

International Narcotics Research Conference

14 – 19 July 2013

Pullman Cairns International
Cairns, Australia

CONFERENCE HANDBOOK

www.dcconferences.com.au/inrc2013/

WELCOME

Welcome and thank you for participating in INRC 2013 in Cairns. This is the 44th meeting of the INRC. INRC has not met in Australia for 26 years (Adelaide in 1987). This current meeting in Australia acknowledges the contributions Australian scientists have made to our field. In recognition of this, plenary lectures this year include presentations from two outstanding Australian scientists, Bernard Balleine and Arthur Christopolous.

The highlight of our meeting as always is the Founder's Lecture. This year the award goes to Dr. Graeme Henderson of the University of Bristol in the UK, for his outstanding research on opioid receptor signaling in single neurons. Also this year we have our first Young Investigator Awardee, Dr. Louis Gendron of the Université de Sherbrooke, Canada.

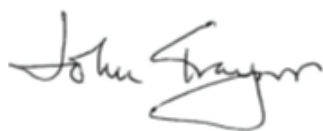
You will see from the program that we have a great line up of symposia and submitted abstracts that attests to the vitality of our field – as well as the ever popular data-blitz. Please note that the symposium on “Endogenous Opioids” will be held in honor of one of INRC's founding fathers, Avram Goldstein, who passed away in 2012.

I know you will enjoy the meeting and get involved in the scientific debate that makes our field so cutting-edge, lively and exciting and inspires new ideas and collaborations.

And ... don't forget the business meeting on Thursday afternoon. The executive committee is very open to new ideas. This is your meeting and we want to know your views.

Finally, I would like to thank Mark Connor and Macdonald Christie for putting together such a great program and for all the behind the scenes logistic work in getting this meeting off the ground. Of course, this meeting would not have been possible without our sponsors – the INRC thanks you.

Have a great meeting!

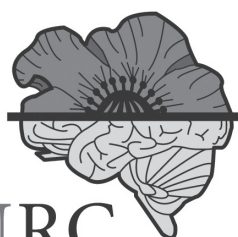


President, INRC

THANKS TO OUR SUPPORTERS



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INRC

Special thanks to the Local Organising Committee

- **Mark Connor**, Co-Chair, Macquarie University, Australia
- **Mac Christie**, Co-Chair, University of Sydney, Australia
- **Andrew Lawrence**, Florey Institute/University of Melbourne, Australia
- **Ernie Jennings**, James Cook University at Cairns, Australia
- **Andrew Somogyi**, University of Adelaide, Australia
- **Alfreda Stadlin**, Chungbuk National University, South Korea

Special thanks to the Scientific Program Committee

- **Charles Chavkin**, University of Washington, United States
- **Ian Kitchen**, University of Surrey, United Kingdom
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- **Susie Ingram**, Oregon Health and Science University, United States
- **John Traynor**, University of Michigan Medical School, United States
- **Louis Gendron**, Université de Sherbrooke, Canada
- **Lan Ma**, Fudan University Pharmacology Research Center, China
- **Minoru Narita**, Hoshi University School of Pharmacy and Pharmaceutical Science, Japan

GENERAL INFORMATION

Venue

Pullman Cairns International

17 Abbott Street

CAIRNS QLD 4870

T: +61 7 4031 1300

F: +61 7 4031 1801

W: www.pullmancairnsinternational.com.au

Registration Inclusions

Delegate registration includes:

- Attendance to all scientific sessions
- Satchel (including conference materials)
- Welcome Reception (Sunday evening)
- Morning tea, lunch and afternoon tea (Monday–Thursday and morning tea on Friday)
- Conference handbook

Registration Desk Opening Hours

Sunday 14 July: 4.00pm – 6.00pm

Monday 15 July: 7.30am – 5.30pm

Tuesday 16 July: 8.00am – 6.00pm

Wednesday 17 July: 8.00am – 12.30pm

Thursday 18 July: 8.00am – 5.00pm

Friday 19 July: 8.00am – 12.30pm

Speaker's Preparation – Tully Room

Opening hours

Sunday 14 July: 4.00pm – 5.30pm

Monday 15 July: 7.00am – 2.00pm

Tuesday 16 July: 7.00am – 2.00pm

Wednesday 17 July: 7.00am – 11.00am

SOCIAL PROGRAM

Welcome Reception

Date: Sunday 14 July 2013

Venue: Daintree Pool Deck, Pullman Cairns International

Time: 6.00pm – 8.00pm

Soak up the delights of tropical Cairns whilst enjoying a refreshing beverage or two on the Daintree Pool Deck. Take time out to catch up with friends and colleagues.

The Welcome Reception is complimentary for all conference delegates. Additional tickets can be purchased for \$60 per person at the registration desk.

Conference Data Blitz

Date: Monday 15 July 2013

Venue: Mossman Ballroom, Ground Floor Pullman Cairns International

Time: 6.30pm – 8.30pm

Price: Complimentary

The INRC Conference Data Blitz offers students and postdocs the chance to introduce results in a casual session. All students and postdocs are welcome to present their data.

The presentation is a starting point for informal discussions with light refreshments to follow.

Conference Dinner

Date: Thursday 18 July 2013

Venue: Thornton Peak, Pullman Cairns International




Time: 7.00pm – 10.30pm

Price: \$80 per person



Join your INRC colleagues for an evening of great food, fine wine and excellent conversation under the stars of a balmy tropical North Queensland evening.

Please visit the registration desk to purchase tickets to the conference dinner.

Sunday, 14 July 2013**Welcome Reception:** 6pm – 8pm Daintree Pool Deck, Pullman Cairns International**Monday, 15 July 2013**

TIME	PRESENTATION	SPEAKER
8.00 – 8.30am	Arrival Tea and Coffee	
8.30 – 9.45am	Welcome <i>Proudly sponsored by the Ian Potter Foundation</i> Plenary 1: Founders Lecture: Opioid research – turning full circle	 Graeme Henderson
9.45am	Symposium 1: Opioid heteromers <i>Chair: Lakshmi Devi</i> <i>Proudly sponsored by the British Pharmacological Society and the British Journal of Pharmacology</i>	 
9.45 – 10.10am	Co-localization of Mu and Delta Opioid Receptors in the Nervous System Using Double Fluorescent Knock-in Mice	Dominique Massotte
10.10 – 10.35am	Opioid receptor trafficking and interaction in nociceptors and its role in opioid tolerance	Xu Zhang
10.35 – 11.15am	Morning Tea	
11.15 – 11.40am	Symposium 1 Continued: Opioid heteromers A μ OR- δ OR Heteromer-biased ligand with antinociceptive activity	Wakako Fujita
11.40am	Symposium 2: Human Aspects of Opioid Use	
11.40 – 12.05pm	Heroin dependence in Asian populations	Alfreda Stadlin
12.05 – 1.00pm	Lunch	
1.00 – 1.25pm	Symposium 2 Continued Opioid-induced hyperalgesia: does the myth need busting?	Andrew Somogyi
1.25 – 1.50pm	Patterns of prescription drug use in Australia	Paul Haber
1.50 – 2.15pm	Afternoon Tea	
2.15 – 3.15pm	Hot Topics 2	Kate Dolan, Alisa Knapman, Shu-Lung Yang, Nayla Chajale
3.30 – 5.30pm	Poster Session I	
6.30pm – 8.30pm	Data Blitz Mossman Ballroom, Ground Floor Pullman Cairns International	

Tuesday, 16 July 2013

TIME	PRESENTATION	SPEAKER
8.00 – 8.30am	Arrival Tea and Coffee	
8.30am	Symposium 3: MOR regulation <i>Chair: Macdonald Christie</i> <i>Proudly sponsored by the British Pharmacological Society and the British Journal of Pharmacology</i>	 
8.30 – 8.55am	Differentiation of opioid drug effects by hierarchical multi-site phosphorylation	Stefan Schulz
8.55 – 9.20am	Modulation of MOR binding affinity by desensitizing agonists	Will Birdsong
9.20 – 9.45am	Acute injury establishes constitutive μ -opioid receptor activity leading to long-term endogenous analgesia and dependence	Bradley Taylor
9.45 – 10.15am	Morning Tea	
10.15 – 11.00am	Hot Topics 3	Arsalan Yousuf, Seksiri Arttamangkul, Janet Lowe
11.00am	Symposium 4: Opioids in pain states <i>Chair: Susan Ingram</i>	
11.00 – 11.25am	Amygdala plasticity as a mediator of diverse persistent pain conditions	Robert Gerreau IV
11.25 – 11.50am	Spinal cord AMPA receptors containing the GluA4 subunit mediate morphine-induced hyperalgesia	Jose Moron-Concepcion
11.50 – 12.15pm	Opioid reward in pain states	Minoru Narita
12.15 – 1.15pm	Lunch	
1.15 – 2.00pm	Hot Topics 4	Karen Tonsfeldt, Benjamin Lau, Kelly Standifer
2.00pm	Symposium 5: Opioids In non-neuronal cells	
2.00 – 2.25pm	Role of glia in opioid-mediated analgesia	Sandra Comer
2.25 – 2.50pm	Microglia-mediated disruption of neuronal CI – homeostasis gates morphine hyperalgesia	Tuan Trang
2.50 – 3.15pm	Morning Tea	
3.15 – 3.40pm	Opioid Actions in Non-Neuronal Cells – The Heart	John Headrick
3.40 – 4.25pm	Hot Topics 5	Marie-Odile Parat, Mei Bigliard, Naoko Kuzumaki
4.30 – 6.00pm	Poster Session 2	

Wednesday, 17 July 2013		
TIME	PRESENTATION	SPEAKER
8.00 – 8.30am	Arrival Tea and Coffee	
8.30 – 9.30am	Plenary 2 <i>Proudly sponsored by the International Society for Neurochemistry</i> Allosteric and bias at G protein-coupled receptors. Implications for drug discovery	 ISN International Society for Neurochemistry
9.30am	Symposium 6: Bias/allosteric <i>Chair: Mark Connor</i> <i>Proudly sponsored by the International Society for Neurochemistry</i>	 ISN International Society for Neurochemistry
9.30 – 9.55am	Measurement and mechanism of ligand bias at MOPr	Eammon Kelly
9.55 – 10.20am	Ligand and cell-dependent determinants of cellular responses to delta opioid receptor ligands	Graciela Pineyro
10.20 – 11.00am	Morning Tea	
11.00 – 12.15pm	Hot Topics 6	Meritxell Canals, Andrew Alt, John Traynor, James Zadina, Erin Bobeck
12.15pm	Lunch	
Thursday, 18 July 2013		
TIME	PRESENTATION	SPEAKER
8.00 – 8.30am	Arrival Tea and Coffee	
8.30am – 9.30am	Plenary 3: Forebrain mechanisms of opioid action: Reward and stimulus control of choice and decision-making	Bernard Balleine
	Symposium 7: Endogenous Opioids <i>Chair: Larry Toll</i> <i>Proudly sponsored by The American Society for Pharmacology and Experimental Therapeutics in memory of Avram Goldstein</i>	 aspet
9.30 – 9.55am	Endogenous Opioids in the Amygdala	Elena Bagley
9.55 – 10.20am	Opioid peptides induce long-term depression at glutamatergic synapses in the dorsal striatum	Brady Atwood
10.20 – 10.50am	Morning Tea	
10.50 – 11.20am	Hot Topics 7	Brian Cox, Aya Matsui
11.20 – 11.25am	Symposium 8: INRC Young Investigator Symposium <i>Chair: John Traynor</i>	
11.25 – 12.00pm	Roles and regulation of the delta opioid receptor in pain	Louis Gendron
12.00 – 12.25pm	β -arrestin 1 control of delta receptor and ORL signaling	Wendy Walin
12.25 – 2.00pm	Lunch and Poster Presentations	
2.00 – 2.25pm	DOR maturation and trafficking	Ulla Petaja-Repo
2.25 – 3.10pm	Hot Topics 8	Erica Levitt, Karim Nagi, Bronwyn Kivell
3.10 – 3.45pm	Afternoon Tea	
3.45 – 5.00pm	INRC Business Meeting	
7.00 – 10.30pm	Conference Dinner	
Friday, 19 July 2013		
TIME	PRESENTATION	SPEAKER
8.00 – 8.30am	Arrival Tea and Coffee	
8.30am	Symposium 9: Hypocretin/Orexin and Addiction <i>Chair: Ingrid Nylander</i>	
8.30 – 8.55am	Morphine-induced plasticity of Ventral Tegmental Area dopamine neurons is gated by Hypocretin/Orexin receptor 1 signaling	Stephanie Borgland
8.55 – 9.20am	The hypocretin-dynorphin system in reward and drug-seeking	John Muchamp
9.20 – 9.45am	The role of Orexins in the VTA in ethanol and sucrose self-administration	Selena Bartlett
9.45 – 10.15am	Hot Topics 9	Lih-Chu Chiou Andrew Lawrence
10.15 – 10.45am	Morning Tea	
10.45am	Symposium 10: Mechanisms of opioid synergy <i>Chair: Thomas Tzschentke</i> <i>Proudly sponsored by Grunenthal</i>	 GRUNENTHAL
10.45 – 11.10am	A subset of spinal analgesic synergy requires activation of protein kinase C-epsilon	George Wilcox
11.10 – 11.35am	Cannabinoid/opioid interactions: mechanisms for improving pain relief	Adie Wilson-Poe
11.35 – 12.00pm	Exploiting between-opioid differences to enhance analgesia and improve tolerability	Maree Smith
12.00 – 12.15pm	Hot Topics 10	Mahsa Sadeghi

PLENARY 1**OPIOID RESEARCH – TURNING FULL CIRCLE****Henderson, G.***School of Physiology & Pharmacology, University of Bristol, BS8 1TD, UK*

The aim of this lecture will be to describe how the use of electrophysiological recording techniques have contributed to our understanding of the mechanisms underlying the profound effects of opioid drugs on the central nervous system.

While reflecting on what to include I was struck by how, in some areas, we are still seeking to answer the same fundamental questions that were being posed in the 1970s when I began my scientific career. Of course nowadays we have much more detailed information, crystal structures even, and use more sophisticated tools but the same questions still remain to be answered.

PLENARY 2**ALLOSTERY AND BIASED AGONISM AT GPCRS: RELEVANCE TO NOVEL DRUG DISCOVERY****Christopoulos, A.***Monash Institute of Pharmaceutical Sciences & Dept. of Pharmacology, Monash University, VIC, 3052, Australia*

G protein-coupled receptors (GPCRs) constitute the largest family of drug targets. In the new millennium, two important paradigms have emerged that are having an enormous impact on drug discovery approaches at this receptor family. The first is that of biased agonism (or functional selectivity), which posits that different ligands engender unique receptor conformations such that only a subset of pathways linked to a given receptor are recruited. The second paradigm is that of allosteric modulation, which exploits topographically distinct binding sites on GPCRs to modify the affinity, efficacy and/or selectivity of classic orthosteric ligands. Both paradigms have substantial implications with respect to screening for, or interpreting structure-function studies of, novel GPCR-targeting small molecules. The development of novel analytical approaches for quantifying allosteric and biased ligand effects on GPCRs, as well as new breakthroughs in GPCR structural and computational biology, promise to facilitate new approaches to enriching structure-activity relations and structure-based drug design at this therapeutically important receptor family.

PLENARY 3**OPIOID PROCESSES IN REWARD AND DECISION-MAKING****Balleine, B.***Brain & Mind Research Institute, University of Sydney, NSW, Australia*

There is considerable research supporting a general role of opioid-related processes in reward. The specifics of this involvement are, however, much less well understood, whether the focus is at a behavioral, neural systems or cellular level. Although often construed in terms of hedonics, reward signals mainly regulate the evaluative processes through which the goals of goal-directed actions are acquired on which decision-making processes operate. As a consequence, it would appear likely that opioid-related processes play a central role in decision-making. I will describe two recent series of experiments that support this claim in which we have assessed the role of opioids, specifically mu- and delta- opioid receptor-related processes, in (i) the learning processes through which specific rewards are acquired and (ii) the way reward and reward prediction modulate decision-making at both a behavioural and a cellular level.



SYMPOSIUM I**CO-LOCALIZATION OF MU AND DELTA OPIOID RECEPTORS IN THE NERVOUS SYSTEM USING DOUBLE FLUORESCENT KNOCK-IN MICE****Massotte, D.***Dept Neurobiology and Translational Medicine, IGBMC, Illkirch, France*

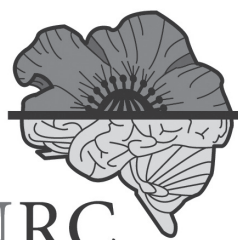
We have generated double knock-in animals expressing delta opioid receptors in fusion with the green fluorescent protein and mu receptors in fusion with a red fluorescent protein. We have used these animals to map the two receptors in the brain, spinal cord and dorsal root ganglia and create a virtual atlas of their distribution. Special attention was given to the profile of mu-delta co-expression at the cellular level. Our data indicate that neurons co-expressing the two receptors are indeed present in a number of brain structures. This suggests potential physiological relevance and prompts to investigate the reality of in vivo functional mu-delta heteromers to assess them as new potential therapeutic targets.

