, PRL-BUP significantly increased tail withdrawal latency and locomotion at 1 day (Ps<0.01), but not any following time point. Analysis showed peak buprenorphine blood concentrations of 92 ± 7 ng/mL(wild-type) and 89 ± 9 ng/mL (MOPr -/-) at 1 day post administration. In wild-types, buprenorphine brain concentration was observed at 38 ± 4 ng/mL (3 day), and 21 ± 5 ng/mL (6 day) before a steady state was achieved (11 ± 6 ng/mL) until day 21. Peak blood norbuprenorphine was observed at 11 ± 5 ng/mL at 1 day, with a steady state of 4.5 ng/mL until day 21. Interestingly, no norbuprenorphine was observed in the brain at any timepoint. There was no significant effect of sex or strain on concentrations of buprenorphine and its metabolites at any dose or time point tested.

Conclusion

These data show an initial hyper locomotor and analgesic response to PRL-BUP in C57BL/6 mice, loss of these activities at later time points. This contrasts with our findings that after repeated daily administration, these behaviours are maintained and decouples the previously observed correlation between pharmacokinetics and observable behaviours.

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B11 - Using ultra-large DNA-encoded chemical libraries to discover novel opioid modulators

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The μ-opioid receptor (μOR) is a well-established G-protein coupled receptor (GPCR) target for analgesia, yet conventional opioid receptor agonists suffer from serious adverse effects, notably addiction, tolerance, and respiratory depression, the latter contributing significantly to the mortality of the current opioid overdose epidemic. While the opioid antagonist naloxone (Narcan™) serves as a powerful clinical tool to reverse opioid overdoses, its short half-life and fast off-rate, along with serious withdrawal effects, limit its efficacy against powerful opioids like fentanyl. The body can exquisitely target endogenous opioid peptides to areas that need pain relief, yet this system could potentially be improved as a way of targeted analgesia. To those ends, we have several large DNA-encoded libraries (DELs) against active and inactive μOR to "steer" selections towards functional positive and negative allosteric modulators (PAMs and NAMs) that could "boost" the activity of endogenous opioids as pain relievers or naloxone's ability to reverse overdoses. We discovered a panel of functional modulators of the μOR of all flavors ranging from μM affinity PAMs to low single-digit nM affinity NAMs. We used cryo-electron microscopy (cryoEM) to demonstrate the mechanisms of action for the most potent of our new ligands, while initial results in vivo show that the NAMs and a potent novel antagonist are capable of reversal of opioid agonist effects. Together, the allosteric modulators presented here represent a detailed structural description of negative allosteric modulatory mechanisms within the μOR, as well as highlight the power of leveraging ensemble selection principles in "steering" DEL selections towards mechanisms of interest.

B12 - In vitro and in vivo pharmacological characterization of a new N/OFQ dimeric derivative

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Nociceptin/orphanin FQ (N/OFQ) is the endogenous ligand of the N/OFQ peptide (NOP) receptor, a G protein- coupled receptor that shares similarities with classical opioid receptors but has a distinct pharmacological profile. This system regulates several functions including pain transmission, sleep and mood. In this study a series of dimeric N/OFQ derivatives were generated by linking two peptides via a cysteine sulfur bridge in positions 7 to 17. From this series [[Cys^{14}]N/OFQ(1-17)-NH_2]$_2$ was selected and further investigated in vitro and in vivo.
[[Cys^{14}]N/OFQ(1-17)-NH_2]$_2$ has been investigated in vitro using (i) a calcium mobilization assay performed in cells stably expressing chimeric G proteins and NOP or classical opioid receptors, (ii) a bioluminescence resonance energy transfer (BRET) assay for detecting NOP/G protein interaction, (iii) the electrically stimulated mouse vas deferens (mVD) assay. In vivo the compound has been tested in mice for its ability to induce loss of righting reflex (RR).
[[Cys^{14}]N/OFQ(1-17)-NH_2]$_2$ acted as a potent full NOP receptor agonist in the calcium mobilization assay, showing pEC_{50} value of 9.14, similar to N/OFQ(1-17)NH_2. In parallel experiments, [[Cys^{14}]N/OFQ(1-17)-NH_2]$_2$ resulted completely inactive up to 1 μM in cells expressing the classical opioid receptors. In the BRET assay the compound behaved as a NOP agonist with pEC_{50} of 9.91 and α of 0.80. In the mVD assay [[Cys^{14}]N/OFQ(1- 17)-NH_2]$_2$ induced a concentration-dependent inhibition of the electrically induced contraction, with pEC_{50} of 7.29, similar to N/OFQ (7.61). When the experiments were performed with tissues taken from NOP knockout (NOP(-/-)) mice both N/OFQ and [[Cys^{14}]N/OFQ(1-17)-NH_2]$_2$ resulted completely inactive. In vivo, the icv injection of [[Cys^{14}]N/OFQ(1-17)-NH_2]$_2$ induced a long-lasting loss of the RR in mice, being active from the dose of 3 nmol and exhibiting a threefold higher potency than N/OFQ. Notably, while the effects of N/OFQ lasted only a few minutes, those of [[Cys^{14}]N/OFQ(1-17)-NH_2]$_2$ persisted for 7 hours. Again the compound (as well as N/OFQ) was completely inactive in NOP(-/-) mice.
In summary, [[Cys^{14}]N/OFQ(1-17)-NH_2]$_2$ is a potent, full, and selective NOP receptor agonist. Dimerization significantly increased in vivo potency and extended duration of action, highlighting its potential as a strategy for developing novel NOP-targeting compounds.

B13 - Peripherally Acting Mu Opioid Receptor Antagonists (PAMORAs): Investigating a Novel Strategy for Opioid Overdose Reversal

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Fentanyl continues to devastate American communities and is the primary driver of the surge in opioid overdose deaths over the past decade. The leading cause of death from fentanyl is opioid- induced respiratory depression (OIRD). Due to its high potency at mu-opioid receptors (MORs), fentanyl rapidly suppresses cardiorespiratory function and induces loss of consciousness, making timely and effective intervention challenging. Naloxone (Narcan), a centrally acting MOR antagonist, is the first-line treatment for reversing opioid overdoses. It is widely available, easy to administer, and suitable for use in prehospital settings by both bystanders and first responders. However, while naloxone effectively reverses OIRD, its broad MOR antagonism often triggers severe withdrawal symptoms, which can be highly aversive to individuals recovering from an overdose. We recently demonstrated that naloxone methiodide, a peripherally restricted MOR antagonist, can reverse OIRD without inducing aversive behaviors. This finding suggests that peripherally restricted MOR antagonists may represent a viable therapeutic strategy for managing opioid overdoses without eliciting withdrawal. In the present study, we evaluated whether FDA- approved peripherally acting MOR antagonists (PAMORAs) could be repurposed to reverse OIRD. Catheterized rats received intravenous fentanyl followed by intravenous or subcutaneous administration of methylnaltrexone, naloxegol, or naloxone. Cardiorespiratory parameters were measured using a pulse oximeter collar, and locomotor activity and somatic withdrawal behaviors were assessed before and after antagonist administration. As expected, naloxone rapidly reversed OIRD, followed by the emergence of withdrawal behaviors and increased locomotor activity. In contrast, methylnaltrexone and naloxegol also reversed OIRD and increased locomotor activity but did not induce somatic withdrawal signs. These results suggest that PAMORAs can rapidly and effectively reverse fentanyl-induced respiratory depression without triggering withdrawal, supporting their potential repurposing for managing opioid overdoses. Moreover, this approach could improve outcomes for individuals recovering from an overdose and reduce the burden on those administering MOR antagonists.

B14 - Chronic administration of NC-2800, a novel δ-opioid receptor agonist, exhibited antidepressant-like effects in mice without inducing tolerance

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Abstract

The delta opioid receptor (DOP) is an attractive target for novel antidepressants owing to its potential for rapid action with minimal adverse effects. We previously suggested that single administration of a selective DOP agonist KNT-127 and SNC80 exert antidepressant-like actions through facilitation of neuronal excitability in the mouse IL-PFC, which is implicated in mammalian target of rapamycin (mTOR) signal transduction in parvalbumin-positive interneurons (Yoshioka et al., Mol Psychiatry. 2025;30:2038.). On the other hand, we recently succeeded in synthesizing a novel small molecule DOP agonist, NC-2800, which exhibits potent antidepressant-like effects in rodents. In this study, we examined the effects of chronic administration of NC-2800 on antidepressant effects in a mouse model. Chronic oral administration of NC-2800 twice daily for 5 days showed antidepressant-like effects in a mouse learned helplessness model. These effects were further validated using a forced swimming test in mice. In immunoblotting assays, chronic administration of NC-2800 significantly elevated the phosphorylation level of the mTOR signal–related proteins Akt and p70S6 kinase in the mouse medial prefrontal cortex. Furthermore, the phosphorylation of p70S6 was inhibited by pretreatment with the mTOR signaling pathway inhibitor rapamycin. We propose that chronic administration of NC-2800, a novel DOP agonist, exhibits antidepressant-like effects via PI3K- Akt-mTORC1-p70S6 kinase signal transduction in mice without inducing tolerance.

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflicts of interest.

B15 - Novel oxymorphone analogues, as bifunctional mu/delta opioid receptor agonists, produce antinociception without the risks of analgesic tolerance and physical dependence in mice

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An adequate pain management, particularly chronic pain, is still an area of unmet medical need. Opioids are highly effective painkillers. However, chronic use of opioids is associated with analgesic tolerance, physical dependence and addictive potential. Opioids mediate their biological effects via activation of opioid receptors, mu (MOR), delta (DOR), kappa (KOR) and nociceptin (NOP) receptors. The MOR is the primary target for the therapeutic analgesia, but also for the severe side effects. Currently, alternative chemical and pharmacological strategies are explored to mitigate the deleterious effects of conventional opioids (i.e. morphine, fentanyl, oxycodone, as MOR agonists), and to limit their abuse and misuse, amongst which are the multifunctional drugs. In this study, we present the design and pharmacology of a series of oxymorphone analogues that emerged as bifunctional MOR/DOR agonists. In vitro binding studies showed the new oxymorphone derivatives to bind with high affinities (picomolar-to- subnanomolar range) at the MOR, DOR and KOR. They were potent and full agonists at the MOR and DOR and partial agonists at the KOR. In vivo, the oxymorphone analogues displayed antinociceptive efficacy in mouse models of acute nociception and inflammatory pain after s.c. administration. Their antinociceptive effect was demonstrated to be selectively mediated by both MOR and DOR. Chronic s.c. drug treatment of mice did not cause antinociceptive tolerance and naloxone-induced withdrawal syndrome. In conclusion, we show that targeted structural modifications on the oxymorphone scaffold resulted in significant alterations in opioid activity by influencing the pharmacological properties. Oxymorphone, a potent and selective MOR agonist, was converted into bifunctional MOR/DOR agonist ligands. The dual activation of the MOR and DOR using the novel oxymorphone analogues produces effective antinociception without the CNS-mediated risks of analgesic tolerance and physical dependence. These findings pave the way to new pain therapeutics with limited side effects following both acute and chronic use.

