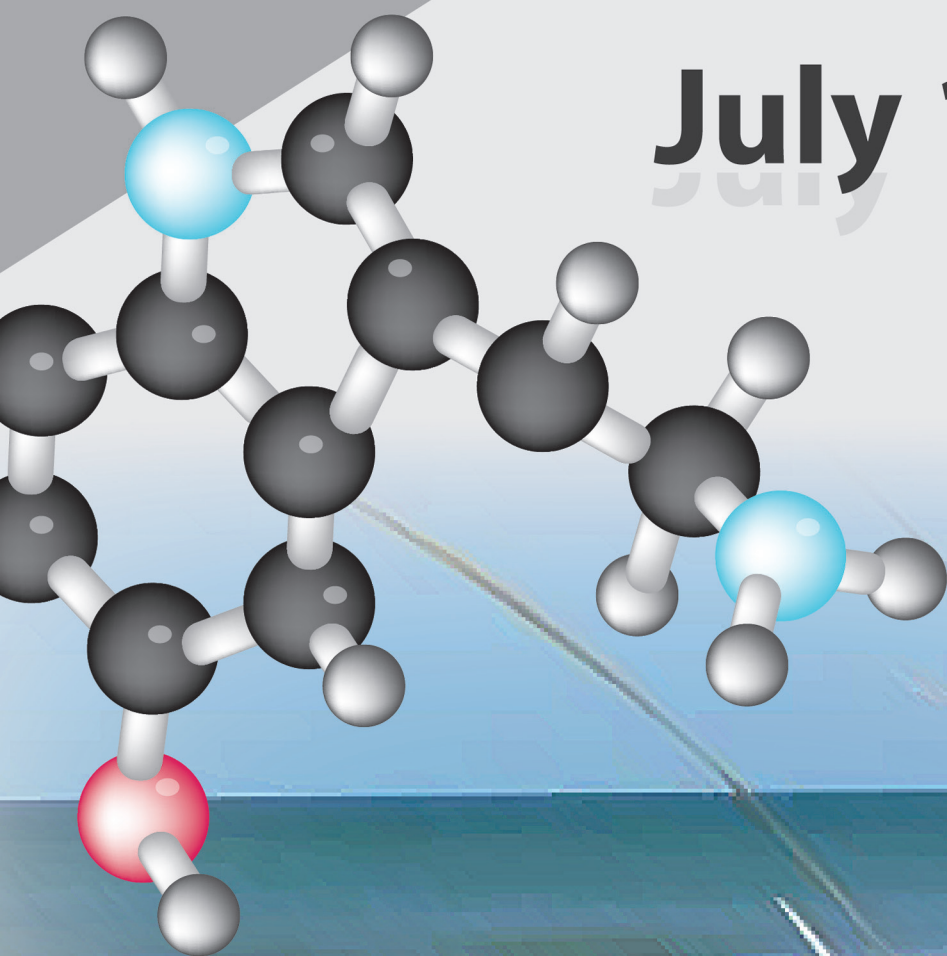


SEROTONIN CLUB MEETING

Faculty of Medicine
Montpellier, France

July 10 - 12



SEROTONIN
A place in the sun

2012

ANNIVERSARY

1987



25th

2012





SEROTONIN CLUB MEETING

2012

July 10 - 12
Faculty of Medicine
Montpellier, France

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WELCOME

Dear registrants,

we wish you a very warm welcome to the 2012 Serotonin Club Meeting in beautiful Montpellier, France. We are also celebrating the 25th anniversary of the Serotonin Club, which was brought into being by a small but determined group of serotonin researchers in Sydney, Australia in 1987. Since then, the Serotonin Club has grown into a dynamic research society with over 450 members from around the world.

This is the 3rd EPHAR Serotonin Satellite Meeting, and we are delighted to offer such an outstanding program. This year's theme is «Serotonin: a place in the sun». Serotonin continues to be the focus for research important to substance abuse, pain, cardiovascular function, digestive processes, and mental health. The program includes leading researchers in the field of serotonin, and highlights the most recent advances in multiple aspects of serotonin research. This year's meeting is different in that the majority of the symposia will be presented separately except for the last day, in which the sessions will run concurrently. We hope you will take full advantage of this unparalleled opportunity to hear from established and junior investigators alike.

We hope that you have a wonderful meeting and enjoy the beauty of Montpellier as well. Montpellier is vibrant, elegant and artistic, a lively and surprising city in the heart of the Languedoc-Roussillon region. We are delighted to have you visit this beautiful area of France, along the shores of the Mediterranean Sea in southern France.

We wish you safe travels and look forward to greeting you.

Sincerely,

Local Organizers,

Joël Bockaert
Philippe Marin
Michel Hamon

President,

Julie Hensler



SEROTONIN CLUB MEETING

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Montpellier, France

The Serotonin Club's Silver Anniversary

The Serotonin Club (www.serotoninclub.org) is an international association for biomedical scientists who are interested in research on any aspect of the neurotransmitter serotonin. Serotonin remains a key neurotransmitter that continues to be the focus for research important to substance abuse, pain, cardiovascular function, digestive processes, and mental health.

The Serotonin Club was brought into being over dinner by a small group of serotonin researchers in Sydney, Australia in 1987. This group gathered after a symposium on serotonin during the 10th International Union of Pharmacology Congress. Their objective was to form a professional society to promote scientific communication among serotonin researchers. The rapidly increasing interest in serotonin had resulted in much closer contacts between serotonin pharmacologists worldwide, and led Paul Vanhoutte in July 1985 to propose the formation of a serotonin club to a group of colleagues [1]. The officers of this new Serotonin Club were Paul Vanhoutte, president, Ray Fuller and Pramod Saxena, vice presidents, and Ewan Mylecharane secretary/treasurer. Councillors were Efrain Azmitia, Marlene Cohen, I.S. de la Lande, John Fozard, Richard Green, Patrick Humphrey, David Nelson, Elaine Sanders-Bush, N. Toda and JM Van Nueten.

In the early 1980s there was accumulating evidence for several subtypes of receptor for serotonin. However, there was little agreement on nomenclature and classification of these receptors. In 1984, a working group was formed to address the growing controversy. In 1986, PB Bradley and colleagues proposed a classification for serotonin receptors into three main groups, ie. 5-HT₁, 5-HT₂ and 5-HT₃ receptors [2]. This initial framework for classification served as a template that was later expanded to accommodate subsequent discoveries. Because of the complexity of the serotonin field, the Serotonin Club established its own nomenclature committee very early on. Eventually, the basic principles and guidelines developed for the classification of a sometimes seemingly overwhelming number of serotonin receptor subtypes served as a template adopted by the entire field of pharmacology, and led to the creation of the IUPHAR Committee on Receptor Nomenclature and Drug Classification (www.iuphar.org/nciuphar.html) [3].

This year marks the 25th anniversary of the Serotonin Club. This dynamic research society has grown and currently has a membership of over 450 persons. The Serotonin Club sponsors an international meeting on serotonin every other year, appropriate for such a rapidly developing scientific field. These meetings bring together the best in serotonin scientists from academia and industry, junior and senior investigators, and are often official satellite meetings to IUPHAR, or the Federation of European Pharmacological Societies (EPHAR). As the field advances, the Serotonin Club continues to promote the work of young investigators, future leaders in serotonin research.

1. Green AR, Marsden CA and Mylecharane EJ. (2008) *The Serotonin Club: coming of age*. *Trends Pharmacol Sci* 29:431-432.
2. Bradley PB, Engel G, Feniuk W, Fozard JR, Humphrey PP, Middlemiss DN, Mylecharane EJ, Richardson BP, Saxena PR. (1986) *Proposals for the classification and nomenclature of functional receptors for 5-hydroxytryptamine*. *Neuropharmacology* 25:563-576.
3. Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP. (1994) *International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin)*. *Pharmacol Rev.* 46:157-203.





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"All conference materials (promotional materials, agenda, publications and internet sites) related to this project must include an acknowledgement of NIH grant support and a disclaimer stating the following:
Funding for this conference was made possible (in part) by 1R13DA033783-01 from the National Institute on Drug Abuse. The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention by trade names, commercial practices, or organizations imply endorsement by the U.S. Government."





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ORGANIZATION

Scientific program organised by officers and councilors of the Serotonin Club

President:	Julie G. Hensler (<i>San Antonio, TX, USA</i>)
Past President:	Theresa A. Branchek (<i>Teaneck, NJ, USA</i>)
Vice Presidents North America:	Lynette C. Daws (<i>San Antonio, TX, USA</i>)
Vice Presidents Europe:	Frances Artigas (<i>Barcelona, Spain</i>)
Secretary/Treasurer:	Sheryl Beck (<i>Philadelphia, PA, USA</i>)

Organizing committee

Joël Bockaert (*IGF, Montpellier, France*)
Philippe Marin (*IGF, Montpellier, France*)
Michel Hamon (*Pitié-Salpêtrière, Paris, France*)

Secretariat

Aurore Castillo (*IGF, Montpellier, France*)
Sylvie Pagan (*CNRS, Montpellier, France*)

Communication / Iconography

Monique Anoal (*IGF, Montpellier, France*)
Muriel Asari (*IGF, Montpellier, France*)



2012

July 10 - 12
Faculty of Medicine
Montpellier, France

Localization: Faculty of Medicine



TRAM STOP
Place Albert 1^{er}

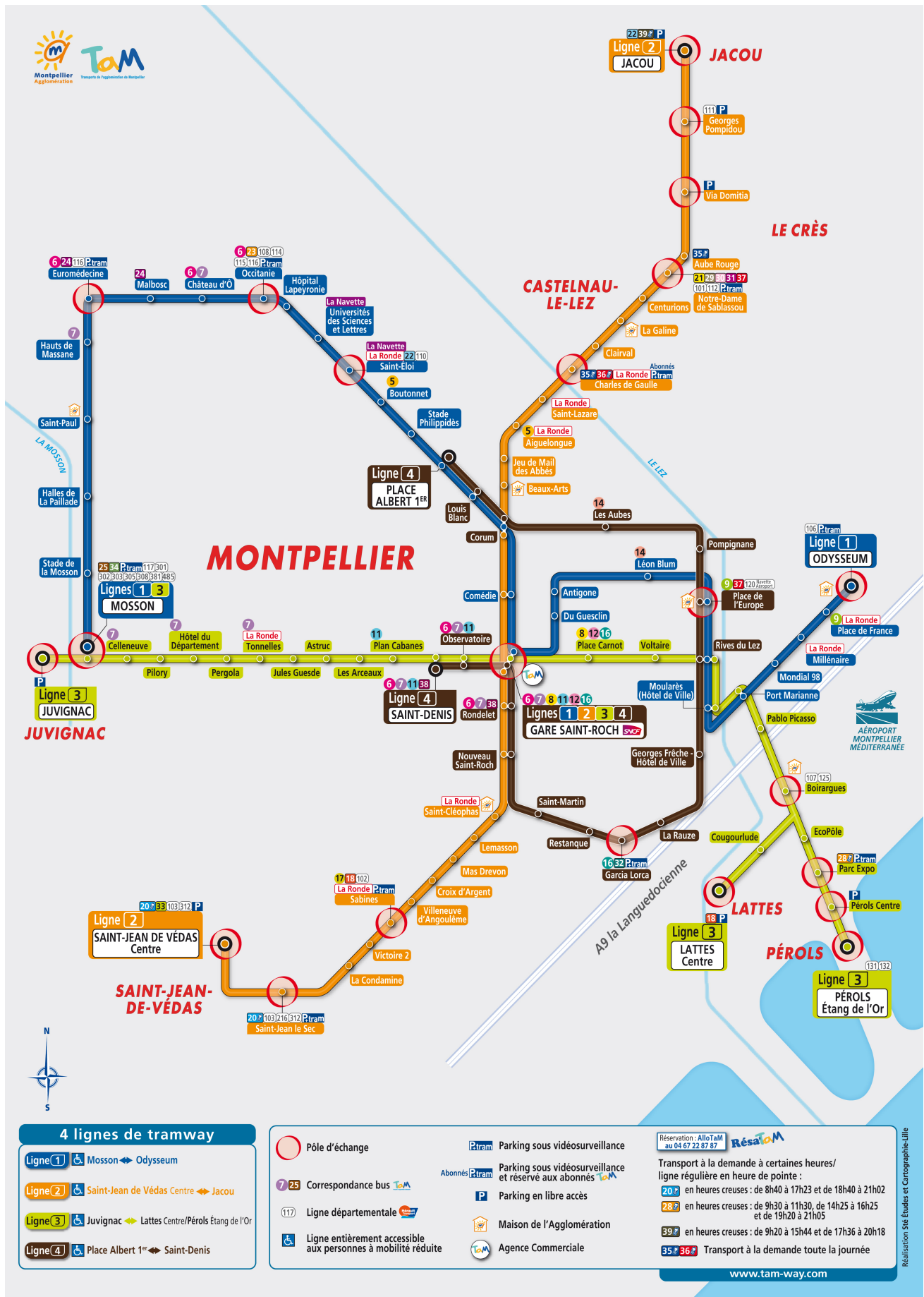




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Taxi

ALLO TAXI 34

06 800 805 98
04 67 200 100

TAXI BLEU

04 67 03 20 00

TAXI TRAM

0467581010

AÉRO TAXI MONTPELLIER

06 87 52 42 60

Wifi

Exhibitor list (*Salle Dugès*)

1 - ASCENTSCIENTIFIC AN ABCAM COMPAGNY

Elizabeth Day
Adrian Kinkaid

2 - BD BIOSCIENCES

Romain Guegan

3 - SIGMA-ALDRICH

Julie Mautord

4 - TOCRIS BIOSCIENCES

Marilyn Duncan Crawford

5 - VIEWPOINT

Bruno Girier



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The Medical University of Montpellier/Nimes

The Montpellier medical University founded in 1220 Medical Doctors were coming from all Europe (Saint-Jacques de Compostelle).

Arabian and jewish medical doctors persecuted in Spain came to teach in Montpellier. They had translated the texts from Bagdad medical University one of the oldest in the world. Since the 1740 the Medical University is established in this place, a benedictus monastery related the Saint Pierre Cathedral; It is deeply molded by physicians who acquired universal renown such as Rabelais, Lapeyronie, Chaptal, Arnaud de Villeneuve, and Gui de Chauliac. For centuries, generations of physicians have been inspired by its high achieving scientific tradition and have continued to honor this reputation. Today, it ranks with Paris as one of the two most attractive medical schools in France for students from all geographic horizons.

Conference rooms

Anatomy Auditorium
Theatrum Anatomicum



Dissertation Room
Salle des Actes



Dugès Hall
Salle Dugès





SEROTONIN CLUB MEETING

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Montpellier, France

PROGRAM: Tuesday, July 10, 2012

From 7:00 AM	REGISTRATION: "Salle du Conseil"
7:30 - 8:45 AM	BREAKFAST NIDA Travel Awardees : "Salle Fonds Jaume"
9:00 - 9:15 AM	<i>Theatrum anatomicum</i> Welcome and Irvine Page lecture presentation
9:15 - 10:15 AM	<i>Theatrum anatomicum</i> Irvine Page Lecture Elaine Sanders Bush, Vanderbilt University School of Medicine, Nashville, USA
10:15 - 10:45 AM	COFFEE BREAK 
10:45 - 12:45 PM	SYMPOSIUM 1 <i>Theatrum anatomicum</i> "Regulation of 5-HT neurochemistry and behavior by unsuspected "villains": Implications for psychiatric disorders and drug abuse." Chair: Lynette C. Daws, University of Texas Health Science Center, San Antonio, USA 1 - "Impact of feeding conditions on sensitivity to drugs acting on 5-HT and DA systems: Implications for drug abuse and psychotherapeutics." Charles P. France, University of Texas Health Science Center, San Antonio, USA 2 - "Serotonin transporter genotype and early life stress interactions in psychiatric disorders and addiction: Are organic cation transporters to blame?" Lynette C. Daws, University of Texas Health Science Center, San Antonio, USA 3 - "Peripheral immune system regulation of brain serotonin homeostasis." Randy D. Blakely, Vanderbilt University, Nashville, USA 4 - "The proaddictive effects of stress exposure are mediated by increased serotonin transporter function through kappa receptors activation of p38alpha MAPK in the ventral striatum." Abigail Schindler, University of Washington, Seattle WA, USA NIDA Travel Awardee
12:45 - 2:00 PM	LUNCH  (1:00-2:00 PM Business Meeting)



SEROTONIN CLUB MEETING

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2:00 - 4:00 PM

SYMPOSIUM 2 *Theatrum anatomicum*

"Putting the Pieces Together: Intergrating Dopamine, Serotonin, Reward and Aversion."

Chair : **John Neumaier**, *University of Washington, Seattle, USA*

1 - "Striatal 5-HT₆ receptors: Opposing dopamine."

John Neumaier, *University of Washington, Seattle, USA*

2 - "5-HT_{2A}R and 5-HT_{2C}R balance in decision-making and cocaine reward."

Kathryn A. Cunningham, *University of Texas Medical Branch, Galveston, USA*

3 - "Affective and decision functions of serotonin and dopamine."

Molly Crockett, *University of Zurich, Switzerland*

4 - "The influence of serotonin-1B receptors on cocaine-abuse related behaviors."

Nathan Pentkowski, *Arizona State University, Arizona, USA*

NIDA Travel Awardee

4:00 - 4:30 PM

COFFEE BREAK



4:30 - 6:00 PM

Theatrum anatomicum

FRONTIERS IN SEROTONIN RESEARCH: PIONEERS AND PRODIGIES

Chairs and discussants : **Elaine Sanders-Bush** and **Ewan Mylecharane**

1 - "Postnatal antidepressant treatment vs constitutive SERT deficiency produce opposing changes in presynaptic 5-HT_{1A} responses and emotion-related behavior in adolescent and adult mice."

Stephanie Altieri, *Pennsylvania State University, University Park, Pennsylvania, USA*

NIDA Travel Awardee

2 - "Decynium-22 enhances SSRI-induced antidepressant effects in mice: Uncovering new targets to treat depression."

Deana Apple, *University of Texas Health Science Center, San Antonio Texas, USA*

NIDA Travel Awardee

3 - "Autoinhibition in dorsal raphe is mediated by local release of serotonin."

Daniel Huereca, *Wayne State University School of Medicine, Detroit, MI, USA*

NIDA Travel Awardee

4 - "Synergistic suppression of cocaine-evoked elevations in motility and cortical serotonin 5-HT_{2C} receptor (5-HT_{2C}R) expression by combined administration of a selective 5-HT_{2A}R antagonist plus a 5-HT_{2C}R agonist."

Sarah Swinford, *University of Texas Medical Branch, Galveston, Texas, USA*

NIDA Travel Awardee

5 - "Schizophrenia-like disruption of sensory gating by serotonergic stimulation in rats: comparison with dopamine and NMDA receptors."

Shane Thwaites, *University of Melbourne, Melbourne, Australia*

NIDA Travel Awardee

6:30 - 8:30 PM

POSTER SESSION *Salle Dugès* with wine and cheese



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PROGRAM: *Wenesday, July 11, 2012*

8:00 - 9:00 AM

Theatrum anatomicum

Maurice rapport lecture

Ewan Mylecharane, University of Sydney, Sydney, Australia

9:00 - 11:00 AM

SYMPOSIUM 3 *Theatrum anatomicum*

"Serotonergic neurons and adaptative responses to emergency situations: a focus on autonomic and nociceptive systems."

Chair: **Véronica Fabre**, *Université Pierre et Marie Curie-Paris 6, Paris, France*

1 - "Involvement of lateral paragigantocellular serotonergic cells in nociceptive and cardiovascular processing."

Jean-François Bernard, *Université Pierre et Marie Curie-Paris 6, Paris, France*

2 - "Regulation of breathing by medullary raphe serotonergic neurons in conscious or anesthetized rodents."

Patrice Guyenet, *University of Virginia School of Medicine, Charlottesville, USA*

3 - "Central chemoreception by serotonin neurons of the raphe."

George B. Richerson, *The University of Iowa, Iowa City, USA*

4 - "Inducible, repeatable and specific inhibition of serotonergic neurons in vivo reveals their essential roles in mammalian physiology and behavior."

Susan Dymecki, *Harvard Medical School, Boston, USA*

11:00 - 11:30 AM

COFFEE BREAK



11:30 - 1:30 PM

SYMPOSIUM 4 *Theatrum anatomicum*

"Multiple facets of serotonin:placenta, blood, bone connection."

Chair: **Francine Côté**, *CNRS UMR 8142, Hôpital Necker, Paris, France*

1 - "Vital source of serotonin: The placenta."

Cathy Vaillancourt, *University of Québec, Laval, Canada*

2 - "Key function of serotonin in erythrocyte production and survival."

Edouard Kouassi, *University of Montréal, Montréal, Canada*

3 - "Serotonergic system and bone cells : New paracrine autocrine pathways."

Corinne Collet, *Hôpital Lariboisière, Paris, France*

4 - "Serotonin receptor 5-HT2B potentiates interferon- γ production by activating T lymphocytes."

Marie-Eve Koué, *University of Montréal, Montréal, Canada*

NIDA Travel Awardee

1:30 - 2:30 PM

LUNCH



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2:30 - 4:30 PM

SYMPOSIUM 5 *Theatrum anatomicum*

"Serotonin Transporter Redux 2012."

Chair: **Kathryn A. Cunningham**, *University of Texas Medical Branch, Galveston, USA*

1 - "A Novel Approach for Defining the Role of Serotonin in Drug Action: SERT Ile172Met Mice."

Randy D. Blakely, *Vanderbilt University, Nashville, USA*

2 - "Role of PIP2 and PLC in mediating amphetamine-evoked alterations in SERT function."

Harald Sitte, *Medical University of Vienna, Vienna, Austria*

3 - "SERT as a therapeutic target for cocaine dependence."

F. Gerard Moeller, *University of Texas Health Science Center at Houston, Houston, USA*

4 - "Examining the interaction between 5-HT1B receptors and SERT"

Yusha (Katie) Liu, *University of Washington, Seattle, USA*

NIDA Travel Awardee

4:30 - 5:00 PM

COFFE BREAK 

5:00 - 7:00 PM

SYMPOSIUM 6 *Theatrum anatomicum*

"Serotonin in the cardiovascular system: New findings on an old transmitter."

Chair: **Luc Maroteaux**, *Hôpital Pitié-Salpêtrière, INSERM U839, Paris, France*

Co-Chair: **Stephanie Watts**, *Michigan State University, East Lansing, USA*

1 - «The serotonin transporter (SERT) in blood pressure regulation."

Stephanie Watts, *Michigan State University, East Lansing, USA*

2 - "The 5-HT4 serotonin receptor: Physiological and pathophysiological roles in the heart ventricle."

Finn Olav Levy, *University of Oslo, Oslo, Norway*

3 - "Role of serotonin and 5-HT2 receptors in cellular processes involved in cardiac valve remodeling."

Laurent Monassier, *Faculté de Médecine, Strasbourg, France*

3 - "Refining the serotonergic contribution to central chemosensitivity."

Rachael Brust, *Harvard Medical School, Boston, USA*

NIDA Travel Awardee

7:10 PM

DEPARTURE FOR BANQUET





SEROTONIN CLUB MEETING

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PROGRAM: Thursday, July 12, 2012

8:30 - 10:30 AM

SYMPOSIUM 7 *Theatrum anatomicum*

"New roles of 5-HT₆ receptor in neuro-developmental and cognitive processes."

Chair: **Joël Bockaert**, University of Montpellier, Montpellier, France

Co-Chair: **Mark Millan**, Institut de Recherches Servier, Croissy sur Seine, France

1 - "Developmental role of the 5-HT₆ receptor in neuronal migration."

Alexandre Dayer, Geneva University Hospital, Geneva, Switzerland

2 - "Proteomic analysis of the 5-HT₆ receptor complex: Identification of a new signaling pathway underlying modulation of cognition by 5-HT₆ ligands."

Phillippe Marin, University of Montpellier, Montpellier, France

3 - "Functional role of 5HT₆ receptor in the neuro-developmental cognitive deficits seen in schizophrenia."

Kevin C.F. Fone, University Nottingham Medical School, Nottingham, UK

4 - "Effect of the 5-HT₆ receptor antagonist, SB-399885, on cognition, hippocampal cell proliferation and protein expression in the neurodevelopmental model of schizophrenia."

Madeleine King, University of Nottingham, Nottingham, UK

NIDA Travel Awardee

8:30 - 10:30 AM

SYMPOSIUM 8 *Salle des Actes*

"The 5-HT₃ receptor system: Therapeutic potential and advantages from novel ligands."

Chair: **Nicholas Barnes**, University of Birmingham Medical School, Birmingham, UK

Co-Chair: **Beate Niesler**, University of Heidelberg, Heidelberg, Germany

1 - "The therapeutic potential of targeting individual 5-HT₃ receptor subtypes."

Beate Niesler, University of Heidelberg, Heidelberg, Germany

2 - "The therapeutic potential of 5-HT₃ receptor partial agonists."

Nick Moore, AMRI Inc, Albany, USA

3 - "Tickling the 5-HT₃ receptor; molecular studies and therapeutic potential of allosteric 5-HT₃ receptor ligands."

Nicholas Barnes, University of Birmingham Medical School, Birmingham, UK

4 - "The native serotonin 5-HT_{5A} receptor: Electrophysiological characterization in rodent cortex and 5-HT_{1A}-mediated compensatory plasticity in the knockout mouse."

Nathalie Goodfellow, University of Toronto, Toronto, Canada

NIDA Travel Awardee

10:30 - 11:00 AM

COFFEE BREAK





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11:00 - 1:00 PM

SYMPOSIUM 9 *Theatrum anatomicum*

"Recent advances in understanding the interaction between the glutamatergic and serotonergic systems."

Chair: **Mark A. Geyer**, *University of California, San Diego, La Jolla, USA*

Co-Chair: **Adam Halberstadt**, *University of California, San Diego, La Jolla, USA*

1 - "Antipsychotic signaling in a 5HT_{2A}-mGlu₂ receptor heterocomplex."

Javier Gonzalez-Maeso, *Mount Sinai School of Medicine, New York, USA*

2 - "Is RNA editing relevant for serotonergic transmission in the cortex in health and disease?"

Dan Urban, *University of Chapel Hill, Chapel Hill, USA*

3 - "Interactive effects of 5-HT_{2A} and mGlu₅ receptors on behavior in mice."

Adam Halberstad, *University of California, San Diego, La Jolla, USA*

4 - "Higher-order interactions of GABA and glutamate axons in the rat dorsal raphe nucleus visualized by array tomography."

Mariano Soiza-Reilly, *Harvard Medical School, Boston, USA*

NIDA Travel Awardee

11:00 - 1:00 PM

SYMPOSIUM 10 *Salle des Actes*

"Molecular genetics of serotonin neuron development, physiology and pharmacology."

Chair: **Evan Deneris**, *Case Western Reserve University, Cleveland, USA*

1 - "Contribution of serotonin_{1A} auto and heteroreceptors to depression and antidepressant response."