OPIOID RECEPTOR TRAFFICKING AND INTERACTION IN NOCICEPTORS AND ITS ROLE IN OPIOID TOLERANCE**Zhang, X.***Institute of Neuroscience and State Key Laboratory of Neuroscience, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, 200031, China*

The excitatory neurotransmission of nociceptive afferent terminals in the dorsal horn of spinal cord can be modulated by the inhibitory opioid peptides released from the local neurons and exogenously applied opioid analgesics such as morphine through activating the presynaptic δ - and μ -opioid receptors (DORs and MORs), respectively. We find that MORs and DORs are co-expressed in a considerable population of nociceptive neurons in the dorsal root ganglion (DRG). However, these types of opioid receptors have distinct subcellular distributions. MORs are mainly localized on the cell surface, while DORs mostly distribute in the cytoplasm and are often localized in the membrane of neuropeptide-containing large dense-core vesicles. The vesicular localization of DORs allows activity-dependent membrane insertion of DORs and interaction with MORs in the plasma membrane. The function of MORs could be negatively regulated by DORs. Activation of DORs in the DOR/MOR complex results in co-internalization and degradation of DORs and MORs. Morphine analgesic tolerance could be reduced by treatment with DOR antagonists or disruption of the DOR/MOR interaction in the nociceptive afferent terminals.

A μ OR- δ OR HETEROMER-BIASED LIGAND WITH ANTINOCICEPTIVE ACTIVITY***Fujita, W.¹, Gomes, I.¹, Gupta, A.¹, Hodder, P.², Roberts, E.³, and Devi, L.A.¹***¹ Department of Pharmacology and Systems Therapeutics, Icahn School of Medicine at Mount Sinai, New York, NY 10029, ² Department of Molecular Therapeutics, The Scripps Research Institute, Jupiter, FL 33458 and, ³ Scripps Research Institute Molecular Screening Center, La Jolla, CA 92037.*

Emerging studies suggest that dimerization of G protein-coupled receptors (GPCRs) including opioid receptors is necessary for receptor maturation, signaling and trafficking. Heteromerization between opioid receptor (OR) types is thought to affect the pharmacological effects of opiates. We have previously shown that μ OR and δ OR form heteromers whose presence can be detected in brain regions involved in pain processing using μ OR- δ OR heteromer-selective antibodies. We also reported that chronic morphine administration leads to an upregulation of these heteromers and that the heteromer exhibits signaling distinct from that of μ OR or δ OR alone. These results suggest that the μ OR- δ OR heteromer could serve as a novel therapeutic target for the treatment of chronic pain. Here we report the identification of compounds specifically targeting μ OR- δ OR heteromers by high-throughput screening of a library of small molecules using a β -arrestin recruitment assay. We find that 125 of the ~335,461 compounds tested exhibit receptor activation only in cells expressing μ OR- δ OR, but not in cells expressing μ OR or δ OR alone. Since, 14 of 125 compounds exhibited less than 30% structural similarity to known opioids, these were repurchased and reanalyzed using in vitro and in vivo assays. Among the top 3 candidates, we found CYM51010 to be the most potent μ OR- δ OR-biased ligand. Furthermore, intrathecal administration of CYM51010 leads to potent antinociceptive activity that can be blocked by μ OR- δ OR heteromer selective antibodies. Interestingly, while the antinociceptive activity of systemically administered CYM51010 is similar to that of morphine, it exhibits lesser antinociceptive tolerance compared to morphine. These results suggest that CYM51010, a μ OR- δ OR-biased ligand, could serve as a scaffold for the development of a novel type of (heteromer-biased) drug that is as potent as the conventional clinical opioids with reduced side-effects. Supported by NIH grants DA008863 and DA019521 (L.A.D.) and by the NIH Molecular Library Screening Center Network Grant MH084512 (P.H. and E.R.).

**INRC**

SYMPOSIUM 2**IMPULSIVITY PERSONALITY TRAITS AND GENE POLYMORPHISMS ASSOCIATED WITH HEROIN DEPENDENCE**

Dissabandara, L.O.^{1,2}, Loxton, N.J.³, Ho, A.MC.², Dodd, P.R.⁴, Daghli, M.², *Stadlin, A.⁵

¹ School of Medicine, Griffith University, Gold Coast; ² School of Medicine, ³ Psychology and ⁴ Chemistry & Molecular Biosciences, The University of Queensland, Herston, Australia; ⁵ Chungbuk National University School of Medicine, Cheongju, South Korea

Impulsivity is a risk factor associated with all facets of addiction from initial predisposition, through the maintenance phase and as a predictor of relapse risk. Genes encoding molecular pathways of the reward system are implicated in both addiction and impulsivity. A complex interplay of these genes is likely to contribute to the risk of addiction, both directly and indirectly, mediated via the intermediate phenotype of impulsivity. We assessed the impulsivity personality traits and nineteen candidate polymorphisms of the GABA, opioid and dopamine pathways of the reward system in an ethnically distinct and homogenous population sample of 157 Sinhalese, male dependent heroin users and 155 age- and ethnicity-matched extreme controls with no life time drug use and negligible nicotine or alcohol consumption, using direct, indirect and epistatic association analyses. Results showed that the use of a two-factor model of impulsivity provided additional, novel important information about substance-use behaviours. The diagnosis of dependence was associated with both higher rash-impulsiveness and reward sensitivity, while heroin dependence-associated sub-phenotypes such as high-risk behaviour that included escalating heroin consumption, injecting heroin use, hazardous drinking, low treatment-seeking, and risky sexual behaviour were associated only with high rash-impulsiveness. An early onset of drug use was associated with reward sensitivity. By applying different analytic strategies based on direct, indirect and epistatic associations to previously reported and/or functionally relevant candidate variants we can enhance the biological plausibility of their associations and increase the chances of revealing subtle polygenetic effects. GABA receptor SNPs (*GABRG2*-rs3219151 and *GABRG2*-rs211013) showed both individual and haplotype association with heroin dependence. Two opioid mu-receptor SNPs (*OPRM1*-rs1799971 and *OPRM1*-rs563649) also showed individual association with heroin dependence. The same GABA SNPs and *DRD2*-rs1079597 were associated with impulsivity-related traits, which mediated the effect of these polymorphisms on heroin dependence. Further, we found a statistically significant and biologically relevant epistatic interaction between *GABRG2* and *OPRM1* for risk of heroin dependence. These findings portray novel and plausible potential mechanisms of genetic predisposition to heroin dependence.

OPIOID-INDUCED HYPERALGESIA: DOES THE MYTH NEED BUSTING?

Somogyi, A.

Discipline of Pharmacology, School of Medical Sciences University of Adelaide, Adelaide 5005 Australia

Opioid-induced hyperalgesia (OIH) is a phenomenon whereby opioids seem to induce a paradoxical increase in pain sensitivity (and more diffuse pain) and is seen after chronic opioid dosing in dependence and pain treatments and sometimes in the acute perioperative setting. The somewhat "mythical" nature of OIH is in contrast to opioid-induced tolerance (OIT) whereby an increase in

dose is needed to achieve the same analgesic effect. Using the cold-pressor model, 'tolerance' time is markedly reduced in methadone and buprenorphine maintained subjects and in chronic pain patients after all opioids tested. IOH and IOT can be detected also after prolonged intraoperative dosing with high potency opioids (fentanyl and remifentanyl). For OIH, its clinical existence is debated in terms of patient group (acute versus cancer versus noncancer pain), detection methods, and relationship to opioid dosing route and regimen.

As not all people develop IOH (or IOT), the question of a genetic risk factor has been raised but without resolution or research. Nevertheless for both IOH and IOT, the landscape of the molecular mechanism has mainly involved the neuronal system (NMDA receptor, glutamate, dynorphin, mu G-protein). However, the role of the CNS innate immune system and especially proinflammatory cytokines may be important, as many animal and human studies and case reports have shown that ketamine (the so-called NMDA antagonist) can attenuate to a certain extent both IOH and IOT during acute and chronic opioid administration. Ketamine has anti-proinflammatory effects via stereoselective and possibly stereospecific effects on TLR4/MD2 and reduction in LPS-induced IL-6 secretion. The busting of the myth of OIH and its mechanism(s) may herald new approaches to how opioids are used in the clinical setting.

INCREASING USE OF OPIOIDS AND OTHER PSYCHOACTIVE MEDICATIONS IN AUSTRALIA

***Haber, P.¹, Blanch, B.²**

¹ Drug Health Services, Royal Prince Alfred Hospital, University of Sydney, NSW Australia ² School of Pharmacy, University of Sydney, NSW Australia

The use of pharmaceutical opioid drugs in the community is controversial with evidence of both significant under-utilization and over-utilization. There is some evidence to support efficacy for chronic non-malignant pain, but community and individual harms are increasingly reported. There is an increasing range of other psycho-active medications that may contribute to therapeutic benefit and to overdose risk. The aim of this presentation is to review availability of opioid and other psycho-active prescription patterns through the subsidized Pharmaceutical Benefits Scheme in Australia from 1992 to the present. Methods: Interrogation of the Pharmaceutical Benefits Scheme online statistics data from 1992 onwards. Item numbers for all available preparations were identified, and frequency of dispensing was collected and collated. Results: The number of medication classes, and the number of preparations within each class increased during this period, in the case of opioids from 11 to 70 preparations. The total number of PBS opioid prescriptions increased from 6.0 million in 1992 to 18 million in 2012, a three-fold increase. There were dramatic and continuing increases in prescription of oxycodone, buprenorphine and fentanyl but little change in use of morphine and methadone. There were also dramatic increases in dispensing of olanzapine, quetiapine and risperidone. Conclusion: There is increasing use of all classes of medications examined. There is a pressing need to implement strategies to improve the quality usage of opioids and other psycho-active medications, individually and in combination.

HOT TOPICS 2**SIX-MONTH FOLLOW-UP OF IRANIAN WOMEN IN METHADONE TREATMENT: DRUG USE, SOCIAL FUNCTIONING, CRIME, AND HIV AND HCV SEROINCIDENCE****Dolan, K.¹, Salimi, S.², Nassirimanesh, B.³, Mohsenifar, S.², Allsop, D.¹, Mokri, A.²***¹Program of International Research and Training, National Drug and Alcohol Research Centre, Australia; ²Iranian National Center for Addiction Studies, Tehran University of Medical Sciences, Iran; ³Persepolis Centre, Tehran, Iran*

Background: In general, information about women who use drugs comes from studies performed in the West. Whether women in countries such as Iran are likely to enter drug treatment or how they will respond is not known.

Purpose: To examine the short-term impact of methadone maintenance treatment (MMT) on drug use, dependence, social functioning, crime, and human immunodeficiency virus (HIV) and hepatitis C virus (HCV) risk behavior and seroincidence in female drug users in Iran.

Methods: Women were eligible for inclusion in the study if they were assessed as dependent on opiates according to the International Statistical Classification of Diseases and Related Health Problems, tenth revision (ICD-10). The sample comprised 78 female heroin or opium users who attended the Persepolis women's drug treatment clinic in Tehran between 2007 and 2008. Participants were followed up in 2009/2010. Heroin and the use of other drugs, social functioning, involvement in crime, and involvement in HIV and HCV risk behavior were measured by self-report. The prevalence and incidence of HIV and HCV were measured by serology and self-report.

Findings: Of the 78 women recruited, 40 were followed up, and this occurred approximately 7 months later. One in four women reported a history of drug injection. At follow-up there were significant reductions in self-reported heroin use on ICD-10 dependence scores. Subjects with more severe drug dependence at baseline were significantly more likely to be criminally active than less severely dependent subjects. Baseline prevalence for HIV and HCV was 5% and 24%, respectively. At follow-up, no one had acquired HIV infection, but one participant had acquired HCV, giving an incidence rate of 7.1 per 100 person-years.

Conclusion: This research provides the first evidence that Iranian female drug users can enter MMT and respond well. Within a few months of entering MMT, improvements occurred in heroin use, levels of dependence, social functioning, and HIV risk behavior. While the incidence of blood-borne viral infections was low, there was a serious risk of HIV transmission among this cohort and also to participants' needle and sexual contacts. In a country with high levels of drug use, the high levels of HCV among female drug users require more women to enter drug treatment if an HIV epidemic is to be avoided. Many participants had a chronic drug problem and had had little or no previous exposure to MMT. The introduction or expansion of women only drug treatment services is urgently needed in order to engage more women in treatment.

THE EFFECT OF μ -OPIOID RECEPTOR POLYMORPHISMS ON RECEPTOR FUNCTION***Knapman, A., Connor, M.***The Australian School of Advanced Medicine, Macquarie University, Sydney, NSW, Australia*

There is significant variation in individual response to opioid drugs, one cause of which is likely to be polymorphisms on the opioid receptors themselves. The μ -opioid receptor (MOR) is the primary site of action for most analgesic opioids. Previous studies have identified a number of naturally occurring single nucleotide polymorphisms (SNPs) in the coding region of the MOR. The A118G SNP variant, present in various populations at allelic frequencies ranging from 10 – 40%, has been associated with differences in the requirement for post-operative opioid analgesics, and for drug dependence, although these results are not consistent. In vitro, MOR-A118G has been reported to exhibit an altered signalling profile compared with MOR including different β -endorphin potency and alterations in N-type Ca²⁺ channel inhibition, however these findings are also not consistent. Few studies have examined the effect of MOR polymorphisms on receptor function, or potential ligand functional selectivity. We assessed the relative potency and efficacy of a range of opioid ligands in Chinese hamster ovary (CHO) cells stably transfected with human wild type MOR and five naturally occurring MOR variants, including A118G. Receptor expression levels were similar for all mutants examined. MOR mediated adenylyl cyclase (AC) inhibition was measured using a novel, fluorescence based membrane potential assay. Treatment of CHO cells with the AC activator forskolin (FSK) hyperpolarized CHO-MOR cells with a pEC₅₀ of 7.3±0.1 to a maximum of 52±1.7% from baseline. The hyperpolarization induced by FSK (300nM) was inhibited in a dose-dependent manner by the addition of a range of MOR agonists, including DAMGO, morphine, β -endorphin and buprenorphine (n ≥ 5). In MOR-A118G cells the maximal buprenorphine inhibition of AC was reduced to 12 ± 2% (compared with 30±4% in MOR-WT, P < 0.05), without a change in potency, while responses to morphine and B-end were unaffected. Cells expressing MOR-C17T showed significantly reduced E_{max} and pEC₅₀ for morphine but not DAMGO. By contrast, MOR-C253A cells showed significantly increased E_{max} for most ligands, with no change in potency. CHOMOR-C541T was confirmed as non-functional. The differences in inhibition of AC observed between MOR variants suggest that MOR SNPs may contribute to individual variability in the response to opioid analgesics, and we are exploring this at a range of signalling pathways.

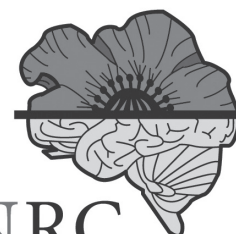
HOT TOPICS 2**THE EVALUATION ON THE EFFECTIVENESS OF SCHEDULE I AND SCHEDULE II DRUG ADDICTS' TREATMENT UNDER JUDICIAL SUPERVISION IN TAIWAN****Yang, Shu-Lung***Crime Research Center, National Chung Cheng University, Chia-Yi, Taiwan*

The effectiveness of drug addicts' two different models (i.e., institutional treatment-compulsory treatment in drug abuser treatment center and deferred prosecution for drug addicts' treatment in the community) for schedule I offenders (majorly Heroine) has been empirically tested and evaluated by the author (2012). Specifically, the study sample 437 drug addicts from drug abuser treatment center, and 362 clients under deferred prosecution order, for the evaluation of their effectiveness in terms of family attachment, friend attachment, addiction severity index and Short Form-36 (Physical, mental and social assessment) for the year of 2012. This study found that the two groups in normal family attachment, normal friend attachment and quality of life were significantly improved after treatment, but also significantly reduce addiction, as most studies expected. But the two groups in an attempt to reduce both drug addicts' family attachment, and drug addicts' friend attachment, to improve get rid of the motivations of drug use and quality of health are not statistically significant. Nevertheless, both of institutional treatment and deferred prosecution treatment are generally effective.

Despite these, the effectiveness of such comparison has not yet been empirically verified for schedule II offenders (majorly Amphetamine) in Taiwan. The current study attempts to fill such void, and provided further evidence. This study conducts a series fieldwork surveys to analyze the outcomes and cost-benefit effectiveness of two model of traditional detention-detoxification group (schedule II drug addicts of Taipei Detention Center and Taipei Women's Detention Center) and deferred prosecution and addiction treatment group (schedule II drug addicts of Taipei District Prosecutors Office and Taipei City Hospital Songde branch). After analyzing the data, the policy implications of two judicial models can be derived.