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B16 - Discovery of Phenyltriazole-Based Dual KOR Antagonists and MOR Agonists via Structure-Based Virtual Screening

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Kappa opioid receptor (KOR) agonists effectively reduce pain, but their clinical application is often limited by adverse effects such as dysphoria, sedation, and reduced dopamine release. In contrast, KOR antagonists show therapeutic potential in the treatment of major depressive
disorder, anxiety, anhedonia, and substance use disorders. These divergent effects justify continued efforts to discover new KOR ligands with defined functional profiles.
In our structure-based virtual screening campaign targeting KOR-active agents, in the initial stage, we used Triazole 1.1 as a template—a selective KOR agonist with high affinity and G- protein-biased signaling over β-arrestin recruitment. This compound retains the analgesic and antipruritic efficacy typical of KOR agonists while avoiding sedative and dopaminergic side effects. Next, we employed SilicoPharm, an in-house developed screening platform
(Polypharmacological In Silico Screening Platform For The Next Generation Drugs, www.silicopharm.eu) to conduct a structure-based virtual screening of approx. 3-million- compound library from commercial databases, focusing on molecules containing triazole moieties. Six top-ranked hits were selected for in vitro binding assays. Among them, one phenyltriazole derivative exhibited moderate affinity for KOR (98 nM) and MOR (162 nM), and low affinity for DOR (>2.5 μM). The remaining five compounds showed low affinity for all three opioid receptors (>10 μM). In the next phase, ten analogs of the phenyltriazole hit were obtained via synthesis or selected based on the substructure search from commercial sources. One compound from this series demonstrated nanomolar affinity for KOR (57 nM) and MOR (6 nM). Functional studies revealed that a representative compound from this series acted as a KOR antagonist and MOR agonist. Additionally, in silico binding mode analyses were conducted for two phenyltriazole derivatives.
This study demonstrates that our virtual screening protocol enabled the identification of a novel scaffold featuring a phenyltriazole moiety with nanomolar affinity for KOR and MOR, selectivity over DOR, and a unique dual pharmacological profile. These findings provide a promising starting point for the development of novel opioid receptor modulators with improved therapeutic potential.

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B17 - Design and Synthesis of Pyrazolomorphinan Derivatives as Novel Delta Opioid Receptor Agonists

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Among the opioid receptor subtypes, the delta opioid receptor (DOR) subtype is a promising drug target because the activation of the receptor produces not only analgesic effect but also antidepressant or anxiolytic effects without serious side effects like drug dependence or aversive effect which are sometimes observed in mu or kappa receptor agonists. We designed pyrazolomorphinan derivatives as a novel DOR selective agonist with an unprecedented chemotype according to the drug design concept for the DOR selective agonist KNT-127 which encompassed the message-address concept, the accessory site concept, and the conversion of an indole ring into a quinoline ring. The designed compounds as well as their regioisomers showed selective agonist activities for the DOR. Among the tested compounds, SYK-1106 bearing a cyclohexyl substituent was the most potent and efficacious agonist (EC_{50} = 0.089 nM, E_{max} = 111%). SYK-1106 showed dose-dependent and DOR antagonist NTI reversible antidepressant- like effects at 0.3 mg/kg, s.c. in the mouse forced-swimming tests without an effect on locomotor activity and with no convulsive effects. SYK-1106 is expected to be a promising lead compound for antidepressants.

B18 - Structure-Activity Exploration of Delta Opioid Receptor Positive Allosteric Modulators

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Chronic pain and depression are two of the most experienced medical ailments worldwide. Approximately 12 million Americans suffer from this cooccurrence. The endogenous opioid system has been shown to regulate pain and mood; however, the long-term administration of clinically used opioids is associated with adverse effects. The delta opioid receptor (DOR) is a promising target for the treatment of chronic pain and depression. DOR expression is upregulated in chronic pain states and its activation does not carry the adverse effects associated with mu opioid receptor (MOR) activation. However, orthosteric agonists of DOR can cause convulsions. Positive allosteric modulators (PAMs) of DOR, exemplified by the xanthene-dione BMS-986187, bind to a topographically distinct location from the orthosteric binding site to increase the affinity and/or efficacy of orthosteric agonists. Importantly, BMS-986187 does not promote the convulsive activity of the DOR agonist SNC80 in mice. Although BMS-986187 is DOR-preferring, this molecule is not selective and has PAM activity at the mu and kappa opioid receptors (KOR). Here, we report a structure-activity study of BMS-986187 at DOR and MOR to probe the allosteric pharmacophore, while simultaneously improving DOR selectivity. Concentration-response curves of BMS-986187 analogs were obtained using a β-arrestin recruitment assay in the presence of an EC20 concentration of Met-enkephalin. We identified a potent and DOR-selective PAM (AP- 513) that displays negligible functional activity at MOR. However, AP-513 acts as a silent allosteric modulator at KOR, hinting at a conserved allosteric site across these receptors. Global molecular docking experiments with BMS-986187 reveal interactions with a conserved cholesterol binding site. In on-going work, we are examining how mutations around this proposed region affect the activity of AP-513. Further experiments seek to examine this compound's selectivity for analgesia and antidepressant-like activity without causing convulsions. This work seeks to validate a new strategy for therapeutically targeting DOR. Supported by R37 DA039997 and T32GM1402233.

B19 - Novel Hydroxynorketamine Analogs for Chronic Neuropathic Pain Management

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Chronic neuropathic pain remains a critical therapeutic challenge, as current analgesics frequently provide inadequate relief accompanied by substantial side effects, including significant abuse potential. Hydroxynorketamine (HNK), a metabolite of ketamine, is a promising non-opioid analgesic without dissociative effects associated with NMDA receptor antagonism. One potential mechanism of action of novel analogs of (2R,6R)-HNK (HNK-SPs) is via positive allosteric modulation (PAMs) at the mu opioid receptor (MOR), enhancing endogenous enkephalin activity without direct agonism or dependence risk. Another possible mechanism of action is through activation of AMPA receptors. This study evaluates in vivo efficacy of novel analogs and examines potential mechanisms of action.

We assessed mechanical allodynia in mice using the spared nerve injury (SNI) model following administration of HNK analogs. Preliminary data demonstrate that select HNK-SPs effectively reduce mechanical hypersensitivity, achieving analgesic efficacy comparable to or exceeding that of 2R,6R-HNK. Specifically, Compound 1, identified as a MOR PAM without direct agonist activity, exhibited robust antiallodynic effects without impairing locomotor function. Activities of additional compounds with differing activity at the mu receptors as well as receptor antagonist studies will be presented. These findings highlight the therapeutic potential of HNK-SPs, offering potent analgesia for chronic neuropathic pain management with a significantly reduced risk profile compared to traditional opioids.

B20 - TRPing receptors – modulatory effect of TRP channels on MOR

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Introduction

Opioids are still the mainstay treatments for pain relief. However, their use is associated with severe adverse effects such as tolerance and respiratory depression and the search for safer opioids has not been successful yet. This highlights the need for novel strategies that promote antinociceptive signalling while minimising adverse effects[1]. Transient receptor potential vanilloid 1 (TRPV1) channels are often co-expressed with mu-opioid receptors (MORs) in dorsal root ganglia neurons (DRGs) and have been proposed to modulate MOR signalling and promote opioid analgesic properties under acute inflammation[2]. However, the cellular mechanisms involved in MOR/TRPV1 regulation are not yet understood.

Methods

Our study used HEK 293 cells transiently transfected with a range of bioluminescence resonance energy transfer (BRET) sensors and membrane receptors to investigate the effect of TRPV1 activation on MOR signalling responses. Calcium mobilisation experiments were performed using a Fluo-4AM dye. Experiments were performed in 96-well plates coated with poly-D-lysine, 48h post-transfection.

Results

BRET assays showed that TRPV1 activation by capsaicin fully inhibits agonist-induced arrestin-3 and GRK2 recruitment to the MOR, without preventing the recruitment of a conformationally selective nanobody (Nb33) or mini Gi protein. TRPV1 activation also affected the regulation of other GPCRs including all the other opioid (delta, kappa and nociceptin), neurokinin NK1 and dopamine D2 receptors. Activation of transient receptor potential ankyrin 1 (TRPA1) channel modulated MOR signalling similarly to TRPV1 activation. Using a calcium-free buffer abolished capsaicin effects and a non-specific intracellular calcium increase induced by ionomycin or activation of Gq-coupled muscarinic acetylcholine M1 and M3 receptors using carbachol failed to modulate MOR signalling.

Conclusion

Our results suggest a pan-regulatory mechanism by which localised Ca^{2+} signalling from TRP channels can modulate GPCR signalling. Understanding this mechanism further will provide new avenues for rational drug design in complex diseases, such as inflammatory or neuropathic pain.

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B21 - Mapping Brain-Wide Neuronal Activation in Chronic Neuropathic Pain and Opioid-Mediated Analgesia

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Chronic neuropathic pain severely diminishes quality of life, and many patients rely on opioid analgesics for relief. While peripheral pain pathways are well understood, the brain circuits and cellular mechanisms that mediate pain perception remain less explored. In this study, we used a genetically engineered mouse model and an optimized tissue-clearing technique to enable whole- brain 3D visualization of activated neurons. We identified regions of high neuronal activation during chronic neuropathic pain and analyzed their spatial distribution. We also assessed the effects of two analgesics—morphine (a mu-opioid receptor agonist) and Ro 64-6198 (a NOP receptor agonist)—on brain region activation. Each treatment produced distinct patterns of activity in regions involved in nociceptive processing. These findings highlight brain areas involved in both chronic pain and its modulation by opioid and NOP receptor pathways, and advance our understanding of where pain and analgesia are encoded in the brain—potentially guiding the development of more targeted pain therapies.

B22 - Potentiation of opioid analgesia and delay of tolerance by ultramicronized palmitoylethanolamide: involved cells, underlying mechanisms, and proteomics insights

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Chronic pain profoundly impairs physical, psychological, and cognitive functions. Although opioids are effective, their clinical utility is constrained by adverse effects and the development of tolerance, underscoring the need for adjunctive strategies. Palmitoylethanolamide (PEA) is a naturally occurring fatty-acid amide produced on demand in response to tissue injury and exerts multiple pro-homeostatic functions. PEA has been shown to be safe and effective for pain relief, with ultramicronized formulation (PEAum) offering superior bioavailability and pharmacokinetics compared to naïve PEA. This in vitro and in vivo study aimed to investigate PEAum as a candidate to improve opioid efficacy and delay tolerance, with the underlying mechanisms being explored and proteomics being used for the first time in this context. Repeated PEAum administration delayed morphine tolerance in both physiological and neuropathic (i.e., sciatic nerve ligation) murine pain models. Moreover, PEAum prolonged opioid analgesia, enabling sustained low-dose morphine regimens. Mechanistic investigations showed that PEAum prevented morphine- induced activation of astrocytes and microglia at the spinal level and reduced mast cell degranulation. In vitro studies confirmed that PEAum down-modulated mast cell degranulation and attenuated the pro-inflammatory effects of mast cell-conditioned medium (c.m.) in organotypic spinal cord slices. Notably, c.m. from PEAum- pretreated mast cells activated astrocytes while preserving neuronal integrity and significantly decreased the release of calcitonin gene-related peptide, a key pro-nociceptive mediator. Proteomic analysis of spinal cord slices treated with morphine and mast cell c.m. showed that PEAum upregulated pathways associated with cell survival as well as neuronal development and proliferation, while down-regulating those related to apoptosis and necrosis. These findings shed new light on the mechanisms underlying the anti-neuroinflammatory functions of PEAum through the modulation of mast cell–astrocyte crosstalk. Moreover, our results highlight the ability of PEAum to mitigate opioid-induced neuroinflammation, delay the development of tolerance and enhance opioid analgesia, supporting its therapeutic potential as a neuroprotective adjuvant in pain management.