Eduardo Leonardo, *Columbia University, New York, USA*

2 - "Transcriptional control of serotonin neurons across the lifespan."

Evan Deneris, *Case Western Reserve University School of Medicine, Cleveland, USA*

3 - "Raphe neuron development and its perturbations by altered transcription and receptor deletion."

Sheryl Beck, *Children's Hospital of Philadelphia, Philadelphia, USA*

4 - "Shaping patterns of serotonin neurotransmission via feedback circuits."

Kathryn Commons, *Harvard Medical School, Boston, USA*

1:00 - 2:00 PM

LUNCH 🍴



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2:00 - 4:00 PM

SYMPOSIUM 11 *Theatrum anatomicum*

"From Ligand Functional Selectivity to Brain Region Selectivity: Differential Pharmacological Targeting of Serotonin System."

Chair: **Kelly A. Berg**, *University of Texas Health Science Center, San Antonio, USA*

Co-Chair: **Adrian Newman-Tancredi**, *NeuroAct Communication, Castres, France*

1 - "Biased agonism at serotonin 5-HT_{1A} receptors : Preferential postsynaptic activity for improved therapy of CNS disorders."

Adrian Newman-Tancredi, *NeuroAct Communication, Castres, France*

2 - "Agonist-directed signaling at 5-HT_{2A} receptors in cortex."

Laura M. Bohn, *The Scripps Research Institute, Jupiter, USA*

3 - "Central 5-HT_{2B} receptors exert a differential control of mesocorticolimbic and nigrostriatal dopamine pathway activity: An in vivo microdialysis study in the rat."

Umberto Spampinato, *Université Victor Seglan Bordeaux 2, Inserm U862, Bordeaux, France*

4 - "Characterization of serotonin neurons in the raphe based on molecular and electrophysiological identity."

Sebastian Fernandez, *INSERM, Paris, France*

NIDA Travel Awardee

2:00 - 4:00 PM

SYMPOSIUM 12 *Salle des Actes*

"Ovarian Sex Steroids and Serotonin Function."

Chair: **Alan Frazer**, *University of Texas Health Science Center, San Antonio, USA*

1 - "Estrogen receptor GPR30 mediates desensitization of serotonin_{1A} receptor signaling."

Nancy Muma, *University of Kansas School of Pharmacy, Lawrence, USA*

2 - "Serotonin transporter function: Interaction among ovarian steroids and antidepressants."

Alan Frazer, *University of Texas Health Science Center, San Antonio, USA*

3 - "Estradiol modulation of 5HT_{1A} and 5-HT_{2A} receptors and SERT in brain of female hemiparkinsonian monkeys after long-term ovariectomy."

Therese Di Paolo, *Faculty of Pharmacy, Laval University, Laval, Canada*

4 - "Influences of sex hormones and diminished central nervous 5-HT synthesis rate on impulse control related to reward and punishment processing in young adult females."

Katrin Helmbold, *RWTH Aachen University, Aachen, Germany*

NIDA Travel Awardee

4:00 PM

CLOSING REMARKS AND FAREWELL





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ORAL PRESENTATIONS

SYMPOSIUM 1



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Montpellier, France

IRVINE PAGE LECTURE

A LIFE-LONG JOURNEY WITH SEROTONIN: THEN, NOW AND THE FUTURE

Elaine SANDERS-BUSH

Vanderbilt University School of Medicine, Nashville, USA

A brief chronicle of how the explosion of knowledge about serotonin shaped my scientific career. This story moves from early days of fluorescent measurement of brain serotonin to current, genetic strategies for identifying novel targets and mechanisms controlling serotonergic function in brain.



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IMPACT OF FEEDING CONDITIONS ON SENSITIVITY TO DRUGS ACTING ON 5-HT AND DA SYSTEMS: IMPLICATIONS FOR DRUG ABUSE AND PSYCHOTHERAPEUTICS

Charles P. FRANCE

University of Texas Health Science Center, San Antonio, USA

Serotonin (5-HT) and dopamine (DA) systems play a role in mood, motivation, and reward and they are primary targets for many therapeutic drugs as well as some drugs of abuse. Drugs acting on these neurotransmitter systems can also have marked effects of feeding behavior. Less well studied is how feeding conditions might alter the effects of drugs acting on 5-HT and DA systems. It is well established that food restriction enhances the positive reinforcing effects of a variety of drugs and recent studies demonstrate that food restriction enhances other effects of drugs acting at DA receptors while attenuating effects of drugs acting on 5-HT receptors. These changes in behavioral effects of drugs in food-restricted animals are accompanied by decreases and increases, respectively, in DA and 5-HT clearance. It is becoming clear that not only the amount of food consumed, but also the type of food, impacts the effects of drugs acting on 5-HT and DA systems. For example, eating high fat chow increases the sensitivity of rats to direct-acting DA receptor agonists and to direct-acting 5-HT receptor agonists. Sensitization to the locomotor stimulating effects of indirect-acting DA receptor agonists (e.g. cocaine) is significantly enhanced in rats eating high fat chow, and this enhancement is greater in female as compared with male rats. Converging lines of evidence implicate endocrine hormones (e.g. insulin and leptin) as possible mediators of diet-induced changes in drug effects. Understanding how feeding conditions modify drug effects could improve pharmacotherapeutic outcomes and provide insights regarding vulnerability for substance abuse. CPF is supported by K05DA17918



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SEROTONIN TRANSPORTER GENOTYPE AND EARLY LIFE STRESS INTERACTIONS IN PSYCHIATRIC DISORDERS AND ADDICTION: ARE ORGANIC CATION TRANSPORTERS TO BLAME?

Lynette C. DAWS

University of Texas Health Science Center, San Antonio, USA

Dysfunction of serotonergic neurotransmission is considered a primary factor contributing to a host of psychiatric and addictive disorders, including depression and alcoholism. The serotonin transporter (SERT) controls the strength and duration of serotonin signaling by high-affinity uptake of serotonin. Interestingly, individuals carrying variants of the SERT gene that confer reduced SERT expression and/or function appear to be at greater risk for psychiatric and addictive disorders, particularly when faced with early life stressful events. Related to this, we and others have recently identified a previously unsuspected role for the corticosterone-sensitive organic cation transporter 3 (OCT3) in serotonin uptake in brain. Of note, OCT3 expression and activity is increased when SERT activity is genetically compromised. Likewise, when SERT is pharmacologically inactivated, serotonin uptake by OCT3 appears to limit the increase in extracellular serotonin following administration of selective serotonin reuptake inhibitors (SSRIs), thus providing a mechanistic basis for the sub-optimal therapeutic effects of SSRIs. We also found that the ability of alcohol to increase extracellular serotonin is mediated, at least in part, by alcohol-induced inhibition of serotonin uptake via OCT3, suggesting a putative role for this transporter in the development of alcoholism. Of note, we found that repeated exposure to intermittent increases in plasma corticosterone down-regulated the expression and function of OCT3. Together, these data support an important role for OCT3 in controlling the tone of serotonergic neurotransmission. Given the trophic role of serotonin during brain development, abnormally high levels of serotonin during this critical phase, promoted by stress-induced impairment of OCT3 function, may provide mechanistic insight into the relationship between early life stress and increased predisposition to psychiatric illness.



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PERIPHERAL IMMUNE SYSTEM REGULATION OF BRAIN SEROTONIN HOMEOSTASIS

Randy D. BLAKELY
Vanderbilt University, Nashville, USA

The presynaptic serotonin (5-HT) transporter (SERT) has long been known to serve as the major determinant of synaptic 5-HT clearance in the CNS. The mechanisms that regulate SERT remain an active area of investigation. We have identified signaling through the p38 MAPK pathway as a critical contributor to the rapid regulation of SERT and established that IL-1beta, acting through presynaptic IL-1Rs, rapidly enhances SERT activity through a shift in the transporter's affinity for 5-HT. More recently, we demonstrated that activation of the peripheral, native immune system via a single LPS injection can activate CNS SERT activity and enhance 5-HT clearance rates. These changes in SERT activity are lost when animals are either injected peripherally or centrally with the p38 MAPK inhibitor SB203580. In my presentation, I will describe our ongoing efforts to implicate presynaptic p38 MAPK in the SERT activation produces by inflammatory cytokines and our efforts to establish a mechanism for transport changes using transgenic mice and single molecule recording techniques.



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THE PROADDICTIVE EFFECTS OF STRESS EXPOSURE ARE MEDIATED BY INCREASED SEROTONIN TRANSPORTER FUNCTION THROUGH KAPPA RECEPTORS ACTIVATION OF P38ALPHA MAPK IN THE VENTRAL STRIATUM

Abigail SCHINDLER (1), J.S. Smith(1), D.I. Messinger(1), Selena Schreiber(1),
R.M. Gustin(1), C.E. Hagan(2), J.F. Neumaier(3), C. Chavkin(1)
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Activation of the dynorphin/kappa opioid receptor (KOR) system by stress exposure results in maladaptive behaviors by mechanisms that are not completely understood. In the present study, stress-induced potentiation of cocaine conditioned place preference was not seen in mice lacking GRK3 or p38 α MAPK in serotonergic cells. Furthermore, serotonin transporter (SERT) knockout mice did not show kappa-mediated aversion. Activation of KOR stimulates p38 α through GRK3, and p38 can increase the function of SERT. The current study elucidated mechanisms underlying these KOR-induced behaviors by investigating the role of KOR-activated p38 α on SERT function. Using rotating disk electrode voltammetry, we found that synaptosomes isolated from animals previously exposed to various stressors demonstrated increased serotonin uptake by SERT. This effect was recovered 24hr following stress exposure, and was not apparent after a brief acute stress. Stress exposure did not affect other monoamine transporter function. Michaelis-Menten analysis showed that stress increased SERT V_{max} without affecting SERT K_m. Furthermore, biotinylation studies showed that stress increased the surface expression of SERT. Surprisingly, this stress-induced increase in surface SERT was only seen in the ventral striatum. Using microinjections of the KOR antagonist norBNI, we determined that local KOR activation in the ventral striatum mediated the effect. Finally, increased uptake was not apparent in animals lacking GRK3 or p38 α in serotonergic cells. Together these results support the hypothesis that p38 α in serotonergic afferents to the ventral striatum regulates SERT function following repeated stress exposure, and may underlie adverse consequences of stress.

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SYMPOSIUM 2



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STRIATAL 5-HT6 RECEPTORS: OPPOSING DOPAMINE

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5-HT6 serotonin receptors are densely localized in dorsal and ventral striatum and have been implicated in reward motivated learning and habit behaviors. We have previously found that increased 5-HT6 expression using viral mediated gene transfer in rats inhibits reward motivated learning (in the dorsomedial striatum) but facilitates behavioral flexibility in compulsively responding animals (in the dorsolateral striatum). Since these receptors are localized in medium spiny neurons that contribute to both direct and indirect pathways, as compared to dopamine receptors that are segregated between these pathways, we developed the hypothesis that 5-HT6 signaling in striatum tends to oppose the effects of dopamine by reducing the differential activation and inhibition of direct and indirect pathways by dopamine, respectively. We tested this by developing viral vectors that target the direct or indirect pathway selectively by using dynorphin or encephalin promoters to drive gene expression. We dissected the role of these receptors in striatum by expressing them in either one pathway or the other, and discovered that increased 5-HT6 signaling in direct pathway neurons of dorsomedial striatum facilitated learning whereas increased 5-HT6 signaling in indirect pathway neurons impaired learning. Finally, increased expression of 5-HT6 receptors in indirect pathway neurons of dorsolateral striatum facilitated behavioral flexibility in the omission training model in compulsively responding rats, supporting the idea that the key to understanding these receptors in striatum involves considering the effects of these receptors in direct or indirect pathways, where they have generally opposite effects on behavior. Thus, balanced activation of 5-HT6 receptors in both output pathways from striatum will tend to support stability of behavior and interfere with new procedural learning.



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5-HT_{2A}R AND 5-HT_{2C}R BALANCE IN DECISION-MAKING AND COCAINE REWARD

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Abstinence from cocaine dependence is stymied by relapse precipitated by impulsive behavior upon exposure to cocaine-associated cues (cue reactivity). Our hypothesis is that disrupted homeostasis in serotonin 5-HT_{2A}R and 5-HT_{2C}R function in prefrontal-striatal-thalamic circuits underlies the mechanistic imbalance that drives these phenotypes. We have developed a rat model in which high (HI) and low impulsive (LI) rats are identified in a 1-choice serial reaction time (1-CSRT) task (top and bottom 25% of rats based upon highest and lowest premature responses). HI rats exhibited higher 5-HT_{2A}R and lower 5-HT_{2C}R protein expression in nucleus accumbens (NAc) and medial prefrontal cortex and exhibited differential sensitivity to selective ligands vs. LI rats. When trained to self-administer cocaine (0.25 mg/kg/inf) and exposed to forced abstinence, HI rats exhibited higher cue reactivity (lever presses reinforced by cues previously associated with cocaine delivery) vs. LI rats. Levels of impulsivity and cue reactivity correlate positively in rats, as we have shown in humans (Liu et al., *Addictive Behaviors* 37:193, 2011). These phenotypic data suggest that impulsivity and cue reactivity are related processes possibly mediated by common underlying neurobiology. To study the hypothesis that the underlying neurobiology involves a disruption of a 5-HT_{2A}R:5-HT_{2C}R balance, we are employing adeno-associated viral (AAV) vectors to deplete each receptor using RNA. Rats with a 'knockdown' of the 5-HT_{2C}R (5-HT_{2C}R-shRNA-AAV) in the NAc expressed significantly higher impulsivity and cue reactivity relative to controls (non-silencing RNA-AAV; $p < 0.05$). Pretreatment with the 5-HT_{2A}R antagonist M100907 reversed the enhancement of premature responses observed in NAc 5-HT_{2C}R knockdown rats at doses ineffective in control rats ($p < 0.05$). These results indicate that the loss of NAc 5-HT_{2C}R enhances the stimulatory effects of the 5-HT_{2A}R over inherent impulsivity. Greater understanding of the 5-HT_{2A}R:5-HT_{2C}R balance in these phenotypes will allow optimization of therapeutics for relapse. Supported by DA024157, DA DA020087, DA07287, DA030977



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AFFECTIVE AND DECISION FUNCTIONS OF SEROTONIN AND DOPAMINE

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Updated theoretical accounts of the role of serotonin (5-HT) in motivation propose that 5-HT operates at the intersection of aversion and inhibition, promoting withdrawal in the face of aversive predictions. Meanwhile, dopamine (DA) is posited to operate at the intersection of reward and action, promoting vigour in the face of appetitive predictions. In this talk, I will present an overview of the theoretical framework describing how 5-HT and DA modulate affect, action and decision-making. I will also present a series of empirical studies in humans designed to test the hypothesis that 5-HT plays a key role in translating aversive predictions into behavioral inhibition. In these studies, we examine the specific cognitive mechanisms through which 5-HT modulates such withdrawal behavior. The first study employed a novel task designed to obtain independent measures of motor response inhibition, aversive prediction, and punishment-induced behavioral inhibition. Using acute tryptophan depletion to challenge the 5-HT system in humans, we demonstrated that 5-HT is critical for behavioural inhibition in the face of aversive predictions, but not overall motor response inhibition or sensitivity to aversive outcomes. Our second study examined more deeply the cognitive processes underlying the influence of 5-HT on behavioral inhibition. Behavioral inhibition in response to punishments reflects at least two concurrent processes: instrumental aversive predictions linking stimuli, responses, and punishments, and Pavlovian aversive predictions linking stimuli and punishments irrespective of response. Using a task designed to disentangle the effects of Pavlovian and instrumental aversive predictions on response vigor, we show that acute tryptophan depletion affects response bias and response vigor in a manner best explained by a role for 5-HT in Pavlovian aversive predictions. I conclude by highlighting how theoretical approaches to understanding 5-HT and DA in the context of affect and action have implications for psychiatry.



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THE INFLUENCE OF SEROTONIN-1B RECEPTORS ON COCAINE-ABUSE RELATED BEHAVIORS

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The influence of serotonin-1B receptors (5-HT_{1B}Rs) on cocaine abuse-related behaviors has been inconclusive due to discrepancies between pharmacological and gene knockout approaches, and opposite influences on cocaine reinforcement versus incentive motivation elicited by cocaine-priming injections or exposure to cocaine-paired cues. We hypothesized that 5-HT_{1B}R modulation of these behaviors may vary depending on the stage of the addiction cycle. To test this hypothesis, we examined the effects of increased 5-HT_{1B}R tone at different stages of the addiction cycle via either systemic agonist administration or by viral-mediated 5-HT_{1B}R-gene transfer. 5-HT_{1B}R-gene transfer into medial nucleus accumbens shell neurons during maintenance shifted the dose–response curve for cocaine self-administration upward and to the left on a fixed ratio (FR) schedule of reinforcement and increased break points and cocaine intake on a progressive ratio (PR) schedule, consistent with enhanced reinforcing effects of cocaine. In contrast, following 21 days of forced abstinence 5-HT_{1B}R-gene transfer attenuated break points and cocaine intake on a PR schedule, as well as cue- and cocaine-primed reinstatement of cocaine-seeking behavior. Similarly, following protracted withdrawal the 5-HT_{1B}R agonist CP94253 blunted cocaine intake on both FR and PR schedules, and attenuated cue- and cocaine-primed drug-seeking following 5, but not 1 day of withdrawal. This unique pattern of effects suggests that 5-HT_{1B}Rs undergo a change in modulatory influence over cocaine abuse-related behaviors, with a facilitative influence during periods of active drug use in striking contrast to an inhibitory influence during protracted withdrawal. These findings suggest that targeting 5-HT_{1B}Rs may lead to a novel treatment for cocaine dependence and that the therapeutic efficacy of these treatments may vary depending on the stage of addiction.



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POSTNATAL ANTIDEPRESSANT TREATMENT VS CONSTITUTIVE SERT DEFICIENCY PRODUCE OPPOSING CHANGES IN PRESYNAPTIC 5-HT_{1A} RESPONSES AND EMOTION-RELATED BEHAVIOR IN ADOLESCENT AND ADULT MICE

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Administration of serotonin transporter (SERT) inhibiting antidepressants during a critical postnatal period has been hypothesized to recapitulate changes in behavior arising from constitutive reductions in SERT expression. Both have medical relevance related to neonatal exposure to serotonin reuptake inhibitors (SRIs) and differential SERT expression associated with human SERT gene polymorphisms, respectively. We investigated postnatal administration of the SRIs escitalopram (S-CIT) and fluoxetine (FLX) vs constitutive SERT deficiency on emotion-related behaviors and presynaptic 5-HT_{1A} receptor responses in late adolescent and adult mice. Enhanced anxiety-related responses characteristic of SERT-deficient mice were notably not observed in mice receiving transient postnatal antidepressant treatment. Furthermore, whereas 5-HT_{1A}-mediated decreases in body temperature were absent in

SERT-deficient mice of both sexes, mice receiving postnatal S-CIT or FLX showed pronounced hypothermia after 8-OH-DPAT administration. Moreover, S-CIT but not fluoxetine resulted in 5-HT_{1A} autoreceptor hypersensitivity as adults. In vivo microdialysis studies are ongoing to assess further the ramifications of postnatal SRI administration on extracellular serotonin levels. We conclude that transient vs constitutive SERT deficiency produces opposing changes in 5-HT_{1A} autoreceptor function lasting into adulthood. Persistent changes in presynaptic circuitry might underlie differential emotional phenotypes associated with these two SERT disruption models. Future studies are aimed at understanding the mechanisms of differential 5-HT_{1A} regulation between these models and consequences of altered serotonergic tone. These findings lead to important implications for antidepressant use during pregnancy and genetic influences regarding susceptibility and/or treatment responses in anxiety and mood disorders.



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DECYNIUM-22 ENHANCES SSRI-INDUCED ANTIDEPRESSANT EFFECTS IN MICE: UNCOVERING NEW TARGETS TO TREAT DEPRESSION

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Cano, W Anthony Owens, Melissa Vitela, Georgianna G
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A major problem in the treatment of depression is that many patients do not experience therapeutic benefit from currently available antidepressants. The most commonly prescribed antidepressant drugs are the selective serotonin (5-HT) reuptake inhibitors (SSRIs), which act by blocking the high-affinity 5-HT transporter (SERT). The increase in extracellular 5-HT produced by SSRIs is thought to be critical to initiate downstream events needed for therapeutic effects. A potential explanation for their limited therapeutic efficacy is the recently characterized presence of low-affinity, high-capacity transporters for 5-HT in the brain, (e.g. organic cation transporters [OCTs]), which may limit the ability of SSRIs to increase extracellular 5-HT. Decynium-22 (D-22) is an OCT blocker, and using this compound, we uncovered a significant role for OCTs in 5-HT uptake in SERT knockout mice, raising the possibility that pharmacological inactivation of OCTs might enhance the neurochemical and behavioral effects of SSRIs. Here we show that [3H]D-22 binding sites are richly expressed in mouse hippocampus, and that D-22 enhances the effects of the SSRI fluvoxamine to inhibit 5-HT clearance and to produce antidepressant-like activity. Further, preliminary data with mice lacking the OCT3 subtype shows a decreased sensitivity to D-22-mediated enhancement of the behavioral effects of SSRIs. Our findings provide a mechanistic basis for poor therapeutic outcome following treatment with SSRIs, and point to D-22-sensitive transporters as novel targets for new antidepressant drugs with improved therapeutic potential.



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AUTOINHIBITION IN DORSAL RAPHE IS MEDIATED BY LOCAL RELEASE OF SEROTONIN

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The midbrain dorsal raphe nucleus (DRN) contains a large fraction of the serotonergic neurons projecting to the forebrain. Because these cells have been hypothesized to play a key role in the actions of antidepressants, many studies have addressed the factors controlling their activity. A key hypothesis that has emerged from this work is that DRN serotonergic neurons express inhibitory 5-HT_{1A} autoreceptors which sense locally released serotonin as part of a negative feedback loop controlling serotonin neuron firing. However electrophysiological experiments aimed at directly testing this hypothesis have generally failed to find support for it.

The main problem for experimentally addressing this issue has been the paucity of tools for controlling the activity of DRN at the network level. We bypassed this limitation by selective expression of channelrhodopsin in serotonergic neurons. This allowed us to stimulate serotonergic neurons within the DRN using light. Using fast scan cyclic voltammetry we found that flashes of light were sufficient to cause serotonin release within the DRN in in vitro brain slices. Whole cell recording of DRN serotonergic neurons in slices revealed that the light flashes elicited robust depolarizations which were followed by slower afterhyperpolarizations/spiking inhibitions. Using apamin and the 5-HT_{1A} receptor antagonists WAY100,135/WAY100,635 we could show that the channelrhodopsin-induced afterhyperpolarization/inhibition was mediated by a 5-HT_{1A} receptor-activated potassium current and had a smaller contribution from an apamin sensitive calcium-activated potassium current. Under voltage clamp we could block the channelrhodopsin depolarization to isolate a pure WAY100,135/WAY100,635 sensitive outward aftercurrent. We have also obtained similar results in wild type mice by applying electrical stimuli to the DRN. These findings directly demonstrate 5-HT_{1A} receptor-mediated autoinhibition in the DRN.



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SYNERGISTIC SUPPRESSION OF COCAINE-EVOKED ELEVATIONS IN MOTILITY AND CORTICAL SEROTONIN 5-HT_{2C} RECEPTOR (5-HT_{2CR}) EXPRESSION BY COMBINED ADMINISTRATION OF A SELECTIVE 5-HT_{2AR} ANTAGONIST PLUS A 5-HT_{2CR} AGONIST

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Aims: An excitatory role for the 5-HT_{2AR} and inhibitory role for the 5-HT_{2CR} to control the in vivo effects of cocaine are supported by the literature. We tested the hypothesis that combined pretreatment with the selective 5-HT_{2AR} antagonist M100907 plus 5-HT_{2CR} agonist WAY163909 would synergistically suppress cocaine-evoked hyperactivity. Secondly, we evaluated this combinatorial treatment to block cocaine-evoked modifications of 5-HT_{2AR} and 5-HT_{2CR} expression in prefrontal cortex (PFC), a key site implicated in the modulatory roles of these receptors in the behavioral effects of cocaine.

Methods: Doses of M100907 and WAY163909 without effects on motility alone were employed to determine whether these receptor subtypes act in concert. Male rats were injected with M100907 or vehicle (45 min), WAY163909 or vehicle (30 min), and cocaine or vehicle (15 min) prior to placement in motor activity monitors. A separate group of animals underwent identical pharmacological treatments and were sacrificed 15 min after the last injection; crude membrane fractions of the PFC were analyzed using Western blot for 5-HT_{2AR} and 5-HT_{2CR} protein expression.

Results: M100907 plus WAY163909 synergistically attenuated cocaine-induced hyperlocomotion, but had no combined effect on basal locomotion. There was a trend toward elevated 5-HT_{2CR} protein expression in the PFC following acute cocaine administration; this effect was attenuated by M100907 plus WAY163909. No difference in 5-HT_{2AR} protein expression was observed among treatment groups.

Conclusions: Our observation that M100907 and WAY163909 synergistically suppress cocaine-induced hyperlocomotion raises the possibility that therapeutic advantage might be gained by a bifunctional selective 5-HT_{2AR} antagonist:5-HT_{2CR} agonist.



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SCHIZOPHRENIA-LIKE DISRUPTION OF SENSORY GATING BY SEROTONERGIC STIMULATION IN RATS: COMPARISON WITH DOPAMINE AND NMDA RECEPTORS

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Schizophrenia pathophysiology is associated with aberrant serotonergic, as well as dopaminergic and glutamatergic neuronal activity. Atypical antipsychotic drugs differ from typical antipsychotics by interacting with serotonin receptors in addition to dopamine-D2 receptors. Schizophrenia patients show disruptions in sensory gating, a normal brain filtering mechanism which occurs in response to repeated auditory stimuli. Sensory gating can be assessed by presenting two clicks of sound and measuring the responses with electroencephalography (EEG). In healthy subjects the response to the second click is diminished, however in schizophrenia patients the response is similar for both sounds. In rodent studies this filtering mechanism is referred to as N40 sensory gating, however little is known about the serotonergic mechanisms involved. We therefore investigated the acute effects of several psychoactive compounds that alter serotonergic neuronal activity and compared the effects with those of drugs acting on dopaminergic or glutamate NMDA receptors. Male Sprague-Dawley rats were implanted with cortical surface electrodes and allowed one week to recover. Experiments comprised of 150 presentations of two 85dB bursts of white noise, 500ms apart (S1 and S2). Treatment with the serotonin releaser, MDMA (Ecstasy), the serotonin-2A receptor agonist, DOI, the dopamine releaser amphetamine, the dopamine-D1/D2 receptor agonist, apomorphine, and the glutamate NMDA receptor antagonist, phencyclidine, all caused a dose dependent increase in N40 sensory gating ratios (S2/S1), indicating a disruption of normal sensory gating. The serotonin-1A receptor agonist, 8-OH-DPAT, had bimodal effects on sensory gating depending on the dose. These results enhance our understanding of neural processes that underlie sensory gating. Sensory gating deficits that were caused by serotonergic receptor activation were similar to deficits caused by drugs that increase dopaminergic activity or block glutamate NMDA receptors, suggesting that a common mechanism may be involved.