SOCIAL STRESS ENGAGES OPIOID MODULATION OF THE LOCUS COERULEUS-NOREPINEPHRINE SYSTEM AND INCREASES THE SALIENCE OF REWARD.***Chajale, N., Curtis A.L., Snyder, K., Bhatnagar, S., Valentino, R.J.***Childrens Hospital of Philadelphia, Philadelphia, PA, USA.*

The locus coeruleus-norepinephrine (LC-NE) system is a major stress response system through which stress affects arousal and cognitive function. The LC is co-regulated by stress neuromediators and endogenous opioids. Because social stress (SS) is a common stressor for humans, this study characterized the enduring impact of repeated SS on LC activity. Specifically, we determined whether SS alters the LC-NE system two (Day 7) and ten days (Day 15) after the last SS and evaluated whether these changes in LC-NE system translate to changes in cognitive function. Rats were implanted with a multiwire bundle into the LC and exposed to five sessions of the resident-intruder model of social defeat. On Day 7, LC discharge rate was decreased in stressed rats compared to controls (Day 1: 2.21 ± 0.1 Hz, Day 7: 1.35 ± 0.1 Hz for stress rats and Day 1: 2.23 ± 0.1 Hz, Day 7: 2.20 ± 0.1 Hz, for control rats; $n < 40$). By Day 15, LC rates were comparable between groups. Systemic administration of the opiate antagonist naloxone, robustly increased LC rate selectively in stressed rats when administered on Day 7 (1.18 ± 0.1 Hz pre-naloxone, 2.24 ± 0.1 Hz post-naloxone) or Day 15 (1.87 ± 0.1 Hz pre-naloxone, 4.77 ± 0.2 Hz post-naloxone). This cellular evidence of opiate withdrawal was accompanied by behavioral signs of mild opiate withdrawal. To evaluate whether SS-induced changes in LC activity affect cognitive flexibility, rats were tested in an attention set shifting task (AST) and LC activity was recorded. Stress rats performed better in intradimensional set shifting (IDS): 36.7 ± 4 and 23.8 ± 4 trials to criterion for control and stress rats, respectively ($p < 0.05$). Recordings during IDS showed that LC neurons of stressed rats were reward, but not decision responsive. During reversal learning although overall performance was comparable between groups, stressed rats made less perseverative errors. LC neurons of stressed rats selectively showed decision- and reward-related activation. LC activity also increased for stressed rats only during incorrect trials between decision and recognition of the absence of reward. These results suggest that SS engages endogenous opioid modulation of LC activity and induces a state of opiate dependence. Additionally, SS strengthens the relationship between LC activity and goal-directed behavior. Together these effects of SS on the LC system can increase vulnerability to opiate abuse by promoting the positive and negative reinforcing effects. Supported by T32-NS007413, 58077 LSDRP, MH40008, DA09082.



SYMPOSIUM 3**DIFFERENTIATION OF OPIOID DRUG EFFECTS BY HIERARCHICAL MULTI-SITE PHOSPHORYLATION**

Just, S., Illing, S., Trester-Zedlitz, M., Lau, E.K., Kotowski, S.J., Miess, E., Mann, A., Doll, C., Trinidad, J.C., Burlingame, A.L., von Zastrow, M., Schulz, S.

Institute of Pharmacology and Toxicology (S.J., S.I., E.M., A.M., C.D., and S.S.), Jena University Hospital, Friedrich Schiller University Jena, Drackendorfer Straße 1, D-07747 Jena, Germany.

Departments of Psychiatry (M.T.-Z., E.K.L., S.J.K., and M.v.Z.), Pharmaceutical Chemistry (J.C.T., and A.L.B.), Chemistry (A.L.B.), and Cellular and Molecular Pharmacology (M.v.Z.), University of California, San Francisco, CA 94158, USA.

Differences in the ability of opioid drugs to promote regulated endocytosis of μ -opioid receptors are related to their tendency to produce drug tolerance and dependence. Here we show that drug-specific differences in receptor internalization are determined by a conserved, 10 residue sequence in the receptor's carboxyl-terminal cytoplasmic tail. Diverse opioids induce receptor phosphorylation at S375, present in the middle of this sequence, but opioids differ markedly in their ability to drive higher-order phosphorylation on flanking residues (T370, T376 and T379). Multi-phosphorylation is required for the endocytosis-promoting activity of this sequence and occurs both sequentially and hierarchically, with S375 representing the initiating site. Higher-order phosphorylation involving T370, T376 and T379 specifically requires GRK2/3 isoforms, and the same sequence controls opioid receptor internalization in neurons. These results reveal a biochemical mechanism differentiating the endocytic activity of opioid drugs.

AGONIST-INDUCED RECEPTOR PHOSPHORYLATION INCREASES AGONIST AFFINITY AT THE MU OPIOID RECEPTOR. DOES IT AFFECT DESENSITIZATION?

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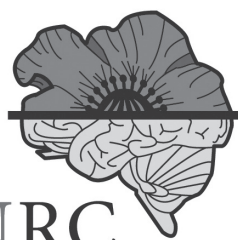
Prolonged exposure to high-efficacy agonists results in receptor phosphorylation at the mu opioid receptor (MOR) and desensitization of downstream signaling. It is unknown what effects desensitizing agonist exposure has on agonist receptor interactions. Using confocal imaging and a fluorescent agonist (dermorphin Alexa 594) we found that prolonged exposure to desensitizing agonists increased the affinity of MOR for dermorphin Alexa 594. This increase in affinity was long lasting, independent of downstream signaling and abolished by mutation of putative phosphorylation sites. Thus, conditions that are known to cause receptor desensitization also result in receptor phosphorylation causing an increased agonist affinity. The role of receptor phosphorylation in opioid signaling will be discussed.

Supported by DA08163, DA026617 (JTW) and DA007262-18 (WTB).

ACUTE INJURY ESTABLISHES CONSTITUTIVE μ -OPIOID RECEPTOR ACTIVITY LEADING TO LONG-TERM ENDOGENOUS ANALGESIA AND DEPENDENCE

Taylor, B.

Injury causes acute pain sensation that resolves with healing; however, the transition to a persistent pathological pain state remains an important clinical problem. Endogenous activation of μ -opioid receptors (MORs) provides relief from acute pain, but the existence of long-term opioid inhibition of pathological pain has not been sufficiently explored. Here we show that acute tissue inflammation produces agonist-independent MOR signaling in the dorsal horn, which tonically represses spinal nociceptive signaling for months. Disruption of this constitutive activity reinstated signs of pain and neuronal sensitization, and precipitated cellular, somatic and aversive signs of opioid withdrawal. This required N-methyl-D-aspartate receptor activation of calcium-sensitive adenylyl cyclase type I. Thus, we have discovered a novel mechanism of long-lasting opioid analgesia that regulates the transition from acute to chronic pain while, in parallel, generates opioid dependence. The prevalence of chronic pain syndromes may result from a failure in constitutive signaling of spinal MOR.



HOT TOPICS 3**THE ROLE OF DIFFERENT PHOSPHORYLATION SITES IN RAPID DESENSITIZATION OF THE μ -OPIOID RECEPTOR***Yousuf, A.¹, Miess, E.², Schulz, S.², Christie, M.J.¹¹Discipline of Pharmacology, University of Sydney, NSW, Australia, ²Institute of Pharmacology and Toxicology, Jena University Hospital, Friedrich Schiller University Jena, Jena Germany

Tolerance and addiction to opioids are serious clinical and social problems that result in part from the loss of μ -opioid receptor (MOR) function when activated by different opioids. Phosphorylation of specific residues in the C-terminal domain of MOR is thought to be a key step in desensitization, endocytosis and loss of MOR function. Sequential phosphorylation of S375 along with the flanking residues T370, T376 and T379 are required for endocytosis (Just et al., Mol Pharmacol (2013) 83:633-639) but their role in rapid desensitization of MOR is unknown. The aim of this study was to determine the influence of C-terminal phosphorylation sites on rapid desensitization of MOR. Wild type mMOR, 3S/T-A (S363A, S370A, S375A), 6 S/T-A (S363A, S370A, S375A, T376A, T379A, T383A) and 11 S/T-A (T354A, S355A, S356A, T357A, S363A, S370A, S375A, T376A, T379A, T383A, T394A) were expressed stably in AtT20 cells. Using perforated patch-clamp recording we examined the effects of MOR activation, desensitization and re-sensitization using activation of GIRK channels by a submaximal concentration of met-enkephalin (10 nM) to measure receptor activity and somatostatin (100 nM) coupling to native SSTR2 (or sst2 according to IUPHAR) to determine heterologous desensitization. MOR desensitization and resensitization produced by 5 min exposure to met-enkephalin (10 μ M) at 37°C did not differ from wild type for 3S/T-A or 6S/T-A but desensitization was abolished in the 11 S/T-A mutant. Desensitization, when detected, was largely homologous. Because 3S/T-A suppresses, and 6S/T-A abolishes MOR endocytosis, these findings suggest that homologous desensitization can occur independently of the phosphorylation and arrestin-dependent mechanisms that drive endocytosis. However, C-terminal phosphorylation sites are necessary for desensitization because mutation of all C-terminal sites (11 S/T-A) abolishes desensitization.

DISRUPTION OF THE ACTIN CYTOSKELETON DELAYS RECOVERY OF MU OPIOID RECEPTOR FROM DESENSITIZATION IN LOCUS COERULEUS NEURONSArttamangkul, S.¹, Schulz, S.² and Williams, J.T.¹¹Vollum Institute, Oregon Health & Science University, Portland, Oregon, USA ²Pharmacology, Jena University Hospital, Jena, Germany

The actin cytoskeleton acts to support cellular structure, but it also plays a role in GPCR signaling and regulation. The network of actin filaments beneath plasma membrane has been shown to confine μ opioid receptors (MOR) into a compartment and restrains global lateral diffusion properties. The present study used latrunculin B (Lat B) to disrupt actin polymerization in HEK293 cells or mouse locus coeruleus neurons (LC). The effect of Lat B on lateral diffusion of Flag-tagged MOR (Flag-MOR) in HEK293 cells was studied by line-scanning fluorescence correlation spectroscopy. In Lat B treated cells, the diffusion rate of Flag-MOR was faster than control. The effect of Lat B on [Met5]enkephalin (ME)-induced receptor internalization was studied in brain slices of locus coeruleus neurons from transgenic mice expressing Flag-

MOR. In the Lat B treated cells the amount of internalized Flag-MOR and the endosome size was increased compared to control following treatment with ME (30 μ M, 10 min). The desensitization measured by the decline in the hyperpolarization induced by ME (30 μ M) was not altered but there was only a partial recovery from desensitization following the wash. The impaired recovery from desensitization correlated with a reduction in the recycling of receptors back to the plasma membrane. Experiments using LC slices obtained from mutant S375A knock-in mice showed that the desensitization and the recovery were not altered by Lat B. This study suggests that the actin cytoskeleton plays a key role in the recovery from desensitization by regulating the receptor trafficking. The study is supported by NIH grant DA08163.

AGONIST-INDUCED DESENSITIZATION OF MU OPIOID RECEPTORS DEPENDS ON THEIR CELLULAR LOCALIZATION.

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A primary site for opioid-induced euphoria is activation of μ -opioid receptors (MOPr) in the ventral tegmental area (VTA). The causes and mechanisms of tolerance to opioid-induced euphoria are poorly understood, but one hypothesis for tolerance in general is agonist-induced MOPr desensitization. In the VTA, MOPr are located on GABAergic interneurons both somatodendritically (ie. on cell bodies and dendrites) and on nerve terminals that innervate dopaminergic neurons. Using whole-cell patch-clamp electrophysiological methods, we have investigated agonist-induced desensitization of both populations of MOPr in mouse VTA slices and found profound differences in their desensitization profile depending on their subcellular localization. In common with many other neuronal cell-types, agonist-induced MOPr desensitization is readily seen at VTA cell bodies, where the DAMGO-induced response declined by approximately 50% during a 10-minute application. In contrast, neither DAMGO, Met-Enkephalin, nor morphine were able to induce rapid MOPr desensitization at nerve terminals even in the absence of a receptor reserve. Because activation of PKC has been shown to enhance morphine mediated desensitization in other brain regions (Bailey et al. 2004), we treated slices with the phorbol ester PMA. Although morphine still did not appear to induce desensitization during a 10 minute application, PMA treatment dramatically reduced the peak morphine response compared to untreated slices suggesting desensitization may have occurred during the slow onset of morphine action (> 5 minutes). PMA did not affect the peak response to DAMGO, nor promote DAMGO desensitization over the 10-minute application. Therefore, PKC selectively enabled morphine-induced desensitization of nerve terminal MOPr. Although the high efficacy MOPr agonists DAMGO and Met-Enkephalin were unable to promote rapid desensitization of nerve terminal receptors, preliminary data suggest that treating slices for 7-10 hours with Met-Enkephalin reduced nerve terminal MOPr function in most cells. Thus, MOPr located at nerve terminals desensitize very slowly to high efficacy agonists, but do desensitize to morphine if PKC is activated. Overall, these results demonstrate profound differences in the mechanism of MOPr desensitization based both on their localization within the neuron and on which agonist is used to activate them. (Funded by MRC)

Bailey CP et al. (2004) Mol. Pharm., 66, 1592-98.

SYMPOSIUM 4**AMYGDALA PLASTICITY AS A MEDIATOR OF DIVERSE PERSISTENT PAIN CONDITIONS**

Gerreau, R. IV

The comorbidity of multiple chronic pain conditions and the presence of enhanced sensitivity at sites far removed from the site of tissue injury are consistent with an anatomical locus of pain amplification within the central nervous system. In this presentation, I will describe studies performed in our lab utilizing anatomy, behavioral pharmacology, and optogenetic approaches to demonstrate a role for mGluR5-dependent signaling in the central nucleus of the amygdala in the context of persistent pain. The data support the hypothesis that plasticity in central amygdala leads to the generation of widespread pain in response to anatomically restricted injury.

A NEW ROLE FOR SPINAL CORD AMPA RECEPTORS IN THE MECHANISMS UNDERLYING MORPHINE-INDUCED PAINCabanero, D.¹, Carlton, S.², *Concepcion, J.M.¹¹Dept Anesthesiology, Columbia University, New York²Dept Neuroscience, University of Texas Medical Branch, Galveston, Texas

Individuals who discontinue opioid use after repeated exposure develop an abstinence syndrome in which one of the core symptoms is an increase in pain sensitivity. In patients treated with opioids, this hypersensitivity can increase the probability of abuse. It is thought that the molecular mechanisms underlying opioid-induced hypersensitivity are regulated by plasticity events that converge at the dorsal horn of the spinal cord. We have recently reported that repeated morphine administration triggers an insertion of GluA2-lacking (Ca²⁺-permeable) AMPA receptors (AMPA) in the hippocampus. This finding together with the reported involvement of AMPAR in the mechanisms underlying inflammatory pain led us to hypothesize a role for spinal AMPAR in opioid-induced pain behavior. Mice treated with escalating doses of morphine showed hypersensitivity to mechanical stimulation. Intrathecal administration of the Ca²⁺-permeable AMPAR selective blocker, naspm, disrupted morphine-induced mechanical sensitivity. Analysis of the expression and phosphorylation levels of AMPAR subunits (GluA1/2/3/4) in homogenates and in postsynaptic density fractions from spinal cord dorsal horns showed an increase in GluA4 expression and phosphorylation in the postsynaptic density after morphine. Co-immunoprecipitation analyses suggested an increase in GluA4 homomers (Ca²⁺-permeable AMPAR) and immunohistochemical staining localized the increase in GluA4 levels in laminae III-V. In addition, the excitatory postsynaptic currents (EPSCs) recorded in laminae III-V showed enhanced sensitivity to Ca²⁺-permeable AMPAR blockers in morphine treated mice. Furthermore, current-voltage relationships of AMPAR-mediated EPSCs showed that rectification index (an indicator of Ca²⁺-permeable AMPAR contribution) is increased in morphine-treated, but not saline-treated mice. Interestingly, these effects could be reversed by infusion of GluA4 antibody through patch pipette. Therefore, we propose that discontinuation of morphine treatment induces pain sensitivity through the synaptic insertion of GluA4-containing Ca²⁺-permeable AMPAR at spinal cord dorsal horn neurons. Overall, this study highlights spinal GluA4-containing AMPAR as new targets to prevent the pain sensitivity that develops following the discontinuation of opioid treatment.

Supported by NIH grant R01DA027460 to JMC.

CHRONIC NOCICEPTIVE STIMULI NEGATIVELY CONTROL THE MESOLIMBIC DOPAMINERGIC TRANSMISSION

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Although morphine and other mu-opioid agonists are the main analgesics for severe pain, these compounds have potential for abuse and/or addiction. However, clinical studies show that when mu-agonist analgesics are appropriately used to control pain, actual abuse or addiction does not usually occur. Clinical experience and animal studies support the contention that opioid euphoria and reward are attenuated by pain. Inflammatory nociception and neuropathic pain resulted in the suppression of activated mesolimbic dopaminergic transmission evoked by mu-opioids. These results indicate that chronic nociceptive stimuli may induce the functional down-regulation of the rewarding network, leading to the depression and a reduction in abuse potential of mu-opioid analgesics. We also found that these phenomena were eliminated in mice that lacked the beta-endorphin gene. Next, we used optogenetic techniques to selectively inhibit VTA-NAc pathway. To evaluate the effect of inhibiting the VTA-NAc pathway on the expression of analgesia produced by morphine, we injected AAV-halorhodopsin into the VTA of TH-Cre mice in vivo. Phasic stimulation decreased VTA dopamine-neuronal excitability and inhibited antinociception produced by morphine during optical stimulation. These findings provide evidence for the crucial role of the activated endogenous mu-opioidergic system in negatively controlling the abuse potential of opioids by chronic nociceptive stimuli. In this symposium, we will discuss our current data and review the molecular mechanisms of pain-induced suppression of opioid dependence.