B23 - Morphine antinociception restored by methadone in the morphine-resistant inflammatory pain state

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The antinociceptive effect of methadone in the morphine-resistant inflammatory pain state was described in the paw-withdrawal test using the complete Freund's adjuvant (CFA)-induced mouse inflammatory pain model. After intraplantar (i.pl.) injection of CFA, thermal hyperalgesia was observed in the ipsilateral paw. The antinociceptive effects of subcutaneous (s.c.) injection of morphine, fentanyl, and oxycodone against thermal hyperalgesia in the inflammatory pain state were reduced in the ipsilateral paw 7 days after CFA pretreatment. On the contrary, the antinociceptive effect of s.c. injection of methadone was maintained in the ipsilateral paw 7 days after CFA pretreatment. The suppressed morphine antinociception in the CFA model mice was bilaterally restored following s.c. treatment with methadone 20 min prior to or 3 days after CFA pretreatment. The suppressed morphine antinociception was also bilaterally restored by intraperitoneal treatment with MK-801 30 min prior to CFA pretreatment; however, the s.c. injection of morphine 30 min prior to CFA pretreatment failed to restore the suppressed morphine antinociception in the CFA model mice. The expression level of mRNA for μ-opioid receptors 7 days after i.pl. pretreatment was not significantly changed by i.pl. pretreatment with CFA or s.c. pretreatment with methadone. In conclusion, methadone is extremely effective against thermal hyperalgesia in the morphine-resistant inflammatory pain state, and restores suppressed morphine antinociception in the inflammatory pain state without altering the expression level of mRNA for μ-opioid receptors.

B24 - Sex-dependent neuronal activation and behavioural dysfunction caused by NTG-induced migraine is reversed by NOP receptor agonist

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Migraine is a debilitating neurological disorder associated with sensory and social impairments. The neural basis of migraine-associated social dysfunction remains insufficiently understood. We aimed to identify the specific brain regions that contribute to migraine-induced pain and social impairments and examine potential sex-dependent differences. Migraine-like symptoms were induced using the nitroglycerin (NTG) model in TRAP2/Ai9 transgenic mice. We demonstrated that NTG induced significant neuronal activation in brain regions associated with pain and social behavior, including the anterior cingulate cortex, amygdala, hippocampus, hypothalamus, and periaqueductal gray in female mice as well as trigeminal nucleus caudalis in male and female mice (at least $p<0.05$). Behavioral analyses included the social novelty test using a three-chambered apparatus, and the von Frey assay for mechanical allodynia. NTG administration led to social avoidance in both male and female mice, as shown by significantly increased time spent in the neutral chamber ($p = 0.0003$). However, the direct contact with familiar (F) ($p < 0.05$) and non-familiar (NF) ($p = 0.0048$) mice significantly decreased in male but not female mice, while total time spent in the chamber with NF was decreased only in female mice ($p < 0.05$). The NOP receptor agonist, Ro 64-6198, reversed both the allodynia and social deficits induced by NTG ($p < 0.0001$ for males; $p = 0.0092$ for females). Consistent with the behavioral results, NTG-induced neuronal activation in these brain regions was significantly reduced in the presence of Ro 64-6198 ($p<0.0001$ for males; at least $p<0.05$ for females). Our findings indicate the role of the presented brain regions in mediating migraine-related behavioral deficits. This provides critical insight into the broader neurobiological impact of migraine and suggests novel targets for therapeutic intervention beyond pain relief.

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B25 - The Effects of Mixed Mu/NOP Agonist on Mechanical Allodynia in NTG-Induced Acute and Chronic Migraine in Mice

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Migraine is a neurological disorder characterized by throbbing headache, and accompanying symptoms including mechanical allodynia - an abnormal pain response to normally non-painful stimuli. Nitroglycerin (NTG) administration induces migraine-like symptoms in mice, including mechanical hypersensitivity in the periorbital region and hind paws. Both the opioid and nociceptive systems are implicated in the pathophysiology of migraine, although their exact role remains under investigation. The objective of the study was to investigate the role of the mu opioid receptor and the NOP receptor in mechanical allodynia associated with acute and chronic migraine in mice. Migraine-like symptoms were induced by single (acute model) intraperitoneal administration of NTG and five injections of NTG every other day (chronic model). Mixed full mu/NOP agonist, classical opioid receptor antagonist – naloxone, and selective NOP receptor antagonist – SB-612111 were used. Mechanical sensitivity was assessed using the von Frey test and up-down method. Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc test, if applicable. We found that administration of a mixed full agonist targeting both NOP and mu opioid receptors significantly attenuated mechanical allodynia in the periorbital region and paw in both male ($p=0.026$, $p=0.0002$, respectively) and female ($p<0.05$; $p<0.0001$, respectively) mice. Pre-treatment with SB-612111 did not significantly diminish the analgesic agonist effect in the paw of both sexes and in the periorbital region of males, except the female periorbital region ($p=0.0098$). In contrast, administration of naloxone, significantly blocked the analgesic effects of the agonist in both the paw and periorbital region in males ($p<0.0001$, $p<0.0001$, respectively), and in the periorbital region in females ($p=0.0033$). Furthermore, preliminary data show that repeated NTG administration induced mechanical allodynia in mice, which was effectively reversed by the tested agonist after single administration. These findings suggest that both mu opioid and NOP receptors contribute to the modulation of sensory component of migraine pain. However, the mu opioid signaling appears to be more important in alleviating mechanical allodynia in migraine induced by NTG administration.

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B26 - Implications of NOP and classical opioid receptor systems in treatment of migraine-like symptoms in mice

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Migraine is an extraordinarily prevalent and disabling headache disorder that affects one billion people worldwide. It is highly sex dependent as 70% of women suffer from migraine. The objectives of this study are to investigate the role of the classical opioids and NOP (Nociceptin OPioid) receptor systems in migraine, such as affective migraine pain, including photophobia and sociability impairments.
Utilizing immunohistochemical analysis with NOP-eGFP knock-in mice, we have observed the presence of NOP receptors on the majority of neurons in the trigeminal ganglia and significant expression in the trigeminal nucleus caudalis. The nitroglycerin (NTG) migraine mouse model was employed to mimic migraine-like symptoms by inducing vasodilation and significant allodynia throughout the body as well as light sensitivity and social impairments.
All behavioral studies were conducted on C57BL6/J male and female mice. NOP receptor agonist and mixed full NOP and mu receptor agonist were used. After co-injections of each compound and NTG, photophobia was tested using a light/dark box, while social behavior was tested using a three-chamber apparatus. Statistical analysis was performed using two-way ANOVA followed by Tukey's post hoc test, if applicable.
We found that selective NOP agonist attenuated NTG- induced social avoidance in male and female mice (at least $p<0.01$), while mixed mu/NOP compound elicit social impairments by itself ($p<0.0001$). Similar effects were found when light aversive behavior was tested. In particular, NOP agonist reversed the NTG-induced decrease of time spent in the light chamber (at least $p<0.05$) in both male and female mice, while mixed mu/NOP compound induced photophobia when administered alone ($p <0.0001$) in male mice.
These results support further exploration of NOP receptor activation as a potential strategy for attenuating migraine-like symptoms.

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B27 - Decoding pain mediated shifts in social behavior following neuropathic injury in mice

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While the peripheral detection of pain is well-characterized, the brain mechanisms that transform nociception into affective pain states—and their influence on social behavior— remain unclear. Further, the role of the endogenous opioid family, which is known to modulate both pain and social interaction via modulation of nucleus accumbens medium spiny and interneuron cellular populations, has yet to be elucidated. To investigate the intersectional dynamics of pain and social behavior, we used an operant social self- administration paradigm in male and female mice paired with the spared nerve injury (SNI) model. Following 8 days of operant training where lever presses were rewarded with the introduction of an affiliative social partner, mice received SNI and were reintroduced to the task either 1 day (immediate) or 5 days (delayed) following surgery. Single-cell RNA sequencing of the nucleus accumbens was performed at the completion of behavioral testing to examine transcriptional changes in specific cellular populations resulting from SNI alone and from operant social intervention for SNI.

We found that access to social interaction significantly shaped behavioral and nociceptive outcomes. Mice with immediate post-injury social access exhibited reduced mechanical allodynia and higher operant social responding compared to those given delayed access. In contrast, delayed-access mice displayed increased social withdrawal and enhanced allodynia. These effects were sex-dependent, with females showing stronger behavioral sensitivity to both pain and social disruption. Transcriptomic analysis revealed broad gene expression shifts, including differential regulation of kappa and mu opioid receptor genes specifically in pair-housed SNI mice who were not given access to the social operant task.

These findings identify a critical recovery window in which social interaction modulates both behavioral and molecular signatures of pain, revealing a sex-dependent, time- sensitive neurobiological mechanism that may inform therapeutic strategies targeting affective components of chronic pain.

B28 - Spinal Lipocalin-2 Contributes to the Development of Central Post-Stroke Pain

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Background

Central post-stroke pain (CPSP) is a chronic neuropathic pain syndrome resulting from damage to the central nervous system following stroke. Despite its prevalence, the pathophysiological mechanisms underlying CPSP remain poorly understood, and current pharmacological treatments are largely ineffective. Consequently, many patients suffer from persistent pain, including hyperalgesia, allodynia, and spontaneous pain. We have previously demonstrated activation of astrocytes and microglia in the spinal cord of mouse models of CPSP. Glial cell activation exacerbates cerebral ischemic pathology by promoting the release of inflammatory mediators; however, the specific role of spinal glial cells in CPSP remains unclear. We hypothesized that glial cell-derived molecules in the spinal cord contribute to the hyperexcitability associated with CPSP. In this study, we investigated glial factors involved in the development of CPSP using a mouse model induced by bilateral common carotid artery occlusion (BCAO).

Methods

Male ddY mice were subjected to 30 minutes of BCAO. Mechanical hypersensitivity in the right hind paw was assessed using the von Frey test. Immunohistochemistry was employed to examine cellular localization in the spinal cord post-stroke. Quantitative real-time PCR was used to assess changes in gene expression.

Results

Mice subjected to BCAO exhibited significant mechanical hypersensitivity and spinal astrocyte activation three days post-treatment. DNA microarray analysis revealed a marked upregulation of lipocalin-2 (LCN2), also known as neutrophil gelatinase-associated lipocalin, in the superficial dorsal horn. LCN2 was colocalized with GFAP, an astrocyte marker, and GFAP-positive cells in the BCAO mice also expressed STAT3. Increased fluorescence intensities of LCN2 and GFAP were attenuated by intrathecal administration of AG490, a JAK2 inhibitor, or anti-LCN2 antibody.

Conclusions

These findings suggest that LCN2 derived from spinal astrocytes contributes to the development of CPSP via JAK2/STAT3 signaling, highlighting a potential therapeutic target for managing post-stroke neuropathic pain.