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SEROTONIN AND THE AUSTRALIAN CONNECTION: THE SCIENCE AND THE PEOPLE

Ewan MYLECHARANE

School of Medical Sciences University of Sydney, Australia

- Discovery of serotonin (5-hydroxytryptamine: 5-HT) in 1946–49 by Maurice Rapport, Arda Green and Irvine Page
- Beginning of my career in pharmacology in 1973 when my PhD was completed, and I joined Professor James Lance at the University of UNSW, Sydney, Australia, where I began my first research on serotonin
- First introduction to migraine research on cranial vascular arterial blood using electromagnet flow probes in monkeys, to respond to vasoactive agents in monkeys
- Moved 10 km to the Department of Pharmacology, University of Sydney in 1978 as a lecturer, and stayed there ever since!
- Studies on receptor mechanisms for 5-HT receptors, in vascular, smooth and cardiac tissues in vitro, as well as in vivo
- New drug discoveries in the early 1980s, as 5-HT receptor nomenclature thrives
- 5-HT modulation of dopamine release from rat nucleus accumbens and striatal slices in vivo
- 5-HT transporter affinities for pethidine and norpethidine, and their role in serotonin toxicity
- A few photos of our club members at Serotonin Club meetings



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SYMPOSIUM 3



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INVOLVEMENT OF LATERAL PARAGIGANTOCELLULAR SEROTONERGIC CELLS IN NOCICEPTIVE AND CARDIOVASCULAR PROCESSING

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The serotonergic neurons of the lateral paragigantocellular reticular (LPGi) nucleus are thought to participate in the modulation of spinal nociceptive transmission. Furthermore, recent data support the idea that LPGi serotonergic neurons activation plays a critical role in the cardiac baroreflex inhibition triggered by noxious stimuli. However, this inference was based only on c-Fos immunolabeling evidence. This led us to re-address this question by means of a direct electrophysiological approach combined with immunohistochemical identification of the serotonergic phenotype of neurons recorded.

Electrophysiological recordings were performed in rats anaesthetized with halothane (0.7% in 50% O₂ – 50% N₂O). Neurons were characterized by their responses to thermal noxious stimuli (44 – 52 °C, 20s), then filled with neurobiotin through the juxtacellular recording micropipette for subsequent phenotypical identification. After a delay of 2 - 6 hours, the brain was perfused then processed to visualize the serotonergic neurons double labeled for both neurobiotin and serotonin.

A total of 77 neurons, located within the serotonergic portion of the LPGi and the adjacent raphe magnus (RMg) nuclei, were recorded in rats subjected to innocuous and noxious thermal stimuli. Most LPGi serotonergic neurons displayed a tonic and sustained increase of firing during the application of noxious stimuli (threshold around 46°C). Interestingly, both the response and the proportion of serotonergic neurons activated by noxious stimuli were higher in the LPGi than in the RMg nucleus.

Our data directly demonstrated that LPGi serotonergic neurons are strongly activated by noxious thermal stimuli ($\geq 48^{\circ}\text{C}$) under conditions leading to baroreflex inhibition. Furthermore, they support the idea that LPGi serotonergic neurons could contribute to a descending feedback control of nociception triggered by such heavy stimuli.



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OPTOGENETIC STIMULATION OF RAPHE OBSCURUS (RO) SEROTONERGIC NEURONS IN VIVO ACTIVATES BREATHING IN MICE

Patrice GUYENET

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RO serotonergic neurons innervate the lower brainstem and spinal cord and contribute somehow to a variety of autonomic, sensory and motor functions, including locomotion and breathing. We used channelrhodopsin-2 (ChR2) optogenetics to ask whether selective activation of RO serotonergic neurons increases breathing in the anesthetized mouse, whether RO serotonergic neurons are activated by increases in PCO₂ in vivo and whether selective activation of RO serotonergic neurons also activate breathing in conscious mice. Injections of a Cre-dependent AAV2 into RO of e-Pet-Cre mice caused selective expression of ChR2 in RO serotonergic neurons. Photostimulation of RO (2-10 Hz; 30s episodes) caused robust increases in diaphragmatic EMG frequency and amplitude in anesthetized mice. ChR2-expressing RO serotonergic neurons, identified by their light evoked discharge pattern in these mice, were unresponsive to elevation of PCO₂. Photostimulation of serotonergic RO neurons in conscious mice (30s episodes) produced a gradually developing increase in breathing frequency and amplitude without inducing overt behavior or untoward motor activity. These experiments also revealed that the ChR2-expressing neurons innervated the phrenic motor nucleus and the region of the medulla oblongata that generates the respiratory rhythm and pattern. These experiments provide direct evidence that increasing the activity of RO serotonergic neurons in vivo activates breathing rate and amplitude. These results are therefore fully consistent with prior evidence that serotonergic neurons enhance breathing. On the other hand, we could not confirm that RO serotonergic neurons have the ability to detect CO₂ in vivo. This property may be restricted to a smaller subset of serotonergic than previously anticipated or might disappear under anesthesia.



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CENTRAL CHEMORECEPTION BY SEROTONIN NEURONS OF THE RAPHE

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Serotonin neurons of the medullary raphe are strong candidates for central respiratory chemoreceptors. They are intrinsically chemosensitive to changes in pH, even after acute dissociation. They increase their firing rate by 300% in response to a decrease in pH from 7.4 to 7.2. They are closely adjacent to the Basilar Artery, the largest artery in the brainstem, where they would accurately measure changes in blood PCO₂ and thus lung ventilation. There have been three main arguments against the proposal that 5-HT neurons are central chemoreceptors. First, different labs found that 5-HT neurons stimulate, inhibit or “modulate” respiratory output. Second, recordings from 5-HT neurons under isoflurane or halothane anesthetized mice in vivo failed to reveal an increase in firing rate in response to hypercapnia. Third, some data suggest that the pH response of 5-HT neurons is mediated by TASK channels and the HCVR was normal in TASK KO mice. There is now definitive evidence against each of these arguments. 1) 5-HT neurons have now clearly been shown to stimulate respiratory output in brain slices and in vivo. Genetic deletion of >99% of them (in *Lmx1^{bf/f/p}* mice) causes a 50% decrease in the HCVR. Older data lacked specificity for 5-HT neurons. 2) Isoflurane and halothane anesthesia have a marked depressant effect on the ventilatory response to CO₂. Other studies in unanesthetized animals in vivo show that 5-HT neurons are pH sensitive. 3) The pH response of mature 5-HT neurons is not mediated by TASK, but by a CAN current. TASK channels are abundantly expressed in 5-HT neurons, but are also found in many neurons that are not pH sensitive. These data strongly support the hypothesis that 5-HT neurons are chemoreceptors that drive breathing and cause arousal from sleep in response to hypercapnia.



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DECODING THE BRAIN SEROTONERGIC SYSTEM: INTERSECTIONAL GENETICS AND FUNCTIONAL PROBING

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Central serotonin-producing neurons are heterogeneous – differing in embryonic origin, final location, morphology, firing properties, and associated clinical disorders – but the underpinnings and functional implications of this heterogeneity are just beginning to be explored. To examine this heterogeneity, we have generated genetic tools for use in mice that allow multiple features of a neuron type to be delineated and linked in vivo, for example, its origin in the embryo, fate in the adult, and function in particular circuits as relates to behavior and physiology. Our starting point has been development of a dual recombinase (Cre and Flpe)-based molecule delivery system with plug-n-play modularity such that most any genetically-encoded lineage tracer or effector molecule can be incorporated and delivered in vivo to most neuron types. Neuron types are defined by combinatorial gene expression, making cell-type specificity high. Using these tools, we have generated a classification scheme for serotonin neurons that is based on genetic programs differentially enacted among serotonergic precursor cells and/or mature neurons and which represents a more mechanistic view of serotonergic neuron heterogeneity than offered by anatomical segregation. Neuronal silencing tools to plot cellular functions to these different serotonergic lineages will be presented. Through these approaches, we are redefining serotonin neuron subtypes and their contributions to the regulation of specific behaviors and physiological processes.



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VITAL SOURCE OF SEROTONIN: THE PLACENTA

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Serotonin is crucial for foetal development. But, where does serotonin come from during foetal development? Our group and other recently have shown that the rate limiting enzymes in serotonin biosynthesis, tryptophan hydroxylase (TPH)-1 and -2, and serotonin transporter are expressed and catalytically active in mice and human trophoblast (the functional placental cells). These findings suggest that both mother and placenta provide the developing foetus with serotonin long before it is able to synthesize its own neurotransmitter. Thus, the placenta which is the key regulatory organ maintaining maternal and foetal homeostasis, can locally produce serotonin, which is essential for maternal health and foetal development. Nevertheless, the question remains for placentologist, what is the role, locally, of this placental serotonin? Data from our team show that serotonin has a crucial auto/paracrine role in trophoblast development and estrogens production as well as placental haema topoiesis. Since the placenta is part of the so-called "foetal life-support system" our data support a vital direct and indirect role for serotonin in embryo and foetal development.



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KEY FUNCTION OF SEROTONIN IN ERYTHROCYTE PRODUCTION AND SURVIVAL

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The ability to store erythrocytes or red blood cells (RBC) for later transfusion to patients has become a highly valuable tool in healthcare. During their in vitro storage, RBC undergo storage lesions, including proteolysis of the major membrane protein band-3 that compromise their in vivo survival post-transfusion, leading to negative clinical outcomes in recipients. Clearly, there is a need for innovative technologies that can improve RBC quality under storage conditions. Recently it has been shown that endogenous 5-HT is essential for efficient RBC production and in vivo survival. Here, we sought to examine whether addition of exogenous 5-HT to RBC nutritive solutions can improve the quality of stored RBC and their in vivo recovery post-transfusion. Objectives and methods : 1) To evaluate the influence of 5-HT (10-100 μ M) on RBC storage lesions in routine blood bank operations with leukoreduced RBC from 6 healthy donors; 2) To evaluate the influence of RBC storage in the presence of 5-HT (100 μ M) on post-transfusion RBC recovery, using the wild-type C57BL/6 mouse model. Results : Addition of 5-HT to the nutritive solution AS-3 consistently delayed band-3 proteolysis during RBC storage. Furthermore, 24-hr RBC in vivo recovery post-transfusion was increased by 29% when RBC were stored for 7 days in the presence of 5-HT, as compared to RBC stored in the absence of 5-HT. Conclusions : 5-HT improves RBC integrity during storage in blood bank conditions, and it improves in vivo recovery post-transfusion. These findings suggest that RBC storage in the presence of 5-HT may provide safer clinical outcomes in patients post-transfusion.



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SEROTONERGIC SYSTEM AND BONE CELLS : NEW PARACRINE AUTOCRINE PATHWAYS

Corinne COLLET

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Recently, bone has also emerged as a target for serotonin. This has resulted in a multiplicity of data that it has been difficult to reconcile. Firstly, several in-vitro studies have reported the existence of serotonin receptors and/or of a functional serotonin transporter in osteoblast cells. Then, the bone phenotype of the 5-HTT^{-/-} mice was described; they presented a decrease bone formation as the 5htr2b^{-/-} mice. However, these studies could not provide any clue about the physiological role of serotonin in bone remodelling in vivo. At this point Yadav et al suggested that circulating serotonin produced by the gut was responsible for the reduction of osteoblast proliferation via the 5-HTR1D receptor on osteoblasts. Besides, we observed an increase in bone mass in the growing Tph1^{-/-} male mice that was resolved at maturity. In both juvenile and mature mice there was evidence of decreased bone resorption with an unchanged bone formation. In ex -vivo studies, we showed that osteoclast differentiation was reduced in Tph1^{-/-} animals. Also, osteoclasts were able to synthesize serotonin and this synthesis was increased by RANKL. These data could not have been observed by Yadav et al, as they reported only specific invalidation of Tph1 in the gut. Our result shows for the first time that there is a source of serotonin in the bone microenvironment. Although our data show that serotonin increases osteoclastogenesis by a paracrine/autocrine mechanism, but the physiological role of serotonin in osteoblastogenesis is less clear. Low serotonin level present in the bone micro-environment produced by osteoclasts would trigger activated proliferation of both osteoclast and osteoblast precursors. The reality is certainly more complex, and many questions, mainly concerning the role of SERT, are still awaiting answers. It is therefore important that clinical and experimental studies using different models should be lead to provide answers to these questions.



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SEROTONIN RECEPTOR 5-HT_{2B} POTENTIATES INTERFERON- γ PRODUCTION BY ACTIVATING T LYMPHOCYTES

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Serotonin (5-HT) is a neurotransmitter and an important immunomodulator. There are at least fourteen 5-HT receptors (5-HTRs) in mice and some of them are expressed in T cells. Among them, 5-HT_{2A} is known to amplify interleukin-2 (IL-2) and interferon- γ (IFN- γ) secretion. Although 5-HT_{2B} receptor has been characterized in several non-immune cell types, little is known about its function in T cells. Hypothesis: 5-HT_{2B} is expressed on murine T lymphocytes and it regulates their function. Objectives: 1) To assess 5-HT_{2B} mRNA expression in T cell subpopulations. 2) To investigate 5-HT_{2B} role in IL-2 and IFN- γ production by T cells. Methods: T cells were sorted from mouse spleen into CD3⁺, CD4⁺, CD8⁺, and CD4-CD8⁻ subpopulations. T cells were activated with phorbol 12-myristate 13-acetate and ionomycin in the presence or absence of selective 5-HT_{2A} or 5-HT_{2B} antagonists. mRNAs were detected by standard RT-PCR or real time PCR, while IL-2 and IFN- γ proteins were detected by intracellular staining followed by flow cytometry analysis. Results: 5-HT_{2A} and 5-HT_{2B} are expressed in all resting or activated T cell subsets. Selective antagonists of 5-HT_{2A} and 5-HT_{2B} decrease mRNA expression of IL-2 and IFN- γ and also reduce secretion of those cytokines in activated T cells, while having no effect on cell death or proliferation. These data are obtained without adding any exogenous 5-HT, corroborating the significance of endogenous 5-HT produced by activated T cells. Conclusion: This is the first demonstration of a functional expression of 5-HT_{2B} in mouse T cells. These results may help to improve understanding of 5-HT and 5-HTRs functions in immunity



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A NOVEL APPROACH FOR DEFINING THE ROLE OF SEROTONIN IN DRUG ACTION: SERT ILE172MET MICE

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Altered serotonin signaling has been recognized as contributing to the actions of antidepressants, including tricyclics, SSRIs, NSRIs, and triple uptake inhibitors, as well as psychostimulants such as cocaine. As these agents are known to interact with many targets, even with said to be «specific» for the serotonin transporter (SERT), the role contributed by altered serotonin signaling in their cellular and behavioral actions remains in further need of clarification. We identified a single amino acid in human SERT that dictates high affinity interaction with antidepressants and cocaine but does not dictate serotonin recognition. We introduced a substitution at this residue in the mouse SERT genomic locus and determined that, as in transfected cell studies, SERT exhibited normal expression and serotonin uptake kinetics, but lost high affinity recognition of fluoxetine, citalopram and cocaine. I will describe these studies, as well as the ongoing use of the mutant mice to investigate how antidepressants and cocaine rely, or not, on altered serotonin signaling for their actions.



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ROLE OF PIP2 AND PLC IN MEDIATING AMPHETAMINE-EVOKED ALTERATIONS IN SERT FUNCTION

Harald SITTE

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Neuronal functions, such as excitability or endo- and exocytosis, require phosphatidylinositol-4,5-bisphosphate (PIP2) since ion channels and other proteins involved in these processes are regulated by PIP2. Monoamine transporters control neurotransmission by removing monoamines from the extracellular space. They also display channel properties, but their regulation by PIP2 has not been reported. The psychostimulant amphetamine acts on monoamine transporters to stimulate transporter-mediated currents and efflux and thereby increases the levels of extracellular monoamines. Direct or receptor-mediated activation of phospholipase-C (PLC) reduced membrane PIP2 and amphetamine-evoked currents through recombinant serotonin transporters; extracellular application of a PIP2-scavenging peptide mimicked this effect. PLC activation also diminished amphetamine-induced reverse transport without altering transmitter uptake. Inhibition of reverse transport by PLC activation was also observed in brain slices and with recombinant dopamine and noradrenaline, but not GABA transporters; rises in intracellular Ca^{2+} or activation of protein kinase C were not involved in these effects. These data demonstrate for the first time PIP2-dependence of reverse transport and current in monoamine transporters.



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SERT AS A THERAPEUTIC TARGET FOR COCAINE DEPENDENCE

F. Gerard MOELLER, Shijing Liu, Joy M. Schmitz, Scott D. Lane, Charles Green, Kathryn A. Cunningham
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Initial placebo controlled studies using SSRIs as treatments for cocaine dependence took place almost 20 years ago. Since that time basic animal and human behavioral studies have shown that all SSRIs are not interchangeable, differing in their effects on other receptors as well as in their interaction with cocaine. Additionally, there has been a growing body of preclinical evidence linking cocaine self-administration with other serotonin receptors. Based on these findings, a clinical trial using the SSRI citalopram combined with the behavioral therapy contingency management for cocaine dependence was completed. Results of that trial showed that cocaine dependent patients treated with citalopram showed significantly greater reductions in cocaine positive urine drug screens compared with cocaine users treated with placebo. Further, our research group showed that those individuals with impaired decision-making did not respond to citalopram. More recently, studies on impulsivity and cue reactivity in human cocaine users have been completed. Results of that study showed that acute doses of 20mg of escitalopram significantly reduced cocaine cue reactivity as measured by attentional bias on the cocaine-Stroop task. However, differences between escitalopram and placebo treated subjects on cue reactivity were not significant after chronic administration of study medication. These findings will be discussed in light of preclinical research on the role of the 5-HT_{2C} and 5-HT_{2A} receptors in cocaine self administration, and potential effects of SERT gene polymorphisms on impulsivity and cue reactivity.



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EXAMINING THE INTERACTION BETWEEN 5-HT_{1B} RECEPTORS AND SERT

Yusha (Katie) LIU

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5-HT_{1B} autoreceptors are optimally positioned to provide immediate feedback and precise spatial and temporal control of synaptic 5-HT levels, affecting the dynamics of serotonergic neurotransmission throughout the entire brain. These autoreceptors regulate 5-HT release but also enhance serotonin transporter (SERT) function; we have developed an in vitro uptake assay with synaptosomes using rotating disk electrode voltammetry (RDEV) to investigate this interaction in detail. Inhibition of 5-HT_{1B} autoreceptors with SB224289 produces a dose-dependent decrease in SERT function in synaptosomes from wild-type (WT) but not 5-HT_{1B} knockout (1BKO) mice. Furthermore, SERT function in WT synaptosomes was significantly greater compared to that in 1BKO synaptosomes. Additionally, viral-mediated 5-HT_{1B} overexpression in the dorsal raphe nucleus (DRN) in rats resulted in increased SERT activity in hippocampal synaptosomes. We are currently defining the optimal conditions to detect 5-HT_{1B} agonist-stimulated SERT activity, which has been elusive due to 5-HT interactions with both the autoreceptor and SERT. We are also examining whether 5-HT_{1B} regulation of SERT involves altered SERT trafficking or catalytic activity. 5-HT_{1B} autoreceptors also modulate emotional behavior. We have developed a novel viral vector that expresses 5-HT_{1B} receptors under the control of the SERT promoter (SERT-1B); stereotaxic DRN injections drive 5-HT_{1B} receptor expression exclusively in serotonergic neurons, thus allowing cell-type specific targeting of the vector to mimic 5-HT_{1B} autoreceptor expression. WT and 1BKO mice were injected with either the SERT-1B or SERT-GFP vector and tested in a contextual fear conditioning paradigm. WT and 1BKO mice receiving SERT-GFP displayed similar levels of conditioned freezing behavior, while WT and 1BKO mice receiving SERT-1B demonstrated significantly reduced freezing, showing that increased 5-HT_{1B} autoreceptor activity reduces conditioned fear expression. The selective manipulation of these receptors has striking behavioral effects and further implicates an important role for 5-HT_{1B} autoreceptors in fear-related psychiatric disorders.



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THE SEROTONIN TRANSPORTER (SERT) IN BLOOD PRESSURE REGULATION

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Though best known as a vasoconstrictor, 5-HT (iv) lowers the blood pressure of the intact animal. This reduction occurs as long as infusion is carried out (up to 30 days tested). Using flow probes, we found that total peripheral resistance is reduced by 5-HT infusion, indicating a reduction in arterial tone. We initially hypothesized that 5-HT reduced TPR through direct vasodilation. However, we have not observed 5-HT-induced relaxation in arteries from the rat (aorta, superior mesenteric artery, mesenteric resistance artery). We reformulated this hypothesis to investigate 5-HT-stimulated withdrawal of sympathetic tone from the vasculature, an event that would support a fall in blood pressure. In isolated superior mesenteric artery prepared for electrical field stimulation (EFS), there was no evidence for presynaptic inhibition of sympathetically-driven contraction by 5-HT (1 nM – 10 μ M). In instrumented animals, we measured pre- and postganglionic splanchnic sympathetic nerve activity (SNA) before and during infusion of 5-HT (iv). 5-HT did not modify activity in either nerve. However, acute 5-HT infusion (iv) significantly reduced SNA controlling brown adipose tissues (BAT) in anesthetized rats. Since BAT SNA changes often parallel those in cutaneous vasoconstrictor SNA, this finding suggests that 5-HT could induce cutaneous vasodilation. Doppler studies support the ability of 5-HT to increase tail artery (cutaneous) flow. The ability of 5-HT, given peripherally, to inhibit BAT activity suggests that 5-HT is entering the CNS to withdraw sympathetic tone. As SERT is the most logical candidate for 5-HT movement into the brain, we tested whether removal of SERT would reduce the ability of 5-HT to reduce blood pressure. We found that the fall in blood pressure caused by 5-HT was reduced 50% in the SERT male KO vs WT rats. Collectively, these data are consistent with peripheral 5-HT entering the central nervous system to inhibit cutaneous vasoconstrictor sympathetic tone.



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THE 5-HT₄ SEROTONIN RECEPTOR: PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL ROLES IN THE HEART VENTRICLE.

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In heart failure (HF), human and rat cardiac ventricles become sensitive to serotonin (5-HT), eliciting positive inotropic (increased contractility) and lusitropic (enhanced relaxation) effects through cAMP-coupled 5-HT₄ serotonin receptors. In chronic HF, treatments increasing cAMP increase mortality, and blockade of cAMP-coupled beta-adrenoceptors (β -AR) improve survival. We evaluated the effect of chronic treatment with the 5-HT₄ antagonist SB207266 (piboserod) in rats and patients with HF, and found effects consistent with HF improvement. Further studies are needed to conclude if such treatment will be useful in HF. The cAMP-dependent effects of 5-HT₄ receptor stimulation in failing ventricles are mainly limited by phosphodiesterase (PDE) 3, with a role of PDE4 demasked by PDE3 inhibition. Since cGMP can competitively inhibit PDE3, we tested if 5-HT₄-mediated effects could be enhanced by cGMP from particulate (natriuretic peptide receptor (NPR) A and B) and soluble (NO-stimulated) guanylyl cyclase. In HF rats (6 weeks post-infarction), NPR-B stimulation (with CNP), but not NPR-A stimulation (ANP, BNP) increased both 5-HT₄- and β 1-AR-mediated inotropic response similar to PDE3 inhibition by cilostamide. Inhibition of nitric oxide (NO) synthesis by L-NAME and soluble GC by ODQ attenuated the 5-HT₄-mediated inotropic response, whereas the NO donor Sin-1 increased this response. The effects were absent during PDE3 inhibition, suggesting cGMP-dependent inhibition of PDE3. In contrast, Sin-1 inhibited, whereas L-NAME and ODQ enhanced the β 1-AR-mediated inotropic response. Thus, cGMP generated both by particulate (NPR-B) and soluble GC increases the 5-HT₄-mediated inotropic response in failing hearts, probably through PDE3 inhibition. β 1-AR and 5-HT₄ receptor signalling are subject to opposite regulatory control by cGMP from soluble GC in failing hearts.



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ROLE OF SEROTONIN AND 5-HT₂ RECEPTORS IN CELLULAR PROCESSES INVOLVED IN CARDIAC VALVE REMODELING

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Cardiac valve degeneration is a current process due to tissue remodelling associated with aging. Some haemodynamic situations and cardiovascular risk factors such as hypertension, dyslipidaemia and diabetes mellitus markedly increase the speed of this natural phenomenon. In front of this “physiological” cardiovascular degeneration, some pathological conditions (rheumatic fever, endocarditis, rheumatoid arthritis ...) affect valve tissue. Apart these classical aetiologies, a recent entity emerged that could be named “serotonergic valve disease”. Lesions exhibit typical features that are common to the so-called carcinoid heart, drug-induced valvulopathy and the canine mitral degenerative valve disease: an important cell proliferation in a dense glycosaminoglycan matrix without inflammation and no osteogenesis. Indirect experiments suggest that 5-HT_{2B} receptors are main targets of 5-HT and drugs (or their active metabolites) to trigger cardiac valve remodelling. However, some other works suggested the involvement of the 2A subtype. The mechanisms by which 5-HT₂ receptors control valvular interstitial cell proliferation and differentiation is also far from being understood. Moreover, a contribution of circulating progenitors is suggested as a contributor to valve degeneration. This talk will discuss all these aspects with a particular emphasis of new potential therapeutic targets in cardiac valve protection.



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REFINING THE SEROTONERGIC CONTRIBUTION TO CENTRAL CHEMOSENSITIVITY

Rachael BRUST , Andrea Corcoran, Russell Ray, Eugene Nattie, George Richerson, Susan Dymecki
Harvard Medical School, Boston, USA

The involvement of brain serotonergic neurons (5HT neurons) in a remarkably wide range of physiological functions and disorders motivates deeper examination of their heterogeneity with respect to developmental origin, functional properties, and molecular expression. We are using these characteristics to subdivide 5HT neurons and study the involvement of molecularly defined subtypes in different functions. Here, we're interested in the role of 5HT neurons in the central respiratory chemoreflex (increasing ventilation in response to elevated CO₂ to restore blood gas/pH to normal). To test this more definitively, we developed a transgenic mouse capable of inducibly and reversibly inhibiting 5HT neuron activity in the awake and behaving mouse, building on the Di system developed by Bryan Roth (Armbruster et al, 2007). We found that suppressing 5HT neuron activity causes a decreased ventilatory response to breathing 5%CO₂ (Ray et al, 2011). We now apply this approach toward silencing 5HT neuron subtypes to identify those that contribute most to the CO₂ respiratory chemoreflex. Using dual recombinase (Cre/Flpe)-based intersectional genetics we have labeled and manipulated subtypes of 5HT neurons defined by developmental gene expression differences. Findings highlight a subset defined by history of transcription factor Krox20 (Egr2) expression and hindbrain rhombomere-5 developmental origin. Using electrophysiology, we find that this same population is chemosensitive in vitro (increase firing rate in response to decreased pH/elevated CO₂, a property not shared among all 5HT neurons). Further, immunohistochemical studies demonstrate projections from eGFP-labeled Krox20-5HT neurons to other brainstem nuclei involved in cardiorespiratory control, including other non-5HT neurons implicated in central chemosensitivity. This work defines a molecular and developmental 5HT neuron subtype involved in CO₂ chemosensitivity and extends our understanding of the role of 5HT neurons in a vital homeostatic network.