HOT TOPICS 4

CHRONIC INFLAMMATION ALTERS GABA SYNAPTIC ACTIVITY AND MU-OPIOID RECEPTOR INHIBITION IN THE PERIAQUEDUCTAL GRAY

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Chronic inflammatory pain can induce neuroadaptations within the central nervous system. Opioid inhibition of GABAergic synaptic activity in the periaqueductal gray (PAG) activates a descending antinociception pathway that reduces nociceptive neurotransmission in the spinal cord. Little is known about changes in synaptic transmission in the PAG following chronic inflammation. Met-enkephalin (ME) inhibition of PAG GABAergic synaptic transmission was examined in male and female rats pretreated with Complete Freund's Adjuvant (CFA) or saline. Recordings from brain slices containing the PAG were made 6-9 days following injections. In females, the CFA-inflamed paw measured 6.8 ± 0.7 mm and was significantly larger than the uninjected hindpaw (3.7 ± 0.2 mm, $t(5) = 6.293$, $p < 0.05$) with no observed change in saline injected hindpaws. Basal GABA mIPSC frequency was increased in CFA (2.2 ± 0.5 Hz, $n = 12$ cells) compared to saline pretreated rats (1.1 ± 0.2 Hz, $n = 8$ cells; Mann-Whitney $U = 26$, $p < 0.05$). Inhibition of mIPSC frequency by ME (300 nM and 10 μ M) was enhanced in CFA-treated rats (26 ± 5 and $59 \pm 5\%$, $n = 11$ cells) compared to saline (14 ± 5 and $35 \pm 7\%$, $n = 9$ cells). These results suggest that CFA induces an increase in GABA release and increases ME inhibition of GABA mIPSC frequency.

Interestingly, different results were observed in male rats pretreated with CFA. Although there was a similar increase in paw diameter with CFA injections (6.0 ± 0.2 mm, $N = 4$) compared to the control paws (3.6 ± 0.2 mm, $t(3) = 14.10$, $p < 0.05$), the basal mIPSC frequency in male rats trended toward decreased frequency in CFA pretreated rats (0.9 ± 0.2 Hz, $n = 8$ cells) compared to saline (2.8 ± 1.3 Hz, $n = 3$ cells). In contrast to the female rats, there was no change in ME (300 nM and 10 μ M) inhibition of mIPSC frequency in CFA pretreated rats (19 ± 6 and $44 \pm 6\%$, $n = 8$ cells) compared to saline (14 ± 12 and $41 \pm 11\%$, $n = 3$ cells). Thus, GABA inhibition in the PAG is modulated differently by CFA-induced inflammation between male and female rats. Supported by DA027625 (SLI).

OPIOID DISINHIBITION OF THE DESCENDING ANALGESIC PATHWAY IN THE PERIAQUEDUCTAL GRAY

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Kolling Institute of Medical Research, Royal North Shore Hospital, University of Sydney

Purpose: The midbrain periaqueductal grey (PAG) is a major site of analgesic action by opioids. These agents have long been hypothesized to produce analgesia via an indirect process of 'disinhibition' in descending analgesic systems. Of particular interest is the descending pathway in the PAG, which projects through the rostral ventromedial medulla (RVM) to modulate nociceptive transmission at the spinal cord dorsal horn. While many studies suggest that μ -opioids activate the PAG-RVM descending pathway via a disinhibitory cellular mechanism, there is no evidence to date directly demonstrating suppression of the inhibitory inputs onto PAG output neurons projecting to the RVM. Thus, there is still a lack of definitive support for disinhibition of the principal neurons involved in pain modulation. The present study aimed to address this issue by examining the cellular actions of opioids specifically on identified PAG-RVM output neurons.

Methods: PAG output neurons projecting to the RVM were retrogradely labelled in Sprague-Dawley rat pups (17-20 days old). Electrophysiological whole-cell patch clamp recordings were then conducted from these identified PAG output neurons, recording evoked inhibitory and excitatory postsynaptic currents (IPSCs/EPSCs). The frequency and amplitude of the individual quantal events underlying the evoked IPSCs/EPSCs were examined using strontium (8 mM) in the external recording solution.

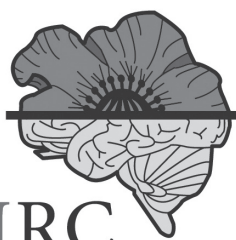
Results: The μ - and κ -opioid agonists, DAMGO and U69593 produced a reduction of both evoked IPSCs and EPSCs in labelled PAG output neurons, which was associated with paired pulse facilitation. In contrast, the δ -opioid agonist, deltorphin-II had no significant effects on synaptic transmission. The response of DAMGO and U69593 on EPSCs was significantly less compared to their effect on IPSCs. DAMGO was also found to reduce the frequency of Sr²⁺-mediated evoked miniature IPSCs in labelled output neurons.

Conclusions: Hence, μ - and κ -opioid agonists act via a presynaptic mechanism to suppress both inhibitory GABAergic and excitatory glutamatergic synaptic transmission onto PAG output neurons. We have previously shown that only a small proportion of output neurons in the ventrolateral PAG respond directly to opioid agonists. Together these findings suggest that opioids act indirectly via a disinhibitory action to activate the PAG-RVM descending pathway. This is consistent with the opioid disinhibition model of analgesia. Furthermore, the observed suppression of excitatory synaptic transmission likely suggests complex effects by opioids in this analgesic system.

HOT TOPICS 4**PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL REGULATION OF IL-6 EXPRESSION BY NOCICEPTIN/ORPHANIN FQ (N/OFQ) PEPTIDE RECEPTOR****Donica, C.L.², Zhang, Y.¹, Simpson-Durand, C.D.¹, *Sherry, D.M.^{2,3}, Gallucci, R.M.^{1,2}, Awwad, H.O.^{1,2} and Standifer, K.M.^{1,2,3}**¹ Department Pharmaceutical Sciences, College of Pharmacy, ² Oklahoma Center for Neuroscience and ³ Department Cell Biology, College of Medicine, University of Oklahoma HSC, OKC, OK, USA

The neuropeptide, N/OFQ, has immunomodulatory actions in addition to modulating nociception, anxiety and many other responses. Expression of Interleukin-6 (IL-6), a cytokine linked to inflammation and pain, is modulated by N/OFQ-N/OFQ peptide (NOP) receptor signaling at the mRNA and protein levels. To better understand how N/OFQ modulates IL-6, IL-6 mRNA levels in U937 human monocyte cells were assessed by quantitative PCR (qPCR). Time course studies revealed that N/OFQ initially increased IL-6 mRNA at 15 min, but decreased IL-6 mRNA over the next 4 hr. Pretreatment with a NOP receptor antagonist blocked the N/OFQ-induced increase in IL-6 mRNA at 15 min, with no effect on the subsequent reduction in IL-6 at 4 hr. N/OFQ-induced up-regulation of IL-6 mRNA also was blocked by inhibitors of NFκB, p38 and Akt signaling, but not by inhibitors of PKC or ERK MAP kinase; suggesting potential mechanisms by which N/OFQ increases IL-6. We previously reported that Single Prolonged Stress (SPS), a PTSD model, produced hyperalgesia and allodynia, and increased N/OFQ levels in serum, CSF and brain. To explore potential relationships between N/OFQ, cytokines and PTSD-related pain, we assessed IL-6 mRNA and protein levels in the spinal cord dorsal horn of male Sprague-Dawley rats exposed to SPS and matching controls by qPCR, multiplex cytokine analysis and immunolabeling. SPS increased IL-6 mRNA in spinal cord and brain between days 14-28 days, but IL-6 levels in sera were unchanged. Preliminary studies indicate that treatment of SPS rats with a NOP receptor antagonist from day 7-21 blocked IL-6 protein up-regulation in spinal cord at day 21 and reversed hyperalgesia and allodynia, suggesting that N/OFQ mediates IL-6 up-regulation during chronic stress and that this up-regulation may contribute to allodynia and hyperalgesia in the PTSD model.

Animal protocol was approved by IACUC and the US Army Medical Research and Materiel Command Animal Care and Use Review Office, complied with the Animal Welfare Act and adhered to the principles described in the Guide for Care and Use of Laboratory Animals.



SYMPOSIUM 5**EFFECTS OF GLIAL INHIBITORS ON OPIOID-MEDIATED RESPONSES IN HUMAN RESEARCH VOLUNTEERS**

***Comer, S.D., Jones, J.D., Mogali, S., Manubay, J., Sullivan, M.A., Cooper, Z.D., Vosburg, S.K., Metz, V., Johnson, K.W.**
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Several recent preclinical studies have suggested that glia may play an important role in opioid-mediated effects, including analgesia, respiration, and reward. Specifically, inhibition of glial activation increases the potency and efficacy of opioid-induced analgesia, and simultaneously reduces opioid-induced reward and respiratory depression. Glia also modulate opioid-induced tolerance and dependence in preclinical models. However, the effects of inhibition of glial activation on opioid-induced responses in humans have not yet been investigated. Two glial attenuators (ibudilast and minocycline) were tested in a laboratory setting in combination with opioids under a variety of experimental conditions. Ibudilast, a non-selective phosphodiesterase-4 and -10 and macrophage migration inhibitory factor (MIF) inhibitor, is used clinically in Asia to treat asthma and post-stroke dizziness, and minocycline, a tetracycline antibiotic, is used to treat acne and other bacterial infections. The effects of ibudilast on opioid withdrawal symptoms in morphine-dependent heroin abusers were investigated, as was the ability of ibudilast to alter oxycodone-induced subjective, analgesic, and physiological effects. The effects of minocycline on oxycodone-induced subjective, analgesic, and physiological effects in non-dependent opioid abusers also were examined. The results of these studies suggest that ibudilast reduced opioid withdrawal symptoms and accentuated oxycodone-induced analgesia without altering the positive subjective effects of oxycodone. Ibudilast also appeared to produce a dose-dependent reversal of tolerance to the miotic effects of oxycodone. Preliminary analyses of the data with minocycline suggest that it produced robust and dose-dependent reductions in the positive subjective effects of oxycodone, as well as drug craving, without altering its respiratory depressant or miotic effects. The potential utility of these medications for treating pain and opioid abuse will be discussed.

Disclosures: We gratefully acknowledge the research support provided by NIDA (DA09236), and the supplemental funding and provision of ibudilast by MediciNova Inc. In addition, SDC consulted for Pfizer and Salix during the past year, and she receives research funding from Reckitt Benckiser and study medication from Omeros. KWJ is an employee of MediciNova. The authors have no other potential conflicts of interest to declare.

MICROGLIA-MEDIATED DISRUPTION OF NEURONAL CL⁻ HOMEOSTASIS GATES MORPHINE HYPERALGESIA

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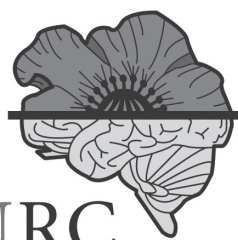
Opiates such as morphine are the gold standard pain relieving drugs, but their use is plagued with debilitating side effects. Two main problems that limit the treatment of pain with opiates are: paradoxical hyperalgesia (increased pain sensitivity) and tolerance (diminished analgesic potency). The prevailing dogma is that both of these problems are inevitable consequences of opiate use, and that tolerance and hyperalgesia reflect a single underlying mechanism. On the contrary, we found that hyperalgesia-inducing treatment with morphine downregulates the K⁺-Cl⁻ cotransporter KCC2, impairing Cl⁻ extrusion and thus causing disinhibition of spinal lamina I pain-transmitting neurons. Restoring anion reversed the morphine-induced hyperalgesia without affecting tolerance. The hyperalgesia was also reversed by ablating spinal microglia with the immunotoxin mac1-saporin. In addition, we found that morphine acting through μ -receptors increased expression of ATP-gated P2X4 receptors in primary microglia which drives the release of brain-derived neurotrophic factor (BDNF). This release was gated by a μ -receptor independent mechanism. Blocking BDNF-TrkB signalling preserved Cl⁻ homeostasis in lamina I neurons and reversed the hyperalgesia. Gene-targeted mice in which BDNF was deleted from microglia, or mice lacking P2X4 receptors, do not develop hyperalgesia to morphine treatment. Yet, neither morphine analgesia nor tolerance was affected in these animals. In summary, we discovered that morphine hyperalgesia, but not tolerance, is mediated by microglia-to-neuron signalling that ultimately increases neuronal excitability by disrupting intracellular Cl⁻ homeostasis. We identified that the core microglia-to-neuron signalling ensemble critically involved in morphine hyperalgesia is the P2X4 receptor-BDNF-TrkB-KCC2 cascade. Collectively, our findings provide a new mechanistic framework for understanding morphine hyperalgesia as being distinct from those of tolerance. This work was supported by grants from the Canadian Institutes of Health Research (CIHR) and the Natural Sciences and Engineering Research Council of Canada (NSERC).

SYMPOSIUM 5**OPIOIDS IN NON-NEURONAL CELLS -
REGULATION AND PROTECTION OF THE
HEART*****Headrick, J.P., See Hoe, L.E., Peart, J.N.***Griffith Health Institute, Griffith University, Southport QLD, Australia.*

Background: Opioids and opioid receptors (ORs) are important in cardiac control, and in governing myocardial tolerance to injurious stressors. The heart produces pro-dynorphin and pro-enkephalin, which are enzymatically processed to active peptides. Such opioids modulate cardiac inotropy and vascular tone via OR-dependent and -independent mechanisms. ORs also modify heart-rate and vagal tone, and negatively control cardiac β -adrenergic responses. Recent work reveals a role for opioids and ORs in cardioprotection, limiting cell damage and dysfunction with ischaemia or hypoxia: exogenous OR agonists induce protection pre- or post-insult, with endogenous OR ligands contributing to conditioning responses and hibernation.

Findings: We show acute morphine and delta- and kappa-OR agonists (BW373U86, U50,488) enhance myocardial tolerance to ischaemia-reperfusion (I-R) via PI3K/Akt signaling and K_{ATP} channel modulation in rodents. These effects appear to involve MMP-dependent EGFR transactivation. Intriguingly, 3- to 5-days of low-level morphine or a delta-OR agonist induces a unique protected state independent of major protective mediators (eg. NOS, PI3K/Akt), and that persists for up to a week post-stimulus. Since I-R occurs primarily in the elderly, and in subjects with common co-morbidities such as diabetes and hypertension, we tested the effects of 5-day morphine conditioning in aged (18-24 mth) mice and a murine model of type II diabetes (T2D; 75 mg/kg STZ and 10 weeks of high fat feeding). Aging and T2D both impaired contractile recoveries and exaggerated infarction in mouse hearts subjected to I-R *ex vivo*. 5-day treatment with subcutaneous morphine (25-75 mg pellets) markedly protected against I-R injury and cell death in both aged and T2D hearts, whereas acute OR responses were ablated (as was protection via ischaemic preconditioning). Protection was associated with improvements in Ca^{2+} -dependent mitochondrial swelling and respiratory function. Chronic morphine also reversed T2D features *in vivo*, improving ventricular contractile function, countering hyperglycaemia and reducing body weight.

Summary: Acute or chronic activation of ORs can exert powerful protective actions in the heart via distinct signalling mechanisms. While effects of acute OR agonism are negated with age and disease, novel protection via chronic morphine persists. This latter response also exhibits unique anti-diabetic features. The OR system warrants further investigation as a means of manipulating cardiovascular responses to diabetes and ischaemic heart disease.



HOT TOPICS 5

MORPHINE MODULATES BREAST CANCER CELL METASTATIC POTENTIALImani, B.A.¹, Baran, J.², Cabot, P.J.¹, *Parat, M.^{1,2}¹ School of Pharmacy, University of Queensland, Woolloongabba, Australia² Department of Anesthesia Research, Cleveland Clinic, Cleveland, OH

Appropriate pain management during and after cancer surgery may play a role in prevention of tumor recurrence and metastasis. Opioids are proven to be highly effective perioperative analgesics and are widely used in cancer surgery patients. Using a mouse syngeneic model of breast cancer, we studied the effect of morphine on tumor growth and dissemination to lungs. I.P. injection of morphine (10mg/kg) to mice (n=8) every 12h for 3 consecutive days, caused a reduction in breast tumor growth and tumor cell dissemination to the lungs as measured 18 days after tumor inoculation. Morphine treatment also caused a reduction in circulating proteolytic enzymes of extracellular matrix (ECM), matrix metalloproteinase-9 (MMP-9) and urokinase-like plasminogen activator (uPA) measured by gelatin and casein-plasminogen zymography, respectively. Furthermore, we tested the effect of morphine on co-cultures of breast cancer cells with stromal cells, either endothelial cells or macrophages. In co-cultures of 4T1 breast cancer cells with murine RAW264.7 macrophages or murine HSV endothelial cells, the level of matrix proteases produced by cells was increased, and so was the level of matrix protease inhibitors TIMPs. Interestingly, morphine treatment of these co-cultures reduced the level of MMP-9 and increased its endogenous inhibitor, TIMP-1, thereby altering the overall proteolytic profile. Morphine affected co-cultures but not cells grown individually. This suggests that anti-tumor effects of morphine are mediated through modulation of paracrine communication between cancer cells and infiltrating cells.