B29 - Understanding the Impact of Inflammatory Pain on Alcohol Use: A Study in Rats with a Focus on Sex and Dose

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In recent years, multiple clinical and epidemiological studies have revealed a strong association between chronic pain and Alcohol Use Disorder (AUD). However, preclinical research on this topic remains limited, and the specific effects of pain on AUD are not yet fully understood. In this study, we aimed to investigate whether the development of an inflammatory pain condition, induced by intraplantar injection of Complete Freund's Adjuvant (CFA), could influence alcohol self-administration (ASA) in animals with a prior history of alcohol exposure.

To this end, male and female rats were first exposed to alcohol in their home cages using the drinking-in-the-dark protocol for two weeks. Subsequently, they were trained to self-administer 20% alcohol. Once stable ASA was established, rats received an injection of CFA or saline into their hind paws. They then underwent a dose-response test consisting of three consecutive sessions for each of the following alcohol concentrations: 20%, 30%, and 50%. The order of presentation was randomized in either ascending or descending fashion.

Our results show that both male and female CFA-treated rats exhibited increased alcohol intake at the highest concentration (50%). Interestingly, males showed a decrease in intake at the lowest dose (20%) compared to controls, a difference not observed in females.

To further assess the impact of inflammatory pain on alcohol-related motivation, a separate cohort underwent behavioral economic analysis. Our data indicate that demand curve analysis is a suitable method for evaluating alcohol motivation.

These findings contribute to a better understanding of the complex relationship between chronic pain, AUD, and potential sex differences. They may also support the development of more personalized treatment strategies for patients with co-occurring chronic pain and alcohol use disorder.

B30 - Convergent state-control of endogenous opioid analgesia

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Pain is a dynamic and nonlinear experience shaped by injury and contextual factors, including expectations of future pain or relief. Increasing evidence suggests that the midbrain periaqueductal gray plays a central role in endogenous analgesia and pain expectancy as the ventrolateral column (vlPAG) receives ascending nociceptive signals from the periphery and descending pain representations arising in forebrain cortical areas. Neurons expressing the μ opioid receptor (MOR+) and the MOR ligand enkephalin (Enk) are crucial to the nociceptive and pain-relieving functions of the vlPAG. However, significant gaps remain in our understanding of the vlPAG cell-type identities, the sub-second neural dynamics involved in pain modulation, the contribution of endogenous peptide neuromodulators, and the contextual factors influencing these processes. We hypothesized that opioidergic signaling dynamics in the vlPAG represent injury- and expectancy-induced pain states that can be targeted for on-demand pain relief. To this end, we used spatial mapping with RNA sequencing of pain-active neurons, alongside activity-dependent and cell type-specific genetic tools for in vivo optical recordings and modulation of neural activity in mice, to identify pain state-related opioidergic signaling in vlPAG. TRAP labeling and Projection-TAGging revealed that fore- and hindbrain projecting MOR+ glutamatergic vlPAG neurons are recruited during acutely nociceptive states, while their real-time calcium activity is modulated by opioid agonists and pain behavior. Enk-releasing afferents densely innervate vlPAG, arising from, among other sources, local neurons. In contrast to the activation of MOR+ neurons, we observed acute pain-related suppression of Enk release detected with the δLight sensor. Interestingly, protracted inflammatory pain, learned predictions of pain onset, and expression of placebo analgesia all produced tonic elevations of Enk. By leveraging the functional effects of pain relief expectation, we used direct optogenetic activation of vlPAG Enk-expressing neurons to drive opioid peptide release onto MOR+ neurons, resulting in a robust reduction in pain. These findings show that diverse biological and contextual factors converge on shared midbrain circuitry that releases endogenous opioids to suppress nociceptive activity and promote analgesia. Harnessing vlPAG Enk signaling offers a promising avenue for future pain therapies that achieve effective pain relief without the harmful side effects associated with prescribed opioid analgesics.

B31 - The kappa opioid receptor (KOR) in paraventricular nucleus of the thalamus (PVT)

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The PVT is the most dorsal nucleus of the thalamic midline nuclei and is involved in stress responses, fear, anxiety, arousal, and reward. The anterior PVT (aPVT) has been linked to appetitive/approach behaviors, whereas the posterior PVT (pPVT) is associated with aversive/avoidance behaviors. In the KOR-tdTomato mouse line, the PVT expressed a moderate level of KOR. In situ hybridization showed that in the PVT, KOR mRNA co- localized substantially with vGluT2 mRNA (but not vGluT1 mRNA) , indicating presence of KOR in glutamatergic projection neurons. As the KOR is also involved in stress responses and anxiety, to further explore the role of KOR-expressing PVT cells we mapped the projection targets of KOR-expressing neurons in PVT. Using KOR-iCre mice and Cre- dependent anterograde tracer AAV-FLEx^{loxP}-mGFP-2A-synaptophysin-mRuby, we found that PVT KOR-expressing neurons projected to many brain regions, with the NAc, CeA, BLA, zona incerta, and BNST among the major projection targets. We conditionally knock- downed KOR (KOR cKD) in the PVT by stereotaxic injection of AAV-eGFP-Cre into aPVT of Oprk1$^{loxP/loxP}$ mice. AAV-eGFP was used as a control. By [^{3}H]U69,593 receptor autoradiography, we found substantial cKD of KOR in the aPVT. In both male and female mice, KOR cKD in aPVT significantly reduced morphine withdrawal-associated jumps precipitated by naloxone and produced anxiolytic-like effects in the elevated plus-maze (EPM) test. However, the KOR cKD attenuated U50,488H (U50)-induced conditioned place aversion (CPA) only in males, with no effect observed in females. Additionally, in either sex KOR cKD in aPVT did not alter the anti-scratching effects of U50or affect immobility in the forced swim test. We will further examine the effects of KOR cKD in the aPVT on additional behavioral endpoints, including morphine withdrawal-induced CPA, U50-mediated inhibition of acetic acid-induced writhing, and fear-elicited positive and negative defensive behaviors. Ongoing work is also examining the effects of KOR cKD in the pPVT. In summary, we showed that KOR in aPVT mediated morphine withdrawal symptom(s) and anxiety-like behaviors in both male and female mice, but contributed to KOR agonist-induced aversion only in males.

Support
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Conflict of interests
None.

B32 - Hypothalamic Vasopressin - Dynorphin Neural Circuit in Endocrine Control of Left-Right Balance

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Traumatic brain injury and stroke often result in contralateral sensorimotor deficits, including asymmetric posture. While these lateralized effects are classically attributed to neural tract disruption, recent evidence supports the existence of a complementary humoral pathway. Our previous work demonstrated that the neurohormone Arg-vasopressin (AVP) mediates contralateral hindlimb responses to left-sided brain injury, whereas dynorphins influence left hindlimb reflexes following right-sided injury.

We hypothesized that AVP- and dynorphin-expressing hypothalamic neuroendocrine circuits function to maintain left–right balance in peripheral regulation, and that selective activation of either neurohormone disrupts this equilibrium, producing asymmetric outcomes.

Using a transgenic AVP rat model expressing hM3Dq and mCherry, we examined AVP–dynorphin co-expression in the paraventricular nucleus (PVN), supraoptic nucleus (SON), and suprachiasmatic nucleus (SCN). AVP was expressed in all three nuclei, while prodynorphin was absent in the SCN. In the PVN and SON, 56% and 96% of mCherry-labeled AVP neurons, respectively, co-expressed prodynorphin. Chemogenetic activation via clozapine-N-oxide (CNO) induced c-Fos expression in ~82% of AVP neurons in these regions.

To assess functional outcomes, we used hindlimb postural asymmetry model as a proxy for lateralized physiological responses. CNO activation of AVP neurons alone did not induce asymmetry. However, following kappa-opioid receptor blockade with nor-binaltorphimine, CNO administration produced right hindlimb flexion in rats with completely transected spinal cords—an effect abolished by AVP antagonists.

These findings reveal antagonistic roles of AVP and dynorphin in postural control and suggest that AVP neurocircuits, which co-express dynorphin, function as molecular regulators of left–right balance in the body.

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B33 - Ventral tegmental area neurons expressing μ-opioid receptor make distal connections

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The ventral tegmental area (VTA) is central to brain reward circuits and a major target for opioids and other drugs of abuse. VTA is famous for dopamine neurons, but also contains GABA- and glutamate- releasing neurons, each of which can mediate different behavioral effects depending on their projection targets. Untangling the connectivity of heterogeneous VTA neurons is thus crucial to understanding mechanisms underlying addiction. Opioids and other addictive drugs share a common effect of increasing dopamine released from VTA projections. The μ-opioid receptor (MOR) is the most important target for both opioid analgesia and addiction. Opioids are known to reduce GABA-mediated inhibition in the VTA, thereby disinhibiting dopamine neurons, which contributes to the reinforcing properties of those drugs. While classical models suggest that dopamine disinhibition in VTA is mediated by MOR-expressing inhibitory interneurons, defined as neurons only making local connections such that their soma and axon are contained in the same brain region, there is no direct evidence that such interneurons exist in VTA. Instead, MOR-expressing projection neurons in VTA could make intra-VTA collaterals. To elucidate whether MOR is a selective marker for interneurons, we selectively expressed a membrane-bound fluorescent protein in MOR-expressing VTA neurons, revealing dense projections to ventral pallidum (VP) as well as to other brain regions. To determine whether MOR-expressing projection neurons make local connections, we used optogenetics-assisted electrophysiology to functionally identify local GABA connections made by projection-target defined VTA neurons. We found that VTA neurons expressing MOR make distal connections, and that these projection neurons also make local intra-VTA synapses. Our findings suggest that VTA projection neurons, rather than VTA interneurons, may be a major target for opioids.

B34 - Medullary Raphe Serotonin Neurons are Influenced by Opioids and Improve Breathing

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Opioid-induced respiratory depression (OIRD) is due to mu-opioid receptor (MOR) activation and can cause fatality in overdose. Serotonin receptor agonists have been shown to stimulate breathing under OIRD. Thus, serotonin receptors may serve as MOR-independent targets for treating OIRD. Moreover, medullary raphe serotonin neurons regulate breathing and other vital autonomic processes. Despite this, opioid regulation of medullary raphe serotonin neurons and their role in OIRD remains elusive. Herein, we hypothesized that medullary raphe serotonin neurons are distinctly regulated by opioids along the rostral-caudal axis and that stimulation of caudal medullary raphe serotonin neurons can improve breathing under OIRD. To determine if serotonin neurons are regulated by opioids at the cellular level, we made whole-cell voltage clamp recordings from TdTomato-labeled medullary raphe serotonin neurons contained in mouse brain slices. Surprisingly, only 6 % of medullary raphe serotonin neurons hyperpolarized to the opioid agonist met-enkephalin. Regardless of location along the rostral-caudal axis, met-enkephalin decreased serotonin neuron spontaneous excitatory postsynaptic current (sEPSC) frequency. Interestingly, spontaneous inhibitory postsynaptic current (sIPSC) frequency was attenuated greater than sEPSC frequency in rostral medullary raphe serotonin neurons. These data suggest that opioids regulate medullary raphe serotonin neurons through presynaptic inhibition of glutamate release onto rostral and caudal serotonin neurons and strong attenuation of inhibitory neurotransmission in rostral regions. Thus, opioids may inhibit caudal serotonin neurons but disinhibit rostral serotonin neurons through presynaptic mechanisms. To determine if stimulation of caudal medullary raphe serotonin neurons can attenuate OIRD, we coupled optogenetic stimulation of serotonin neurons with whole-body plethysmography. Using Pet-cre mice, we virally expressed channelrhodopsin-2 in caudal medullary raphe serotonin neurons and implanted an optical fiber above the raphe obscurus. Depolarizing light was delivered to the caudal medullary raphe in room air and 5% CO2 following morphine (30 mg/kg, i.p.). Stimulation of caudal medullary raphe serotonin neurons significantly improved minute ventilation depressed by morphine. These data suggest that caudal medullary raphe serotonin neurons may provide excitatory drive to the respiratory network and are important for breathing under OIRD.