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SYMPOSIUM 7



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ROLE OF THE 5-HT₆ RECEPTOR IN EMBRYONIC PYRAMIDAL NEURON MIGRATION

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Serotonin (5-HT) is a neurodevelopmental signal that regulates a variety of cellular processes involved in the formation of cortical circuits. Furthermore rodent and human studies indicate that developmental alterations in serotonin signaling leads to vulnerability to psychiatric-related disorders. Recent data in our laboratory indicates that embryonic excess of serotonin affects the migration of upper layer pyramidal neurons. The 5-HT₆ receptor is expressed in the embryonic developing cortex and pharmacological manipulation of this receptor affects pyramidal neuron migration in brain slices. To further determine the role of the 5-HT₆ receptor in pyramidal neuron migration we used an in vivo cell-specific genetic approach. Using in utero electroporations targeted to the E14.5 ventricular zone of the dorsal pallium, we studied the consequences of 5-HT₆ loss of function on pyramidal neuron positioning at E18.5 and P7. We observed that shRNA-mediated down-regulation of the 5-HT₆ receptor significantly altered the morphology and migrating of migrating pyramidal neuron and decreased the percentage of pyramidal neurons reaching the cortical plate. Analysis of brains at P7 indicated that a fraction of 5-HT₆R shRNA electroporated cells were abnormally misplaced in lower cortical layers and expressed the upper cortical layer marker CUX1. These results indicate that the 5-HT₆ receptor regulates pyramidal neuron migration and sheds a new light upon the developmental role of the serotonin system in cortical development.



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5-HT₆ RECEPTORS DISRUPT COGNITION BY RECRUITING MTOR: RELEVANCE TO SCHIZOPHRENIA

Phillippe MARIN¹, Julie Meffre¹, Séverine Chaumont-Dubel¹, Clotilde Mannoury la Cour²,
Florence Loiseau², David J.G. Watson³, Anne Dekeyne², Martial Séveno¹,
Paul Déléris¹, Kevin C.F. Fone³, Joël Bockaert¹, Mark J. Millan²

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Cognitive deficits of schizophrenia seriously compromise quality of life but are poorly controlled by currently available antipsychotic agents. While 5-HT₆ receptor blockade holds special promise, the signalling mechanisms underlying control of cognition by the receptor remain largely unknown. Using a proteomic approach, we found that 5-HT₆ receptors physically interact with several proteins of the mammalian target of rapamycin (mTOR) pathway, including mTOR itself. Further, activation of 5-HT₆ receptors increased mTOR signalling in HEK-293 cells and in rodent prefrontal cortex. 5-HT₆ receptor-elicited mTOR signalling required both the canonical PI3K/Akt/Rheb pathway involved in mTOR activation by growth factors and 5-HT₆ receptor/mTOR physical interaction. Linking this signalling event to cognitive impairment, the mTOR inhibitor rapamycin prevented deficits induced by a 5-HT₆ agonist in models of social cognition. Mimicking the effects of 5-HT₆ receptor antagonists, rapamycin also rescued the deficit of social discrimination in rats treated with phencyclidine at the neonatal stage (well-characterized developmental model of schizophrenia). Moreover, we found in prefrontal cortex of adult phencyclidine-treated rats an enhanced mTOR signalling that was blocked by a 5-HT₆ receptor antagonist. These studies identify a signalling complex physically associated with the 5-HT₆ receptor that underlies its deleterious influence upon cognition. They suggest a critical role of mTOR activation not only in rare autism-related genetic disorders, such as Fragile X mental retardation syndrome and tuberous sclerosis, but also as a factor triggering the currently-untreatable cognitive deficits of schizophrenia, a more common, multi-factorial and debilitating disorder.



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FUNCTIONAL ROLE OF 5HT6 RECEPTOR IN THE NEURO-DEVELOPMENTAL COGNITIVE DEFICITS SEEN IN SCHIZOPHRENIA

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5-HT₆ receptor antagonists reverse drug-induced, time delay, or aged dependent deficits in learning and memory paradigms, such as Morris water maze, novel object discrimination (NOD) or associative learning. However, few studies have examined the ability of 5-HT₆ receptor antagonists to reverse neurodevelopmental cognitive deficits such as produced by rearing rats in social isolation from weaning, which models changes seen in schizophrenia, as reported herein. Male Lister hooded rats weaned on post-natal day 21-24 and housed individually for six weeks post-weaning show increased locomotor activity in a novel arena, and impairment in NOD and conditioned emotional freezing (CER) compared to their group-housed littermates. Acute i.p. injection of 5-HT₆ receptor antagonists, such as SB-271046 (10mg/kg) or SB-258585 (2.5 or 10mg/kg) 30min before each behavioural test reversed NOD and CER deficits in isolation reared rats. Drugs enhancing cholinergic mechanisms also reverse NOD and CER deficits, suggesting this may also contribute to these 5-HT₆ antagonist effects. Interestingly the extent of phosphorylation of mammalian target of rapamycin (mTOR, a protein linked to long-term potentiation) was elevated in the prefrontal cortex of isolation reared rats and like the deficit in NOD this was reduced to levels in group-housed controls 30 min after treatment with SB-258585 or the mTOR inhibitor, rapamycin (10mg/kg). These data show that 5-HT₆ receptor antagonists can reverse neurodevelopmental deficits in learning and memory in a preclinical model of schizophrenia, and suggest that alteration in mTOR signalling may contribute to the acute precognitive effect of these drugs on visual learning and memory.



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EFFECT OF THE 5-HT₆ RECEPTOR ANTAGONIST, SB-399885, ON COGNITION, HIPPOCAMPAL CELL PROLIFERATION AND PROTEIN EXPRESSION IN THE NEURODEVELOPMENTAL MODEL OF SCHIZOPHRENIA

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Post-weaning social isolation in the rat induces a range of behavioural and neurochemical alterations similar to those in schizophrenia. The current study evaluated the effect of a 5-HT₆ receptor antagonist, SB-399885, on cognition and feeding behaviour in this neurodevelopmental model of schizophrenia, then examined the impact of isolation rearing and 5-HT₆ receptor blockade on hippocampal cell proliferation and protein expression.

Male Lister-hooded rats were weaned on post-natal day 21-24 and housed individually or in groups of 3-4. They underwent locomotor activity, novel object discrimination (NOD), prepulse inhibition of acoustic startle and conditioned emotional response (CER) tests, beginning six weeks post-weaning. Rats (n=11/group) received six i.p. injections of saline vehicle (1ml/kg) or SB-399885 (5 or 10mg/kg) 30min before testing (or immediately after CER acquisition). Rats were killed 24h after the final injection and brains collected for Ki67 immunohistochemistry, Western blots and protein microarrays.

Both doses of SB-399885 significantly reduced weight gain across the treatment period, decreased cumulative food (but not water) intake, and reversed isolation rearing-induced NOD deficits ($P < 0.001$). Isolation rearing decreased the number of Ki67 positive cells in the dentate gyrus ($P < 0.05$) and increased hippocampal VGLUT2 ($P < 0.05$) and RAC/SDC42 expression. SB-399885 (10mg/kg) partially reversed these effects, elevated frontal cortical VGLUT3 expression ($P < 0.05$) and decreased hippocampal TAK1, pSTAT3, SEK1/MKK4 and pHSP27 expression.

These results confirm the procognitive effects of 5-HT₆ receptor antagonists are maintained in a neurodevelopmental model of schizophrenia, and suggest possible roles of the 5-HT₆ receptor in MAPK and Jak/Stat signalling pathways, and the regulation of hippocampal cell proliferation.



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SYMPOSIUM 8



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THE THERAPEUTIC POTENTIAL OF TARGETING INDIVIDUAL 5-HT₃ RECEPTOR SUBTYPES

Beate NIESLER

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The only ligand-gated ion channels under the serotonin receptors are serotonin type 3 (5-HT₃) receptors. 5-HT₃ receptors are formed by five subunits of diverse composition. In humans, five subunits have been identified (5-HT_{3A-E}) which are encoded by the genes HTR3A-E. These genes have been found to be expressed in various isoforms. Lately, factors influencing receptor expression, such as chaperones and microRNAs have been reported.

Due to their expression profile and physiological functions, 5-HT₃ receptors have been implicated in irritable bowel syndrome (IBS) and psychiatric disorders. 5-HT₃ receptor antagonists are beneficial in the treatment of these conditions. Interestingly, HTR3 variants have been shown to be associated with these disorders during the last decade. This underlines the potential of 5-HT₃ receptors as therapeutic targets and may enable personalized therapies in the future.

Background on 5-HT₃ receptors and their therapeutic potential as molecular target will be given (to include problems of 'classical' antagonism and also introduce atypical 5-HT₃ receptor antagonists e.g. ricasetron, palonosetron). Potential of 5-HT₃ receptor subtype-selective antagonists - exploiting structural differences at either the subunit interfaces comprising the orthosteric 5-HT binding site arising from the five different 5-HT₃ receptor subunits (5-HT_{3A-E}) or the arising ion channels. An outlook on the potential pharmacogenetic potential will also be addressed as well as alternative options manipulating the 5-HT₃ system by therapeutic microRNAs and antibodies.



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THE THERAPEUTIC POTENTIAL OF 5-HT₃ RECEPTOR PARTIAL AGONISTS

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Irritable bowel syndrome (IBS) is a functional bowel disorder characterized by abdominal pain, discomfort and altered bowel habits, which have a significant impact on quality of life for approximately 6% to 12% of population in western countries. IBS can be divided into three main types IBS-d (diarrhea predominant), IBS-c (constipation predominant) and mixed or alternating IBS. 5-HT₃ receptor antagonism has proved to be an efficacious treatment option for IBS-d. For example; alosetron displays excellent efficacy in the treatment of multiple symptoms, including abdominal pain, discomfort, urgency, stool frequency and consistency. However, significant constipation occurred in approximately 25% of patients, leading to withdrawal of up to 10% of patients in clinical trials^{1,2}.

Targeting compounds with partial agonist activity at the 5-HT₃ receptor represent a mechanistic departure from the classic 5-HT₃ receptor antagonist approach and should result in agents that are applicable to a broader array of IBS patient populations. Attenuation of the activity of the ion channel without completely abolishing its function may control or normalize bowel function without leading to a total block associated with severe constipation. We have identified a new class of selective, orally active 5-HT₃ receptor ligands with high 5-HT₃ receptor affinity and low partial agonist activity currently in preclinical development that should offer a significant advantage over existing therapies.



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TICKLING THE 5-HT₃ RECEPTOR; MOLECULAR STUDIES AND THERAPEUTIC POTENTIAL OF ALLOSTERIC 5-HT₃ RECEPTOR LIGANDS

Nicholas BARNES

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The 5-HT₃ receptor is an established therapeutic target with antagonists providing effective symptomatic relief from emesis (arising from cancer treatment or surgical procedures performed under general anaesthesia) and irritable bowel disease (IBS). Side-effects of constipation and, albeit rarely, ischemic colitis (the latter so far associated solely with the treatment of IBS) have limited the use of 5-HT₃ receptor antagonists. Indeed for most patients suffering from IBS, 5-HT₃ receptor antagonists have been effectively withdrawn leaving the patients essentially without suitable medication.

In common with other ligand-gated ion channels, there is growing awareness that the 5-HT₃ receptor complex is decorated with allosteric receptor sites distinct from the orthosteric 5-HT binding site. Consistent with the increasing interest in drugs that allosterically modulate receptor function – typically providing a more subtle adjustment of physiological activation – 5-HT₃ receptor allosteric modulators offer exciting opportunities for potential therapeutic benefit with a more desirable benefit:risk ratio.

To better understand allosteric modulation of the 5-HT₃ receptor, our studies have identified 5-chloroindole (Cl-indole) as a potent and selective Type II positive allosteric modulator at recombinant (h5-HT_{3A}, h5-HT_{3AB} and m5-HT_{3A}) and native (m5-HT₃) 5-HT₃ receptors. An understanding of the molecular binding site of Cl-indole will allow rationale design of molecules with more desirable pharmaceutical characteristics as drug candidates, which may offer therapeutic benefit over orthosteric 5-HT₃ receptor ligands.



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THE NATIVE SEROTONIN 5-HT_{5A} RECEPTOR: ELECTROPHYSIOLOGICAL CHARACTERIZATION IN RODENT CORTEX AND 5-HT_{1A}-MEDIATED COMPENSATORY PLASTICITY IN THE KNOCKOUT MOUSE

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The 5-HT_{5A} receptor is the least understood serotonin (5-HT) receptor. Here, we electrophysiologically identify and characterize a native 5-HT_{5A} receptor current in acute ex vivo brain slices of adult rodent prefrontal cortex. In the presence of antagonists for the previously-characterized 5-HT_{1A} and 5-HT₂ receptors, a substantial proportion of layer V pyramidal neurons continue to show unidentified 5-HT outward currents in both rats and mice. These 5-HT currents are suppressed by the selective 5-HT_{5A} antagonist, SB-699551, and are not observed in 5-HT_{5A} receptor knockout mice. Further characterization reveals that the 5-HT_{5A} current is activated by submicromolar concentrations of 5-HT, is inwardly-rectifying with a reversal potential near the equilibrium potential for K⁺ ions, and is suppressed by blockers of Kir3 channels. Finally, we observe that genetic deletion of the inhibitory 5-HT_{5A} receptor results in an unexpected large increase in the inhibitory 5-HT_{1A} receptor currents. The abundance of functional prefrontal 5-HT_{5A} receptors in normal rodents along with compensatory plasticity in 5-HT_{5A} receptor knockout mice testifies to the significance of this receptor in the healthy prefrontal cortex.



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ANTIPSYCHOTIC SIGNALING IN A 5HT2A-MGLU2 RECEPTOR HETEROCOMPLEX

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The serotonin and glutamate systems are suspected in the etiology and pathophysiology of schizophrenia, as well as in the mechanism of action of antipsychotic drugs. Atypical antipsychotic drugs all have in common a high affinity to antagonize serotonin 5-HT_{2A} receptors (5HT_{2A}s). Drugs that activate metabotropic glutamate 2 receptor (mGlu₂) represent potentially new antipsychotic medications. We found that 5HT_{2A} and mGlu₂ interact through specific transmembrane domains to form a specific receptor heterocomplex in tissue culture and mouse frontal cortex. Our results suggest that the 5HT_{2A}-mGlu₂ receptor heterocomplex may be responsible for some psychotic symptoms in schizophrenia, and that it is the target of these two different classes of antipsychotic drugs. Our findings also provide evidence that gene-environment interactions and epigenetic mechanisms affect the expression of 5HT_{2A} and mGlu₂ receptors in mouse models of schizophrenia and antipsychotic action



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IS RNA EDITING RELEVANT FOR SEROTONERGIC NEUROTRANSMISSION IN THE HUMAN CORTEX ?

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Previous studies have suggested that A-to-I RNA editing in the human cortex is altered in patients with psychiatric diseases—thereby altering serotonergic neurotransmission. In particular, changes in A-to-I RNA editing of the 5HT_{2C} receptor in the cortex in schizophrenia, major depressive disorder and in suicide have been reported. In addition, studies have demonstrated differences in A-to-I RNA editing of cortical 5HT_{2C} receptors after various pharmacological treatments altering the serotonergic system. However, the results have been highly inconsistent among studies and, given the methodology used, inconclusive. In order to determine whether or not A-to-I RNA editing plays a role in human disease and in serotonergic neurotransmission in the cortex, we utilized a novel ultra high-throughput sequencing method in order to quantitatively examine 29 A-to-I RNA editing sites in subjects with major depressive disorder and schizophrenia. We also examined if a range of genetic and pharmacologic treatments altered A-to-I RNA editing of the 5HT_{2C} receptor in mice. In contrast to prior studies, we did not find any significant differences in the frequency of RNA editing at any of the editing sites in the samples from patients including the 5HT_{2C} serotonin receptor. A few small but likely biologically irrelevant changes in A-to-I RNA editing were observed in the 5HT_{2C} serotonin receptor after pharmacologic treatments in mice. Thus, uHTS is a fast, quantitative and high-throughput method to assess RNA editing in human physiology and disease and that many prior studies of RNA editing have overestimated drug and disease-related variability of RNA editing in the mouse and human brain. In addition to our findings on RNA editing, new data revealing how direct and reversible modulation of serotonergic projections into the forebrain alters behavior may also be highlighted.



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INTERACTIVE EFFECTS OF 5-HT_{2A} AND MGLU₅ RECEPTORS ON BEHAVIOR IN MICE

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Metabotropic glutamate (mGlu) receptors have been suggested to play a role in neuropsychiatric disorders including schizophrenia, drug abuse, and depression. Because serotonergic hallucinogens increase glutamate release and mGlu receptors modulate the response to serotonin (5-HT)_{2A} receptor activation, the interactions between serotonin 5-HT_{2A} receptors and mGlu receptors may prove to be important for our understanding of these diseases. 5-HT_{2A} receptors and mGlu₅ receptors are known to influence several behaviors in mice, and we investigated whether there are behavioral interactions between these receptors. Experiments performed in the Behavioral Pattern Monitor (BPM), which assesses exploratory and investigatory behavior in rodents, confirmed that functional interactions occur between 5-HT_{2A} and mGlu₅ receptors. Male and female mGlu₅ knockout mice on a C57 background showed locomotor hyperactivity compared with their wild-type littermates, and the hyperactivity in the knockouts was attenuated by the selective 5-HT_{2A} antagonist M100907. M100907 also blocked the hyperactivity induced by the mGlu₅-negative allosteric modulator MPEP in C57 mice. These findings demonstrate that hyperactivity induced by a reduction or loss of mGlu₅ signaling is dependent on the 5-HT_{2A} receptor. Additionally, deletion of the mGlu₅ gene increased the behavioral response to the 5-HT_{2A/2C} agonist 2,5-dimethoxy-4-methylamphetamine (DOM), suggesting that mGlu₅ receptors either mitigate the behavioral effects of 5-HT_{2A} agonists or that mGlu₅ knockout mice show an increased sensitivity to 5-HT_{2A} activation. In contrast to our BPM findings, MPEP did not significantly alter the head twitch response induced by 5-HT_{2A} activation in mice, indicating that the behavioral interaction between 5-HT_{2A} and mGlu₅ receptors may be specific to locomotor activity. Taken together, these studies demonstrate that functional interactions occur between mGlu₅ and 5-HT_{2A} receptors. Possible mechanisms for these interactions, as well potential therapeutic implications, will be discussed.



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HIGHER-ORDER INTERACTIONS OF GABA AND GLUTAMATE AXONS IN THE RAT DORSAL RAPHE NUCLEUS VISUALIZED BY ARRAY TOMOGRAPHY

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The dorsal raphe nucleus (DR) controls forebrain serotonin (5-Hydroxytryptamine, 5-HT) neurotransmission to influence emotional states. DR neurons are regulated by a complex network of excitatory, glutamatergic and inhibitory, GABAergic axons. However, organization of the synaptic neuropil within the DR remains poorly understood. The recently developed high-resolution immunofluorescence technique Array Tomography (AT) allows identification of multiple antigens within the same tissue volume and quantitative analysis of their spatial relationships. We used AT to examine associations between glutamate and GABA axons within the rat DR by visualizing glutamatergic (Vesicular Glutamate Transporter, VGLUT1-3) and GABAergic (Glutamate Decarboxylase 65, GAD2) synaptic markers, synapsin I for synaptic boutons and tryptophan hydroxylase for 5-HT cells. Quantitative analysis of rendered volumes containing immunolabeling for all 6 antigens showed that VGLUT2- and GAD2-labeled boutons were more numerous within the DR than those containing VGLUT1 or VGLUT3. Examining the relationship between different types of axons revealed preferential associations between GABAergic axons and each types of glutamatergic axon, suggesting the existence of axo-axonic interactions. Ultrastructural analysis subsequently showed GAD2-immunolabeled axons apposed non-GABA boutons without intervening glia, confirming associations between presynaptic axon terminals. Further using AT, GABA-A receptors were localized to glutamate axons adjacent to GABA axons. Thus in this study AT reveals a unique feature of network architecture of the DR: a population-based presynaptic association between glutamate and GABAergic axons.



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CONTRIBUTION OF SEROTONIN_{1A} AUTO AND HETERORECEPTORS TO DEPRESSION AND ANTIDEPRESSANT RESPONSE

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The serotonin-1A receptor (5-HT_{1A}R) has been implicated in both the etiology of depression and anxiety as well as in mediating the response to antidepressant medications. Indeed, epidemiological data from human studies suggest that a polymorphism in the promoter of the gene encoding the 5-HT_{1A}R is related to increased susceptibility to depression and poor treatment response. Elucidating the biological mechanisms underlying 5-HT_{1A} functions is complicated by the fact that it exists both as an autoreceptor that limits serotonin release throughout the brain and as a heteroreceptor that mediates inhibitory responses to serotonin. Using a transgenic mouse approach that allows independent manipulation of either auto or heteroreceptors, we have found that these receptor sub-populations have different roles in developing compared to adult animals. Specifically, suppression of autoreceptors beginning in early development affects adult levels of anxiety without affecting adult depression-like behavior. Additionally, suppression of heteroreceptors beginning in early development results in a depression-like phenotype in adulthood without affecting anxiety levels. In contrast, manipulation of autoreceptor levels in adult animals alters the response to antidepressant treatment without affecting baseline anxiety levels.



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SEROTONERGIC TRANSCRIPTIONAL NETWORKS CONTROLLING DORSAL RAPHE CYTOARCHITECTURE AND FUNCTION

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5-HT neuron development comprises a brief stage of neurogenesis followed by a prolonged maturation stage during which newly born 5-HT neurons migrate to form the various raphe nuclei, extend axonal projections to form the ascending and descending 5-HT subsystems and finally integrate with target circuitry by making synaptic connections. A transcriptional network directing serotonergic neurogenesis induces 5-HT neuron signaling through activation of a gene battery encoding 5-HT synthesis, vesicular transport, reuptake and metabolism. Expression of several network factors continues through fetal and postnatal life suggesting functions in maturing and adult 5-HT neurons. We have developed 5-HT neuron-type conditional targeting approaches to investigate the importance of serotonergic transcriptional networks in the maturation and maintenance of the brain 5-HT system. Serotonergic gene targeting is enabled with a constitutive cre recombinase line, ePet-Cre, that becomes active during fetal life in immature postmitotic 5-HT neurons and an inducible line, ePet-CreER, that can be activated at different stages of life. Targeting of Pet-1, a transcription factor that induces 5-HT neuron identity, showed that it is required during the maturation stage for dorsal raphe (DR) expression of Hrt1a and Hrt1b autoreceptors. Pet-1 deficiency prevented expression of the autoreceptor genes in nearly all DR 5-HT neurons suggesting Pet-1 is indispensable in most of these neurons. Targeting of Pet-1 in adult 5-HT neurons showed that it is required to maintain normal anxiety-like behaviors. Conditional targeting of the Engrailed paralogs, En1 and En2, homeodomain factors required for serotonergic progenitor specification, identified a subsequent stage of intrinsic En function during which En1/2 regulate DR cytoarchitecture, perinatal maintenance of serotonergic identity, and 5-HT neuron survival. Our 5-HT neuron-type conditional targeting approaches are revealing maturation-stage specific functions of intrinsic serotonergic transcriptional networks.



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RAPHE NEURON DEVELOPMENT AND ITS PERTURBATIONS BY ALTERED TRANSCRIPTION AND RECEPTOR DELETION

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Increasing evidence suggests that mood disorders have a developmental component. Behavioral symptoms of mood disorders are present in children as early as 8 years old; early life stress is linked with increased incidence of anxiety and depression as adults. The serotonin (5-HT) system is a major focus of research in anxiety and depression because alterations in this system leads to mood disorders. Early life stress or treatment with antidepressants during the first three weeks of life leads to anxiety-like behavior in adults. Time-specific gene targeting experiments showed that the 5-HT_{1A} auto receptor deletion during the first three weeks of life is important for normal anxiety behavior. Anxiety phenotype is also present in the Pet-1 knockout mouse. Using whole cell electrophysiology, 5-HT neurons in the dorsal raphe, which provide 5-HT to most of the forebrain, have an immature phenotype four days after birth (P4). The neurons are depolarized, hyper-excitable, have increased membrane resistance, lack synaptic activity, and show no response to 5-HT_{1A} receptor activation. By P21, 5-HT neurons were hyperpolarized, less excitable, show synaptic activity, and a marked 5-HT_{1A} mediated outward K⁺ current. Examination of the cell physiology of 5-HT_{1A} knockout mice at P12 and P21 demonstrated an immature phenotype similar to that seen in normal P4 neurons. Neurons from the Pet-1 knockout adult mice also exhibited immature physiological characteristics. We conclude that 5-HT neurons are vulnerable early in development because they are immature after birth; genetic perturbations or environmental stressors lead to the lack of development of innate ion channels and/or receptor mediated responses in the first 21 days, providing targets for alterations that lead to mood disorders.



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SHAPING PATTERNS OF SEROTONIN NEUROTRANSMISSION VIA FEEDBACK CIRCUITS

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Long known, serotonin neurons in the brain are anatomically heterogeneous. Their projections across the neuroaxis, although complex, are topographically organized. That is, different groups of serotonin neurons have distinct sets of preferred targets. Moreover, with different behavioral or pharmacological manipulations, different groups of serotonin neurons express markers of activation such as the immediate early gene product Fos. These patterns of activation correlate with region-dependent changes in extracellular serotonin levels across the brain. Taken together these observations suggest the anatomical topography of serotonin neurons underlies a functional topography.

The heterogeneity and topography of the serotonin system raises questions about how feedback inhibitory pathways work to control serotonin-network activity. Autonomous feedback, where groups of functionally similar serotonin neurons regulate themselves, could underlie a homeostatic function. Non-autonomous feedback, where there is cross-talk between distinct groups of serotonin neurons, could provide for 'lateral inhibition' and influence patterns of activation. We summarize a series of experiments aimed at understanding how 5-HT_{1A}-receptor-dependent feedback inhibition works to control activation of serotonin neurons. Overall the results show that endogenous feedback inhibition provided by 5-HT_{1A} receptors is regionally organized and depends on behavioral state. Furthermore there are pathways of communication between different groups of serotonin neurons that could contribute to cross-talk. While these observations don't exclude an autoregulatory function, they provide evidence for non-autonomous feedback regulation within the serotonin system that could function to shape patterns of activation.