EFFECT OF DELTA-OPIOID RECEPTORS ON HUMAN KERATINOCYTE ADHESION AND MIGRATION DURING WOUND HEALINGBigliardi-Qi, M.¹, Teo, Y.L.¹, Neumann, C.¹, Bigliardi, P.L.^{1,2}¹Institute of Medical Biology, Agency for Science, Technology & Research (A*STAR), Singapore²Department of Internal Medicine, School of Medicine, National University Hospital, 5 Lower Kent Ridge Road, Singapore 119074

Abbreviations: Beta-1 Integrin, ITGB1; Beta-Catenin, β -Cat; Delta-opioid receptor, DOR; Desmoglein, DSG; Desmoplakin, DSP; Extracellular signal-regulated kinases, Erk; Intermediate filaments, IF; Met-Enkephalin, Met-Enk; Naltrindole, Nal; Overexpression, OE; Wild type, WT; Protein Kinase C, PKC

In addition to its analgesic functions, the peripheral opioid receptor system affects skin homeostasis by influencing cell differentiation, migration, and adhesion. Previous studies have shown that wound healing and skin homeostasis are significantly altered in delta-opioid receptor knockout mice (DOR^{-/-}). Extending these observations we now show that DOR activation in human keratinocytes regulates intercellular adhesion molecules facilitating cell migration in vitro. Desmoglein 1 (DSG-1) and DSG-4 expression was up-regulated in skin from DOR^{-/-} mice, and down-regulated in DOR-overexpressing (DOR-OE) N/TERT-1 keratinocytes. The expression pattern of the desmosomal plaque proteins beta-catenin (β -Cat) and desmoplakin (DSP) was also altered, resulting in less stable intercellular adhesion. Consequently, DOR-stimulated migration

and wound recovery were enhanced in DOR-OE cells in an in vitro scratch assay. DOR effects on cell migration and adhesion were mediated by the PKC signalling pathway, and could be antagonized by specific PKC α/β inhibition. Finally, DOR-OE cells build long and characteristic protrusions containing filamentous actin and DOR at their free edges, indicating a migratory phenotype. In conclusion, these results from transgenic mice and human immortalized keratinocyte cell lines show that the opioid receptors affect cell-cell adhesion and wound healing mechanisms, underlining the importance of skin-nerve interactions in wound healing and skin homeostasis.

ROLE OF OPIOID SYSTEM IN THE CELL DIFFERENTIATION FROM PLURIPOTENT STEM CELLS*Kuzumaki, N.^{1,2}, Yamamizu, K.^{3,4}, Narita, M.¹, Okano, H.², Narita, M.¹¹ Dept. Pharmacol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan² Dept. Physiol., Keio Univ. Sch. Med. Tokyo, Japan³ NIH, NIA, Developmental Genomics and Aging Section, Laboratory of Genetics, MD, USA⁴ Laboratory of Stem cell Differentiation, Stem Cell Research Center, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan

Since the advent of human embryonic stem cells (ESCs), stem cell research has been developing at a breathtaking pace. The pluripotent stem cells have the ability to differentiate into any cell types including into those with therapeutic potential, after practically unlimited self-renewal in the stem cell state. The pluripotent stem cells hold enormous promise as tools for understanding normal development and disease, and just as importantly, for cell therapy applications to treat devastating and currently incurable disorders. However a potential mechanism that is poorly understood entails the role of endogenous hormones, growth factors and neurotransmitters in influencing stem cell fate decisions. Therefore, it is important to identify the intrinsic and/or extrinsic factors that regulate the underlying molecular mechanisms involved in ESC self-renewal, proliferation and differentiation.

Here we profiled the expression of opioid-related genes in undifferentiated ES cells and differentiated neural stem cells. We found that delta opioid receptor (DOR), but not mu opioid receptor (MOR) or kappa opioid receptor (KOR), plays a role in neurogenesis and neuroprotection in neural stem cells. We also demonstrated a novel mechanism for the regulation of EC differentiation and vascular formation through the opioid system. Especially, KOR was highly expressed in embryonic stem cell-derived Flk1+ vascular progenitors. Addition of KOR agonists to ES cells-derived Flk1+ vascular progenitors inhibited endothelial cell differentiation, indicating that the KOR system is, thus, a new regulator of vascular development that simultaneously modifies EC differentiation and vascular pathfinding. We conclude that opioids may be responsible for ES cells differentiation at the developmental stage.

SYMPOSIUM 6**MEASUREMENT AND MECHANISM OF LIGAND BIAS AT THE μ -OPIOID RECEPTOR****Kelly, E.***School of Physiology and Pharmacology, University of Bristol, Bristol BS8 1TD, UK*

Drugs acting at the μ opioid receptor (MOPr) are amongst the most important therapeutic agents available, but much remains to be understood about the molecular signals that arise following activation of this G protein coupled receptor by opioid agonists. It is likely that signalling via both G protein- and arrestin-dependent pathways is important for MOPr function. However, the relative abilities of MOPr agonists to signal via these two pathways may not be the same for all agonists, a phenomenon termed ligand bias or functional selectivity. By identifying MOPr ligands that display G protein or arrestin bias at MOPr, it may be possible to develop drugs which have a better therapeutic profile with, for example, fewer adverse effects, less liability to promote tolerance to analgesic effects, or less likelihood to induce dependence.

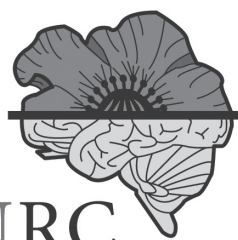
In this presentation I will discuss how cellular assays for G protein- and arrestin-dependent signalling pathways can be used to determine the presence of ligand bias for a series of MOPr ligands. I will discuss how the Operational Model of Agonism, developed by Black and Leff over a quarter of a century ago, is becoming an increasingly important means to analyse agonism and detect ligand bias. In addition, I will discuss potential mechanisms that underlie ligand bias at MOPr, such as ligand-dependent phosphorylation of multiple residues in the COOH-terminus of the receptor. Finally, I will discuss the possible advantages and disadvantages of developing ligands with either G protein or arrestin bias at MOPr, and indeed whether or not we know enough about the MOPr signalling pathways in brain to actually know what sort of bias may be preferable.

LIGAND AND CELL DEPENDENT DETERMINANTS OF DELTA OPIOID RECEPTOR (DOR) LIGAND SIGNALLING.**Pineyro, G.***Department of Psychiatry and Department of Pharmacology, University of Montreal. Ste-Justine Hospital Research Center. Montreal, Quebec, Canada*

Opioid receptor agonists have potent analgesic actions but these effects are restricted by the development of tolerance. However, tolerance is not the same for all ligands and these differences have been attributed, at least in part, to biased agonism. This notion is based on the observation that ligands with similar G protein-mediated responses may display distinct potential for tolerance depending on their internalization profiles. A problem with this notion is that some of these observations have relied on comparison of E_{max} values rather than estimation of relative efficacies. Here we sought to measure whether DOR agonists displayed bias in cyclase and internalization responses. To do so, we used the operational model of Black and Leff to generate transduction coefficients ($\log\tau/K_A$) for each of the responses of interest. These coefficients capture differences both in ligand efficacy (τ) and "functional affinity" (K_A), which intuitively corresponds to ligand affinity for the receptor state(s) mediating the response of interest. Comparison of transduction coefficients obtained in HEK293 cells indicated that TIPP and UFP512 were respectively 54- and 112-fold more efficient in cyclase than internalization assays as compared to DPDPE. SNC-80 was similarly biased, being 41-fold more efficient in cyclase than internalization assays.

Importantly, molecular determinants of internalization were not the same in HEK293 cells and neurons, raising the question whether bias identified in one cell type could be relevant to the other. In particular, β arrestins contributed to DOR internalization in the two cellular backgrounds, but PKC and GRK2 (G protein-coupled receptor kinase 2) were only involved in neurons. These differences resulted in higher relative E_{max} internalization values for TIPP and UFP512 but not for SNC-80 (DPDPE was the standard). Moreover, the extent to which different ligands relied on β arrestins and PKCs to induce maximal internalization in neurons were both correlated with transduction coefficients for cyclase inhibition in HEK293 cells, implying a common substrate for these responses that could be evidenced across cellular backgrounds. GRK2 contribution to ligand-induced internalization was unrelated to the other signals, suggesting that the activity of this kinase could determine internalization bias in neurons.

Taken together these observations indicate that for bias to be independent of cellular background, it should be measured on actual signals and not phenotypic cellular responses.

**INRC**

HOT TOPICS 6

QUANTIFICATION OF FUNCTIONAL SELECTIVITY OF ENDOGENOUS OPIOIDS AT THE MU-OPIOID RECEPTOR

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Functional selectivity, or biased agonism, refers to the paradigm whereby different ligands, binding to the same receptor, engender distinct receptor conformations linked to divergent signalling pathways and physiological outcomes. The description of numerous functionally selective ligands in the last decade has prompted the idea that functional selectivity represents a novel direction for drug discovery whereby drugs can be “pathway selective” in addition to “receptor selective”. As such, biased ligands may allow for the fine-tuning of receptor signalling towards desired pathways and away from unwanted signalling cascades such as those mediating adverse side effects. However, functional selectivity has broader implications for GPCR-directed therapeutics when considering systems in which multiple endogenous ligands target a given receptor, as it raises the possibility of “endogenous (natural) functional selectivity” mediating specific physiological responses.

Unfortunately, with few exceptions, most descriptions of biased agonism have been based on qualitative comparisons between ligands, and the number of studies quantifying bias in a statistically robust manner is still very low. However, in order to apply functional selectivity therapeutically and in the most effective manner, it is necessary to incorporate parameters that describe the degree of bias in a manner that can inform structure activity studies and drug candidate selection matrices. We have developed an analytical method, based on the operational model of agonism, that can be applied to standard concentration-response data to derive “bias factors” for each agonist. These bias factors can be evaluated statistically in a system-independent manner.

The current study applied this method to the quantification of functional selectivity of endogenous opioids at the mu-opioid receptor (MOR) in cellular model systems. This has allowed the generation of the first activity profiles for the endogenous opioid family and the detection of divergent signal fingerprints for this class of ligands. Unravelling unappreciated activity patterns of endogenous opioid peptides can provide essential information regarding the mechanisms behind opioid-induced physiological responses (from analgesia to respiratory depression and constipation) that can then be exploited to design novel drug targets with effective analgesia but with minimal side effects. As such, future studies will address the potential for the bias profiles to be predictive in physiological systems and to further validate functional selectivity *in vivo*.

IDENTIFICATION OF ALLOSTERIC MODULATORS OF OPIOID RECEPTORS BY HIGH-THROUGHPUT SCREENING

Burford, N.T.¹, Wehrman, T.S.², Clark, M.J.³, Gerritz, S.W.¹, Banks, M.¹, O’Connell, J.¹, Traynor, J.R.³ and *Alt, A.¹

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Allosteric modulators of G protein-coupled receptors have a number of potential advantages over traditional orthosteric agonists and antagonists, including potential improvement in tolerance, safety, and side-effect profiles.

A high-throughput screen (HTS) of >1 million compounds was designed to identify positive allosteric modulators (PAMs) of opioid receptors. The HTS measured the enhancement of endomorphin-1 and Leu-enkephalin-mediated recruitment of β -arrestin-2 in U2OS human osteosarcoma cells expressing μ - and/or δ opioid receptors; the assay was designed to simultaneously identify PAMs of μ - and/or δ opioid receptors in a single screen. The HTS used an N-terminal deletion mutant of β -galactosidase fused to the C-terminus of stably expressed β -arrestin-2 and a mutated amino-terminal fragment of β -galactosidase fused to the carboxyl terminus of the appropriate opioid receptor. Binding of β -arrestin-2 to activated opioid receptor results in a complementation of the enzyme and reconstitution of enzyme activity. From the HTS screen we identified two selective μ -opioid receptor PAMs: (E)-N-(2,6-dichlorophenyl)-4-(4-nitrophenyl)-2,3-dihydro-1,3-thiazol-2-imine (BMS-986121) and 2-(3-bromo-4-methoxyphenyl)-3-(4-chlorobenzenesulfonyl)-1,3-thiazolidine (BMS-986122). *In vitro*, both compounds produced concentration-dependent and saturable leftward shifts in concentration-response curves for the μ -opioid receptor agonist endomorphin-1 to recruit β -arrestin-2 producing an approximate 7-fold maximal leftward shift in agonist response, with K_b (shift₅₀) values of 2-5 μ M. While BMS-986121 behaved as a “pure” PAM, BMS-986122 displayed allosteric agonist activity, behaving as a PAM at lower concentrations but exhibiting its own intrinsic agonist activity at higher concentrations.

Structure-activity relationships within the thiazolidine chemotype were explored and the effects of chemical modifications to BMS-986122 on μ -PAM activity will be described; however none showed improved PAM activity over the original hit. While BMS-986122 was seen to be selective for the μ -opioid receptor, some analogs were found to exhibit weak PAM activity at δ -opioid receptors. Additionally, two analogs were identified as “silent” allosteric modulators (SAMs) of the μ -opioid receptor and acted as competitive antagonists for the allosteric site to which BMS-986122 binds, confirming the presence of a discrete allosteric binding site on the μ opioid receptor.

HOT TOPICS 6**POSITIVE ALLOSTERIC MODULATION OF THE MU OPIOID RECEPTOR****Clark, M.J.¹, Burford, N.T.², Livingston, K.E.¹, Mahoney, J.¹, Alt, A.², *Traynor, J.R.¹**¹ Department of Pharmacology, University of Michigan, Ann Arbor, MI, USA,² Molecular Sciences and Candidate Optimization, Bristol-Myers Squibb, Wallingford, CT, USA

Allosteric ligands for G protein coupled receptors (GPCRs) bind to a site on the receptor that is distinct from the site that binds orthosteric (or endogenous) agonists. Positive allosteric modulators (PAMs) generally lack intrinsic efficacy, but increase the binding affinity and/or efficacy of orthosteric agonists. This enhancement may be agonist- and signaling pathway-dependent. Thus, PAMs active at the mu-opioid receptor (MOR-PAMs) could promote activation of select signaling pathways, thereby potentially separating beneficial and unwanted effects. In addition, MOR-PAMs might augment the action of endogenous opioid peptides and so avoid compensatory mechanisms that are triggered by sustained receptor activation produced by exogenous opioids. Here, we report an initial characterization of a MOR-PAM, BMS-986122, identified by a high-throughput screen. BMS-986122 improved the binding affinity of the MOR agonist DAMGO by 6-fold in C6 cells expressing MOR (C6MOR) without altering binding of the antagonist diprenorphine. The potency of DAMGO to stimulate [35S]GTP γ S binding in C6MOR cells and mouse brain membranes was increased by 4-7-fold in the presence of BMS-986122. With the partial agonists morphine and endomorphin-1 there was a 3-fold increase in potency together with a 1.7-fold increase in maximal [35S]GTP γ S stimulation. Thus, BMS-986122 is both an affinity and an efficacy modulator. The affinity of BMS-986122 calculated using an allosteric ternary complex model was 5 μ M. A close analog of BMS-986122 acted as a competitive antagonist of the PAM, but not diprenorphine, indicating the presence of a distinct allosteric binding site. To confirm the allosteric binding site is on MOR we used purified MOR protein in complex with G protein heterotrimer and GDP. This complex was incorporated into a phospholipid bilayer in the form of recombinant high density lipoprotein particles. In this preparation only a single low affinity low DAMGO binding site (K_i = 500nM) was observed. BMS-986122 caused the appearance of a high affinity site for DAMGO (K_i = 20nM) that was lost in the presence of unlabelled GTP γ S. These studies provide proof-of-concept for the development of novel allosteric modulators of MOR that may have therapeutic potential in improving pain management. Supported in part by MH083754.

ENDOMORPHIN ANALOGS WITH REDUCED ABUSE LIABILITY, RESPIRATORY DEPRESSION, MOTOR IMPAIRMENT, TOLERANCE AND GLIAL ACTIVATION RELATIVE TO MORPHINE.***Zadina, J.E.^{1,2,3,4}, Nilges, M.R.⁴, Morgenweck, J.², Zhang, X.^{1,2}, Hackler, L.^{1,2}, Fasold, M.B.^{1,2}**¹ SE Louisiana Veterans HCS, NEW ORLEANS, LA and Depts of ² Medicine, ³ Pharmacol., ⁴ Neurosci. Program, Tulane Univ. Sch. of Med., New Orleans, LA, USA

Opioids acting at the mu opioid receptor (MOR) are the most effective analgesics, but adverse side effects severely limit their use. Abuse liability results in major medical, societal, and economic problems, respiratory depression is the cause of death from overdose, and tolerance complicates treatment and increases the risk of side effects. Motor and cognitive impairment are especially problematic for older adults. Morphine-induced glial activation is well characterized in tolerance and may contribute to additional side effects. The endomorphins (EMs) are potent and selective agonists at the mu opioid receptor. Three metabolically stable EM analogs were synthesized and tested for their antinociceptive and side-effect profiles in the rat. In receptor and GTP γ S assays, the analogs showed high affinity, selectivity, and efficacy at MOR relative to delta and kappa opioid receptors. In tests of reward, morphine induced significant conditioned place preference and self-administration, while the analogs did not, consistent with reduced abuse potential. At equianalgesic doses, respiratory depression was significantly reduced for all analogs relative to morphine. Analog 1 did not induce significant respiratory depression at a dose producing significantly longer antinociception than morphine. Motor impairment on a rotorod was significantly less, and antinociception significantly longer, after analog 2 than after morphine. Tolerance was determined with cumulative dosing before and after intrathecal administration of morphine or analogs for 7 days with an osmotic minipump. Analog 1 was initially 30-fold more potent than morphine and, compared to a 61-fold shift in the dose-response curve by morphine, the average shift for the analogs was only 13-fold, consistent with significantly less tolerance. Morphine produced significant glial activation, as measured by increased GFAP, Iba1 and pp38 staining, while the analogs did not. In summary, our novel EM analogs show a highly favorable profile relative to morphine as potent analgesics with reduced 1) reward/abuse liability, 2) respiratory depression, 3) motor impairment, 4) tolerance and 5) glial activation. Funded by the VA, ONR, and DOD.