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B35 - Functions of zona incerta MOR neurons in the control of hedonic balance in mice

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The mu opioid receptor (MOR) is main target of opiate, such as morphine, and is involved in reward and addiction[1]. MORs are expressed throughout the brain including in the Zona Incerta (ZI). ZI is implicated in eating[2], and emotional behaviors, including anxiety regulation[3]. The functions of the neurons expressing MOR in ZI (ZI-MOR) remains poorly understood.

To explore their role, we used the MOR-Cre mouse model, enabling selective optogenetic manipulation of ZI-MOR neurons. Specifically, MOR-Cre male and female mice were injected into the ZI with cre-recombinase dependent viral vectors expressing the inhibitory opsin, GtACR2, and implanted with fiber optics for precise temporal inhibition. We determined ZI- MOR neurons functions via behavioral tests, including aversive and reward paradigms. Our results show that inhibition of ZI-MOR neurons induces an aversive response, suggesting their involvement in the modulation of negative affect. In the real-time place preference test (RTPP), inhibited mice showed significant avoidance behavior ($n = 38$; $p = 0.004$). Furthermore, inhibition of ZI-MOR neurons affects anxiety regulation, as evidenced by the Elevated Plus Maze (EPM) test, where GtACR2 female mice show more anxious behavior compared to the control group ($n = 27$; $p = 0.005$). These results underline that ZI-MOR neurons mediate motivation and emotional states, in particular the regulation of negative emotional responses and anxiety.

In conclusion, our findings reveal that ZI-MOR neurons play a key role in the regulation of hedonic balance. And thus a dysfunction of ZI-MOR neurons may be involved in disorders such as compulsive eating, mood and addiction.

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B36 - Excitatory synaptic transmission is differentially modulated by opioid receptors along the claustro-cingulate pathway

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The anterior cingulate cortex (ACC) plays a pivotal role in processing pain and emotion, communicating with both cortical and subcortical regions involved in these functions. The claustrum (CLA), a subcortical region with extensive connectivity to the ACC also plays a critical role in pain perception and consciousness. Both ACC and CLA express Kappa (KOR), Mu (MOR), and Delta (DOR) opioid receptors, yet whether and how opioid receptors modulate this circuit is poorly understood. This study investigates the effects of opioid receptor activation on glutamatergic signaling in CLA-ACC circuitry using spatial transcriptomics, slice electrophysiology, optogenetics, and pharmacological approaches in mice of both sexes. Our results demonstrated that excitatory inputs generated by the CLA onto layer 5 pyramidal cells (L5 PYR) in the ACC are reduced by KOR, MOR, and DOR agonists. However, only KOR agonists reduce monosynaptic transmission from the CLA onto L5 ACC PYR cells, highlighting the unique role of KOR in modulating the CLA-ACC pathway. MOR agonists had a heterogeneous effect on optically-evoked excitatory postsynaptic currents (oEPSCs), significantly reducing longer-latency excitatory responses while only modestly inhibiting the short latency excitatory postsynaptic currents. DOR agonists only reduce slower, longer-latency recurrent excitatory responses. These findings provide new insights into how opioid receptors regulate the claustro-cingulate circuit and demonstrate the distinct, receptor-specific modulation of synaptic transmission within this network.

B37 - Autophagy in Reversing Neuronal Damage from HIV and Morphine

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The goal of this project is to explore the role of autophagy in the mechanism of neuronal injury and cognitive decline associated with combined exposure to HIV and opioid use. We have extensively studied the autophagy protein Beclin1 and demonstrated that this protein plays a crucial role in HIV replication, the secretion of inflammatory molecules by HIV-infected glial cells, and the regulation of HIV-Tat-induced calcium flux, dendritic spine loss, and neuronal injury exacerbated by morphine exposure. Beclin1 acts at the initiation stage of autophagy as part of a lipid kinase complex that stimulates the formation of autophagosomes. Beclin1 contains a BH3 domain that interacts with anti-apoptotic members of the Bcl-2 family, inhibiting autophagy induction. Here, we sought to examine whether treatment with ABT-737, a BH3 peptidomimetic agent that has previously been shown to induce autophagy by disrupting the Bcl-2-BECN1 binding, can revert the adverse interactive effects of HIV, antiretroviral therapy (ART), and opioid use. Our in vitro studies, using the latent infected monocytic U1 cells exposed to ABT-737, resulted in an increase in autophagy that was Beclin1-dependent, alongside a decrease in ART ± morphine-induced apoptosis. For our in vivo studies, we used an EcoHIV mouse model on ART ± morphine. Interestingly, treatment with ABT-737 led to a significant increase in the expression of synaptic proteins and autophagy. Our studies demonstrate the feasibility of using BH3 mimetics as modulators of autophagy and apoptosis and the expression of proteins associated with synaptic plasticity.

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Keywords
EcoHIV, Morphine, BH3 mimetics, Autophagy, and Synaptic plasticity

B38 - A novel form of spatiotemporal bias – using mathematical models and electrophysiology in C57 mice to compare pre- and post-synaptic MOPrs

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Mu-opioid receptors (MOPrs) are present on neurons both pre- and post-synaptically. Presynaptic MOPrs constantly diffuse along the membrane, in and out of the active zone (Jullié et al., 2020), whereas post-synaptically MOPrs are largely static within the post-synaptic density. We therefore hypothesise that agonists with a slower off-rate at MOPr (i.e. have a longer dwell-time) are more likely to successfully signal at presynaptic MOPrs than shorter dwell-time agonists, and so would be 'biased' towards signalling through presynaptic receptors. Mathematical modelling of agonist-receptor signalling, based on Hoare et al. (2020), enabled determination of factors affecting potency at
mobile and immobile receptors. Whole-cell brain-slice electrophysiological recordings from mouse locus coeruleus neurons were performed to quantify potency of agonists with different dwell times at pre- and post-synaptic receptors.

Computational modelling showed dwell-time to be the most influential factor when comparing relative potencies at pre- and post-synaptic MOPrs, followed by agonist concentration and binding on-rate. Using brain-slice electrophysiology the pre- and post-synaptic potencies of a short (met-enkephalin) and longer (etorphine) dwell-time agonist were determined. Met-Enkephalin logEC50s at pre- and post-synaptic receptors were -6.0 ± 0.3 and -5.7 ± 0.2 respectively. Etorphine log EC50s at pre- and post- synaptic receptors were -10.6 ± 0.4 and -7.4 ± 0.5. Therefore, while etorphine was ~45x more potent than Met-Enkephalin at postsynaptic receptors it was ~36,000x more potent at presynaptic receptors. Deltalog (tau/kA) analysis (van der Westhuizen et al., 2014) showed this to be highly significant ($p<0.001$). Thus etorphine (a longer dwell-time agonist) was relatively more potent at presynaptic MOPrs than met-enkephalin (a shorter dwell-time opioid). We are in the process of testing a longer dwell-time opioid and other short and medium opioids. These findings strongly support our hypothesis that longer dwell-time agonists are biased towards signalling through presynaptic receptors. As there is evidence that pre- and post-synaptic receptors play differential roles in inducing analgesia and respiratory depression this may lead to a new approach for designing safer analgesics.

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B39 - Immunohistochemical and mRNA detection of opioid receptors and their endogenous ligands in human dorsal root ganglion neurons

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Introduction

Peripheral analgesic effects of opioids has been shown for animals and humans. Corresponding opioid receptor mRNA, protein and signalling have been shown in dorsal root ganglia of animals. Here we show for the first time the distinct mRNA and protein expression of all components of the opioid system (receptors and their endogenous ligands) and their respective location within certain subtypes of dorsal root ganglion neurons.

Methods

All human tissue samples were supplied by AnaBios Corporation which received IRB approval of research. Real-Time PCR, western Blot, and immunohistochemistry were performed according to previously published protocols.

Results

Mu-, delta-, and kappa-opioid receptors were abundantly expressed (mRNA and protein) in lumbar human dorsal root ganglion neurons. Expression level for mu-opioid receptors were highest compared to delta-, and kappa-opioid receptors. In addition, the opioid precursor peptides proopiomelanocortin, proenkephalin, and prodynorphin were all expressed in lumbar human dorsal root ganglion neurons with proenkephalin revealing the highest level of expression compared to proopiomelanocortin and prodynorphin. Opioid receptors were mainly located in nerve growth factor-dependent subpopulations of lumbar human dorsal root ganglion neurons co-localizing with well-known pain signalling molecules.

Conclusion

Peripheral opioid receptors and their endogenous ligands are shown to be expressed in human dorsal root ganglion neurons. The relative expression levels might give some indication for the clinical use of locally applied opiods.

B40 - Role of central amygdala protein kinase C-δ, corticotropin-releasing factor, and somatostatin neurons in opioid-related mice behavior

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Opioid use disorder (OUD) is a chronic psychiatric condition with high morbidity and mortality. We used a mouse model of OUD to investigate the role of protein kinase C-δ (PKC-δ), corticotropin-releasing factor (CRF), and somatostatin (SST) neurons in the central nucleus of the amygdala (CeA) in opioid self -administration and withdrawal-related behavior. We hypothesize that these distinct CeA neuronal populations influence different behavioral aspects of opioid withdrawal. Using fentanyl vapor self-administration with short- access (ShA; 1 h/day) versus long-access (LgA; 6 h/day), we modeled nondependent and opioid-dependent states. In fentanyl-dependent mice, μ-opioid receptor mRNA (oprm1) levels were reduced (multiple comparisons, $p < 0.01$), whereas the expression of PKC-δ (prkcd), CRF (crh) and SST (sst) mRNA levels remained unchanged (multiple comparisons, $p = 0.87$, $p = 0.97$ and 0.54). In dependent mice, chemogenetic inhibition of $CeA^{PKC\text{-}\delta}$ alleviated opioid withdrawal-induced hyperalgesia ($F_{(1,12)} = 7.13$, $p = 0.02$) and reduced fentanyl intake ($F_{(1,12)} = 5.79$, $p = 0.03$), inhibition of CeA^{CRF} reduced irritability ($F_{(1,12)} = 12.8$, $p = 0.003$) and somatic withdrawal signs ($F_{(1,8)} = 7.38$, $p = 0.02$) and activation of CeA^{SST} reduced somatic withdrawal signs ($F_{(1,11)} = 7.01$, $p = 0.02$). We did not find sex differences in these effects. Altogether, these findings suggest that distinct CeA neuronal subpopulations uniquely regulate different aspects of opioid dependence and withdrawal.