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BIASED AGONISM AT SEROTONIN 5-HT_{1A} RECEPTORS : PREFERENTIAL POSTSYNAPTIC ACTIVITY FOR IMPROVED THERAPY OF CNS DISORDERS

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Therapeutic efficacy of SSRIs in depressed patients is delayed until serotonin 5-HT_{1A} autoreceptors are desensitized, enabling a normalizing of serotonergic neurotransmission. However, 5-HT_{1A} receptors are also expressed post-synaptically, and their activation is a common mechanism of action of antidepressants. Therefore, it would be desirable to preferentially activate post-synaptic 5-HT_{1A} receptors without activating 5-HT_{1A} autoreceptors. Recent findings indicate that different brain regions exhibit distinct 5-HT_{1A} receptor-mediated intracellular signaling cascades allowing pharmacological targeting of post-synaptic cortical 5-HT_{1A} receptors by "biased" (or "functionally-selective") agonists.

F15599 is a prototypical 5-HT_{1A} biased agonist that exhibits a distinctive signaling profile, potentially eliciting ERK1/2 phosphorylation in vitro. F15599 potentially stimulates rat medial pre-frontal cortex pyramidal neuron electrical activity and dopamine release (controlled by post-synaptic 5-HT_{1A} receptors) at low doses that do not inhibit raphe serotonergic neuron electrical activity or hippocampal 5-HT release (controlled by 5-HT_{1A} autoreceptors). F15599 also preferentially stimulates c-Fos expression and ERK1/2 phosphorylation in rat pre-frontal cortex, with less pronounced effects in raphe [1,2].

F15599 exhibits potent anti-depressant-like activity in the forced swim test, inhibits stress-induced ultrasonic vocalization, and attenuates phencyclidine-induced cognitive impairments in reversal learning, in novel object recognition and in a hole-board spatial memory model. At "antidepressant" doses in rat, F15599 does not induce serotonin syndrome, does not disrupt attentional performance, does not impair working memory and does not inhibit pre-pulse inhibition of startle response [3].

Biased agonism at post-synaptic 5-HT_{1A} receptors, exemplified by F15599, is a promising therapeutic strategy to treat CNS disorders involving dysfunctional cortical serotonergic neurotransmission.

[1] Newman-Tancredi A. et al. (2009) Br J Pharmacol. 156(2):338-53.

[2] Lladó-Pelfort L. et al. (2010) Br J Pharmacol. 160(8):1929-40.

[3] Newman-Tancredi A. et al. (2011) Neuropsychiatry 1(2): 149-164.



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AGONIST-DIRECTED SIGNALING AT 5-HT_{2A} RECEPTORS IN CORTEX

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Agonist-directed signaling, also referred to as functional selectivity, describes events whereby different ligands can promote different signaling events downstream of a particular GPCR. In addition to the nature of the chemical ligand binding to the receptor, the proteins associated with the receptor may also dictate the directionality of signaling that ensues. Therefore, the endogenous environment in which a receptor is expressed, and the diverse protein-protein associations that assemble, may ultimately contribute to drug and hormone responsiveness in vivo. In this presentation, we will discuss our efforts to evaluate functional selectivity of serotonin 2A receptor signaling in cortex in response to exogenous and endogenous ligands and how the intracellular scaffolding protein, β arrestin2, effects serotonin 2A receptor signaling and ligand responsiveness in cortex and in cortical neuron cultures as well in mouse behavior, the head twitch response. In summary, while serotonin activates the serotonin 2A receptor to promote signaling and head twitches using β arrestin2, PI3K, Src and Akt, the serotonin metabolite, including N-methyl serotonin and the analog 5-MeO-DMT, activate signaling and subsequent head twitches in a manner that does not appear to involve these signaling elements. Interestingly, clozapine, which also activates Akt downstream of this receptor, is considered an antagonist at the serotonin 2A receptor. A greater understanding of the ligand-directed signaling nodes downstream of this receptor may point to means to develop improved therapies for treating psychiatric disorders. Funding from NIH/NIDA: DA025158.



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5-HT_{2B} RECEPTORS EXERT A DIFFERENTIAL CONTROL OF DOPAMINE ASCENDING PATHWAY ACTIVITY: AN IN VIVO MICRODIALYSIS STUDY IN THE RAT

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Several studies have recently suggested that the central serotonin_{2B} receptor (5-HT_{2B}R) may represent a new pharmacological target for pathological conditions depending upon mesolimbic DA dysfunction, such as drug addiction. However, the role of 5-HT_{2B}Rs in the control of DA ascending pathways remains weakly investigated to date. This study was therefore aimed at evaluating the influence of selective 5-HT_{2B}R antagonists (LY 266097, RS 127445) on DA release, measured, using in vivo microdialysis, in the rat striatum, nucleus accumbens (NAc) and medial prefrontal cortex (mPFC).

LY 266097 (0.63 mg/kg, i.p.) or RS 127445 (0.16 mg/kg, i.p.) had no effect on striatal DA release, but significantly reduced and increased basal DA release in the NAc and the mPFC, respectively. LY 266097, reduced significantly the increase in accumbal DA outflow induced by haloperidol (0.01 mg/kg, s.c.) administration, but failed to alter haloperidol-induced DA outflow in the striatum. Conversely, the effect of haloperidol on mPFC DA release was significantly potentiated by RS 127445 administration.

These findings demonstrate that 5-HT_{2B} receptors exert a region-dependent modulation of DA ascending pathways, by providing, specifically, no effect in the striatum, and opposite facilitatory and inhibitory controls on NAc and mPFC DA release. Also, they highlight the therapeutic potential of 5-HT_{2B}Rs for pathological conditions requiring an independent modulation of DA pathways, such as schizophrenia or Parkinson's disease.



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CHARACTERIZATION OF SEROTONIN NEURONS IN THE RAPHE BASED ON MOLECULAR AND ELECTROPHYSIOLOGICAL IDENTITY

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The median and dorsal raphe nucleus contain a high density of serotonin (5-HT) neurons that give rise to the majority of the projections to the forebrain, and are implicated in a large number of physiological functions. Accumulating evidence suggests that 5-HT neurons are morphologically, electrophysiologically and biochemically diverse in nature; however, a conclusive classification of 5-HT neuronal types has not been achieved. We aim to combine anatomical, physiological and molecular characteristics at the single-cell level to establish an unbiased categorization of 5-HT neurons with forebrain target-specific projections. We performed electrophysiological recordings in acute brain slices followed by single-cell nested PCR to identify patterns of molecular expression in 5-HT⁺ neurons. Patch-clamp recordings were directed to Pet1-EGFP cells in the mouse median raphe, and the dorsomedial and lateral wing areas of the dorsal raphe. A post-hoc analysis combining the three elements for each cell (anatomy, electrophysiology and molecular) was used to create an unsupervised clustering of members with similar characteristics. We show that at least three different 5-HT cell-types exist in the raphe nucleus and their distribution transcends the traditional anatomical classification of raphe subfields. In addition, we combined the abovementioned techniques with retrograde tracing injections in order to probe 5-HT neurons projecting to three different forebrain areas: the basolateral amygdala, the hippocampus and the medial prefrontal cortex. Our results suggest that these regions are innervated by 5-HT neurons with distinct characteristics, forming a highly organized circuit. This work represents a first step towards identifying functional specific axonal projections from the raphe to forebrain targets that are involved in controlling emotional behaviors.



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ESTROGEN RECEPTOR GPR30 MEDIATES DESENSITIZATION OF SEROTONIN_{1A} RECEPTOR SIGNALING

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Although depression is a common debilitating disorder, current medications do not alleviate depression for a large percentage of patients and it takes on average 6-7 weeks of therapy for patients to respond to the medications. The long-term goal of our studies is to develop selective estrogen receptor (ER) agonists as adjuvant treatments for selective serotonin reuptake inhibitors (SSRIs) to hasten the onset of their therapeutic effects. Hyperactivity of hypothalamic-pituitary-adrenal-mediated hormone responses is a feature of depression that is normalized with clinical improvement during drug therapy. Desensitization of 5-HT_{1A} receptor signaling in the hypothalamus may be an underlying mechanism for the therapeutic effects of estrogens and SSRIs, such as fluoxetine for depression. Although SSRIs require 7-14 days to desensitize 5-HT_{1A} receptor signaling, and 17 β -estradiol benzoate (EB), a nonselective ER agonist, produces rapid effects but only partially desensitizes 5-HT_{1A} receptors, we found that EB and fluoxetine work together to produce more rapid and complete desensitization of 5-HT_{1A} receptor signaling in the paraventricular nucleus of the hypothalamus (PVN). To begin to determine the mechanisms by which estrogens desensitize 5-HT_{1A} receptor signaling and hasten the effects of SSRIs, we used selective agonists and siRNA to determine the roles of ER β and GPR30. We found that GPR30 does but ER β does not mediate the estradiol-induced desensitization of 5-HT_{1A} receptor signaling in the PVN. Alterations in components of the 5-HT_{1A} receptor system that control release of hormones could underlie the changes in hormone response with EB and anti-depressant therapy. We found that EB prevented the fluoxetine-induced increase in 5-HT_{1A} receptor protein and resulted in a decrease in G α_z protein; these changes in 5-HT_{1A} receptor and G α_z could contribute to the more rapid desensitization. These data provide evidence that estrogens may be effective as a short-term adjuvant to SSRIs to accelerate the onset of therapeutic effects.



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SEROTONIN TRANSPORTER FUNCTION: INTERACTION AMONG OVARIAN STEROIDS AND ANTIDEPRESSANTS

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The effectiveness on depressed mood of either hormone replacement (HR) or estrogen replacement (ER) therapy during the postmenopausal period is controversial. Using chronoamperometry, we have shown that acute systemic administration of either estradiol benzoate (EB) and/or progesterone (P) blocked the ability of SSRIs such as fluvoxamine to inhibit the function of the serotonin transporter (SERT). Also, EB itself, but not P, blocked the function of the SERT. Thus, EB had two effects: (1) a possibly beneficial antidepressant (AD)-like effect in blocking the SERT; (2) a deleterious effect by blocking the inhibitory effect of SSRIs on the SERT. The behavioral consequences of such effects were investigated using the FST. The decreased immobility and increased swimming caused by fluvoxamine in the FST was blocked in rats treated acutely with either EB and/or P. We also studied effects of local administration of hormones into the CA3 region of the hippocampus on the clearance of 5-HT. Local application of 17 β -estradiol (E2), but not P, slowed the clearance of 5-HT and either hormone blocked the ability of fluvoxamine to inhibit the clearance of 5-HT. Additionally, time course studies, the use of hormone receptor agonists and antagonists and hormone-BSA conjugates revealed that the effects of estradiol were mediated by activation of both membrane and nuclear receptors whereas the effect of P is due solely to activation of intracellular P receptors. Further, use of subtype specific receptor agonists indicated that the inhibitory effect of E2 alone on the SERT involved ER β and GPR30 whereas its blockade of fluvoxamine's effect was mediated by ER α . The behavioral consequences of long-term administration of these receptor agonists is currently being studied. Targeting ER β or GPR30 might be a way to permit beneficial behavioral effects of estrogen without its deleterious effect on the actions of SSRIs.



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ESTRADIOL MODULATION OF 5HT1A AND 5-HT2A RECEPTORS AND SERT IN BRAIN OF FEMALE HEMIPARKINSONIAN MONKEYS AFTER LONG-TERM OVARECTOMY

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Estradiol modulates serotonin neurotransmission of intact rodents and monkeys and has yet to be characterized in monkey models of Parkinson's disease where serotonin plays an important role in dopamine neurotransmission. Hence, monkeys were ovariectomized and received a month later a unilateral lesion with the toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to model Parkinson's disease and four years later 17 β -estradiol for one month. These monkeys displayed extensive unilateral loss of striatal dopamine. 17 β -estradiol increased bilaterally striatal Akt levels as well as Akt and GSK3 β phosphorylation state. The lesion, but not 17 β -estradiol treatment, increased GPER1 levels in the putamen. [3H]8-OH-DPAT specific binding to 5-HT1A receptors was decreased in frontal cortex while [3H]Ketanserin specific binding to 5-HT2A receptors was increased in frontal cortex and striatum of monkeys treated with 17 β -estradiol in both brain hemispheres. R-(+)-8-OH-DPAT stimulated [35S]GTP γ S specific binding showed reduced stimulation in frontal cortex of monkeys treated with 17 β -estradiol in both brain hemispheres and in dorsal raphe nucleus. [3H]Citalopram specific binding to the serotonin transporter increased in anterior cerebral cortex of monkeys treated with 17 β -estradiol in both brain hemispheres. In another group of monkeys ovariectomized for one month, 17 β -estradiol treatment for one month increased [3H]Citalopram specific binding in cortex and in striatal regions associated with increased striatal serotonin concentrations. In these monkeys 17 β -estradiol also increased striatal Akt levels as well as Akt and GSK3 β phosphorylation state. In conclusion, brain serotonin neurotransmission is responsive to estrogens in parkinsonian monkeys and a response is observed after a short and a long period of ovariectomy modeling hormonal conditions of peri and post-menopause.



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INFLUENCES OF SEX HORMONES AND DIMINISHED CENTRAL NERVOUS 5-HT SYNTHESIS RATE ON IMPULSE CONTROL RELATED TO REWARD AND PUNISHMENT PROCESSING IN YOUNG ADULT FEMALES

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Objective: Serotonin has been shown to be related to affective disorders and impulse control. While repeatedly discussed, influences of sex hormones on central nervous neurotransmission are rarely taken into account in behavioral studies. We aimed to examine the effects of diminished central nervous 5-HT synthesis rate on reward and punishment related impulse control in young adult females.

Methods: Eighteen healthy females (aged 20 to 31 years), free of any hormonal medications including oral contraceptives, participated in a randomized double blind within subject repeated measures study, with two separate days of assessment spaced at least one individual menstrual cycle apart. On one day participants were subjected to acute tryptophan depletion (ATD) lowering central nervous 5-HT synthesis. On a further day they received a tryptophan balanced amino acid load (BAL) serving as a control condition. Both measurements took place during the early follicular phase of participants' menstrual cycle. Baseline hormonal status (FSH, LH and estradiol) was assessed on each study day. Impulse control was assessed using a reward/punishment implementing Go/NoGo task.

Results: A linear trend was detected indicating reduced impulsivity under ATD associated with co-varying increased FSH levels.

Conclusions: Endocrinological parameters such as FSH may operate as protective factors against punishment related impulsive behavior triggered by reduced central nervous 5-HT synthesis.



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POSTER PRESENTATIONS



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N°01

PLACENTAL SEROTONIN ROLE IN THE FETAL PROGRAMMING OF MENTAL DISORDERS

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Serotonin (5-HT), synthesized from the essential amino acid tryptophan (Trp), is a trophic factor for the fetal brain and disruption of 5-HT signaling during early development has long-term consequences on adult brain function and behavior. The recent discovery that the placenta, by converting maternal Trp through the tryptophan hydroxylase 1 (TPH1) pathway, provides a source of 5-HT to the fetal brain during early pregnancy suggests that placental Trp metabolism may constitute a new molecular pathway for the fetal programming of neurodevelopmental disorders. During pregnancy, maternal infections lead to placental inflammation that triggers the activation of a Trp metabolic pathway that protects the fetus from maternal immune response. Thus, in early gestation simultaneous alternative pathways for maternal Trp metabolism in the placenta provide not only a source of trophic support (5-HT) for the fetal brain, but also an immune protection for the fetus. Importantly, both inflammation and genetic alterations of Trp metabolism during development are associated with increased risk of neurodevelopmental disorders in humans, including schizophrenia. In this study we test the hypothesis that maternal inflammation affects the balance of Trp metabolism through alternative placental pathways, resulting in compromised serotonergic modulation of fetal brain development. The impact of inflammation on placental Trp metabolism is quantified at different times of gestation using a new technology that provides mid-throughput analytical capabilities of mouse placenta metabolic pathways *ex vivo*. This technology, combined with *in vivo* approaches, is applied to defining how inflammation can impact fetal brain development placental through Trp metabolism.



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N°02

BALANCE BETWEEN 5-HT_{1A} AND 5-HT_{2A} RECEPTORS TO MAINTAIN A NEGATIVE INFLUENCE ON 5-HT NEURONS IN RESPONSE TO SSRI

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SSRIs are commonly prescribed for the treatment of major depression (MD). However, 50% of depressive patients do not respond adequately to these medications. Although evidence incriminate 5-HT_{1A} autoreceptors in this poor response, it is possible that other(s) mechanism(s) are involved, particularly when these autoreceptors are desensitized.

This study was aimed at determining the impact of 5-HT_{2A} receptors in the effects of SSRIs in mice displaying a pharmacological or genetic inactivation of 5-HT_{1A} receptors.

As previously demonstrated, the SSRI escitalopram decreased the firing rate of dorsal raphe (DR) 5-HT neurons in wild-type (WT), whereas this effect was reversed by the selective 5-HT_{1A} receptor antagonist WAY100635. Interestingly, this response remained intact in 5-HT_{1A}^{+/+} mice pretreated with WAY100635 or in 5-HT_{1A}^{-/-} mice, demonstrating that another serotonergic receptor type was involved in the inhibitory effect of escitalopram. Accordingly, we showed that the 5-HT_{2A} receptor antagonist MDL100907 reversed escitalopram-induced decrease in DR 5-HT neuronal activity in these mice with their 5-HT_{1A} receptors inactivated. Remarkably, the effect of the preferential receptor agonist DOI on DR 5-HT neuronal activity was more pronounced in 5-HT_{1A}^{-/-} mice than in WT. To explore the possibility of interplay between 5-HT_{1A} and 5-HT_{2A} receptors, we also studied the functional status of 5-HT_{1A} receptors in 5-HT_{2A}^{-/-} mice. The 5-HT_{1A} receptor agonist 8-OHDPAT-induced hypothermia or decrease in DR 5-HT neuronal activity was potentiated in 5-HT_{2A}^{-/-} mice.

Hence, the inactivation of one receptor is counterbalanced by a hypersensitization of the other to maintain a negative influence on the serotonergic system. This study supports the interest of combining SSRIs and antipsychotics in MD in order to produce a more robust antidepressant effect.



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N°03

HETERODIMERIZATION OF SEROTONIN RECEPTORS 5-HT1A AND 5-HT7 DIFFERENTIALLY REGULATES RECEPTOR FUNCTIONS DURING DEVELOPMENT

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Serotonin receptors 5-HT1A and 5-HT7 are highly co-expressed in brain regions implicated in depression. However, their functional interaction has not been established. In the present study we show that 5-HT1A and 5-HT7 receptors form heterodimers both in vitro and in vivo. Resonance energy transfer-based assays revealed that, in addition to heterodimers, homodimers composed either by 5-HT1A or 5-HT7 receptors together with monomers co-exist in cells. The highest affinity to form the complex was obtained for the 5-HT7-5-HT7 homodimers, followed by the 5-HT7-5-HT1A heterodimers and 5-HT1A-5-HT1A homodimers. Functionally, heterodimerization decreases 5-HT1A receptor-mediated activation of Gi-protein without affecting 5-HT7 receptor-mediated signalling. Moreover, heterodimerization markedly decreases the ability of the 5-HT1A receptor to activate G-protein gated inwardly rectifying potassium channels in a heterologous system. The inhibitory effect on such channels was also preserved in hippocampal neurons, demonstrating a physiological relevance of heteromerization in vivo. In addition, heterodimerization is critically involved in initiation of the serotonin-mediated 5-HT1A receptor internalization and also enhances the ability of the 5-HT1A receptor to activate the mitogen-activated protein kinases. Finally, we found that production of 5-HT7 receptors in hippocampus continuously decreases during postnatal development, indicating that the relative concentration of 5-HT1A-5-HT7 heterodimers and, consequently, their functional importance undergoes pronounced developmental changes.

Generally, our data suggest that the regulated and balanced ratio of homo- and heterodimerization on pre- and postsynaptic neurons may be critically involved in both, the onset as well as response to treatment of psychiatric diseases such as depression and anxiety.



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N°04

SEX-SPECIFIC AND REGION-SPECIFIC CHANGES IN BDNF-TRKB SIGNALLING IN THE HIPPOCAMPUS OF 5-HT_{1A} RECEPTOR AND BDNF SINGLE AND DOUBLE MUTANT MICE.

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SSRIs are commonly prescribed for the treatment of major depression (MD). However, 50% of depressive patients do not respond adequately to these medications. Although evidence incriminate 5-HT_{1A} autoreceptors in this poor response, it is possible that other(s) mechanism(s) are involved, particularly when these autoreceptors are desensitized.

This study was aimed at determining the impact of 5-HT_{2A} receptors in the effects of SSRIs in mice displaying a pharmacological or genetic inactivation of 5-HT_{1A} receptors.

As previously demonstrated, the SSRI escitalopram decreased the firing rate of dorsal raphe (DR) 5-HT neurons in wild-type (WT), whereas this effect was reversed by the selective 5-HT_{1A} receptor antagonist WAY100635. Interestingly, this response remained intact in 5-HT_{1A}^{+/+} mice pretreated with WAY100635 or in 5-HT_{1A}^{-/-} mice, demonstrating that another serotonergic receptor type was involved in the inhibitory effect of escitalopram. Accordingly, we showed that the 5-HT_{2A} receptor antagonist MDL100907 reversed escitalopram-induced decrease in DR 5-HT neuronal activity in these mice with their 5-HT_{1A} receptors inactivated. Remarkably, the effect of the preferential receptor agonist DOI on DR 5-HT neuronal activity was more pronounced in 5-HT_{1A}^{-/-} mice than in WT. To explore the possibility of interplay between 5-HT_{1A} and 5-HT_{2A} receptors, we also studied the functional status of 5-HT_{1A} receptors in 5-HT_{2A}^{-/-} mice. The 5-HT_{1A} receptor agonist 8-OHDPAT-induced hypothermia or decrease in DR 5-HT neuronal activity was potentiated in 5-HT_{2A}^{-/-} mice.

Hence, the inactivation of one receptor is counterbalanced by a hypersensitization of the other to maintain a negative influence on the serotonergic system. This study supports the interest of combining SSRIs and antipsychotics in MD in order to produce a more robust antidepressant effect.



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N°05

PERIPHERAL SEROTONIN IN HEART FAILURE PATIENTS WITH AND WITHOUT DEPRESSION

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Congestive heart failure (CHF) and depression are characterized by dysfunctions in monoaminergic systems affecting serotonin, norepinephrine and dopamine. Recent epidemiological research has shown that congestive heart failure and depression each independently increase risk for the other disorder. Mechanisms linking congestive heart failure and depression remain unclear. In the present study we evaluated the whole blood serotonin and final metabolite of serotonin - 5-hydroxyindoleacetic acid (5-HIAA) by high-affinity liquid chromatography with electrochemical detection in CHF patients with current drug-free depression (according to the DSM-IV criteria) and CHF patients without depression. The control group is a people without cardiovascular diseases. Values are expressed as means \pm SEM. T-test was used when comparing means. $p < 0.05$ was considered statistically significant. We established that CHF patients ($n=20$) with and without depression showed significant rises of whole blood serotonin level in comparison healthy control ($1694,58 \pm 344,29$ ($p < 0,01$); $2102,02 \pm 512,95$ ($p < 0,05$) respectively versus control $375,19 \pm 158,08$ nmol/L). Patients with CHF with and without depression have the high blood level of 5-HIAA in comparison control ($198,96 \pm 31,37$ ($p < 0,001$); $222,32 \pm 56,57$ ($p < 0,01$) respectively versus control $25,66 \pm 5,94$ nmol/L). In summary, congestive heart failure patients with depression have high level of whole blood serotonin, 5-HIAA in comparison with control patients. Whole blood serotonin and 5-HIAA did not differ in congestive heart failure patients with depression compared to congestive heart failure patients without depression. Thus, congestive heart failure and depression have common peripheral pathophysiological mechanisms that may link these two disorders. These results clearly demonstrate that significant changes in peripheral serotonin metabolism occur in congestive heart failure patients without depression, that may be used as novel diagnostic approaches and at the same time serve as therapeutic targets.



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N°06

DIFFERENTIAL EFFECT OF CATHINONES AND MDMA ON BODY TEMPERATURE AND BRAIN NEUROCHEMISTRY IN THE RAT

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Recreational users compare the psychoactive effects of 4-methylmethcathinone (mephedrone) to those of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy). MDMA induces well-characterised, and potentially fatal, changes in body temperature due to complex monoaminergic effects on central thermoregulation, peripheral blood flow and thermogenesis (Docherty & Green 2010 Br. J. Pharmacol. 160 1029). However, there are few preclinical data on the acute temperature effects of mephedrone or the other cathinones.

The present study examined the acute effect of cathinone, methcathinone and mephedrone (4 or 10mg/kg HCl salt, i.p.) on rectal and tail temperature in adult male Lister hooded rats (215-305g, n=5-6/group), with (±)-MDMA included for comparison. Animals were killed 2h after drug administration and frontal cortex, hippocampus, striatum and hypothalamus collected for quantification of 5-HT and 5-HIAA using HPLC with electrochemical detection. Striatal dopamine and its metabolites were also measured.

At normal room temperature ($20.5 \pm 1.5^{\circ}\text{C}$) MDMA caused a sustained decrease in both rectal and tail temperature. Mephedrone caused a transient decrease in rectal temperature (20min post-injection) followed by a prolonged decrease in tail temperature (40-120min post-injection). Cathinone and methcathinone both caused sustained increases in rectal temperature (40-80 and 40-120min post-injection, respectively) without altering tail temperature. MDMA produced the expected acute decrease in 5-HT and/or 5-HIAA levels in several brain regions and reduced striatal dopamine metabolite concentrations. Mephedrone increased 5-HT levels in the frontal cortex only ($P < 0.05$). Cathinone and methcathinone increased striatal 5-HIAA and HVA ($P < 0.01/P < 0.001$), and cathinone also increased hypothalamic 5-HT content ($P < 0.05$).

The cathinones clearly have differential effects to each other and to MDMA on both thermoregulation and brain monoamines. It is unclear whether these differences are primarily pharmacodynamic or pharmacokinetic, but they do not correlate with the modest variations in molar dose (186, 200, 214 and 230 mmol/kg respectively for cathinone, methcathinone, mephedrone and MDMA at 4mg/kg).



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BOTH PERIPHERAL AND NEURONAL TRYPTOPHAN HYDROXYLASES PARTICIPATE IN HUMAN TROPHOBLASTIC SEROTONIN PRODUCTION: IMPLICATION OF FETAL SEX

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CONTEXT: Altered maternal serotonin levels are observed in pregnancy complications (preeclampsia) associated with trophoblastic changes, suggesting a role for this neurohormone in placental function and development. Moreover, serotonin and its receptors and transporter have been found in placental villi and trophoblasts. Although placental functions (hormone production and gene expression) has been shown to be affected by the sex of the fetus, studies involving trophoblast primary cells often overlooked the potential effect of fetal sex. However, whether human placenta synthesizes serotonin *de novo*, and if this production varies between placentas from male or female fetuses, has never been studied.

OBJECTIVE: We propose that the human placenta produces serotonin *de novo* and that this production is affected by the sex of the fetus. To verify this hypothesis, the expression and activity of tryptophan hydroxylase (TPH), the rate-limiting enzyme in serotonin synthesis, was determined according to fetal sex.