HOT TOPICS 6**LIGAND-BIASED MECHANISMS OF OPIOID ANTINOCICEPTION*****Bobeck, E. N.¹, Ingram, S. L.², & Morgan, M. M.¹**¹ Dept. of Psychology, Washington State University, Vancouver, WA, USA² Dept. of Neurological Surgery, Oregon Health & Science University, Portland, OR, USA

Opioids such as morphine bind and activate the mu-opioid receptor (MOPr) which leads to activation of G-proteins. Inhibitory G-proteins have been shown to be important in antinociception. Inhibition of these G-proteins with pertussis toxin (PTX) causes a greater reduction in morphine antinociception than that produced by other MOPr agonists. MOPr agonists also differ in their ability to recruit β -arrestin and induce MOPr internalization. Morphine produces little internalization, whereas DAMGO and fentanyl produce rapid internalization. Although much is known about these ligand-biased signaling pathways, little is known about the behavioral significance of activation of these signaling pathways. The purpose of this experiment was to test the hypothesis that the signaling pathway (i.e., G-protein and internalization) that contributes to antinociception differs depending on whether the MOPr is bound by morphine, DAMGO, or fentanyl. All drugs will be injected into the ventrolateral periaqueductal gray (vlPAG) so as to link specific signaling pathways to antinociception. To evaluate the role of G-proteins in antinociception, PTX (5 or 50 ng/0.5 μ L) was microinjected into the vlPAG prior to cumulative doses of the opioid. Pretreatment with 5 or 50 ng PTX caused a rightward shift in the morphine dose-response curve. However, only 50 ng PTX attenuated antinociception to DAMGO, and neither dose altered fentanyl-induced antinociception. To investigate the role of internalization in antinociception dynamin-dominant negative peptide (dyn-DN; 2 μ g/0.5 μ L) was administered into vlPAG to prevent receptor internalization. Pretreatment with dyn-DN did not alter the morphine dose-response curve, but did cause a rightward shift in the DAMGO dose-response curve and a leftward shift in the fentanyl dose-response curve. These data indicate that morphine uses a G-protein mediated process to induce antinociception, whereas DAMGO uses both G-protein and dynamin-dependent mechanisms. The mechanism underlying fentanyl antinociception is unclear. However, these studies suggest that receptor internalization may terminate the MOPr signaling that causes antinociception. These results suggest that antinociception can be caused by distinct mechanisms depending on the agonist. Funding by NIH grant DA 015498.



SYMPOSIUM 7**ENDOGENOUS OPIOIDS IN THE AMYGDALA**
Bagley, E.

Fear and anxiety are adaptive responses that allow us to defend ourselves against harm. Neural circuits in the amygdala are key mediators of fear memory acquisition and storage, but also reduce the fear response when the threat has passed (extinction). Disturbance of these circuits is thought to underlie a range of anxiety disorders in humans, including phobias, PTSD and panic disorders. One possible modulator of extinction is the endogenous opioid system, as deletion of enkephalin produces mice that are highly anxious and fearful. The extinction of the fear response relies on greater activity of a group of GABAergic interneurons in the amygdala, the intercalated cells inhibiting the fear response. Intercalated neurons express very high levels of enkephalin and the μ -opioid receptor, strongly suggesting that intercalated neurons release enkephalin that acts locally to regulate cellular excitability and ultimately alters expression of the fear response. Our data shows that in the amygdala endogenously released enkephalin acts in several different ways to modify neuronal excitability and synaptic transmission. These actions of the endogenous opioids occur with minimal stimulation indicating that these peptides are significant modulators of amygdala function and are likely to modulate the fear response. This work may ultimately provide insights into the physiological role of enkephalin in the intercalated cells of the amygdala, especially during fear extinction and provide the basis for better therapeutic approaches to anxiety disorders.

OPIOID PEPTIDES INDUCE LONG-TERM DEPRESSION AT GLUTAMATERGIC SYNAPSES IN THE DORSAL STRIATUM**Atwood, B.K., Kupferschmidt, D.A., Xiong, W. and Lovinger, D.M.***National Institute on Alcohol Abuse and Alcoholism, Rockville, MD, USA*

As prescription opioid analgesic abuse rates rise, so does the need to understand the long-term effects of opioid exposure on brain function. The dorsal striatum, with roles in skill learning and habit formation, is a site of importance for opioid-induced neuronal plasticity. Opioid peptides, their target receptors and the peptidase enzymes that are responsible for terminating their actions are abundantly expressed in this brain region. However there is a paucity of data concerning opioid roles in long-term synaptic plasticity in the dorsal striatum. We utilized whole-cell patch clamp electrophysiological recordings of medium spiny neurons in the dorsal striatum in an acute brain slice preparation to determine whether opioids could produce long-lasting changes in synaptic plasticity at excitatory synapses. We discovered that opioid agonist-induced inhibition is long-lasting and is able to be blocked, but not reversed, by opioid receptor antagonists. We refer to this long-lasting suppression of excitatory neurotransmission as opioid peptide-induced long-term depression (OP-LTD). We also found that endogenous opioid peptides, released locally in the striatum, could produce OP-LTD. Mu and delta opioid-mediated forms of OP-LTD are dissociable, in that they summate, differentially occlude LTD mediated by endocannabinoids, and are affected differently by *in vivo* exposure to the opioid analgesic oxycodone. These data demonstrate a novel form of long-lasting synaptic plasticity in the dorsal striatum that is induced by a brief exposure to opioid peptides.

This research was supported by the Division of Intramural Clinical and Basic Research of the National Institute on Alcohol Abuse and Alcoholism, US National Institutes of Health.

HOT TOPICS 7**REPEATED AVERSIVE STRESS EXPOSURE REDUCES MU OPIOID RECEPTOR EXPRESSION IN THE INTERCALATED NUCLEI OF THE AMYGDALA OF RATS**

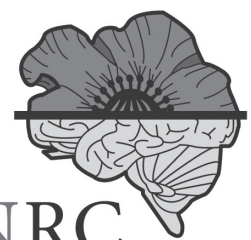
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Mu receptors (MOPr) are strongly expressed in the cell clusters of the intercalated nuclei (ITC) that surround the lateral (LA) and basolateral (BLA) nuclei in rat amygdala (Likhtik et al, 2008). ITC neurons may play a role in regulating activity in the neuronal connections from the BLA, which mediates the integration of conditioned and unconditioned noxious stimuli to the central amygdaloid nuclei (CeA), the major output nucleus of the amygdala regulating motor and autonomic responses (Pape & Pare, 2010). We hypothesized that activation of amygdaloid pathways by exposure to repeated aversive stimuli might change the extent of activation or the level of expression of MOPr in the ITC. We therefore determined the effects of repeated aversive stress in rats on the expression of MOPr in the ITC and on opioid peptide expression in amygdala nuclei, using a stressful stimulus (restraint for 2 hr with intermittent unpredictable tail shock, repeated on three consecutive days) that has been used as an experimental model for post-traumatic stress disorder (Manion et al, 2007). At 3 hr and 24 hr after the end of the third stress exposure, the level of MOPr expression was determined by fluorescence immuno-histochemistry in individual ITC cell groups at three A-P levels through the amygdala. The level of MOPr in ITC nuclei was reduced by about 50% at 3 hr and by about 40% at 24 hr after the third day of stress exposure. Endogenous opioids are also expressed in amygdala, with the highest levels of expression in the CeA. No significant stress related changes in the expression of pro-enkephalin mRNA were observed, but there was a significant increase in the expression of prodynorphin mRNA and of dynorphin A(1-8) immunoreactivity (DynA-ir) in the paracapsular subdivision of the CeA. Dyn A-ir fibers were also observed in close proximity with some ITC cell clusters. These results suggest that MOPr in ITC are down-regulated after repeated aversive stress. It is possible that peptide products of the prodynorphin gene participate in this regulation. Further study is required to elucidate the role of MOPr in ITC in response to aversive stimuli. Supported by grant PT-73729 from the US Dept of Defense.

OPIOID INHIBITION OF GABA INPUTS FROM RMTG AND INTERNEURONS IN THE VTA AFTER CHRONIC MORPHINE TREATMENTMatsui, A.¹, Jarvie, B.C.², Hentges, S.T.², Williams, J.T.¹¹Vollum Institute, Oregon Health and Science University, Portland, OR, 97239, USA²Department of Biomedical Sciences, Colorado State University, Fort Collins, CO, 80523, USA

The activity of dopamine neurons is modulated by the balance between excitatory and inhibitory inputs. Opioids increase dopamine neuron activity through inhibition of GABA input to dopamine neurons. The recent discovery of a dense opioid-sensitive GABA inputs originating from the rostromedial tegmental nucleus (RMTg) brings into question the role of GABA interneurons in the ventral tegmental area (VTA). The present study utilized an optogenetics in rats to examine a relative opioid sensitivity of GABA inputs from interneurons in VTA and RMTg neurons. The results show a relative insensitivity of VTA interneuron input to opioids. When animals were treated with morphine continuously for 1 week, the inhibition of GABA-A IPSCs by MOR agonists was reduced, indicating development of tolerance particularly the GABA input from RMTg. This study demonstrates that GABA inputs from VTA interneurons were less sensitive to opioids compared to RMTg neurons and less tolerance of this pathway was observed. The functional connection from the RMTg to the VTA is the key structure in the MOR dependent regulation of dopamine neurons. Supported by DA08163, DA04523 (JTW).



INRC

SYMPOSIUM 8**ROLE AND REGULATION OF THE DELTA OPIOID RECEPTOR IN PAIN****Gendron, L.***Department of Physiology and Biophysics, Université de Sherbrooke, Sherbrooke, Quebec, Canada*

We have previously shown that the analgesic efficacy of delta opioid receptor (DOPr) agonists can be enhanced by prolonged morphine treatment and by chronic inflammatory pain. Over the years, we have demonstrated that the mu opioid receptor (MOPr) is an important regulator of the membrane expression and of the functions of DOPr in spinal cord and dorsal root ganglia (DRG) neurons. The mechanisms involved in this regulation are still unclear but heterodimerization between MOPr and DOPr was postulated to play an important role in this phenomenon. Admittedly, to physically interact with each other these receptors have to be co-expressed in the same cells. This, however, remains highly controversial. Indeed, in spinal cord and DRG, MOPr and DOPr were found to be segregated in different population of cells, at least in the mouse. This segregation between MOPr and DOPr is further supported by recent findings showing that activation of MOPr and DOPr specifically inhibits distinct pain modalities. To address this controversy and to determine whether or not MOPr and DOPr are expressed in substance P-containing neurons, we have combined behavioral tests, in vivo electrophysiology and immunolabeling and found that both spinal MOPr and DOPr attenuate noxious behavior and inhibit substance P release induced by intraplantar formalin and capsaicin. We also found that activation of these receptors potentially inhibits diffuse noxious inhibitory controls (DNIC) induced by thermal and mechanical noxious stimuli applied to the hindpaw, an effect also accompanied by an inhibition of substance P release in the spinal cord. Our results therefore suggest that MOPr and DOPr are both expressed in a subpopulation of primary afferents containing substance P where physical interactions between them might participate in the regulation of DOPr. Most importantly, our findings indicate that activation of spinal MOPr and DOPr similarly inhibits DNIC evoked either by noxious thermal or mechanical stimuli. The specific role of the MOPr-DOPr heterodimer in the regulation of DOPr trafficking remains to be addressed.

This work was supported by the FRQ-S, the CIHR and the NSERC. LG is the recipient of a J1 salary support from the Fonds de la Recherche du Québec – Santé.

G-PROTEIN COUPLED RECEPTORS SUCH AS THE DELTA OPIOID AND ORL-1 RECEPTORS ARE ABLE TO MODULATE AGONIST SIGNALING VIA A ROCK, LIMK AND β -ARRESTIN 1 PATHWAY.**Mittal, N.¹, Roberts, K.¹, Pal, K.², Bentolila, L.A.^{3,4}, Cahill, C.⁵, Pradhan, A.¹, Conner, D.⁶, DeFea, K.², Evans, C.¹, Walwyn, W.¹**

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Ligands of G-protein coupled receptors (GPCRs) transduce signals from the external environment across the plasma membrane to induce an appropriate cellular response. We have found a novel mechanism whereby some GPCRs are able to amplify or reduce

such signaling. This mechanism allows agonists of the delta opioid receptor or Opioid Receptor-Like 1 (ORL-1) to activate cofilin, an actin modulating protein, through ROCK and LIMK in a β -arrestin 1 dependent manner. This controls actin polymerization and regulates receptor export from the Golgi to the cell membrane. Receptor function, as determined by receptor inhibition of voltage-dependent Ca²⁺ channels in DRG neurons, and the behavioral effects of delta opioid receptor and ORL1 agonists are both influenced by these mechanisms. Furthermore the upregulation of delta opioid receptor function following a chronic inflammatory injury can also be explained by this ROCK-activated pathway. In summary this novel pathway allows specific GPCRs to rapidly influence the magnitude of agonist signaling and provides further insight into THE mechanisms by which GPCRs signal.

DELTA OPIOID RECEPTOR PROCESSING AND MATURATION**Petäjä-Repo, U.***Department of Anatomy and Cell Biology, Institute of Biomedicine, University of Oulu, FI-90014 Oulu, Finland*

The delta opioid receptor (DOR) is one of the three opioid receptors involved in pain and mood modulation. The human (h) DOR carries a non-synonymous single nucleotide T80G polymorphism that replaces Phe at position 27 with Cys in the receptor N terminus. The hDOR variants display markedly different behavior during their “life cycle” that governs the number of functionally active receptors at the cell surface. The variants mature with similar slow kinetics but differ in maturation efficiency. The nascent receptors are subjected to N-glycan dependent quality control that retains the precursors in the endoplasmic reticulum (ER) and eventually targets a large percentage of the Cys27 variant precursors, but not those of the Phe27 variant, to ER-associated degradation (ERAD). In addition, the Cys27 variant precursors that exit the ER and reach the cell surface are unstable and are internalized constitutively. These differences result in a lower steady-state expression level of the Cys27 variant at the plasma membrane. The dissimilarities are increased further when the two N-glycosylation sites (Asn18 and Asn33), flanking the polymorphic site, are mutated, resulting in expression of ligand-binding incompetent Cys27 variant at the cell surface. Importantly, in co-transfected cells the Cys27 variant acts in a dominant negative manner and impairs cell surface delivery of the Phe27 variant. It impairs conversion of the Phe27 variant precursors to the mature form and redirects them to ERAD. This occurs via dimerization in the ER. Nevertheless, the unpaired Cys residue in the N terminus of the Cys27 variant does not cause a major folding defect because pharmacological characteristics of the variants are indistinguishable and opioid antagonists, acting as pharmacological chaperones, are able to enhance maturation of both variants, whether expressed individually or simultaneously. The hDOR is thus a G protein-coupled receptor that is predisposed to premature targeting to ERAD, most likely because of its relatively slow folding rate in the ER. It is amenable to rescue by pharmacological chaperones that stabilize newly-synthesized receptors, and eventually enhance their ER export and increase the steady-state receptor level at the plasma membrane.

HOT TOPICS 8

MU OPIOID RECEPTORS HYPERPOLARIZE RESPIRATORY-CONTROLLING KÖLLIKER-FUSE NEURONSLevitt, E.S.¹, Abdala, A.P.L.², Paton, J.F.R.², Williams, J.T.¹¹Vollum Institute, Oregon Health & Science University, Portland, OR, USA²School of Physiology and Pharmacology, University of Bristol, Bristol, UK

Respiratory depression is the primary cause of death from opioid overdose. The preBotzinger complex in the medulla has traditionally been considered the main area responsible for opioid-induced respiratory depression. However, in the rat working heart-brainstem preparation, injection of the mu opioid agonist DAMGO (60 – 120 nl of 1 mM) into the Kölliker-Fuse (KF) area of the pons caused disruption of the normal respiratory cycle. The primary effect was a loss of post-inspiratory activity, which is characteristic of silencing the KF. In whole-cell recordings from KF neurons contained in rat brain slices, activation of mu opioid receptors hyperpolarized KF neurons by opening G protein-coupled inwardly rectifying potassium (GIRK) channels. The outward GIRK current produced by [Met⁵]enkephalin (ME) was concentration-dependent, reversed at the potassium equilibrium potential and was blocked by BaCl₂. In locus coeruleus neurons, a saturating concentration of ME causes robust desensitization of the mu opioid receptor-induced current. In KF neurons, a saturating concentration of ME caused only modest desensitization. The current produced by a saturating concentration of ME declined by 27 ± 1 % during a ten-minute perfusion while the effectiveness of an EC₅₀ concentration of ME was reduced by 30 ± 2 %. The current produced by an EC₅₀ concentration of ME recovered gradually over 30 minutes. Pretreatment with the protein kinase C activator PDBu (100 nM) did not alter desensitization or recovery from desensitization. Mu opioid receptors on respiratory-controlling neurons in the KF only modestly desensitize, which correlates with the modest tolerance to respiratory depression observed during prolonged opioid treatment.