B41 - Dynamic Resilience: Stress Phenotypes and Their Impact on Opioid-Taking Behavior and Brain Activity

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Stress is a universal experience, but while some individuals develop maladaptive behaviors and stress-related disorders others return to normal functioning and are considered resilient. Resilience is defined as the ability to adapt positively to significant stressors, involving a dynamic process of returning to baseline functioning over time. Thus, assessing stress-related behaviors at a single time point may overlook key aspects of resilience. We used chronic social defeat stress (CSDS) to examine phenotypic stability and found mice distributed into the expected susceptible/resilient ratio at both 24 hours and 3 weeks, with most maintaining their phenotype over the 3-week period. After three weeks, resilient mice show a broad distribution of social interaction (SI) ratios, while the SI values in susceptible mice are tightly clustered and shifted lower. While stress is known to impact substance use disorders (SUDs), few studies have examined the relationship between stress resilience and opioid use. To address this gap, we examined intravenous self-administration (IVSA) of remifentanil at 1 or 3 weeks following CSDS. Regardless of phenotype, remifentanil fixed ratio 1 (FR1) intake was increased one week after CSDS compared to unstressed controls (susceptible $p=0.0189$, resilient $p=0.0093$). However, after 3 weeks, resilient mice showed FR1 intake comparable to unstressed controls, whereas susceptible mice showed continued elevated intake (compared to resilient $p=0.0245$ and control $p=0.0007$). To explore neural correlates of these behavioral shifts, we quantified cFOS levels in the basolateral amygdala (BLA) and nucleus accumbens (NAc) as representative stress- and reward-related brain regions. cFOS was increased compared to controls in the BLA in susceptible mice at one ($p=0.0095$) and three weeks ($p=0.0375$) consistent with the sustained stress phenotypes. In the NAc, both susceptible and resilient mice showed increased activity compared to controls 1-week post-CSDS ($p=0.0004$ and $p=0.0009$, respectively). At 3 weeks, cFOS normalized in resilient mice but remained elevated in susceptible mice ($p=0.001$), paralleling their persistent increased opioid intake. Our findings highlight resilience as a dynamic process, with behavioral and neural responses evolving over time. By assessing multiple time points, we provide novel insights into how stress phenotypes shape opioid use trajectories and identify brain regions involved in these differences.

B42 - Characterization of SNC80 Behaviors in a DORfl/fl Parvalbumin Cre Mouse Line

Marie Walicki

The delta opioid receptor (DOR) represents a target for the analgesic, anxiolytic and antidepressant-like behaviors with a reduction in addiction liability. However, the synapse, circuit and behavioral-level aspects that drive DOR-mediated behaviors remain unknown. To address which cell-types and circuits mediate the behavioral aspects of DOR agonists we used behavioral pharmacology, behavior, optogenetics and whole-cell electrophysiology to uncover which circuits mediate the behavioral effects of DOR agonists. Using a floxDOR PV-Cre mouse line we assessed how a conditional knockdown of DOR signaling on PV interneurons alters synaptic transmission in thalamo-cortical circuits. Additionally, we probed how a loss of DOR signaling on PV interneurons alters SNC80-mediated behaviors. We found a reduction in the convulsant, and anxiolytic effects of the DOR agonist SNC80. Whereas the pro-locomotor, analgesic and pro-respiratory breathing effects of DOR agonist in these floxDOR PV-Cre animals. We are currently using a conditional knock-in DORKi mouse model to selectively re-express DOR in selective regions like the anterior cingulate cortex(ACC), thalamus(Thal) and striatum(Str),. Using this approach we assessed how DOR signaling on PV cells within distinct circuits are involved in SNC80-mediated behaviors. We hypothesized that a rescue of DOR function on PV interneurons in the ACC may be responsible for the pro-anxiolytic effects of SNC80 while the proconvulsant effects of SNC80 may be driven through the thalamo-cortical circuits. Overall, this work highlights how DOR signaling in cortical circuits alters synaptic transmission and DOR-mediated behaviors.

B43 - Neurological deficits induced by brain injury: bipartite ipsilateral opioid signaling via humoral pathway

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Contralateral effects of brain injury are set through crossed descending neural tracts. A novel topographic neuroendocrine system (T-NES) that operates via a humoral pathway can also mediate the left-right side-specific effects of unilateral brain lesions. In rats with completely transected thoracic spinal cords, unilateral injury to the sensorimotor cortex produced contralateral hindlimb flexion, a proxy for neurological deficit. Here, we investigated in acute experiments whether T-NES consists of left and right counterparts and whether they differ in neural and molecular mechanisms. We demonstrated that left- and right-sided hormonal signaling is differentially blocked by the δ-, κ- and μ-opioid antagonists. Left and right neurohormonal signaling differed in targeting the afferent spinal mechanisms. Bilateral deafferentation of the lumbar spinal cord abolished the hormone- mediated effects of the left-brain injury but not the right-sided lesion. The sympathetic nervous system was ruled out as a brain-to-spinal cord-signaling pathway since hindlimb responses were induced in rats with cervical spinal cord transections that were rostral to the preganglionic sympathetic neurons. Analysis of gene-gene co-expression patterns identified the left- and right- side-specific gene co-expression networks that were coordinated via the humoral pathway across the hypothalamus and lumbar spinal cord. The coordination was ipsilateral and disrupted by brain injury. These findings suggest that T-NES is bipartite and that its left and right counterparts contribute to contralateral neurological deficits through distinct neural mechanisms, and may enable ipsilateral regulation of molecular and neural processes across distant neural areas along the neuraxis.

B44 - Critical Windows of Endogenous Opioid Influence on Breathing Development

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After nine months in the aqueous uterine environment, a newborn's first breath of air is a major transition for the respiratory system. Unfortunately, newborn breathing problems are common and apneas in premature infants can be life-threatening. Multiple factors contribute to slow breathing at birth, including developing neuromodulatory systems. Administration of the non-selective opioid receptor antagonist naloxone increases breathing rate in neonatal mammals including human infants. Therefore, I hypothesized that endogenous opioid peptides suppress breathing during early life and shape the development of control of breathing networks. To test this hypothesis, I measured breathing in mice lacking mu-opioid receptors (MOR-KO) and wild type littermate control mice at multiple developmental time points. Breathing in postnatal day 0 (P0) to P14 mice was measured using a small piezoelectric sensor placed beneath the chest. For P21 mice, whole-body plethysmography was used to measure breathing. Both MOR-KO and wild type mice followed similar developmental trajectories; breathing faster and more regularly as they aged. At P0 and P21, MOR-KO mice breathed faster than wild type littermates. However, breathing rate was not different between P4-P14. These results suggest that P0 and P21 are critical windows for endogenous opioid regulation of breathing in mice. Next, I wanted to determine how the loss of mu opioid receptors influenced respiratory-controlling neurons in the brainstem respiratory network. Kölliker-Fuse neurons undergo postnatal maturation from P0- P21, therefore I hypothesized that endogenous opioids influence KF neuron development. To test this, I performed brain slice electrophysiology experiments and recorded action potential firing in KF neurons from adult mice. Preliminary data show that KF neurons from MOR-KO mice spontaneously fire action potentials whereas KF neurons from wild type mice rarely fire action potentials at rest. These exciting data suggest that the lack of mu opioid receptors has long-term consequences for KF neuron excitability. Future work will examine breathing development and respiratory neuron function in mice with conditional deletion of proenkephalin from neurons that express mu opioid receptors. Understanding the fundamental role of the endogenous opioid system in respiratory development will help inform new strategies aimed at improving breathing rate in premature infants and in other hypoventilation conditions.

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B45 - Isolating the role of endogenous μ-opioid activity in the Ventral Tegmental Area during natural reward

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Opioid use disorder and opioid overdose death rates in the United States reached unprecedented levels during the COVID-19 pandemic. Understanding the critical role of endogenous opioid activity in natural reward behaviors is essential for understanding opioid use disorder, yet the fundamental mechanisms by which opioids affect these behaviors in the brain remain elusive. Here, we used a combination of genetic and molecular tools to isolate the role of μ-opioid receptor (MOR) activity in the ventral tegmental area (VTA) and identified the Lateral Hypothalamus (LH) as a source of endogenous opioid release onto these receptors. To investigate the role of endogenous opioid activity on VTA^{GABA} neurons, we first used ex vivo 2-photon slice imaging. Preliminary results reveal that endogenous opioid peptides exert heterogenous effects on VTA^{GABA} neurons which can be blocked using the MOR antagonist, CTAP. Then, using fiber photometry and $Oprm1^{fl/fl}$ mice, we found that VTA MOR knockout decreased dopamine release to the cue but increased dopamine release to reward consumption in the nucleus accumbens (NAc) during Pavlovian conditioning. Next, using in situ hybridization and viral tracing methods, we identified enkephalin-containing neurons in the LH that project to the VTA. Optical stimulation of these projections ex vivo elicited inhibitory post-synaptic currents, indicating that these projections are primarily inhibitory. In vivo optical stimulation of these enkephalinergic projections during real-time place preference and intracranial self-stimulation suggests these projections play a role in reward and reinforcement. These results provide insight into the role of endogenous opioids in the VTA in modulating dopamine activity and motivated behavior. Current work is focused on further elucidating the role of endogenous μ-opioid activity in the VTA on GABA interneurons and determining how enkephalinergic projections from the LH to the VTA modulate natural reward. Ultimately, these studies lay the groundwork for understanding how opioid drugs of abuse elicit maladaptive changes in endogenous opioid systems to drive opioid use disorder.

B46 - Inhibitors of Glycine Transporter 1 Reduce the Development of Morphine Antinociceptive Tolerance in Rats

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Introduction

Opioid analgesic tolerance (OAT) remains an obstacle, as achieving adequate pain relief requires dose escalation, which is limited by central and peripheral side effects. N- methyl D-aspartate receptor (NMDAR) blockers have been reported to delay OAT through mechanisms involving extrasynaptic NMDARs with the GluN2B subunit. The activation of NMDARs requires glutamate alongside the co-agonist glycine. The extrasynaptic levels of glycine are regulated by glycine transporter-1 (GlyT-1), thus GlyT-1 inhibitors impact NMDAR effects.

Methods: Male Wistar rats (180–250 g) treated with subcutaneous (s.c.) morphine HCl (mor) alone or in combination with NFPS, a GlyT-1 inhibitor for 10 days. The test doses (mg/kg) were 10 and 0.6 mg mor and NFPS, respectively. Saline or DMSO served as the vehicle. Mor was administered twice daily, while NFPS was given once daily. Mor antinociceptive tolerance (MAT) was assessed by the rat tail-flick (RTF) assay. Glutamate and glycine levels in the cerebrospinal fluid (CSF) from treated animals were determined by capillary electrophoresis. Motor function was evaluated using the Rotarod test.

Results

The pain threshold in the RTF assay on day 1 for mor and the mor/0.6 mg NFPS combination was 8.0 ± 0.0 and 7.9 ± 0.05, respectively, and after 10 days of treatment, it was 4.56 ± 0.35 and 6.92 ± 0.35, respectively 30 min after sc. injections. These results indicate the development of MAT, which was inhibited by NFPS. No differences were found between the groups treated with vehicles. In the experiment measuring glycine and glutamate levels in CSF, significant increases were observed both in animals treated with morphine/NFPS and morphine alone. Chronic treatment with 0.6 mg/kg NFPS failed to affect rats' motor function 30 min after sc. injections (mor peak effect), whereas mor alone or in combination with NFPS induced significant motor dysfunction.

Conclusion

GlyT-1 inhibitors likely delay MAT through mechanisms involving NMDAR functioning and support our prior findings that increased glycine and glutamate levels reduce NMDA receptor responsiveness. To the best of our knowledge, we are demonstrating the impact of GlyT-1 inhibitors on MAT for the first time. Nevertheless, future studies are required to elucidate the exact mechanisms.