METHODS AND RESULTS: Placentas from uncomplicated pregnancies were obtained following vaginal delivery (term) or therapeutic abortion (1st trimester). Primary cultures of term (villous) and first trimester (villous and extravillous) trophoblasts were isolated and purified using a magnetic cell sorter. Immunohistochemical and expression (qRT-PCR, Western Blot) analyses showed that villous cytotrophoblast, syncytiotrophoblast, foetal capillaries (from both 1st trimester and term placentas) and extra-villous cytotrophoblasts express TPH1 and TPH2. Serotonin production *de novo* by primary trophoblast cells was measured by LC-MS/MS and increased along with differentiation of term villous cytotrophoblast into syncytiotrophoblast, with no difference dependent on fetal sex (student t-test).

CONCLUSION: This study demonstrates for the first time that the human trophoblast expresses both peripheral TPH1 and brain-specific TPH2 and produces serotonin *de novo* with no apparent difference due to fetal sex. Our findings suggests an important autocrine/paracrine role for serotonin in placental function and development and, consequently, in pregnancy and foetal well-being.



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N°08

5-HT₄ RECEPTOR SIGNALING IN PORCINE LEFT ATRIUM: INFLUENCE OF PHOSPHODIESTERASES AND CROSS-TALK WITH cGMP

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Phosphodiesterase (PDE)3 and PDE4, degrading cAMP, in concert regulate the fade of inotropic responses to 5-HT₄ receptor activation in pig left atrium. Elevation of cGMP mediated by stimulation of particulate guanylyl cyclase (pGC) or soluble guanylyl cyclase (sGC) can influence responses to 5-HT via cross-talk with cAMP. This cross-talk is potentially mediated by the cGMP-inhibited PDE3 and the cGMP-activated PDE2. The role of PDEs and elevated cGMP in controlling responses to 5-HT was investigated.

Porcine left atrial muscles were mounted in organ baths, received the pGC stimulator C-type natriuretic peptide (CNP), the NO-donor Sin-1 (stimulating sGC) and/or specific PDE inhibitors followed by 5-HT and were freeze-clamped for further processing. Phosphorylation of the protein kinase A targets troponin I (TnI) and phospholamban (PLB) was determined by immunoblotting and cAMP was measured by enzyme immunoassay.

5-HT increased cAMP content and phosphorylation of PLB, and this was significantly further enhanced under PDE3 and PDE4 inhibition. TnI phosphorylation was significantly increased by 5-HT only when PDE3 and PDE4 were inhibited. These results are in line with the functional data. Functional responses to 5-HT in the presence of CNP plus PDE2- and PDE4-inhibitors, ensuring maximal cross-talk with cAMP, were significantly enhanced. In this condition, cAMP content and PLB phosphorylation were significantly increased compared to 5-HT alone; however this was not significantly different from 5-HT under PDE4 inhibition. Sin-1 significantly hastened fade of the response to 5-HT in functional experiments; this was not mirrored in cAMP content.

PDE3 and PDE4 control the cAMP response to 5-HT₄ receptor activation, bringing about a dampening of downstream mediators of this pathway. The enhancing effect of pGC stimulation on functional responses to 5-HT is not fully mimicked in cAMP content. Stimulation of sGC hastens the fade of the response to 5-HT by a mechanism unrelated to changes in cAMP content.



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A 5-HT₄ RECEPTOR/APP/ADAM10 MEMBRANE BOUND COMPLEX CONTRIBUTES TO PROMOTE NON-AMYLOIDOGENIC PATHWAY

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According to the amyloid hypothesis, a promising therapeutic approach in Alzheimer's disease (AD) could be to enhance the protective α -secretase activity. However, the α secretase cleavage regulatory mechanisms of the amyloid precursor protein (APP) are still poorly understood. Here, we showed a new mechanism by which the 5-HT₄ serotonin receptor (5-HT₄R) stimulates α -secretase-dependent cleavage of APP, thus leading to the extracellular release of the non-amyloidogenic neuroprotective secreted APP α (sAPP α) in both HEK 293 and neuronal cells. 5-HT₄R-mediated secretion of sAPP α was, for a great part, «constitutively» associated with receptor expression levels at the plasma membrane. In addition and as previously described, stimulation of 5-HT₄R by an agonist further stimulated secretion of sAPP α . Both «constitutive» and stimulated 5-HT₄R mediated production of sAPP α were reduced by a metalloproteinase inhibitor (TAPI1) and siRNA specific for ADAM10 expression, demonstrating that 5-HT₄R-mediated release of sAPP α was mainly dependent on the disintegrin and metalloprotease ADAM10. Our data further demonstrate that 5-HT₄R physically interacted directly or indirectly with ADAM10 (mature form) but also with APP and promoted the targeting of both proteins to the plasma membrane. Among several G-protein coupled receptors (GPCRs), these interactions were only observed with the 5-HT₄R. If the 5-HT₄R agonist-induced sAPP α production was related to cAMP/EPAC signalling, the «constitutive» 5-HT₄R-induced secretion of sAPP α was independent of cAMP production. Our findings add to the understanding of AD cell biology in particular to the complex and highly regulated sorting of APP and secretases.



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N°10

IMPACT OF SEROTONIN DEFICIENCY ON EMOTIONAL STATE IN MICE

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Every fifth European suffers from depression or anxiety disorders. However, molecular mechanisms underlying the origin of these psychological states are only partially understood.

Tryptophan hydroxylase 2 (TPH2) converts tryptophan to 5-hydroxytryptophan (5-HTP) and is a limiting enzyme of serotonin (5-HT) synthesis in the central nervous system. Recently, by genetically ablating Tph2 we generated mice completely lacking central serotonin (Tph2^{-/-}). These mice exhibit high aggressiveness, decreased anxiety-like and increased depression-like behavior. Tph2 C1473G is a single nucleotide polymorphism, which leads to the substitution of the aminoacid Pro447 by Arg447 and a subsequent reduction in TPH2 activity. This polymorphism was discovered between C57Bl/6 (Tph2^{C/C}) and DBA (Tph2^{G/G}) mouse strains and was suggested to be the reason for behavior differences between these strains.

In this study we generated congenic mice carrying the G allele on C57Bl/6 background (C57Bl/6_Tph2^{G/G}) and crossed these mice to Tph2^{-/-} animals to obtain C57Bl/6_Tph2^{G/-} mice. To elucidate if a reduction in TPH2 activity affects serotonin turnover in vivo, we evaluated serotonin synthesis rate by 5-HTP accumulation after pharmacological inhibition of its further conversion to 5-HT. Tph2^{G/G} and Tph2^{G/-} mice exhibited around 35 and 60% reduction in brain serotonin synthesis in comparison to C57Bl/6 animals, leading to a subsequent 10 and 20% lowering in brain serotonin level in both strains, respectively. We then evaluated an impact of partial serotonin depletion on mouse behavior. Despite considerable decrease in brain serotonin content there were no evident differences between C57Bl/6, Tph2^{G/G}, or Tph2^{G/-} mice in aggression, anxiety-, and depression-like behavior.

Our data suggest that partial reduction in TPH2 activity in comparison to complete depletion does not contribute to the abnormal emotional behavior in mice.



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N°11

RED BLOOD CELL STORAGE IN THE PRESENCE OF SEROTONIN REDUCES POST-TRANSFUSION INFLAMMATION LEADING TO INCREASED RBC IN VIVO SURVIVAL IN MICE

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Background: During their storage in blood bank, red blood cells (RBC) undergo different modifications called «storage lesions» that cause inflammatory reactions post-transfusion leading to decreased RBC in vivo survival. Serotonin (5-HT) is known to improve the survival of various cell types. In this study, we investigated the impact of RBC storage in the presence of 5-HT on subsequent inflammation and RBC in vivo survival post-transfusion. Hypothesis: 5-HT attenuates inflammation and increases RBC survival after transfusion. Specific objectives: 1) To evaluate the effects of 5-HT on local inflammation in the peritoneum; 2) To evaluate the impact of 5-HT on RBC in vivo survival. Methods: RBC were isolated from male C57BL/6 mice and stored at 4 ° C in CPDA-1 with added 5-HT (10-4M) or without 5-HT (control). To evaluate the inflammation, RBC were transfused into the peritoneum and after 2 hrs, peritoneum cells and the spleen were collected. The recruitment of inflammatory cells was analyzed by immunophenotyping. Post-transfusion RBC in vivo survival was analyzed with CFSE-stained RBC injected by i.v. route and assessed by flow cytometry. Results: RBC stored without 5-HT were rapidly cleared from the bloodstream and conveyed by monocytes/macropages into the spleen as reflected by the darkening area found on the spleen, which was reduced with RBC stored in the presence of 5-HT. RBC storage with 5-HT decreased the recruitment of neutrophils (14% vs. 42% for control), and of monocytes/macrophages (4% vs. 17%). RBC 24-hr recovery was 28% higher when RBC were stored for 7 days with 5-HT as compared to control RBC. Conclusion: The addition of 5-HT to the storage medium could improve the quality of transfused RBC.



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FUNCTIONAL CROSSTALK AND HETERODIMERIZATION OF THE 5-HT_{2C} WITH THE GHRELIN (GHS-R1A) AND THE DOPAMINE D₁ RECEPTOR

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G-protein coupled receptors (GPCRs), including the serotonin 2C receptor (5-HT_{2C}) and the ghrelin receptor (GHS-R1a) are well-known for their key role in the homeostatic control of food intake and energy balance. In addition, the 5-HT_{2C} receptor has been identified in the regulation of reward-related behaviours and has been shown to exert tonic inhibitory influence over dopamine (DA) neurotransmission within the mesolimbic pathway. Moreover, recent studies have identified a pivotal role for the ghrelinergic system in reward signalling as well. In addition, both ghrelin as well as the 5-HT_{2C} receptor have been shown to be involved in the secretion of corticotrophin releasing factor (CRF) and hypothalamic-pituitary-adrenal (HPA) axis activation. Moreover, elevated serotonin (5-hydroxytryptamine, 5-HT) levels have been shown to effectively block ghrelin's orexigenic effects. Finally, both the 5-HT_{2C} receptor and the GHS-R1a receptor are expressed in overlapping neurocircuits, reinforcing a potential functional interaction of these GPCRs. In this study, we identify a novel heterodimerization of the 5-HT_{2C} receptor with the DR1 receptor as well as with the GHS-R1a receptor in vivo, using combined immunocytochemistry and histochemistry approaches. In addition, dimerization of the unedited 5-HT_{2C}-INI, but not the edited 5-HT_{2C}-VSV isoform, attenuated ghrelin mediated signalling. Similarly, DR1-induced cAMP signalling was reduced in cells co-expressing both isoforms of the 5-HT_{2C} receptor. The 5-HT_{2C} receptor mediated attenuation of both GHS-R1a and DR1 signalling was fully restored following pharmacological blockade of the 5-HT_{2C} receptor using the specific antagonist, RS102221.



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MODULATORY ROLE OF POSTSYNAPTIC 5-HT_{1A} RECEPTORS IN 8-OH-DPAT-INDUCED HYPERPHAGIA IN MICE

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Brain serotonin (5-HT) is involved in the control of food intake. The ingestive effects of 5-HT are mediated by a number of different receptor subtypes. It has been shown that 5-HT_{1A} receptor agonists increase intake of food in non-food deprived rodents. The aim of this study was to determine whether this hyperphagic effect is mediated by presynaptic 5-HT_{1A} autoreceptors in the raphe nuclei or by postsynaptic 5-HT_{1A} heteroreceptors in serotonergic terminal structures. Here we studied feeding in young-adult and adult, non-food-deprived male NMRI and transgenic mice, characterized by an exclusive overexpression of postsynaptic 5-HT_{1A} receptors. 5-HT_{1A} receptor full agonist 8-OH-DPAT increased food consumption in NMRI wildtype mice. In young-adult transgenic mice the feeding response occurred at lower doses with an earlier onset whereas adult transgenic mice failed to respond to 8-OH-DPAT. These results confirm a pivotal function of 5-HT_{1A} receptors on feeding control in mice, in which the hyperphagic effect of 8-OH-DPAT is mediated by 5-HT_{1A} autoreceptors located in the raphe nuclei. However, the specific and suppressed effects of 8-OH-DPAT in transgenic mice indicate a modulatory role of postsynaptic 5-HT_{1A} receptors in control of feeding.



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THE NOVEL ANTIDEPRESSANT LU AA21004: IMPLICATIONS OF ITS MULTIMODAL MECHANISM OF ACTION IN PRECLINICAL FUNCTIONAL AND BEHAVIORAL MODELS

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Lu AA21004, an investigational multimodal antidepressant, is a 5-HT₃ and 5-HT₇ receptor antagonist, 5-HT_{1B} receptor partial agonist, 5-HT_{1A} receptor agonist and inhibitor of 5-HT reuptake. In microdialysis studies in rat brains Lu AA21004 increased extracellular 5-HT, NE, DA, acetylcholine and histamine. The recovery of dorsal raphe nucleus 5-HT neuronal firing was much faster with Lu AA21004 than with fluoxetine (1 vs. 14 days). Locus coeruleus NE and ventral tegmental area DA neuronal firing was unaffected by Lu AA21004, whereas SSRIs/SNRIs decreased the firing rate. Quantitative EEG analyses demonstrated increases across power bands with Lu AA21004, whereas escitalopram and duloxetine had no effect. Lu AA21004 showed antidepressant-like activity in standard monoaminergic behavioral models and a progesterone withdrawal model that was insensitive to fluoxetine. Lu AA21004 improved episodic memory in the rat novel object recognition (NOR) test and contextual memory in the rat fear conditioning test. A 5-HT₃ or 5-HT₇ receptor agonist counteracted the effects of Lu AA21004 in the NOR test. In contrast to escitalopram and duloxetine, Lu AA21004 normalised deficits in episodic and spatial memory induced in rats by 5-HT depletion in the NOR and spontaneous alteration tests. Lu AA21004 increased cell proliferation in the ventral hippocampus after 1 and 3 days' treatment whereas a 2 weeks treatment was required for fluoxetine. Thus, Lu AA21004 exerts its antidepressant and memory-enhancing effects via mechanisms beyond 5-HT transporter inhibition.



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MKC-733 STIMULATES GASTRIC EMPTYING AND ANTRODUODENAL MOTILITY THROUGH 5-HT₃ RECEPTORS, PROBABLY LOCATED IN THE INTESTINAL WALL

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MKC733 stimulates gastric emptying (GE) and intestinal motility in dogs. We determined whether intestinal 5-HT₃ receptors are involved by comparing oral and intravenous administration.

In conscious fasted dogs (7-16 kg; n=3-6), GE of 250 mL water was assessed by determining the volume of the gastric content and a marker concentration at several time points after administration (gastric content was collected and reintroduced through a pre-implanted gastric cannula). Lidamidine (0.63 mg/kg sc; to delay GE) and test drugs were administered 30 minutes before GE was determined. Blood samples were collected to determine the MKC733 plasma concentration using HPLC. In a separate set of dogs antroduodenal contractions were recorded using pre-implanted serosal force transducers. Results are expressed as mean±SEM.

When dosed orally 0.0025-0.16 mg/kg MKC733 dose-dependently accelerated delayed GE up to 90±7% (P<0.05) with an approximate ED₅₀ of 0.005 mg/kg, an effect that could be blocked with granisetron. Although MKC733 significantly accelerated GE, MKC733 blood concentrations were below detection limit. When dosed intravenously 0.04-0.16 mg/kg MKC733 accelerated delayed GE with maximally 61±14% (P<0.05) and an approximate ED₅₀ of 0.04 mg/kg. In contrast to oral administration MKC733 blood concentrations reached after intravenous administration clearly increased with a maximum measured concentration of 57±8 and 327±17 ng/ml 5 minutes after administration of 0.04 and 0.16 mg/kg respectively. MKC733, at doses (0.04-0.63 mg/kg po) that did not result in measurable blood levels, dose-dependently increased antral and duodenal contraction amplitudes with 411±275% and 69±12% respectively (P<0.05), an effect blocked by pre-treatment with granisetron (1 mg/kg sc).

In conclusion, the selective 5-HT₃ receptor agonist MKC733 accelerates GE and stimulates antroduodenal motility through 5-HT₃ receptors, probably located in the intestinal wall.



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PARTIAL AGONIST EFFICACY OF EMD386088, A 5-HT₆ RECEPTOR LIGAND, IN FUNCTIONAL ASSAYS IN VITRO

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EMD386088 (5-chloro-2-methyl-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole hydrochloride) has been described by Mattsson et al. [2005] as a high affinity ($IC_{50} = 7.4$ nM) 5-HT₆ receptor ligand. In a functional assay measuring production of cyclic AMP, EMD 386088 behaved as a full agonist ($EC_{50} = 1.0$ nM). To extend that study, we set up two different functional in vitro procedures. Efficacy of EMD386088 was determined by measurement of cAMP by homogeneous TR-FRET immunoassay and calcium flux assay by aequorin detection technology using cells expressing the human serotonin 5-HT₆ receptor (PerkinElmer). Three independent experiments were performed in duplicates. EMD386088 was also tested in an additional cellular functional assay conducted by CEREP (Celle L'Evescault, France). Thus three experimental procedures with different recombinant cell lines, modulated pathway and detection methods were used to analyze the efficacy of EMD386088. In all experimental assays its maximal effect was markedly lower than that of serotonin (5-HT). In two models based on cyclic AMP formation maximal efficacy (E_{max}) values for EMD386088 were $65,7 \pm 6,6\%$ and $33,5 \pm 9,8\%$. In a model based on calcium response the studied compound showed $39,7 \pm 3,6\%$ of maximal 5-HT signal. The observed potency of EMD386088 slightly varies depending on experimental conditions. Moreover, EMD386088 antagonized the 5-HT response in a concentration-dependent manner. Increasing concentrations of EMD386088 from 10^{-9} to 10^{-6} M shifted to the right a 5-HT concentration-response curve.

Concluding, the present in vitro results reveal that EMD386088 behaves as a potent partial agonist of 5-HT₆ receptor under conditions used.

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DIFFERENCES IN THE EFFECTS OF NEW DESIGNER CATHINONE DERIVATIVES ON HUMAN SEROTONIN AND DOPAMINE TRANSPORTER FUNCTION IN VITRO

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The number of abused designer cathinone derivatives ("legal highs", "research chemicals") increased markedly. Cathinones are amphetamine analogues characterized by a β -keto group. The mechanism of action of the cathinones in comparison to classic amphetamines or cocaine is not well characterized. We investigated the effects of mephedrone, methylone, MDPV, naphyrone, cathinone, methcathinone, and flephedrone on the human serotonin (5-HT) and dopamine (DA) transporter in HEK cells in vitro. MDPV inhibited the DA transporter (DAT) very potently and had no effect on the 5-HT-transporter (SERT). Cathinone, methcathinone and flephedrone resembled amphetamine and methamphetamine in their uptake inhibition profile with more potent effects on DAT than on SERT. Unlike MDPV, these drugs also induced transporter-mediated DA release. With mephedrone, naphyrone, and methylone a third group was identified. Their 5-HT and DA uptake inhibition profiles were comparable with the one of cocaine, inhibiting both SERT and DAT with equal potency. Mephedrone and methylone, but not naphyrone, also induced both 5-HT and DA release through SERT and DAT, respectively and similar to methylenedioxymethamphetamine (MDMA, "ecstasy"). Mephedrone, flephedrone, and methcathinone also exhibited 5-HT_{2A} receptor binding similar to MDMA. None of the cathinones inhibited SERT more than DAT, a profile unique to MDMA. Based on the general higher inhibition potency of DAT versus SERT, we concluded that all cathinones are stimulant-like drugs with addictive potential similar to methamphetamine or cocaine. However, there is considerable diversity in the pharmacological profiles of these compounds which will likely result in different psychotropic effects and relative risks for addiction in humans.



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FUNCTIONAL PROPERTIES OF GAD67-CONTAINING 5-HT NEURONS IN THE RAT DORSAL RAPHE NUCLEUS

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The serotonergic (5-HTergic) system originating from the dorsal raphe nucleus (DRN) is implicated in behavioral and physiological responses to environmental stressors. The DRN is topographically organized and contains several anatomically and functionally distinct groups of serotonergic neurons. Furthermore, subsets of 5-HTergic neurons are known to co-express other transmitters, including GABA, glutamate, or neuropeptides, thereby generating further heterogeneity. However, despite the growing evidence for functional variations among DRN subnuclei, relatively little is known about how they map onto neurochemical diversity of 5-HTergic neurons. In the present study, we characterized functional properties of GAD67-expressing 5-HTergic neurons (5-HT/GAD67 neurons) in the rat DRN, and compared with those of neurons expressing 5-HTergic molecules (5-HT neurons) or GAD67 (GAD67 neurons) alone. While 5-HT/GAD67 neurons were absent in the dorsal (DRD) or ventral (DRV) parts of the DRN, they were selectively distributed in the lateral wing of the DRN (DRL), constituting 12% of the total DRL neurons. They expressed plasmalemmal GABA transporter 1, but lacked vesicular inhibitory amino acid transporter. By using whole-cell patch-clamp recording, we found that 5-HT/GAD67 neurons had lower input resistance and firing frequency than 5-HT neurons. As revealed by c-Fos immunohistochemistry, neurons in the DRL, particularly 5-HT/GAD67 neurons, showed higher responsiveness to exposure to an open field arena than those in the DRD and DRV. By contrast, exposure to contextual fear conditioning stress showed no such regional differences. These findings indicate that 5-HT/GAD67 neurons constitute a unique neuronal population with distinctive neurochemical and electrophysiological properties and high responsiveness to innocuous stressor.



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SEROTONIN SIGNALING IS REQUIRED FOR WNT-DEPENDENT GRP SPECIFICATION AND LEFTWARD FLOW IN XENOPUS

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In vertebrates, most inner organs are asymmetrically arranged with respect to the main body axis. Symmetry breakage in vertebrate embryos depends on cilia-driven leftward flow of extracellular fluid during neurulation. Flow induces the asymmetric Nodal cascade which governs asymmetric organ morphogenesis. In the frog *Xenopus* an alternative laterality-generating mechanism involving asymmetric localization of serotonin at the 32-cell stage has been proposed. However, no functional linkage between this early localization and flow at neurula stage has emerged. Here we report that serotonin signaling via a type 3 receptor (5-HT₃) is required for specification of the superficial mesoderm giving rise to the ciliated gastro-coel roof plate (GRP) where flow occurs. Flow and asymmetry was disturbed following down-regulation of serotonin signaling. Serotonin, which we found uniformly distributed along the main body axes in the early embryo, was required for Wnt signaling, which provides the instructive signal to specify the GRP. The importance of Wnt signaling on early development can easily be demonstrated in the frog, as misexpression results in the formation of siamese twins. Importantly, serotonin itself and 5-HT₃ was required for Wnt-induced double axis formation. Our data confirm flow as primary mechanism of symmetry breakage and suggest a general role of serotonin as competence factor for Wnt signaling during axis formation in *Xenopus* (Curr.Biol. 2012).



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A PROTEOMIC AND FUNCTIONAL ANALYSIS REVEALS THAT 5-HT₆ RECEPTORS MODULATE NEURONAL DIFFERENTIATION BY RECRUITMENT OF CYCLIN-DEPENDENT KINASE 5

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The serotonin 5-HT₆ receptor is predominantly expressed in CNS regions involved in the pathogenesis and control of psychiatric disorders like schizophrenia. It has become a promising target for the treatment of the cognitive deficits, as blockade consistently enhances mnemonic performance in a broad range of procedures in rodents. Paradoxically, little is known about the 5-HT₆ receptor-associated signalling pathways, an issue addressed by a proteomic approach to systematically identify its protein partners. We detected a physical association of the receptor with a network of proteins, including Cyclin-dependent kinase (Cdk)5, a protein kinase involved in the control of actin cytoskeleton dynamics and known to modulate neurodevelopmental processes. Expressing the 5HT₆ receptor in NG108-15 cells triggered morphological and functional changes associated with neuronal differentiation. These effects were not further enhanced by exposure of NG108-15 cells to an agonist and were prevented when cells were treated with SB258,585 (1 mM), a selective 5HT₆ antagonist. Expression of a dominant negative mutant of Cdk5 or treating cells with roscovitine or asterpauillone, two pharmacological inhibitors of Cdk5, also inhibited NG108-15 cell differentiation induced by 5-HT₆ receptor expression. Finally, SB258585 strongly impaired interaction between the 5HT₆ receptor and Cdk5, suggesting that this interaction was necessary for the induction of differentiation. This study offers strong evidence that the 5HT₆ receptor promotes neuronal differentiation and suggests a critical role for Cdk5 in this process. It provides new insight into the molecular mechanisms underlying neurodevelopmental effects of 5-HT₆ receptors, the pathophysiological relevance of which will be important to establish.



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THE DOPAMINERGIC AGONIST QUINPIROLE TRIGGERS 5-HT_{2C} RECEPTOR-DEPENDENT CONTROLS ON BOTH PURPOSELESS ORAL MOVEMENTS AND THE ACTIVITY OF THE HYPERDIRECT PATHWAY IN BASAL GANGLIA.

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Excessive dopamine transmission in associative/limbic areas of basal ganglia is thought to underline a variety of behavioral disorders including dyskinesia. The excessive DA tone induces alterations on other neurochemical pathways and numerous authors have suggested that serotonergic controls, notably via the 5-HT_{2C} receptor, are triggered in case of DA changes.

Here, we studied in rats the contribution of 5-HT_{2C} receptors using the 5-HT_{2C} antagonist SB243213 in the effects elicited by the dopaminergic agonist quinpirole on purposeless oral movements, c-Fos expression in basal ganglia nuclei and the electrophysiological activity of substantia nigra pars reticulata (SNr) neurons, the main output of basal ganglia, responding to the electrical stimulation of the cingulate cortex. The results showed that SB243213 (1mg/kg i.p.), without effect by itself, blocked the purposeless oral movements induced by 0.5 mg/kg i.p. quinpirole. The levels of the protein c-Fos, barely affected by quinpirole or SB-243213, were significantly increased in the subthalamic nucleus (STN) when the treatments were combined. Similarly, in urethane-anesthetized rats, SB-243213 unmasked a facilitatory effect of quinpirole on the spontaneous discharge of SNr neurons. Interestingly, the effect elicited by the electrical stimulation of the cingulate cortex, leading to an excitatory-inhibitory-excitatory response, was subtly changed by the drugs. Quinpirole enhanced the amplitude of the early excitatory response, involving the hyperdirect pathway, and this effect was abolished by SB-243213.

In conclusion, these results extend previous evidence that excessive DA tone triggers 5-HT_{2C} receptors-dependent controls in basal ganglia. The interaction occurs likely on the hyperdirect pathway in line with the role of the STN in mediating the purposeless oral movements induced by DA and 5-HT_{2C} agonists



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INVESTIGATION OF THE EFFECTS OF TIME OF DAY AND AGING ON 5-HT7 RECEPTOR BINDING IN THE HIPPOCAMPUS.