MOLECULAR MECHANISMS OF TOLERANCE TO OPIOID: INWARD RECTIFYING POTASSIUM CHANNELS KIR3.1/3.2 AND DELTA OPIOID RECEPTORS FORM CONSTITUTIVE SIGNALLING COMPLEXES THAT ARE MODULATED BY β-ARRESTIN2.*Nagi, K.^{1,2}, Charfi, I.^{1,2}, Hébert, T.⁴, Pineyro, G.^{1,2,3}¹ Ste-Justine Hospital Research Center, ² Dept Pharmacology and ³ Dept Psychiatry, University of Montreal and ⁴ McGill University, Montreal, Quebec, Canada

Monitoring of G-protein coupled receptor (GPCRs) interactions has revealed that receptors, G proteins and downstream effectors reach the membrane as a signalling unit. These signalling complexes maintain their integrity during early stages of receptor activation implying that proteins responsible for receptor regulation are most probably recruited to the complex rather than to isolated receptors. Here we were interested in the regulation of DOR-Kir3 signalling complexes. In a first series of experiments, we used BRET and co-immunopurification assays, to establish if Kir3.1/3.2 channels constitutively associate with heterotrimeric G protein subunits and delta opioid receptors (DORs). We then showed that all complex components remained associated after sustained activation (30 min) of the receptor with SNC-80 or DPDPE (1 μM). We further observed that DOR stimulation (SNC-80 1 μM; 30 min) induced βarr2 recruitment towards DORs, Gβγ subunits and

Kir3.1/3.2 channels, and established that receptors and channels expressed in primary neuronal culture were both internalized by SNC-80, DPDPE and morphine. However, the internalization of Kir3 channels was specific to DOR and was not observed following stimulation of GABAB receptors which also use Kir3 channels as effectors. Moreover, receptors and Kir3 channels colocalized with each other after stimulation with SNC-80 and morphine agonists but only SNC-80 induced colocalization of βarr2 with DORs and Kir3 channel subunits. Taken together, these data show that DORs and Kir3 channels form constitutive complexes that remain associated during late stages of receptor activation and indicate that regulatory proteins such as βarr2 recognize these complexes as a unit, causing simultaneous internalization of the receptor and the effector.

NOVEL KAPPA OPIOID RECEPTOR ACTIVATING SALVINORIN A ANALOGUES MOM SAL B AND EOM SAL B ATTENUATE DRUG-SEEKING BEHAVIOURS IN THE RAT AND MODULATE DOPAMINE TRANSPORTER FUNCTION.Simonson, B.¹, Morani, A.¹, Ewald, A.¹, Walker, L.¹, Prisinzano, T.², *Kivell, B.¹¹ School of Biological Sciences, Victoria University of Wellington, New Zealand,² Department of Medicinal Chemistry, School of Pharmacy, University of Kansas

Activation of kappa-opioid receptors (KOPr) by Salvinorin A (Sal A) attenuates cocaine prime-induced drug-seeking in rats with fewer side effects compared to traditional arylacetamide KOPr agonists. In the present study, we investigate the behavioural anti-addiction effects and side effect profile of two potent, longer acting, Sal A analogues, 2-methoxy-methyl Salvinorin B (MOM Sal B), and ethoxymethyl salvinorin B (EOM Sal B) and evaluate their ability to modulate the function of the dopamine transporter (DAT). Our aim is to identify novel anti-addiction compounds with reduced side effects and identify their mechanism of action. Both MOM Sal B and EOM Sal B significantly attenuate cocaine prime-induced drug-seeking behaviour in cocaine self-administering rats at a dose of 0.3 mg/kg. However, increased immobility and decreased swimming in the forced swim test indicates depressive side effects similar to those seen in the parent compound, Sal A. MOM Sal B rapidly increases DAT function in tissue taken from the rat striatum, nucleus accumbens and medial prefrontal cortex using rotating disk electrode voltametry techniques. The dopamine uptake kinetics in the nucleus accumbens showed a significant increase in V_{max} (1592 ± 176) with no change in K_m (1.6 ± 0.4) compared to controls (V_{max} (1026 ± 181) and K_m (1.5 ± 0.6) (p<0.05)), indicating an increase in the number of functional transporters with no change in dopamine binding affinity. The effects on DAT function were reversed by pre-incubation with KOPr antagonist nor-BNI. Both MOM Sal B (p<0.01) and EOM Sal B (p<0.05) rapidly phosphorylate ERK in vitro in a KOPr-dependent manner using phospho specific antibodies and Western blotting techniques. Inhibition of ERK activation by U0126, a MEK inhibitor also prevented the KOPr mediated increases in DAT function. This study shows two novel potent Sal A analogues that have behavioural anti-addiction effects and modulate DAT function in a KOPr and ERK dependent manner.

Supported by Health Research Council of New Zealand, Neurological Foundation of New Zealand, and Wellington Medical Research Foundation.

SYMPOSIUM 9**HYPOCRETIN MODULATION OF MORPHINE-INDUCED SYNAPTIC PLASTICITY IN THE VENTRAL TEGMENTAL AREA****Baimel, C. and Borgland, S.L.***Department of Anesthesiology, Pharmacology and Therapeutics, University of British Columbia, Vancouver, Canada*

Dopamine neurons in the ventral tegmental area (VTA) are a key target of addictive drugs and neuroplasticity in this region may underlie some of the core features of addiction. All drugs of abuse induce an LTP-like potentiation of excitatory inputs to VTA dopamine neurons. Hypocretin (hcrt), also known as orexin, is a lateral hypothalamic neuropeptide released into the VTA that exerts modulatory effects on a variety of behaviors produced by drugs of abuse. Acute application of hcrt potentiates excitatory synaptic transmission in the VTA, and inhibition of hcrt signaling blocks both cocaine-induced plasticity and behavioral sensitization. However, the role of hcrt on the plasticity induced by other classes of abused drugs is unknown. We determined if hypocretin action was necessary for morphine-induced synaptic plasticity in VTA dopamine neurons using whole-cell patch clamp electrophysiology in rat horizontal brain slices including the VTA. Morphine potentiated glutamatergic synapses by a pre-synaptic increase in glutamate release and by a post-synaptic change in AMPAR number or function, likely including a switch in subunit composition. Systemic or intra-VTA administration of SB 334867, a hcrt receptor-1 antagonist, blocked a morphine-induced increase in the AMPAR/NMDAR ratio, morphine-induced increases in AMPAR mEPSC frequency and amplitude, as well as morphine-induced AMPAR redistribution measured by a change in rectification. These results support a role for hcrt signaling in both pre- and post-synaptic potentiation of glutamatergic transmission in the VTA by morphine. Because hcrt signaling is required for plasticity induced by both morphine and cocaine, hcrt may function as a gate keeper for drug induced plasticity of dopamine neurons.

THE HYPOCRETIN-DYNORPHIN SYSTEM IN REWARD AND DRUG-SEEKING**Muschamp, J.W. Ph.D.***Department of Psychiatry, Harvard Medical School, McLean Hospital, Belmont, MA 02478*

Hypocretin (orexin) and dynorphin are neuropeptides with opposing actions on motivated behavior. Orexin is implicated in maintaining arousal and in the rewarding effects of food, sex, and drugs of abuse. Conversely, dynorphin is implicated in the dysphoric effects of stress that often accompany or precipitate depressive-like states. We have found that, despite their opposing actions on mood and behavior, orexin and dynorphin are packaged in the same synaptic vesicles within the hypothalamus, the major source of orexin in the mammalian CNS. Pharmacologic or genetic disruption of orexin function attenuates the rewarding effects of rewarding lateral hypothalamic (LH) electrical stimulation, eliminates cocaine-induced motor impulsivity, and markedly

reduces cocaine self-administration. These behavioral deficits are reversed by concomitant disruption of dynorphin signaling at kappa opioid receptors. We also demonstrate that concomitant orexin and dynorphin exert opposing effects on in vitro excitability of ventral tegmental area (VTA) dopamine (DA) neurons, a prominent target of orexin-containing neurons, and that intra-VTA orexin antagonism produces deficits in cocaine intake that are reversed by dynorphin blockade. Our findings identify a novel cellular mechanism that regulates the function of midbrain DA systems, disruption of which could contribute to the pathophysiology of disorders characterized by dysregulation of motivated behavior such as addiction and depression.

THE ROLE OF OREXIN RECEPTORS IN THE DEVELOPMENT OF ADDICTION TO SUGAR AND ALCOHOL**Srinivasan, S.², Simms, J.A.², Hopf, F.W.², Bonci, A.³, *Bartlett, S.E.¹***¹ Translational Research Institute, Queensland University of Technology, Brisbane, Queensland, Australia ² Ernest Gallo Clinic and Research Center, University of California San Francisco, CA ³ National Institute of Drug Abuse, Maryland, USA*

Orexin containing neurons project from the lateral hypothalamus to the ventral tegmental area (VTA) and nucleus accumbens (NAc), two brain regions that comprise the mesolimbic 'reward pathway'. Many investigators have shown that orexin receptors (Ox-Rs) in the VTA play a role in the motivational drive for addictive substances such as morphine, cocaine and alcohol. Furthermore, the OX1R plays a specific role in ethanol self-administration, cue and stress-induced relapse, with a more limited role for OX2R being shown. We use two distinct methods to model ethanol and sucrose reinforcement and consumption. The ethanol-self administration (SA) protocol allows us to explore the motivational salience/reinforcing properties of ethanol and the intermittent access (IA) protocol models voluntary 'binge-like' ethanol consumption in the home cage. We have shown that the dual OX1/2R antagonist, almorexant, reduced both ethanol and sucrose self-administration and consumption in Long-Evans rats and that intra-ventral tegmental area (VTA) infusions, but not intra-substantia nigra infusions, of almorexant reduced ethanol but not sucrose self-administration (Srinivasan et al., 2012). To begin to determine the mechanism of action of orexins in the mesolimbic circuitry, we are using electrophysiological recordings in naive and ethanol trained animals. We show that Ox-A (100 nM), but not Ox-B, increased firing in presumed dopaminergic neurons in VTA neurons in Long-Evans rats and the firing was reduced by the OXR antagonist almorexant. As the effects of almorexant in the VTA to decrease ethanol self-administration were only ~ 20%, this suggests that other brain regions are also involved. The results demonstrate that orexin/hypocretin receptors in distinct brain regions regulate ethanol and sucrose mediated behaviors.

HOT TOPICS 9**OREXIN-ENDOCANNABINOID SIGNALING IN STRESS-INDUCED COCAINE RELAPSE**

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Hypothalamic orexin neurons project to the ventral tegmental area (VTA) whose dopaminergic activity is crucial for reward seeking. Activation of hypothalamic orexin neurons has been reported to be involved in stress-induced reward seeking. Here, we have revealed a novel mechanism for stress-induced cocaine seeking, involving orexin-induced endocannabinoid (eCB) signaling in the VTA. Orexin A inhibited GABAergic transmission in VTA slices via a presynaptic mechanism. This effect was antagonized by OX1 (SB 334867) and CBI (AM 251), but not OX2 (Compound 29), antagonists, mimicked by a CBI agonist (WIN 55,212-2) and prevented by internally applied GDP- β S and a phospholipase C inhibitor (U73122). JZL 184, an inhibitor of monoglycerol lipase that hydrolyses 2-arachidonoylglycerol (2-AG), an eCB, potentiated and prolonged the effect of orexin A in VTA slices. Consistently, the effect of orexin A was prevented by tetrahydrolipstatin, an inhibitor of diacylglycerol lipase, a 2-AG synthesizing enzyme. In a conditioned place preference (CPP) test in mice, a 30 min-restraint stress, which increased the number of c-Fos-containing orexin neurons in the hypothalamus, significantly reinstated extinguished cocaine CPP. This acute stress-induced cocaine reinstatement was prevented by SB 334867 and AM 251, and was abolished in CBI receptor-knockout mice. These results suggest that acute restraint stress activates hypothalamic orexin neurons to release orexins, which then activate postsynaptic OX1 receptors on VTA dopaminergic neurons, through a Gq-protein coupled phospholipase C and diacylglycerol lipase enzymatic cascade, generating 2-AG. This eCB then retrogradely inhibits GABA release through presynaptic CBI receptors, leading to disinhibition of VTA dopamine neurons and cocaine seeking behavior. (Supported by the grants NSC101-2325-B002-048, NSC101-2321-B002-081 and NHRI-EX102-10251NI).

THE ROLE OF OREXIN1 VS OREXIN2 RECEPTORS IN ETHANOL SELF-ADMINISTRATION AND ETHANOL-SEEKING

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We were the first to demonstrate a role for orexins in both ethanol consumption and cue-induced reinstatement of ethanol-seeking. These effects were specific, with a differential effect of orexin1 receptor (OX1R) antagonism on the motivational strength of ethanol compared to sucrose. Moreover, we also found evidence for a role of OX1R in a model of reinstatement following extended abstinence. Fos studies suggested that the prelimbic cortex was a potential locus where ascending orexinergic input could modulate relapse-like behaviour. We therefore tested the role of prefrontal cortical OX1R in cue-induced ethanol-seeking. iP rats were trained to self-administer ethanol (10% v/v) and then implanted with indwelling guide cannulae bilaterally targeting the prelimbic cortex. After restabilisation of ethanol self-administration, rats were subjected to extinction. Immediately prior to cue-induced reinstatement, rats were microinjected with either vehicle or SB-334867 (3 μ g/side; 300nl/side). Antagonism of prelimbic OX1R with SB-334867 significantly attenuated cue-induced reinstatement of ethanol-seeking in an anatomically specific manner, with no effect on sucrose-seeking. While a role for OX1R has been established in both ethanol reinforcement and ethanol-seeking behaviour, the role of orexin2 receptors (OX2R) in these behaviours is less clear. We therefore sought to determine the role of central OX2R in ethanol-taking and ethanol-seeking behaviour. iP rats were trained to self-administer ethanol (10% w/v) or sucrose (0.7-1% w/v) in the presence of reward-associated cues before being implanted with indwelling guide cannulae. The selective OX2R antagonist TCS-OX2-29 was administered intracerebroventricularly (ICV) to assess its effect on operant self-administration and cue-induced reinstatement following extinction. Following icv injection TCS-OX2-29 reduced self-administration of ethanol, but not sucrose. Despite reducing ethanol self-administration, TCS-OX2-29 had no impact on cue-induced reinstatement of ethanol-seeking. To determine where in the brain OX2R were acting to modulate ethanol self-administration, TCS-OX2-29 was microinjected into either the shell or core of the nucleus accumbens (NAc). Intra-NAc core, but not shell, infusions of TCS-OX2-29 decreased responding for ethanol. Thus, OX2R in addition to OX1R may represent a potential therapeutic target for the treatment of alcohol use disorders. However, unlike OX1R, no impact of OX2R antagonism was observed on cue-induced reinstatement, suggesting a more prominent role for OX2R in ethanol self-administration compared to cue-conditioned ethanol-seeking.

SYMPOSIUM 10**A SUBSET OF SPINAL ANALGESIC SYNERGY REQUIRES ACTIVATION OF PROTEIN KINASE C-EPSILON**

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We recently showed that spinal synergistic interactions between delta opioid receptors (delta-ORs) and alpha2A adrenergic receptors (alpha2A-ARs) require protein kinase C (PKC). To identify which PKC isoforms contribute to analgesic synergy, we evaluated the effects of various PKC isoform-specific peptide inhibitors on synergy between delta-ORs and alpha2A-ARs using the tail flick assay of thermal nociception in mice. Only a PKC-epsilon inhibitor abolished synergy between a delta-OR agonist and an alpha2A-AR agonist. We tested a panel of combinations of opioid and adrenergic agonists in PKC-epsilon knockout mice and found that all four combinations of a delta-OR agonist and an alpha2A-AR agonist required PKC-epsilon for antinociceptive synergy. None of the combinations of a mu-OR agonist with an alpha2-AR agonist required PKC-epsilon. However, screening combinations of a broad range of mu- and delta-OR agonists in the PKC-epsilon knockout mice revealed a subset that required PKC-epsilon for analgesic synergy; only three delta agonists, deltorphin I and II and oxymorphone, showed PKC-epsilon-dependent synergy with morphine, the only mu-OR agonist to show this dependence. Immunohistochemistry confirmed that PKC-epsilon could be found in the population of peptidergic primary afferent nociceptors where delta-ORs and alpha2A-ARs have been found to extensively co-localize. Immunoreactivity for PKC-epsilon was found in the majority of dorsal root ganglion neurons, and intensely labeled laminae I and II of the spinal cord dorsal horn. Protein kinase C-epsilon is widespread in the spinal nociceptive system, and in peptidergic primary afferents it appears to be specifically involved in mediating the synergistic interaction between delta-ORs and both mu-ORs and alpha2A-ARs.