Funding

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B47 - The Role of AT1 Receptor and PPARγ in Opioid Antinociceptive Tolerance

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Introduction
Opioid analgesics are the mainstay in the management of moderate to severe pain. Their effectiveness is hampered by side effects and analgesic tolerance. Studies indicated that AT1 receptor blockers (ARBs), notably telmisartan, have shown antinociception in chronic pain and delayed morphine tolerance (MT), yet activates peroxisome proliferator-activated receptor gamma (PPARγ).

Aim
Exploring whether morphine analgesic tolerance is mediated by AT1 or PPARγ, telmisartan (TEL) and losartan (LOS) were assessed using in vivo and in vitro assays.

Methods
The rat tail-flick assay was used to test the antinociceptive effects of sc. morphine (31.08µmol/kg), po. TEL (20µmol/kg), or LOS (50µmol/kg), either individually or in combination with morphine 60, 90, 150 and 210 min after treatment on day 1, 4 and 10 in the presence or absence of PPARγ antagonist GW9662. At the end of the 10th day, the animals' spinal cords were removed for histological analysis. In colocalization studies, naïve rats were used. In the in vitro tolerance assay, mouse vas deferens (MVD) was used. MVD was treated three times with vehicle, 1µM morphine alone or in combination with LOS, and then the effect of 0.5µM morphine was tested.

Results
In vivo by day 10 morphine lost its antinociceptive effect compared to day 1 (4.2s vs. 8s). The cotreatment with TEL or LOS attenuated the development of MT (TEL: 6.2s, LOS: 6.6s vs. 4.2s). The effect of ARBs was partially blocked by GW9662. ARBs alone failed to show any antinociceptive effect. Chronic morphine treatment increased spinal IBA1 expression (897 vs. 402 cells+/mm2), which was reduced by TEL and LOS (450 and 576 cell+/mm2 respectively). That was reversed by GW9662. Colocalization of AT1, MOR and CGRP on small to medium-sized primary afferent neurons was observed. Compared to vehicle-treated MVD, the effect of 0.5µM morphine significantly decreased in tissues exposed to repeated 1µM morphine treatment (60.9% vs. 27.2%), indicating the development of MT, LOS restored the effect of 0.5µM morphine (52.9% vs. 60.9%).

Conclusion
Simultaneous activation of MOR and inhibition of AT1 delayed morphine tolerance in vivo and in vitro assays. The involvement of PPARγ in this scenario was proved.

B48 - Inhibition of histone demethylase LSD1 alleviates opioid-induced tolerance and hyperalgesia

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As an essential chromatin modifier, lysine-specific demethylase 1 (LSD1) functions as a transcriptional coregulator by modulating histone methylation. Its participation in opioid use disorder remains unexplored. This study aimed to investigate whether LSD1 mediates epigenetic maladaptation induced by chronic opioid. Mice were treated with morphine by intracerebroventricular (i.c.v.) injections. We found repeated brain administrations of sub- analgesic dose of morphine (2 nmol/day, i.c.v., 5 days) elicited opioid tolerance in mice as determined by the tail-flick assay. Meanwhile, we observed the presence of evoked hypersensitivity to mechanical and thermal stimuli in chronic morphine treated mice by the von Frey filaments assessment and Hargreaves radiation heat test, respectively. Therefore, antinociceptive tolerance and opioid-induced hyperalgesia (OIH) co-occurred in mice after chronic central exposure of morphine. In this mouse model of opioid tolerance and hyperalgesia, treatment with RN-1 (3 nmol/day, i.c.v., 3 days), a selective irreversible inhibitor of LSD1, significantly attenuated opioid antinociceptive tolerance. In addition, RN-1 treatment effectively reduced mechanical allodynia and heat hyperalgesia, indicating substantial alleviation of OIH. To complement pharmacological inhibition, we further targeted LSD1 by siRNA knockdown. LSD1 siRNA (3 nmol/day, i.c.v., 3 days) dramatically reduced both tolerance and hyperalgesia induced by chronic morphine. These results demonstrated for the first time that LSD1 serves as a key epigenetic regulator of opioid-induced tolerance and hyperalgesia. Our findings reveal the functional participation of LSD1 in the development of both analgesic tolerance and OIH, which highlighted the possibility of a new intervention target to treat these adverse conditions associated with chronic opioid use.

B49 - Presynaptic modulation of transmitter release in striatopallidal afferents by the μ Opioid receptor

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Although opioid ligands are potent and valuable analgesics for acute pain management, chronic use results in decreased analgesic response (tolerance) which necessitates dose escalation. This repeated and escalating use increases the risk of abuse and dependence. The μ opioid receptor (MOR) is the primary receptor responsible for the analgesic and rewarding effects of opioids, implicating it in the development of tolerance as well as drug dependence. Upon ligand binding, MOR agonists activate second-messenger signaling cascades and regulatory mechanisms that lead to desensitization and internalization of the receptor.

Postsynaptic desensitization and tolerance following application of supersaturating concentrations of agonists have been well characterized. Recent work has revealed tolerance at presynaptic terminals following chronic agonist application, though desensitization is yet to be observed. While both pre- and postsynaptic MORs undergo phosphorylation-dependent endocytosis, a prevailing theory is that a pool of laterally diffusible receptors replaces internalized MORs at presynaptic terminals, preventing measures of acute desensitization. The purpose of this project is to understand the regulatory mechanisms that shape MOR activity at presynaptic sites following acute and chronic agonist application. To do this, a Cre-dependent excitatory opsin was virally expressed in the dorsal striatum (DS) of Oprm1-Cre rats, and GABA release from DS terminals was optically evoked and measured as $GABA_A$ mediated inhibitory post-synaptic currents (IPSCs) recorded using whole-cell voltage-clamp in the globus pallidus externus (GPe). Application of the endogenous MOR agonist, [Met]^{5}enkephalin decreased DS to GP IPSC amplitudes in a dose dependent manner with an ec_{50} of 134 nM (N=12, 4-6 cells/concentration). Ongoing experiments are examining the presynaptic adaptations of MOR- mediated inhibition of the DS-GPe pathway using slices from rats chronically treated with morphine for 6-7 days (60 mg/kg). This highlights the value of the novel Oprm1-Cre rat to selectively probe the activity, regulation, and adaptations following chronic activation of MORs at the presynapse. The long-term goal of this work is to identify the mechanisms of presynaptic tolerance by targeting G-protein coupled receptor regulatory proteins using pharmacological and Cre-dependent viral tools.

B50 - Investigation of Pregabalin, Tolperisone, and Naloxone in the Context of Morphine-Induced Tolerance and Constipation in Rats

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Introduction
Morphine (Morph) is a mainstay in the management of mild to severe pain. However, long-term use causes morphine antinociceptive tolerance (MAT) and constipation, potentially impeding treatment. These effects stem from various mechanisms associated with both opioid and non-opioid systems, including glutamate. N-methyl-D-aspartate receptor (NMDAR) antagonists have been approved to delay the development of MAT. NMDAR activation requires binding of glutamate and co-agonists such as glycine or D-serine.

Aims and methods
This study investigates the effects of oral pregabalin (PGB) and tolperisone (TOLP) at a dose of 100 mg/kg on MAT (10 mg/kg, sc., BID, 10 days) and their mechanisms in male Wistar rats, using the rat tail-flick test to assess MAT. Cerebrospinal fluid (CSF) D-serine and glycine levels were assessed by capillary electrophoresis. For Morph-induced constipation (MIC), different doses of oral naloxone (NX) were combined with Morph to investigate the role of NX in reversing MIC, which was assessed by the charcoal meal test.

Results
BID Morph and tested compounds (TOLP, TOLP/Morph, PGB/Morph, vehicle) chronic administration induced Maximum possible effect (MPE)(%) of 22.4, 10.6, 13.3, 56.7, and -1.8, respectively, at 60min, which indicates that only PGB, when combined with Morph, delayed MAT. This was associated with a decrease in the CSF D-serine but not in glycine levels (1.0 vs vehicle 1.8). In the charcoal meal test, charcoal travel distance (%) of vehicle, Morph, and Morph/NX 0.3, 1, 5, or 10 mg/kg was 80.1, 31.5, 20.7, 24.9, 44.7, and 70.4, respectively which indicate that only 10 mg/kg NX when combined with Morph acutely abolished MIC. In addition, 50 mg/kg PGB acutely evoked constipation 68.9 vs vehicle 83.9%.

Conclusion
Combining PGB with Morph delayed the development of MAT by a mechanism involving D-serine, which acts as a co-agonist on the NMDAR, thus decreasing the receptor activity. In accordance with the literature, NX can alleviate MIC. It remains to be investigated whether oral NX, either alone or with PGB, can delay MAT development when administered at constipation-relieving doses. This may shed light on the role of OIC in MAT development.

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B51 - Morphine-induced mechanical hypersensitivity in mice requires prokineticin receptors

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Morphine is used clinically for the treatment of chronic pain, but its long-term effect is compromised by tolerance and hypersensitivity, with chemokines playing a crucial role.
The chemokine Prokineticin 2 (PK2), which acts at two receptors prokineticin receptor 1 (PKR1) and 2 (PKR2), triggers pain in inflammatory conditions that are directly associated with an increase in the PK system in both the central and peripheral nervous system.
We investigated the contribution of PK2/PKRs to the development of opioid tolerance and morphine- induced hypersensitivity (MIH) in inflamed mice.
Inflammation was induced in wild-type (WT) and PKR1-/- mice by intraplanar administration of Complete Freund's Adjuvant (CFA). Mice were injected daily with morphine 10 mg/kg s.c. (or saline s.c.) for 15 days after CFA and mechanical sensitivity was assessed using a dynamic aesthesiometer. Results showed that CFA-induced hypersensitivity in both WT and PKR1−/− mice occurred on day 1 (4.1±0.54 and 3.74±0.23, respectively) and ceased on day 9 (5.7±0.24 and 5.5±0.64, respectively). Daily administration of morphine reduced hypersensitivity in WT on day 1 compared to controls (7.01±0.35 and 4.66±0.81, $p<0.001$), which returned on day 9 (5.69±0.46). In contrast, PKR1-/- mice injected with morphine showed no recurrence of hypersensitivity reactions (6.8±0.39). The same results were obtained in WT mice injected with both morphine and PC1, the PKRs antagonist, 150 μg/kg s.c. twice/day for 15 days; there was no MIH in these mice either (8.56±0.70, $p<0.001$).We analysed the mRNA expression of PK2 in the L4-L5 dorsal root ganglia (DRG) and in the spinal cord by RT-PCR. The results showed that PK2 expression increased in WT mice treated daily with morphine on day 9 after CFA (DRG: 3.79±0.39; spinal cord: 3.64±1.3-fold increase), suggesting that restoration of basal sensitivity in WT was accompanied by increased PK2 expression. Conversely, PK2 expression was low in PKR1-/- mice treated daily with morphine (DRG: 1.06±0.95; spinal cord: 0.96 ±0.73-fold increase), consistent with the absence of MIH. Taken together, these data indicate that the PK system is involved in the development of opioid tolerance and hyperalgesia in inflamed mice and that blocking PKR1 may represent a strategy to counteract MIH in inflammatory pain conditions.