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5-HT7 receptors regulate many processes including circadian rhythms, REM sleep, memory, and depression. 5-HT7 receptors exhibit down-regulation by chronic antidepressant treatments that increase extracellular serotonin levels. Because endogenous serotonin release exhibits a daily rhythm with higher levels at night, we hypothesized that 5-HT7 receptors exhibit 24-h variations characterized by lower nighttime expression. Also, because aging decreases 5-HT7 receptors in the dorsal raphe nucleus, a brain region in which these receptors modulate circadian rhythms and REM sleep, we investigated whether aging decreases 5-HT7 receptors in the hippocampus, a likely substrate for the effects of 5-HT7 receptor compounds on memory and depression. Male hamsters (young, 3-5 mos; old, 17-21 mos) exposed to a light:dark cycle were euthanized at 4 times of day (zeitgeber times [ZT] 1, 6, 13, & 19; ZT12=time of lights:off). 5-HT7 receptors receptor autoradiography was conducted on hippocampal sections using [³H]8-OH-DPAT [2 nM] as the radioligand and SB-269970 [1 µM] to define nonspecific binding. Slide-mounted sections and radioactive standards were apposed to X-ray films; the resultant autoradiograms were assessed by computer-assisted microdensitometry. The results showed robust specific 5-HT7 receptor binding in the dentate gyrus (DG), CA1, and CA2 but not CA3. In the CA1 and DG, specific 5-HT7 receptor binding exhibited 24-h rhythms with troughs at night ($P < 0.005$; $P < 0.05$, respectively). Aging did not significantly affect specific 5-HT7 receptor binding in any region, nor were significant time and age interactions observed. These findings suggest that the therapeutic effectiveness of 5-HT7 drugs may vary with time of day of administration but not with the age of the recipient.



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A QUANTITATIVE PHOSPHOPROTEOMIC APPROACH REVEALS DIFFERENTIAL PHOSPHORYLATION OF SEROTONIN 2A RECEPTORS UPON ACTIVATION BY HALLUCINOGENIC VERSUS NON-HALLUCINOGENIC AGONISTS

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The 5-HT_{2A} receptor is a primary target of psychedelic hallucinogens such as LSD, which reproduce some of the core symptoms of schizophrenia. An incompletely resolved paradox is that only some 5-HT_{2A} receptor agonists exhibit hallucinogenic activity, whereas structurally related agonists with comparable affinity and activity do not. Using a quantitative phosphoproteomic approach combining stable isotope labelling by amino acids in cell culture (SILAC), phosphopeptide enrichment by hydrophilic interaction chromatography (HILIC)/immobilized metal affinity chromatography (IMAC) and high resolution mass spectrometry, we compared the phosphoproteome in HEK-293 cells transiently expressing the 5-HT_{2A} receptor under three conditions: exposure to sham treatment, exposure to the phenethylamine hallucinogen 1-[2,5-dimethoxy-4-iodophenyl]-2-aminopropane (DOI) and exposure to the non-hallucinogenic 5-HT_{2A} agonist lisuride. Among the 5,996 identified phosphopeptides (FDR<1%), 454 sites were differentially phosphorylated upon exposure to DOI vs. lisuride. These included a serine located in the i3 loop of the 5-HT_{2A} receptor, a region important for desensitization. Correspondingly, exposure to hallucinogens induced a less pronounced desensitization of receptor-operated signalling (increase in intracellular Ca²⁺ and Erk1,2 phosphorylation) than exposure to non-hallucinogenic agonists. Moreover, mutation of the serine into aspartate (to mimic phosphorylation) reduced receptor desensitization by non-hallucinogenic agonists, while its mutation to alanine increased the ability of hallucinogens to desensitize the receptor. This study reveals that 5-HT_{2A} receptor stimulation by hallucinogenic vs. non-hallucinogenic agonists induces contrasting phosphorylation patterns that may reflect their distinct behavioural responses upon acute and long-term treatment. It also provides one of the first demonstrations of differential phosphorylation of a G protein-coupled receptor upon stimulation by "biased" agonists.



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ACTIVATION OF 5-HT₆ RECEPTORS MODULATES SLEEP-WAKE ACTIVITY AND HIPPOCAMPAL THETA OSCILLATION

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Modulatory role of 5-HT neurons and a number of various 5-HT receptors has been well documented in regulation of sleep-wake cycle and hippocampal activity. High level of 5-HT₆ receptors expression is present in the rat hippocampus, and pharmacological activities related to hippocampal function have been shown of both 5-HT₆ agonists and antagonists. In the current study potential involvement of 5-HT₆ receptors in control of hippocampal theta rhythm and sleep-wake cycle has been investigated. Hippocampal activity was monitored by intracranial hippocampal electrodes in both anesthetized (n=13) and in freely moving rats (n=9). Theta rhythm was monitored in different sleep-wake states in freely moving rats and was elicited by stimulation of the brainstem reticular formation under anesthesia. Changes in theta frequency and power were analyzed before and after injection of 5-HT₆ antagonist (SAM-531) and agonist (EMD386088). In freely moving rats, EMD386088 suppressed sleep for several hours and significantly decreased theta peak frequency. In anesthetized rats, peak frequency of elicited theta significantly decreased after injection of EMD386088 but there was no change in theta power. SAM-531 did not change sleep-wake pattern and had no effect on theta parameters in either experiment. The decrease in theta frequency induced by 5-HT₆ agonist corresponds to previously described electrophysiological pattern shared by all tested anxiolytic drugs, and it is in line with its behavioral anxiolytic profile. The 5-HT₆ antagonist, however, did not show potentiation of theta power characteristic for many pro-cognitive substances, indicating that 5-HT₆ receptors might not tonically modulate hippocampal oscillation and sleep-wake pattern.



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TARGETED MANIPULATION OF TPH2

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Serotonin is one of the most important neurotransmitters in the brain with proposed functions in numerous neurophysiological circuits and crucial influence on mood and behaviour. Therefore, it is still the main target of neuropharmacological approaches targeting major depressive disorders. The identification of a second TPH-gene in 2003 unravelled the existence of two independent serotonergic systems in vertebrates with clearly distinct effects of central (TPH2/brain-derived) and peripheral (TPH1/gut-derived) serotonin. The existing knockout mouse models for TPH2 have several limitations, one main problem are possible compensatory mechanisms in the serotonergic system which may occur during early development.

To overcome these limitations we generated two different transgenic rat models for targeted and inducible TPH2 manipulation. In order to evaluate the role of serotonin for the functionality of the rat brain, one line is expressing a Doxycycline-inducible shRNA against TPH2. First experiments with homozygous animals show a modest, yet significant decrease in brain serotonin levels after 14 days of Doxycycline treatment. Another transgenic rat line expresses a codon-optimized GFP-tagged nitroreductase (GNTRo) under the control of the TPH2 promoter, which allows drug-inducible ablation of serotonergic neurons. This animal model will be employed to monitor the consequences of postnatal degeneration of serotonergic neurons and for a comparison of brain serotonin-deficient and serotonergic neurons-depleted rats. We additionally performed an enzyme-activity based high-throughput screen looking for pharmacologically active TPH modulators and found, amongst others, a subset of apparently specific TPH2 inhibitors. These structurally related compounds were further characterized and optimized in order to generate a scientific tool helping to unravel the TPH2 structure and for the analysis of induced TPH2 dysfunction.



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A POSITIVE ALLOSTERIC MODULATOR OF THE 5HT_{2C}R FOR OBESITY: PROMISE AND PITFALLS.

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Targeting the 5-HT_{2C} receptor is a promising intervention for the treatment of obesity. The most direct demonstration of the promise had been provided by the clinical trials data from the selective 5-HT_{2C} agonist Lorcaserin, which was found to lead to clinically significant weight loss. However, one of the main obstacles that has plagued this approach in general and Lorcaserin also has been getting truly selective compounds. The related "off-target" effects on the 5-HT_{2A} and 5-HT_{2B} receptors are associated with unacceptable CNS and cardiac risks which also may have lead to dose limitations that restricted access to the full potential of Lorcaserin for weight management.

A different approach to the stimulation of 5-HT_{2C} receptors is the development of PAMs for this target. This approach should lead to better selectivity, although it has not been clear how to find them. Using our proprietary structural design and high sensitivity automated flow cytometry screening platform, we have identified several series of PAMs for the 5-HT_{2C} receptor. One of our initial series yielded hits with modest potency (5 μ M) as PAMs, good selectivity, and clear in vivo activity, although poor drug-like properties. One of these candidates, VIVIA012, was found to be very active in feeding models in rodents. Its acute administration reduced food intake in 18 h food-deprived animals, without inducing malaise as revealed by the lack of induction of taste aversion. Subchronic administration (1 dose per day prior to the onset of the dark phase of the light cycle) reduced both food intake and body weight gain.



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ANTIPSYCHOTIC AGENTS: BIASED AGONISM AT SEROTONIN 5-HT_{1A} RECEPTORS

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The selective agonists, F13714 and F15599, exhibit “biased agonism” (functional selectivity) at 5-HT_{1A} receptors, with distinctive in vitro signaling profiles [1]. This translated to preferential in vivo activation of pre- or post-synaptic 5-HT_{1A} receptors, respectively [2]. However, it is unclear whether antipsychotics possessing 5-HT_{1A} receptor agonism demonstrate biased agonism.

We therefore data-mined literature describing in vitro 5-HT_{1A} receptor agonism of the marketed and exploratory antipsychotics: clozapine, ziprasidone, aripiprazole, adopraxine (SLV313), bifeprunox, SSR181507, F15063 and sarizotan. The effects of these drugs were compared on G protein activation [3], cAMP inhibition [3], ERK1/2 phosphorylation [4] and receptor internalization [5] assays.

Distinct rank orders of potency were observed, consistent with biased agonism. Clozapine: GTP > cAMP = internalization > ERK1/2. Ziprasidone: GTP > internalization > ERK > cAMP. Aripiprazole and SSR181507: internalization > GTP > ERK > cAMP. Bifeprunox: internalization > GTP = ERK > cAMP. Adopraxine and F15063: internalization > GTP > cAMP = ERK. Sarizotan: GTP = internalization > ERK > cAMP. Interestingly, varying degrees of efficacy were also seen across the different assay systems.

These data indicate that antipsychotics exhibit biased agonism at 5-HT_{1A} receptors in vitro and suggest that they may differentially activate 5-HT_{1A} receptor sub-populations in vivo [2]. It may be speculated that biased agonism at 5-HT_{1A} receptors could ultimately serve to target antipsychotics to specific symptom domains of schizophrenia, such as negative symptoms or cognitive deficits.

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2 Newman-Tancredi A, *Neuropsychiatry* 1, 149-164 (2011).

3 Newman-Tancredi A, et al. *Int J Neuropsychopharmacol* 8, 341-56 (2005).

4 Bruins Slot LA, et al. *Eur J Pharmacol* 534, 63-70 (2006).

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THE ASOCIAL MOUSE: CONSEQUENCES OF CENTRAL SEROTONIN DEFICIENCY.

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Depression is characterized by lack of motivation, severe anhedonia and social indifference. Decreased serotonin (5-HT) activity in the brain is discussed to be a major cause leading to the development of depressive disorders in humans. Tryptophan hydroxylase 2 (TPH2) is the rate limiting enzyme of 5-HT synthesis. Consequently, TPH2-deficient mice (TPH2^{-/-}) represent a model of brain 5-HT deficiency. Here, we analyzed TPH2^{-/-} mice and wildtype littermate controls for social and communication behavior.

Mice emit distinct types of ultrasonic vocalizations (USVs), which occur during social and sexual interactions of juvenile and adult mice and serve important communicative functions. Analysis of social interaction in juvenile mice revealed loss of interest in establishing social contacts in TPH2^{-/-} mice, which was evident from the reduced amount of physical contacts. However, USVs did not differ between TPH2^{-/-} mice and controls. Interestingly, females of both genotypes emitted significantly more USVs than males. Next, we evaluated social behavior in mice at the age of 16 weeks during exposure to sexual cues, i.e. female and male urine, before and after social contact to both sexes. Whereas control males exhibited normal vocalization behavior, TPH2^{-/-} mice failed to show typical vocalization responses to sexual cues. In contrast, behavioral activity displayed in response to both urine samples was not affected by genotype.

In conclusion, deficiency in brain serotonin leads to a visible loss of interest in social communication during lifetime. Moreover, despite being able to produce offspring, TPH2^{-/-} males fail to vocalize towards sexual cues, independently of their sexual experience. These results indicate that central serotonergic signaling is a critical modulator of social and sexual behaviour in mammalian brain.



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RESPONSE TO CLOZAPINE REVEALS LINK BETWEEN SCHIZOPHRENIA RISK PROTEINS EGR3 AND 5HT2AR.

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The immediate early gene early growth response 3 (EGR3) is associated with schizophrenia and expressed at reduced levels in patients' brains. We previously reported that Egr3^{-/-} mice are resistant to the sedating effects of clozapine compared with wildtype (WT) littermates, paralleling the heightened tolerance of schizophrenia patients to antipsychotic side effects. We have used a pharmacologic dissection approach to identify a neurotransmitter receptor defect in Egr3^{-/-} mice responsible for this effect. We find that this response is specific to second-generation antipsychotic agents (SGAs), and not seen with first generation antipsychotics. It is replicated by selective serotonin 2A receptor (5HT2AR) antagonists ketanserin and MDL-11939, which repress activity in WT mice at dosages that fails to suppress activity of Egr3^{-/-} mice. This appears to result from a nearly 70% reduction in 5HT2AR expression in the prefrontal cortex of Egr3^{-/-} mice (measured by radioligand binding). Egr3^{-/-} mice also exhibit a decreased head-twitch response to 5HT2AR agonist 1-(2,5-dimethoxy 4-iodophenyl)-2-amino propane (DOI). These findings provide a mechanism to explain the reduced sensitivity of Egr3^{-/-} mice to the locomotor suppressive effects of SGAs, and suggest that 5HT2ARs may also contribute to the sedating properties of these medications in humans. Moreover, since the deficit in cortical 5HT2AR in Egr3^{-/-} mice aligns with numerous studies reporting decreased 5HT2AR levels in the brains of schizophrenia patients, and the gene encoding the 5HT2AR is itself a leading schizophrenia candidate gene, these findings suggest a potential mechanism by which putative dysfunction in EGR3 in humans may influence risk for schizophrenia.



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SEROTONIN 5-HT_{2A} RECEPTOR ACTIVATION BLOCKS TNF- α MEDIATED INFLAMMATION IN VIVO, AND PREVENTS ASTHMA

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We previously discovered that activation of serotonin 5-HT_{2A} receptors has potent anti-inflammatory activity against TNF- α mediated inflammation in primary cell cultures of vascular tissues. We have recently translated these findings to the whole animal, and show here that systemic activation of 5-HT_{2A} receptors with the agonist (R)-DOI blocks the effects of systemic TNF- α in mice. This includes blockade of TNF- α induced expression of proinflammatory marker genes for cell adhesion molecules (ICAM1, VCAM1), cytokines (IL6), and chemokines (MCP-1, CX3CL1), as well as circulating levels of IL6. The anti-inflammatory effects are most potent in aortic arch and intestine. Significantly, we have further translated our findings to a mouse model of the human inflammatory disease allergic asthma. In the ovalbumin sensitization model, mice are sensitized and challenged with inhaled ovalbumin to induce a phenotype typically observed in human asthma including airways hyperresponsiveness in response to methacholine, mucus hyperproduction, and pulmonary inflammation characterized by eosinophilia. We have found that inhaled (R)-DOI, at levels as low as 0.01 mg/kg, completely blocks both inflammation and the symptoms associated asthma in this model. Airways hyperresponsiveness, mucus overproduction, and pulmonary inflammation are all prevented by inhaled (R)-DOI administration. Significantly, recruitment of eosinophils to the airways is also blocked. Our results indicate that agonism of 5-HT_{2A} receptors is a novel approach towards developing therapeutics for inflammatory diseases, especially for those of the vasculature, intestine, and lung, and those involving TNF- α .



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LACK OF BRAIN SEROTONIN AFFECTS POSTNATAL DEVELOPMENT AND SEROTONERGIC NEURONAL CIRCUITRY FORMATION

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Despite increasing evidence suggests that serotonin (5-HT) can influence neurogenesis, neuronal migration and circuitry formation, the precise role of 5-HT on central nervous system (CNS) development is only beginning to be elucidated. Moreover, how changes in serotonin homeostasis during critical developmental periods may have etiological relevance to human mental disorders, remains an unsolved question. In this study we address the consequences of 5-HT synthesis abrogation on CNS development using a knock-in mouse line in which the tryptophan hydroxylase 2 (Tph2) gene is replaced by the eGFP reporter. We report that lack of brain 5-HT results in a dramatic reduction of body growth rate and in 60% lethality within the first 3 weeks after birth, with no gross anatomical changes in the brain. Thanks to the specific expression of the eGFP, we could highlight the serotonergic system independently of 5-HT immunoreactivity. We found that lack of central serotonin produces severe abnormalities in the serotonergic circuitry formation with a brain regionspecific effect. Indeed, we observed a striking reduction of serotonergic innervation to the suprachiasmatic and thalamic paraventricular nuclei, while a marked serotonergic hyperinnervation was found in the nucleus accumbens and hippocampus of Tph2 mutants. Finally, we demonstrated that BDNF expression is significantly up-regulated in the hippocampus of mice lacking brain 5-HT, unmasking a possible regulatory feedback mechanism tuning the serotonergic neuronal circuitry formation. On the whole, these findings reveal that alterations of serotonin levels during CNS development affect the proper wiring of the brain that may produce long-lasting changes leading to neurodevelopmental disorders.



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CORRELATIONAL APPROACH OF MONOAMINERGIC FUNCTION IN CEREBRAL STRUCTURES INVOLVED IN COGNITION

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The widely spread nature of the dopamine (DA), noradrenalin (NA) and serotonin (5-HT) monoaminergic systems is the main difficulty to foresee their functions and their interactions. We sought to undertake the functional relationships between brain areas within a particular monoamine system network and between distinct monoaminergic systems in various brain areas involved in decision-making, through a global correlational approach of the monoamine tissue content.

Bilateral punches of twenty brain regions were taken on a cryostat from each frozen Sprague-Dawley rat brains (n=35). NA, DA and 5-HT tissue contents were measured using a sensitive HPLC/electrochemistry system. Significant correlations were searched for between the monoamine content of brain regions.

NA and 5-HT were present in all selected regions at concentrations approximately ranging from 0.07 to 1 ng/mg. DA tissue content, less homogeneous, was higher in dorsomedial striatum (8.6 ng/mg) compared to extrastriatal tissues (<0.5 ng/mg). We found some significant correlations between paired regions within a particular monoamine system (22/190 possible correlations for 5-HT; 16/190 for NA and 12/152 for DA). Correlations were exclusively positive for intracortical relationships. Negative correlations emerged from few cortico-subcortical and subcortical associations (4/6 and 3/9 for DA or 5-HT, respectively). We did not find any correlation between some adjacent brain regions for any monoamine (prelimbic/infralimbic cortex; core/shell accumbens; basolateral/central amygdala). When looking at the correlations between monoamines tissue content within brain areas, we found a higher degree of significant associations.

This approach of monoamine function reveals intriguing anatomical correlations that corroborate and extend functional relationships described in the literature of decision-making. These patterns could sustain large inter-individual differences in behavior and adaptability.



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THE ROLE OF SEROTONIN NEURON IN PATIENCE OF WAITING FOR DELAYED REWARDS

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Classic theories suggest that central serotonergic neurons are involved in the behavioral inhibition that is associated with the prediction of negative rewards or punishment. Failed behavioral inhibition can cause impulsive behaviors. However, the behavioral inhibition that results from predicting punishment is not sufficient to explain some forms of impulsive behavior. Here, we propose that the forebrain serotonergic system is involved both in “waiting to avoid punishment” for future punishments and “waiting to obtain reward” for future rewards. We found previously that 5-HT efflux in the dorsal raphe nucleus increases when rats perform a task that requires waiting for a delayed reward (Miyazaki et al., Eur J Neurosci 2011). We also found that serotonergic neurons increase their tonic firing rate when rats await food and water rewards and conditioned reinforcer tones. The rate of tonic firing during the delay period was significantly higher when rats were waiting for rewards than for tones, and rats were unable to wait as long for tones as for rewards (Miyazaki et al., J Neurosci 2011). Furthermore, the inhibition of serotonin neural activity by the local application of the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) in the dorsal raphe nucleus impaired rats’ patience of waiting for delayed rewards. These results indicate that the activation of dorsal raphe serotonin neurons is necessary for waiting for long delayed rewards and suggest that elevated serotonin activity facilitates waiting behavior when there is the prospect of forthcoming rewards. We hypothesize that increase in 5-HT neural activity during waiting for delayed rewards contributes to the regulation of the “waiting to obtain reward”.



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EFFECTS OF COMBINED MONOAMINE DEPLETION (CMD) ON BRAIN SEROTONERGIC AND DOPAMINERGIC METABOLITES IN C57BL/6 MICE

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Dietary depletion of different amino acid precursors (such as TYR, TRP, and PHE) has been used in several studies to investigate the effects of serotonin and dopamine in neuropsychiatric disorders. Here the neurochemical effects of a novel amino acid mixture that is suggested to lead to a combined depletion (CMD) of serotonin and dopamine were studied in male C57BL/6 mice, a mouse strain that was previously shown to respond to acute tryptophan depletion with decreased central nervous serotonin. A new CMD mixture consisting of large neutral amino acids and lacking the dopamine precursors PHE and TYR as well as the serotonin precursor TRP were examined by HPLC determination of serotonergic and dopaminergic metabolites in serum and several brain regions (hippocampus, amygdala, frontal cortex, caudate, nucleus accumbens). Food-deprived mice were gavaged with either a balanced (BAL) control formula containing large neutral amino acids or the CMD mixture. At 2.5 hours after gavage, mice were anesthetized and blood was collected by cardiac puncture. Brain regions were dissected and immediately frozen. The frontal cortex exhibited the largest effect of CMD treatment, and TRP and 5-HIAA levels were globally affected by CMD. The levels of their respective precursors and metabolites -TRP and 5-HIAA for serotonin and HVA for dopamine- were significantly reduced. In conclusion, these preliminary data support the notion that CMD administration attenuates serotonergic and dopaminergic metabolite levels in the brain.



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ANTAGONIST-MEDIATED DOWN-REGULATION OF 5-HT₇ SEROTONIN RECEPTORS IS REGULATED BY THE C-TERMINAL DOMAIN AND INTERACTION WITH GASP1

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The human 5-HT₇ serotonin receptor is a G-protein-coupled receptor that activates adenylyl cyclase constitutively and upon agonist-activation. Some inverse agonists towards the 5-HT₇ receptor can induce both homo- and heterologous desensitization, similar to agonist-stimulation, while others can induce receptor internalization, and a subset of these targeted 5-HT₇ receptors for lysosomal degradation. These results demonstrated that various ligands differentially activate regulatory processes governing receptor desensitization, internalization and degradation in addition to signal transduction, providing support for the concept of functional selectivity at the 5-HT₇ receptor, where different ligands stabilize different receptor conformations leading to differential effects.

The important atypical antipsychotics clozapine and olanzapine blocked G-protein activation (as expected), and surprisingly induced both internalization and lysosomal degradation of 5-HT₇ receptors. We therefore wanted to determine the mechanism of clozapine- and olanzapine-mediated internalization and lysosomal targeting of 5-HT₇ receptors.

In the C-terminus of the 5-HT₇ receptor we identified two important YXXΦ motifs and two conserved residues (LR) as potential sites involved in receptor internalization and recruitment of lysosomal sorting proteins, such as GPCR-associated sorting protein 1 (GASP1). Mutating one or both YXXΦ motifs or the LR residues inhibited lysosomal sorting of 5-HT₇ receptors. Over-expression of the C-terminus of GASP1 (previously demonstrated to interact with G-protein-coupled receptors) inhibited clozapine-mediated degradation of 5-HT₇ receptors, indicating that GASP is recruited to these domains of the 5-HT₇ receptor and involved in lysosomal sorting. Exactly how the motifs are involved in internalization remains to be determined.



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SEROTONIN TRANSPORTER DEFICIENT MICE EXHIBIT STATE DEPENDENT AND CONDITIONED AVERSION TO KAPPA OPIOID RECEPTOR ACTIVATION

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The dysphoria induced by activation of kappa opioid receptors (KORs) has been proposed to be mediated by the serotonin transporter (SERT). To further investigate the role of SERT in KOR-induced dysphoria, the KOR agonist U 50488 was used to produce conditioned place aversion (CPA) in mice with varying levels of SERT expression. Male SERT+/+, SERT+/-, and SERT-/- littermates underwent eight sessions conditionally pairing drug or saline injections with one respective side of a two sided conditioning chamber. On the ninth day, mice were not injected but given free access to both sides. Both SERT+/+ and SERT-/- mice showed significant decreases in time spent on the drug-paired side (DPS) compared to saline treated mice, indicating CPA to the DPS. Interestingly, SERT+/- mice did not exhibit CPA. On the tenth day, state-dependent conditioning was evaluated by injecting mice with saline or drug just prior to free access to both sides. On this testing day, mice of all three genotypes (including SERT+/- mice) receiving U 50488 spent significantly less time on the DPS vs saline-treated mice. Results of the state-dependent trial suggest that the lack of conditioning of SERT+/- mice on day 9 does not result from an attenuated drug effect. These findings demonstrate that SERT is not necessary for KOR-mediated dysphoria. Investigations into the molecular mechanisms of dysphoria could help to understand the potential for using KOR antagonists as SERT-independent antidepressants.



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DECODING THE ROLE OF SEROTONIN IN SLEEP USING OPTICAL- AND CHEMICAL-GENETIC APPROACHES

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Serotonin (5-HT) has long been considered to be a part of the ascending arousal system, which triggers transitions from sleep to wakefulness. The 5-HT system, first described as a neuromodulator of sleep, is now generally defined as a wake-promoting system based on the concomitant increase of the electrical activity of 5-HT neurons and 5-HT liberation at the cortical and thalamic level during waking, as opposed to the decrease observed during slow-wave sleep. However there is still no direct evidence indicating whether 5-HT neurons are required to promote and maintain arousal.

We are particularly interested in the individual role of 5-HT sub-groups in the modulation of the electroencephalographic activity and state transitions in different aspects of sleep regulations. Dysfunction of the 5-HT system is associated with severe diseases such as sudden infant death syndrome and sleep apnea syndrome both related to defective arousal response during sleep.

Here, we have explored the direct contributions of the dorsal raphe nucleus (DRN) and raphe magnus (RMg) to sleep regulation using a combination of behavioral and optogenetic methods. Specifically we expressed the light-activated cation channel channelrhodopsin-2 (ChR2) in DRN and RMg 5-HT neurons in order to evaluate the effects of in vivo photostimulation of both 5-HT sub-groups on sleep onset and sleep-to-wake transitions as well as changes in EEG power spectrum.

Furthermore, by using chemical-genetic approaches to deactivate the 5-HT neurotransmission in behaving mice, we have examined the correlation between vigilance states or sleep disturbances with the impaired respiratory and body temperature control upon acute perturbation of 5-HT neuron activity (Ray et al., 2011).