B52 - Sex-specific molecular alterations in mesolimbic brain region following methadone administration in morphine tolerant rats

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Morphine is one of the most effective analgesic drugs for managing various types of pain. However, its prolonged use leads to the development of analgesic tolerance, limiting its clinical usefulness. Opioid rotation (e.g. switching morphine/methadone) is often adopted in clinical settings to achieve analgesia. The development of morphine tolerance involves complex mechanisms across multiple brain regions. Emerging evidence suggests that chronic morphine treatment affects inflammatory processes and molecular pathways in specific brain areas, including the ventral tegmental area (VTA), a central hub for opioid reward and addiction. However, the role of VTA in the molecular adaptations underlying morphine tolerance remain poorly understood. Moreover, cellular alterations occurring with opioid rotation are still unclear and little is known about sex-related differences in this respect.

To elucidate these aspects, male and female adult rats were administered with morphine (10 mg/kg i.p., twice daily) for 7 days. Analgesic tolerance was assessed using the tail-flick test, and at the end of treatment, the expression of inflammation-related genes (TLR4, PPARγ) and of pDYN/KOR mRNA levels were measured in the VTA. The same parameters were also evaluated at day 10 in a separate group of 7 days morphine-treated animals receiving methadone (5 mg/kg i.p, twice daily) from day 8 to 10.

Results showed a progressive reduction in the analgesic effect of morphine in both male and female treated rats, with tail-flick latency values comparable to the vehicle group at day 7 ($p > 0.05$ vs. vehicle). On day 8, methadone administration induced analgesia in both male ($p < 0.05$) and female ($p < 0.001$) morphine-tolerant rats. However, on day 10, methadone analgesic effect was present in males only ($p < 0.01$). At molecular levels, a significant decrease of anti-inflammatory nuclear PPARγ gene expression ($p< 0.05$) was detected in male morphine tolerant rats receiving methadone but not in females. Methadone administration did not affect the down-regulation of TLR4 ($p < 0.01$) and pDYN ($p < 0.01$) gene expression induce by chronic morphine treatment in females.

Overall, these findings show that methadone influences morphine-induced behavioral and molecular changes in a sex-specific manner. These data underline the importance of considering sex as a biological variable to develop more effective and personalized strategies for managing opioid tolerance.

B53 - CaMKIIβ in opioid tolerance, dependence, opioid-induced hyperalgesia, and neuropathic pain

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CaMKII are multifunctional Ca^{2+}/calumodulin activated serine/threonine protein kinases. Similar to CaMKIIα, CaMKIIβ is largely restricted to neurons; however, less is known about CaMKIIβ. We investigated the role of CaMKIIβ in driving opioid tolerance, dependence, opioid-induced hyperalgesia (OIH), and neuropathic pain. CaMKIIβ immunoreactivity is mostly found in the superficial laminae I-III in the spinal cord. Unlike wildtype mice, morphine did not induced OIH in CaMKIIβ null mice (1.52±0.28 vs 0.05±0.00, $p<0.001$). Opioid physical dependence was absence in CaMKIIβ null mice (66.3±14.5 vs 18.2±2.6, $p<0.001$), although opioid antinociceptive tolerance was only partially reduced ($p<0.05$). CaMKIIβ was also activated in the spinal cord and dorsal root ganglion in neuropathic pain states (e.g., sickle cell pain, chemotherapy-induced peripheral neuropathy). CaMKIIβ siRNA (i.t.) or gene deletion also diminished signs of neuropathic pain. Taken together, these data implicate a critical role of CaMKIIβ as a cellular mechanism for opioid dependence, OIH, and neuropathic pain.

B54 - Pharmacological characterization and evaluation of health risks of bovine BCM-7

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Consumption of milk and dairy products has been proposed as potential health risk, including gastrointestinal disturbances, diabetes, autism and cancer. These effects have been attributed to β-Casomorphin-7 (BCM-7), released during digestion of β-casein. Although BCM-7 has been identified and characterized as an exorphine over 50 years ago by in-vitro assays, it has not been proven yet, whether it could be responsible for the detrimental effects of milk consumption currently postulated. Thus, the aim of the present study was to provide basic pharmacological data for BCM-7 using the cloned human μ-opioid receptor (hMOR) and a porcine intestinal preparation as a prerequisite for risk assessment.

A triple HA-tagged version of the hMOR was stably expressed in HEK 293 cells (HEK-hMOR-3HA). Ligand affinities were determined by radioligand binding studies using [^{3}H]-Naloxone as the radioligand. Intrinsic activities were determined by agonist stimulated regulation of ERK 1/2 and adenylyl cyclase. The ligand profile of bovine BCM-7 was compared to human BCM-7, which differs in two amino acids (bBCM-7: YPFPGPI; hBCM-7: YPFVEPI). Met-enkephalin served as a control for endogenous opioid peptides.

Radioligand binding studies revealed that BCM-7 indeed represents an opioid ligand. However, comparted to Met-enkephalin (Ki = 29 nM), the affinities of bovine and human BCM-7 at least 1,000fold lower (bBCM-7: Ki = 39 μM; hBCM-7: Ki = 290 μM). BCM-7 represents only a partial opioid agonist, because even highest measurable concentrations (100 μM) are not able to induce full agonist activity in ERK1/2 phosphorylation and adenylyl cyclase inhibition comparted to Met-enkephalin. Further studies on isolated porcine jejunal preparations showed, that compared to morphine, fentanyl and Met-enkephalin, BCM-7 fails to elicit opioid effects on electrically stimulated muscle contractions.

Further docking studies using the AlphaFold structure of hMOR revealed that BCM-7 binds in a similar conformation to the binding pocket, but with subtle differences compared to Met- Enkephalin.

These data demonstrate that BCM-7 represents an exorphin with low binding affinity and only partial activity at the hMOR. Regardless of its questionable bioavailability, these data argue against the involvement of BCM-7 in the development of potential health risks after milk consumption mediated through opioid receptors.

B55 - Selective inhibition of hemokinin-1-induced pruritic behavior by non-opioid sendide derivatives

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Hemokinin-1 is a neuropeptide that, similar to substance P, effects neurokinin-1 receptors. When hemokinin-1 and substance P are administered intradermally to mice, pruritic behavior such as scratching the site of administration with hind limbs occurs. In addition to CP99994, neurokinin-1 receptor antagonists include sendide, a substance P (6-11) derivative peptide. We have synthesized derivatives based on the structure of sendide and examined their effects on hemokinin-1 and substance P-induced pruritic behavior.

Mice were male ICR strain, 22-26 g. After intradermal administration of hemokinin-1 and substance P, mice were promptly placed in an observation cage and the cumulative number of seconds of pruritic behavior for 30 minutes was recorded. In addition to our synthesized sendide derivatives, CP99994 and sendide were mixed with hemokinin-1 and substance P, respectively.

The sendide derivative peptides R3-1 and R3-3 attenuated the pruritic behavior induced by intradermal administration of hemokinin-1. The attenuation of hemokinin-1 pruritic behavior by R3-1 and R3-3 was not affected by naloxone. R3-1 and R3-3 had no effect on substance P-induced pruritic behavior. CP99994 and Sendide had no effect on the pruritic behavior of either hemokinin-1 or substance P.

We have identified a sendide derivative that selectively attenuates the pruritic behavior that occurs when hemokinin-1 is administered intradermally. Both hemokinin-1 and substance P affect the neurokinin-1 receptor, but it was suggested that hemokinin-1 and substance P may differ in their binding sites at the neurokinin-1 receptor.

B56 - Aticaprant to the rescue: Targeting the κ-opioid receptor system to shield mood and memory from stress impairments

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Major depressive disorder (MDD) is a leading cause of disability and is often linked to chronic stress. Existing treatments require long-term use and are ineffective for some patients, highlighting the need for new therapies. Dysregulation of the dynorphin/kappa opioid receptor (κ-OR) system has been implicated in MDD pathophysiology, as sustained activation of κ-OR mediates depressive symptoms while κ-OR blockade impedes the effects of stress in animal studies. Aticaprant, a selective κ-OR antagonist in phase III trials, demonstrates antidepressant potential with minimal side effects and low abuse liability.

Our objective is to address the efficacy of chronic aticaprant treatment in reversing behaviors and synaptic structural changes in male mice after exposure to chronic unpredictable stress (CUS) and elucidate the molecular mechanism of its action. Thus, adult male C57BL/6J mice were exposed to six weeks of CUS, consisting of overcrowding, vibrating platform, restraining and hairdryer. After three weeks of stress, aticaprant (10 mg/kg) was administered daily for 21 treatments. Behavioral assessments included the elevated plus maze, open field, forced swim test, novel object recognition and Y-maze.

Our results demonstrate that aticaprant produced an anxiolytic and antidepressant effect and reversed stress-induced impairments in recognition memory. By performing neuronal reconstruction analysis in Golgi-cox-stained neurons we show that aticaprant alleviated stress-induced neuronal and spine loss in stressed animals, supporting its protective role in synaptic plasticity. We also demonstrate that the reduced levels of the hippocampal synaptic proteins spinophilin ($F=2.21$, $p<0.01$) and SNAP25 ($F=9.434$, $p<0.01$) in stressed animals were restored in aticaprant-treated animals. Moreover, aticaprant altered the levels of the autophagic markers in chronic stressed animals compared to naïve ones, with a concomitant alteration of the MAPK and Akt/mTOR signaling pathways. Our findings are further supported by proteomic analysis of hippocampal synaptosomes showing that aticaprant reverses stress-induced changes in cytoskeletal organization and synaptic transmission . These data provides evidence for the mechanism via which aticaprant exerts its therapeutic effects as a putative novel drug to alleviate stress-related symptoms.

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B57 - A cellulose-rich diet disrupts gut homeostasis and leads to anxiety through opioid-mediated gut-brain axis in mice

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It is widely said that a healthy intestinal environment plays an essential role in better mental condition. One known dietary nutrient that maintains the intestinal environment is dietary fiber. A recent study showed that maintaining the intestinal environment with dietary fiber alleviated symptoms of psychiatric disorders in animals. However, such effects have only been reported with soluble fiber, which is highly fermentable and promotes short-chain fatty acid (SCFA) production, and not with insoluble fiber. Therefore, we aimed to verify whether insoluble fiber, such as cellulose, can alter emotion via changes in the gut.

We divided mice into two groups and fed either a standard diet (SD, which contains both insoluble and soluble dietary fibers) or a cellulose-rich diet (CRD, which contains cellulose alone as the dietary fibers). We found that CRD-fed mice display increased anxiety-like behavior in marble burying test. CRD-fed animals also showed decreased intestinal SCFA levels, intestinal permeability, dysmotility, and hypersensitivity. This behavioral and physiological effect of CRD has been completely abolished in vasectomized mice, indicating the direct link between intestinal environment exacerbation to the emotion through the gut-brain axis. Additionally, we found that amygdalar dopamine signaling has been modified in CRD-fed animals, and the opioid antagonist naloxone as well as delta-opioid receptor antagonist naltrindole abolished this dopaminergic modification along with CRD-induced anxiety. Altogether, our findings indicate that consumption of cellulose alone as the dietary fiber may evoke intestinal abnormalities, which fire the vagus nerve, then the delta opioid receptor-mediated opioidergic system, and amygdalar dopamine upregulation, resulting in the enhancement of anxiety.

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