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Elevated 5-HT_{2A}R expression is associated with a predisposition toward inherent impulsivity

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Poor inherent response inhibition, or “action without reflection,” may set the stage for vulnerability to drug abuse and dependence. Serotonin (5-HT) systems play a nuanced role in impulsive action, perhaps mediated by forebrain 5-HT receptors. Selective 5-HT_{2A} receptor (5-HT_{2A}R) antagonists (e.g., M100907) reduce impulsive action with notable efficacy, suggesting that tonic 5-HT_{2A}R signaling supports impulsive behavior. We hypothesized that elevated forebrain 5-HT_{2A}R expression underlies the inherent predisposition to impulsive action. The proposed studies employed the one-choice serial reaction time (1-CSRT) task to identify high (HI) and low (LI) impulsive outbred rats to elucidate the neuromolecular biology of impulsivity. Rats were trained to nose-poke to receive food pellet rewards on a 5-sec inter-trial interval (ITI) schedule; responses during the ITI (premature responses) resulted in further delays of reward presentation. The upper 50% and lower 50% of animals were identified as HI or LI rats, respectively. Rats were sacrificed and the prefrontal cortex (PFC) and nucleus accumbens (NAc) were harvested and crude membrane protein extracted. The HI and LI phenotypes are stable in that premature responses in HI rats remained significantly higher than those of LI rats across 70 days of 1-CSRT task training ($p < 0.001$, Student's t-test). HI rats displayed higher 5-HT_{2A}R expression than LI rats in crude membrane fractions of the mPFC and NAc ($p < 0.05$, Student's t-test). Differences in 5-HT_{2A}R expression between HI and LI rats may be a key feature distinguishing the functional capacity of this receptor and its role in impulsive action. These data suggest that a hyperfunctional 5-HT_{2A}R in the mPFC and/or NAc underlies vulnerability toward impulsive action.



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Serotonin (5-HT) is a mysterious neuromodulator that is linked to a disparate set of functions and disorders. General theories for 5-HT function include at least three main lines: (1) behavioral inhibition/satiety; (2) balance between sensory input and motor output; (3) opponency between dopamine and 5-HT. Yet these theories remain incomplete, with contradictory or ambiguous experimental results which we believe are due in part to the limitations of traditional pharmacological and physiological approaches. We aim to transcend these limitations using an optogenetic toolkit that allows targeting of genetically-defined neural populations and temporally precise optical stimulation, inhibition and recording. We are continuing to refine and validate this strategy using a combination of in vitro and in vivo approaches, including quantification of 5-HT release via microdialysis, cyclic voltammetry and optically-evoked local field potentials (O-LFPs). We are currently testing the impact of 5-HT stimulation in the modulation of olfactory sensory responses, termination of sexual behavior, detection, dopamine opponency and aversive learning.



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CHARACTERIZING PERIPHERAL BLOOD CELL SERT FUNCTION USING A FLUORESCENT TRANSPORTER SUBSTRATE AND FLOW CYTOMETRY

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Objective: In conjunction with studies in our laboratory to determine the feasibility of peripheral blood cells (PBCs) as biomarkers for antidepressant treatment response, we aim to develop a readily translatable clinical assay for ascertaining SERT function in PBCs using flow cytometry.

Methods: The monoamine transporter substrate IDT307, which fluoresces once taken up intracellularly, was used to evaluate SERT function in lymphocytes and platelets isolated from mice, rhesus monkeys, and humans. Mouse platelets were from platelet-rich plasma. Rhesus and human mononuclear cells and platelets were isolated using Ficoll separation protocols. Cells were incubated for 45 min at 37°C in the presence of IDT307 (1 μ M) prior to flow cytometry. Uptake inhibitors were used to assess specificity; cell viability was by propidium iodide staining.

Results/Conclusions: Detection sensitivity using IDT307 and flow cytometry is comparable to previous finding using chronoamperometry, however, flow cytometry has the advantage of assigning relative uptake to separate cell populations. Lymphocytes and platelets exhibit strong IDT307-associated fluorescence, however, SSRIs inhibit IDT307 uptake only in platelet populations. Uptake of IDT307 in lymphocytes is temperature dependent, but DAT and NET independent, inferring that lymphocytic IDT307 uptake is mediated by an unknown transport system. These findings suggest that SERT-mediated uptake is low in lymphocytes compared to platelets, and the latter are better candidates for SERT-based peripheral biomarker assays.



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REPLICATION OF FUNCTIONAL SEROTONIN RECEPTOR TYPE 3A AND B VARIANTS IN BIPOLAR AFFECTIVE DISORDER: A EUROPEAN MULTICENTER STUDY

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Liliana Oruc, Fabio Rivas, Gulja Babadjanova, Maria Grigoriu-Serbanescu, Joanna Hauser,
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Serotonin 3 receptors (5-HT₃) are involved in learning, cognition and emotion, and have been implicated in psychiatric phenotypes. However, their contribution to the pathomechanism of these disorders remains elusive. Three functional SNPs in the HTR3A and HTR3B genes (rs1062613, rs1176744, rs3831455) have been associated with bipolar affective disorder (BPAD) in pilot studies.

We performed a European multicenter study to confirm previous results and provide further evidence for the role of these SNPs to the etiology of neuropsychiatric disorders. This involved analysis of the distribution of the three SNPs among 1804 BPAD cases and 2407 healthy controls. A meta-analysis revealed a pooled odds ratio of 0.881 ($P = 0.009$, 95% CI = 0.802 – 0.968) for the non-synonymous functional SNP HTR3B p.Y129S (rs1176744), thereby confirming previous findings. In addition, three genome wide association study (GWAS) samples showed an over-representation of the p.Y129 in patients, revealing a P-value of 0.048 (OR = 0.934, fixed model) in a meta-analysis including more than 3500 patients and 5200 controls. We also performed expression analyses to gain further insights into the distribution of HTR3A and HTR3B mRNA in the human brain. HTR3A and HTR3B were detected in all investigated brain tissues with the exception of the cerebellum. Interestingly, expression of the B subunit was most prominent in the brain stem, amygdalae, and frontal cortex, regions of relevance to psychiatric disorders.

In conclusion, the present study provides further evidence for the presence of impaired 5-HT₃ receptor function in BPAD.



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MODULATION OF GLUTAMATERGIC SYNAPTIC TRANSMISSION IN PREFRONTAL CORTEX BY 5-HT_{2A} RECEPTORS

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The serotonergic system plays an important role in regulating prefrontal cortex functions such as emotional control, cognitive behaviors and working memory. Among the G protein-coupled receptors activated by serotonin (5-HT), 5-HT_{2A} receptors raise particular interest. Indeed, they are the target of a large number of psychoactive drugs including atypical antipsychotics (antagonists or inverse agonists) and the majority of psychedelic hallucinogens that act as agonists or partial agonists at 5-HT_{2A} receptors.

5-HT_{2A} receptors that are especially abundant in layers V of the prefrontal cortex, with a predominant expression in apical dendrites of pyramidal neurons, have been involved in numerous psychiatric diseases including psychoses such as schizophrenia.

Several studies have shown that activation of 5-HT_{2A} receptors in the prefrontal cortex results in an increase in spontaneous glutamatergic synaptic activity. However, the mechanism of 5-HT_{2A} receptor-mediated modulation of synaptic transmission in prefrontal cortex is still matter of debate. The purpose of this study is to characterize the role of 5-HT_{2A} receptors in the glutamatergic synaptic transmission.

Here, we showed that activation of 5-HT_{2A} receptors by their agonist DOI modulates synaptic transmission of thalamo-cortical input by eliciting a reversible potentiation of NMDA evoked response. We demonstrated that this effect requires presynaptic NMDA receptors, which could lead to an increase of glutamate release. We confirmed this presynaptic effect by performing NMDA paired-pulse facilitation and by recording NMDA or AMPA mediated miniature EPSCs. Moreover, delivering GDP- β -S (a G protein signaling pathway blocker), directly in the post-synaptic neuron did not affect the NMDA response induced by DOI, as well as PLC and PKC blockers, suggesting the involvement of presynaptic 5-HT_{2A} receptors. All the effects of DOI were prevented by ketanserin, a 5-HT_{2A} receptor antagonist, as well as by the genetic invalidation of 5-HT_{2A} receptors.



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INVOLVEMENT OF GIRK2 CHANNELS IN SEROTONERGIC NEUROTRANSMISSION

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Background: Dysfunctional serotonergic neurotransmission is implicated in several neurological diseases such as depression. The serotonergic tone is regulated by 5-HT_{1A} receptors coupled to GIRK channels which are located in dorsal raphe nucleus (DRN). Nowadays, it is well known GIRK channels containing GIRK2 subunits play an important role controlling excitability of several brain areas.

Objective: To study the involvement of GIRK2 channels in the control of spontaneous activity and response to serotonergic drugs of DRN neurons, and behavioral response to anxiety-related situations and antidepressants.

Methods: In vivo single-unit extracellular recordings of DRN neurons and tail suspension test (TST) experiments were performed using wild-type and GIRK2 mutant mice.

Results: The proportion of burst-firing neurons in the DRN from GIRK2 heterozygous and homozygous mice was greater than in wild-type (25% vs 11% in mutant and wild-type mice, respectively). However, Girk2 gene mutation did not alter firing activity or the coefficient of variation of DRN neurons. Dose-response curves of the 5-HT_{1A} receptor agonist 8-OHDPAT (12.5-300 µg/kg, i.p.) were shifted to the right in heterozygous (ED₅₀ 42.15±2.90 µg/kg) and homozygous mice (ED₅₀ 68.65±6.71 µg/kg) compared to that obtained in wild-type (ED₅₀ 30.35±2.92 µg/kg). Similar results were obtained with the antidepressant citalopram (1.05±0.04 mg/kg; 1.29±0.82 mg/kg; 0.85±0.03 mg/kg, ED₅₀ values for heterozygous, homozygous and wild-type mice, respectively). However, the efficacy of both drugs remained unaltered. Heterozygous and homozygous animals showed lower immobility time in the TST (185.0±4.85 s and 151.3±26.67 s) comparing to wild-type group (213.3±6.78 s). Interestingly, citalopram (10 mg/kg, i.p.) was less effective reducing the immobility time in the mutant genotypes (wild-type 89.89±1.47%; heterozygous 41.55±6.99%; homozygous 28.80±5.94%).

Conclusion: Mutation of Girk2 gene altered the neurophysiology of DRN neurons in vivo and improved the behavioral response to anxiety-related situations which could determinate the response to antidepressants.



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CALCINEURIN AND ASCT2: NEW PROTEIN PARTNERS OF SEROTONIN TRANSPORTER

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The plasma membrane serotonin transporter (SERT) plays a critical role in the regulation of serotonergic transmission by enabling serotonin reuptake into the cells. This transporter is of major pharmacological and clinical interest, particularly as it represents one of the primary targets of several widely prescribed antidepressants. It is now well established that SERT does not function as an isolated protein. SERT functional activity is regulated by a combination of multiple mechanisms including both post-translational modifications and association with intracellular proteins. During the last decade, several SERT-interacting proteins have been identified, principally by means of yeast two-hybrid screens. We have recently used a proteomic approach that enabled us to characterize a reciprocal modulation of SERT and neuronal NO Synthase (nNOS) activity mediated by their physical interaction. To get further insight into SERT-associated protein complex, we used high-resolution mass spectrometry to identify novel proteins interacting with SERT C-terminus, or whole SERT protein expressed in two different cell culture models. This shotgun analysis of SERT interactome led us to identify several new partners of SERT. These include Calcineurin, a calcium-dependent serine/threonine phosphatase, ASCT2 (Alanine Serine Cysteine Transporter 2), a neutral amino acid transporter, and a set of proteins related to the SNARE complex, possibly involved in SERT export to the plasma membrane. Moreover, we showed that both physical interaction of SERT with Calcineurin and Calcineurin phosphatase activity increase SERT plasma membrane expression and 5HT uptake via SERT. In addition, co-expression of ASCT2 decreases SERT activity, possibly via modification of its glycosylation status. Lastly, the use of D-Threonine, an inhibitor of ASCT2 activity, demonstrate that ASCT2 transport is required for this inhibitory effect. Collectively, these proteomic studies identify novel regulation mechanisms of SERT activity that might influence serotonergic transmission.



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PROGESTERONE WITHDRAWAL-INDUCED DEPRESSION-LIKE BEHAVIOR IS NOT DEPENDENT ON SEROTONIN LEVELS OR SERT FUNCTION

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Abrupt withdrawal from chronic progesterone administration induces depression-like and anxiety-like behavior in female rats, analogous to symptoms of premenstrual dysphoric disorder (PMDD). However, few studies have systematically compared the efficacy of antidepressants with different mechanisms in women, or in animal models of PMDD. Furthermore, the role of serotonin (5-HT) levels in the etiology of behavioral dysfunction in rodent models of PMDD has not been well studied.

1) Progesterone withdrawal (PWD - i.p. injection of progesterone, 30 mg/kg, for 3 weeks, rats tested 48 hr after final injection) reliably induces depression-like behavior in the forced swim (FS) test.

2) Brain levels of serotonin and 5-hydroxyindoleacetic acid (5-HIAA) and plasma levels of tryptophan (TRP) were not altered during PWD. TRP depletion (via TRP deficient diet) did not augment immobility in the FS test.

3) Neither fluoxetine nor duloxetine reduced depression-like behavior when administered either chronically (14 days) or acutely (2 days). In contrast, acute administration of the 5-HT₃ receptor antagonist, ondansetron, or the 5-HT_{1A} receptor agonist, flesinoxan, reduced depression-like behavior, but these effects were not additive with fluoxetine. Moreover, Lu AA21004, a multimodal investigational antidepressant (5-HT₃ and 5-HT₇ receptor antagonist, 5-HT_{1B} receptor partial agonist, 5-HT_{1A} receptor agonist and inhibitor of the 5-HT transporter in vitro) reduced depression-like behavior after both acute and chronic administration.

These data indicate that antidepressants with 5-HT₃ antagonist and/or 5-HT_{1A} agonist activity reduce immobility during PWD in rats. Furthermore, 5-HT receptor modulation was not additive with an SSRI, consistent with data indicating no primary deficit in serotonin levels in this model.



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SEROTONIN AS A KEY FACTOR FOR RED BLOOD CELL PRODUCTION AND SURVIVAL

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Erythropoiesis is the process through which the hematopoietic tissue produces red blood cells (RBCs). Anemia is defined as a reduction in the number of RBCs or the amount of hemoglobin in the blood. In individuals with ineffective erythropoiesis, a defect prevents erythroid cells from proliferating or differentiating normally, leading to abnormal production of mature RBCs and failure to compensate for anemia. We recently published that mice deficient in peripheral 5-HT display a phenotype of macrocytic anemia associated with inefficient erythropoiesis. In addition, we showed that RBCs from 5-HT-deficient mice are more sensitive to macrophage phagocytosis and have a shortened in vivo half-life. It remained to be determined whether the reduced half-life of TPH1^{-/-} RBCs results from the observed ineffective erythropoiesis, from a direct effect of 5-HT on the RBC, or both. Evidence that 5-HT acts directly on erythroid precursors is provided by the presence of serotonergic effectors on these cells and by in vitro experiments in which supplementing the culture medium of TPH1^{-/-} erythroid precursors with 5-HT restored their proliferative capacity. Also, addition of para-chlorophenylalanine to the culture medium reduced the number of RBCs obtained due to an inadequate proliferation/differentiation process of RBC precursors. In parallel, we established an in vitro model of RBC conservation to test whether 5-HT supplementation improves the viability of stored RBCs. We show that addition of 5-HT to the medium significantly improves RBC survival. Also, our results show that 5-HT addition diminishes hemolysis and improves RBCs in vivo survival after transfusion. These results reveal a crucial role for 5-HT in both RBC production and survival with important clinical implications in treatment of anemia and in transfusion medicine.



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DECISION-MAKING TO EAT AFTER STRESS DEPENDS ON THE SEROTONIN 4 AND 1A RECEPTORS IN THE MEDIAL PREFRONTAL CORTEX AND DORSAL RAPHE

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Feeding anomalies are often attributed to deficits of the homeostatic regulation, but much less to a deficit in decision-making to eat. However, in a stressful context, pathologic decision may trigger the brain to persistently inhibit feeding despite a growing request in energy. Little is known about the neuronal mechanisms associated with decision-making, except that choice depends on the hyperactivity of dorsal raphe nucleus (DRN) serotonergic neurons induced by the medial prefrontal cortex (mPFC). Here, we show that the serotonin 4 receptors (5-HTR4) in the mPFC are required to cause hypophagia following stress. We previously found that the 5-HTR4 knock-out (KO) abnormally persist to eat in response to stress, which is associated with an impaired activity of DRN 5-HT system. Here, siRNA-mediated 5-HTR4 knock-down in the mPFC mimicked the behavioral and neuronal KO phenotype. The viral 5-HTR4 knock-up in the mPFC of 5-HTR4 KO has restored the feeding and DRN 5-HT responses to stress in the KO. Using a large spectrum of techniques, we conclude that stress triggers the stimulation of cortical 5-HTR4, reduces the density of DRN 5-HT transporter, which promotes an increased level of DRN 5-HT in the synaptic cleft. The DRN 5-HT next stimulates 5-HTR1A and avoids that hypophagia lasts and turns into anorexia after stress. Collectively, our findings support that the neuronal network of decision-making to eat after stress involved a 5-HTR4 control in the mPFC of the DRN 5-HTR1A neuronal activity.



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THE ABSENCE OF SEROTONIN 4 RECEPTORS SUPPRESSED ANOREXIA INDUCED BY STRESS AND INCREASED THE NEGATIVE FEED-BACK ON THE STRESS AXIS IN FEMALE

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Anorexia is a deadly mental disease related to restrictive diet despite actual energy requirements and affects more women than men. By modeling anorexia, we have proposed one of the few, if any, therapeutic targets: the serotonin 4 receptors (5-HTR4). Here, female mice lacking 5-HTR4 did not display anorexia following the restraint stress (forced immobilization), a proposed animal model of anorexia nervosa. Inactivating 5-HTR4, after intraperitoneal administration of a 5-HTR4 antagonist in wild-type (WT) female mice, mimicked KO mice phenotype. In neither animal group were changed motor responses to novelty and anxiety-like behavior. We next tested potential changes in the activity of the hypothalamo-pituitary axis in response to stress. Under basal conditions, no changes were detected between mice of both genotypes in adrenocorticotropin-releasing hormone (ACTH) and corticosterone levels under baseline conditions. In contrast, ACTH level was weaker in 5-HTR4 KO than 5-HTR4 WT mice while increases in corticosterone level in plasma were similar between stressed mice of both genotypes. Decreases in ACTH level could ensue to limit corticosterone production and if glucocorticoid receptor (GR) exerts a higher negative feedback influence on stress axis in the absence of 5-HTR4. Injecting GR agonist, dexamethasone, was more effective for increasing food intake in food-deprived 5-HTR4 KO than in WT female mice. The gain-of-function of GR in the absence of 5-HTR4 was associated with increased levels in GR mRNA in the hypothalamus. Accordingly, a 5-HTR4 negative control on the GR negative feedback on the stress axis could favor anorexia induced by stress in female mice.



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BRAIN SEROTONIN DEFICIENCY PROMOTES RESILIENCE TO CHRONIC STRESS

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An increasing body of evidence implicates brain serotonin (5-HT) in gene-environment interactions impacting vulnerability or resilience to neuropsychiatric disorders. Yet, the role of 5-HT in the relationship between (epi)genetic mechanisms in response to environmental adversity and psychopathology remains elusive. To dissect this interaction, we investigated brain-specific 5-HT deficient mice (resulting from Tph2 gene inactivation) and challenged them by environmental stressors.

Compared to wildtype littermates, Tph2-deficient mice (Tph2^{-/-}) display reduced anxiety-like behavior but increased depression-like behavior and conditioned fear. Electrophysiological recordings within amygdala revealed neuronal correlates for the dissociation between enhanced fear learning/retention and low innate anxiety.

In contrast to wildtype mice, Tph2^{-/-} mutants were resilient to unpredictable chronic-mild-stress (CMS) regarding anxiety-like behavior, while depression-like behavior was rescued by CMS. Monitoring of HPA axis function during CMS showed an increased female-specific reactivity which time-dependently normalized by adaptive changes in corticosteroid receptor expression.

Beside, 5-HT deficiency induces brain 5-HT_{1A} and 5-HT_{1B} receptors up-regulation and a reduction of norepinephrine concentrations and neuron number. Nevertheless, raphe serotonergic neurons fully develop and conserve their cellular, molecular and electrophysiological characteristics.

Our findings show that 5-HT mediates adverse stress effects on behavior by facilitating the encoding of negative effects and provide new insight into the role of 5-HT-dependent signaling in the vulnerability to neuropsychopathology and resilience to environmental adversity.



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FRET-BASED EVIDENCE FOR LIGAND-INDEPENDENT PREASSOCIATION OF 5-HT₇ RECEPTORS AND G_s - MAPPING OF POSSIBLE INTERACTION DOMAIN

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Comparison of the G_s-coupled 5-HT₄ and 5-HT₇ serotonin receptors revealed fundamental differences in G protein activation. Whereas 5-HT₄ receptor function is consistent with collision coupling, the pharmacological properties of 5-HT₇ receptors best fit a model with receptor and G_s preassociated without ligand. To test if 5-HT₇ receptors preassociate with G_s, we used Fluorescence Resonance Energy Transfer (FRET) to compare the interaction between fluorescently labeled β ₁-adrenergic, 5-HT₄ or 5-HT₇ receptors and G proteins. Agonist-activation of β ₁ or 5-HT₄ receptors increased FRET. In contrast, 5-HT₇ receptor activation decreased FRET in a concentration-dependent manner. The dissociation of G protein from 5-HT₇ receptors had kinetics identical to G protein activation but slower than recruitment of G protein to β ₁ receptors.

To identify the molecular determinants of the 5-HT₇ receptor responsible for the preassociation with G protein, we constructed a series of mutated and chimaeric receptors. Deletion of the third intracellular loop (ICL) and carboxy-tail did not inhibit preassociation with G protein. Constructing chimaeric 5-HT₇ receptors with different ICLs and carboxy-tails from the 5-HT₄ receptor revealed that a single ICL is not solely important for preassociation with G protein, but some of the ICLs might be important for the orientation of the G protein.

Taken together, there is a stable complex between 5-HT₇ receptors and G_s, but the molecular determinants responsible remain to be further elucidated.



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ROLE OF THE 5-HT SYSTEM IN TINNITUS: A SYSTEMATIC STUDY USING AN ANIMAL MODEL

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Background

Serotonin (5-HT) has multiple functions in the brain, many of which are involved in emotion, attention and mood control. It is also present in the auditory system and has been implicated in tinnitus. Many scattered tinnitus trials with various drugs that target the 5-HT system have produced mixed results with the choice of drugs rather arbitrary. In our research we have started to systematically study the changes in 5-HT receptors and other components of the 5-HT system in various parts of the auditory system of animals with deafness-induced tinnitus.

Materials and Methods

Using a rat model of tinnitus (GPIAS) we have performed a detailed systematic screen of whether the 5-HT system is perturbed following induction of tinnitus. Tinnitus was induced unilaterally and bilateral brain regions were collected and analysed separately by quantitative PCR to determine changes in gene expression.

Results

We have analysed eleven brain regions with a panel of eleven genes and have described significant changes in gene expression of several key 5-HT related genes.

Conclusions

Using sensitive molecular biological techniques we have started to detail the changes in gene expression that occur in different parts of the auditory system following the induction of tinnitus. We have exciting preliminary data showing co-ordinated changes in gene expression in various parts of the brain in these animals. This work holds out the prospect of targeted use of already existing drugs to treat tinnitus and for the longer term, the hope of rational design of specific drug treatments.



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NEW CELL-BASED ASSAYS FOR 5-HT RECEPTOR INVESTIGATIONS USING TAG-LITE TECHNOLOGY

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Cisbio Bioassays presents a new range of innovative products, Tag-Lite, for the study of membrane receptors such as the GPCRs, based on the association of unique cell reagents combined with a specific detection system. Tag-Lite technology is an original and integrated platform to study most of biological interactions by the measurement of protein expression level at the cell membrane, pharmacological compound binding, functional properties and oligomerization assays from a single cell line expressing the receptor of interest. This innovative method combines the detection technology developed by Cisbio Bioassays, HTRF (Homogeneous Time Resolved Fluorescence), specific covalent substrates for labeling of receptors at the surface of living cells using small fusion tags (SNAP-Tag, HALO-Tag...) that covalently interact with the specific substrates conjugated with the HTRF fluorophores, specific ligands coupled with HTRF fluorophores for the screening of many compounds and kits for detection of second messengers production (cAMP, IPone...). HTRF reagents could be easily miniaturized while maintaining their accuracy and reproducibility. Here we present the reagents that we have developed to study several membrane serotonin receptors using Tag-Lite technology: plasmids, fluorescent ligands and kits for detection of second messengers as IP1. We propose plasmids encoding the receptors 5HT1A, 5HT1B, 5HT1D, 5HT1E, 5HT1F, 5HT2A, 5HT2B, 5HT2C, 5HT4, 5HT5A, 5HT6 and 5HT7 including SNAP-tag suicide enzyme. We also have developed plasmids encoding 5HT3A ion channel including HALO-tag suicide enzyme and HA-tag epitope. Also, several specific fluorescent ligands have been synthesized during an on-going collaborative program with the Institut de Génomique Fonctionnelle (IGF) in Montpellier and the Laboratoire d'Innovation thérapeutique (LIT) in university of Strasbourg, and three new fluorescent ligands have been validated to investigate 5HT1A, 5HT1B and 5HT4 receptors (Kd 76nM, 12nM and 25nM respectively) and are already available in our catalogue of products. Recently we discovered new fluorescent ligands to address specifically 5HT2B, 5HT2C 5HT6, and 5HT7 receptors.



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A SEROTONIN-MICROGLIA INTERACTION INVOLVED IN « SICKNESS BEHAVIOR » ?

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The neurotransmitter serotonin (5-HT) is involved in numerous physiological functions (mood, sleep, food intake regulations...) and pathological situations (pain, depression). Most of these physiological functions are transiently dysregulated in case of peripheral infection, resulting in a specific motivation state called "sickness behavior". This transient "depressive-like" state points to a role of the serotonergic system in shaping this behavior. Microglia, brain resident macrophages, can be activated following a peripheral infection.

Sickness behavior following an infection is particularly striking in aged patients, whose microglial cells are thought to be in a "primed" state, thus our hypothesis is that these behavioral effects involve both serotonin-secreting neurons and microglia, in a pathway we want to investigate.

As we observed that microglia express mainly the 5-HT_{2B} receptor, we compared sickness behavior and brain inflammation induced by a peripheral lipopolysaccharide injection in wild-type and 5-HT_{2B} KO mice. Our results indicate that 5-HT_{2B} KO mice react stronger than wild-type to this treatment, which points to a role of serotonin-sensing by microglia in the regulation of sickness behavior.



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