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CANNABINOID/OPIOID INTERACTIONS: MECHANISMS FOR IMPROVING PAIN RELIEF

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Opioids and cannabinoids interact in several clinically useful ways. Co-administration of these drugs produces acute antinociceptive synergy, and there is a bi-directional enhancement of antinociception when one drug is administered prior to the other. Furthermore, cannabinoid administration attenuates the development of opioid tolerance. These effects are at least partially mediated by the midbrain periaqueductal gray (PAG), which is critically involved in descending pain modulation, opioid tolerance, and withdrawal. Therefore, the PAG is an ideal brain region to study the cellular and molecular mechanisms underlying the interactions between opioids and cannabinoids. Within the PAG, mu-opioid and CB1 receptors frequently co-localize in the same neurons. At the presynaptic level, both mu-opioid and cannabinoid agonists inhibit GABAergic synaptic transmission in a concentration-dependent manner. Cannabinoid inhibition of evoked GABAergic inhibitory postsynaptic currents (IPSCs) is unaffected

by chronic morphine treatment (CMT). Furthermore, endogenous cannabinoid modulation of GABAergic transmission is unaffected by CMT. However, CMT produces a rapid depression of evoked IPSCs during high-frequency repetitive stimulation. This short-term synaptic plasticity of synapses to presynaptic bursts is likely to have important functional consequences within PAG, and we are currently examining the effect of cannabinoids on this short-term plasticity.

EXPLOITING BETWEEN-OPIOID DIFFERENCES TO ENHANCE ANALGESIA AND IMPROVE TOLERABILITY

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Oxycodone and morphine are two strong opioid analgesics used clinically to alleviate moderate to severe post-operative pain as well as chronic cancer pain. Although both are members of the benzomorphan class of opioid analgesics, they have subtle structural differences such that oxycodone has a 3-methoxy group, a 6-ketone, absence of the 7,8 double bond and addition of a 14-hydroxy group. Research in my laboratory more than 15 years ago showed significant between-opioid differences in the *in vivo* pharmacological profiles of morphine and oxycodone in rats following supraspinal bolus dose administration, a dosing route that avoids potentially confounding pharmacokinetic effects associated with systemic dosing. These differences were confirmed by supraspinal pretreatment of rats with selective μ -, δ - and κ -opioid receptor antagonists and by that fact that there was no cross-tolerance between supraspinal bolus doses of oxycodone in rats already rendered tolerant to intravenous morphine². Furthermore, co-administration of morphine and oxycodone in rats by supraspinal and systemic dosing routes produced analgesic synergy with markedly reduced central nervous system side-effects compared with equi-analgesic doses of either opioid alone³. More recently, randomized, double-blind clinical studies in patients with moderate to severe post-operative pain administered a dual morphine/oxycodone treatment, morphine alone or oxycodone alone, showed that at equi-analgesic doses, the dual morphine/oxycodone combination treatment had a superior adverse event profile compared with either opioid alone⁴⁻⁶.

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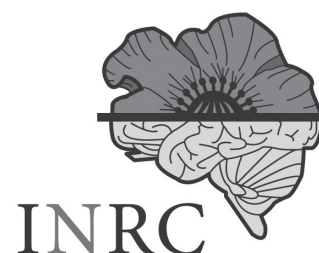
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HOT TOPICS 10 **μ -OPIOID RECEPTOR SIGNALING AND NET INHIBITION BY TAPENTADOL IN RAT LOCUS COERULEUS NEURONS****Sadeghi, M., & Christie, M.J.***Pharmacology DO6, University of Sydney, NSW 2006, Australia*

Opioids acting through μ -opioid receptors (MOR) are an effective treatment option for acute pain states but the side effect profile and limited efficacy in chronic pain states reduces its therapeutic potential. On the other hand, inhibiting the uptake of noradrenaline and/or activating α_2 -adrenoceptors seems to be effective in chronic neuropathic pain states. Tapentadol is a novel analgesic drug that combines both moderate MOR agonism as well as noradrenaline reuptake inhibition in a single molecule (Tzschentke et al. *J.Pharmacol. Exp Ther* 323:265-276, 2007). As such, tapentadol displays analgesic activity in acute and chronic pain states and an improved tolerability profile with less physical dependence liability than morphine.

Aim: to investigate potency and efficacy of tapentadol as a MOR agonist and as a noradrenaline transporter (NET) inhibitor in locus coeruleus (LC) neurons as a model system because LC cells express a homogenous population of noradrenergic cells that express similar levels of MOR and α_2 -adrenoceptors with a smaller contribution of α_1 -adrenoceptors.

Method: We performed whole-cell patch clamp recordings of GIRK potassium channels in LC cells in horizontal brain slices of 2-5 week old rats. The effect of tapentadol as a NET inhibitor was normalized with a supramaximal concentration the well known NET inhibitor; cocaine (10 μ M). Results: Tapentadol is a partial agonist at MOR in LC cells with EC₅₀= 1.8 μ M, approximately 6-fold less than morphine, and an efficacy of 68% relative to morphine. In terms of NET inhibition, tapentadol potentiated the effect of exogenously applied noradrenaline in LC cells with an EC₅₀ = 4 μ M, which was found to be comparable to cocaine in LC cells (EC₅₀= 4 μ M). Thus, there is potential for tapentadol as a novel analgesic drug with lower side effects due to relatively low intrinsic efficacy for MOR with a broader analgesic profile due to a dual mode of action consisting of both MOR activation and NET inhibition.



1. DRUG ADDICT QUALITY OF LIFE ASSOCIATED WITH SUBSTITUTION THERAPY IN INDONESIA

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2. CORTICAL MOTOR DOMINANCE AND MOVEMENT-RELATED CORTICAL POTENTIALS ASSOCIATED WITH FINGER FORCE PRODUCTION

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3. GRK5 GENETIC POLYMORPHISMS ARE ASSOCIATED WITH METHADONE MAINTENANCE TREATMENT DOSE

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4. CHANGES IN WILLINGNESS TO SELF-MANAGE CENTRAL NERVOUS SYSTEM AMONG THE ETHNIC GROUPS OF CHITTAGONG HILL TRACTS BANGLADESH: FINDINGS FROM A POPULATION-BASED COHORT

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5. SEQUENCE OF DRUG USE IN INDONESIA

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6. THE INFLUENCE OF NEUROLEPTICS AND BENZODIASEPIN THERAPY DRUGS ON THROMBOCYTES AGGREGATION IN VIVO IN PATIENT WITH OPIOID DEPENDENCE

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7. THE KINETICS OF μ -OPIOID RECEPTORS AND μ -OPIOID RECEPTORS IN LIVING CELLS

Wang, Y. and Bao, L.*. *State Laboratory of Cell Biology, Institute of Biochemistry and Cell Biology, Chinese Academy of Science, Shanghai, China*

8. CROSSTALK BETWEEN DELTA OPIOID RECEPTOR AND NERVE GROWTH FACTOR SIGNALING MODULATES NEUROPROTECTION AND DIFFERENTIATION IN RODENT CELL MODELS

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9. A NEW POTENT μ -OPIOID RECEPTOR SELECTIVE AGONIST INSPIRED BY TETRAPEPTIDES FROM A MARINE-DERIVED PENICILLIUM PRODUCES ANALGESIA WITHOUT INDUCING RECEPTOR ENDOCYTOSIS

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10. A DELTA-OPIOID RECEPTOR-STAT5B-Gi/G_o PATHWAY LEADS TO NEURITE OUTGROWTH AND NEURONAL DIFFERENTIATION

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11. REPEATED MORPHINE ADMINISTRATION DECREASES ACTIVATION OF EXTRASYNAPTIC GABAA RECEPTORS IN THE PERIAQUEDUCTAL GRAY AREA (PAG)

*Ingram, S.L., Tonsfeldt, K.J., Suchland, K.L. & Li, M. *Dept. of Neurological Surgery, Oregon Health & Science University, Portland, OR*

12. SPONTANEOUS GABA RELEASE DECREASES WITH DEVELOPMENT IN THE ROSTRAL VENTROMEDIAL MEDULLA (RVM)

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13. POSTSYNAPTIC BUT NOT PRESYNAPTIC MU OPIOID RECEPTORS IN THE PREBÖTZINGER COMPLEX DESENSITIZE.

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14. STUDIES ON DETERMINATION OF BIOLOGICAL COMPOSITION OF SEA-WEEDS COLLECTED FROM THE SUNDARBANS WITHIN BANGLADESH

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15. PHOSPHOINOSITIDE 3-KINASE γ INTEGRATES CYCLIC AMP AND AKT SIGNALING OF THE μ -OPIOID RECEPTOR

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16. CORRELATION OF ENDOCYTOSIS WITH KINETICS OF PHOSPHORYLATION AND β -ARRESTIN 2 ASSOCIATION IN μ -OPIOID RECEPTORS

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17. NANOPARTICULATE MORPHINE CONJUGATES FOR THE TREATMENT OF INFLAMMATORY PAIN

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18. OPIOID RECEPTOR COUPLING TO K⁺ CHANNELS IN PERIPHERAL SENSORY NEURONS: RELEVANCE FOR ANALGESIC EFFICACY AND SPECIES DIFFERENCES

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19. TOLL-LIKE RECEPTOR 4 CHANGES OPIOID LIGAND BINDING IN BRAIN HOMOGENATES

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20. BIASED SIGNALLING OF ENDOGENOUS OPIOIDS AT THE MU RECEPTOR

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21. DEVELOPMENT OF MIXED EFFICACY MOR/DOR LIGANDS AND MODIFICATION TO IMPROVE BLOOD/BRAIN BARRIER PENETRATION

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22. A NOVEL MECHANISM FOR RESTRAINT STRESS-INDUCED COCAINE RELAPSE: OREXIN-INDUCED ENDOCANNABINOID RETROGRADE DISINHIBITION IN THE VENTRAL TEGMENTAL AREA

*Tung, L.W.¹, Lu, G.L.¹, Chang, L.Y.¹, Lee, Y.S.⁴, Yu, L.⁶, Lee, H.J.³, Teng, S.F.³, Hwang, L.L.^{4,5}, Hung, M.S.⁷, Mackie, K.⁸, Zimmer, A.⁹ and Chiou, L.C.^{1,2,3}

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23. SPINAL ACTIONS OF TRIPTANS AND OPIOIDS IN AN INFLAMMATORY PAIN STATE

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24. DESIGN, SYNTHESIS AND PHARMACOLOGY OF OPIOID DELTA AGONISTS WITH A NOVEL FUNDAMENTAL OXAZATRICYCLODECANE STRUCTURE

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25. THE ROLE OF DIFFERENT PHOSPHORYLATION SITES IN RAPID DESENSITIZATION OF THE μ -OPIOID RECEPTOR

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26. DESENSITIZATION OF THE μ -OPIOID RECEPTOR IN INTACT ATT20 CELLS: IS C-TERMINAL PHOSPHORYLATION ESSENTIAL?

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27. CAMKII α CONTROLS THE BIOGENESIS OF LET-7 MICRORNAS IN OPIOID TOLERANCE

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28. DISRUPTION OF THE ACTIN CYTOSKELETON DELAYS RECOVERY OF MU OPIOID RECEPTOR FROM DESENSITIZATION IN LOCUS COERULEUS NEURONS

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29. PHOSPHORYLATION OF MU OPIOID RECEPTOR REDUCED MORPHINE ANALGESIA IN INFLAMMATORY PAIN STATE

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30. THE DELTA-OPIOID RECEPTOR CONTRIBUTES TO MORPHINE TOLERANCE IN AN ANIMAL MODEL OF CHRONIC INFLAMMATORY PAIN

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31. ENDOTOXIN ENHANCEMENT OF MORPHINE-INDUCED CONDITIONED PLACE PREFERENCE

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32. ADOLESCENT DRINKING AFFECTS ENDOGENOUS OPIOIDS IN HIPPOCAMPUS AND AMYGDALA INDEPENDENT OF REARING CONDITION

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33. SINGLE HOUSING DURING ADOLESCENCE CAUSE ALTERATIONS IN BASAL LEVELS OF ENDOGENOUS OPIOID PEPTIDES

Roman, E., *Granholm, L., Nylander, I., *Neuropharmacology, Addiction and Behaviour, Department of Pharmaceutical Bioscience, Uppsala University, Sweden*

34. REDUCED MICRO-RNA 200B-429 CLUSTER EXPRESSION IN THE NUCLEUS ACCUMBENS IS INVOLVED IN THE POST-TRANSCRIPTIONAL MODULATION UNDER NEUROPATHIC PAIN

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35. THE TRANSLOCATION OF THE δ -OPIOID RECEPTOR ON CHOLINERGIC INTERNEURONS IN THE STRIATUM

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36. POMC NEURONS IN THE HYPOTHALAMUS MODULATE PERIPHERAL IMMUNE FUNCTION

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37. INVOLVEMENT OF DELTA-OPIOIDERGIC SYSTEM IN ADULT NEUROGENESIS

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38. DIFFERENTIAL EFFECTS OF MORPHINE AND METHAMPHETAMINE ON THE ACTIVATION OF MESOLIMBIC AND NIGROSTRIATAL DOPAMINERGIC SYSTEMS

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39. INVOLVEMENT OF SIGMA-1 RECEPTOR CHAPERONE ON THE EXPRESSION OF WITHDRAWAL SIGNS IN MORPHINE-DEPENDENT MICE

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40. OPIOIDERGIC MECHANISM IS LOCALIZED IN THE DOWNSTREAM OF $\alpha 7$ NICOTINIC ACETYLCHOLINE RECEPTOR, BUT NOT $\alpha 4\beta 2$, IN THE CENTRAL NERVOUS SYSTEM

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41. NOVEL KAPPA OPIOID RECEPTOR ACTIVATING SALVINORIN A ANALOGUES MOM SAL B AND EOM SAL B ATTENUATE DRUG-SEEKING BEHAVIOURS IN THE RAT AND MODULATE DOPAMINE TRANSPORTER FUNCTION

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42. MICROGLIAL ACTIVATION PRECEDES ANTI-OPIOID SYSTEM IN MORPHINE ANALGESIC TOLERANCE

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43. EFFECTIVENESS OF AMIDINO-TAPA AGAINST MORPHINE-RESISTANT NEUROPATHIC PAIN

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44. VOLUNTARY ALCOHOL INTAKE AND CB1 AND MOP RECEPTOR DENSITY IN OUTBRED WISTAR RATS

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45. INVOLVEMENT OF THE LONG-CHAIN FATTY ACID RECEPTOR GPR40 IN CFA-INDUCED INFLAMMATORY PAIN MODEL MICE

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46. G PROTEIN-GATED INWARDLY RECTIFYING POTASSIUM (GIRK) CHANNELS PLAY A PRIMARY ROLE IN THE ANTINOCICEPTIVE EFFECT OF OXYCODONE, BUT NOT MORPHINE AND FENTANYL, AT SUPRASPINAL SITES.

*Ono, H.¹, Nakamura, A.¹, Fujita, M.¹, Shibasaki, M.², Mori, T.², Suzuki, T.², Sakaguchi, G.¹, Hasegawa, M.¹ and Kanemasa, T.¹

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47. HOUSING CONDITIONS AFFECT BASAL AND ALCOHOL-INDUCED OPIOID LEVELS IN ADOLESCENT RATS

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48. THE INTERACTION OF SEVERAL ANTIDEPRESSANT DRUGS WITH ACUTE AND CHRONIC METHADON IN MICE AND POSSIBLE CLINICAL IMPLICATIONS

Schreiber, S.¹, Hostovsky, A.², Barak, Y.², Rubovitch, V.², and Pick, C.G.^{2*} ¹ *Department of Psychiatry, Tel Aviv Sourasky Medical Center & Tel Aviv University Sackler Faculty of Medicine;* ² *Department of Anatomy, Tel-Aviv University Sackler Faculty of Medicine, Tel Aviv, Israel*

49. THE PERIAQUEDUCTAL GRAY CONTRIBUTES TO OXYCODONE, BUT NOT METHADONE ANTINOCICEPTION IN THE RAT

*Reid, R.A. and Morgan, M.M., *Washington State University Vancouver, WA; USA*

50. INCREASED REGIONAL GRAY MATTER VOLUME IN μ -OPIOID RECEPTOR KNOCKOUT MICE AS DETERMINED BY MRI-VOXEL-BASED MORPHOMETRY

Sasaki, K.^{a,b}, Sumiyoshi, A.^b, Nonaka, H.^b, Hall, F.S.^c, Uhl, G.R.^c, Watanabe, M.^d, Kasahara, Y.^a, Ikedae, K., Kawashima, R.^b, Soraa, I.^f

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51. PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL REGULATION OF IL-6 EXPRESSION BY NOCICEPTIN/ORPHANIN FQ (N/OFQ) PEPTIDE RECEPTOR

Donica, C.L.², Zhang, Y.¹, Simpson-Durand, C.D.¹, *Sherry, D.M.^{2,3}, Gallucci, R.M.^{1,2}, Awwad, H.O.^{1,2} and Standifer, K.M.^{1,2,3}

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52. INVOLVEMENT OF μ - AND δ -OPIOID RECEPTOR FUNCTION IN THE REWARDING EFFECT OF (\pm)-PENTAZOCINE

***Suzuki, T., Itoh, T., Saeki, T., Masukawa, D., Shibasaki, M. and Mori, T.** Dept Toxicology, Hoshi University School of Pharmacy and Pharmaceutical sciences, Tokyo 142-8501, Japan

53. NALTREXONE ON TREATMENT OF NEUROPATHIC PAIN IN MICE LOCALLY TRANSFECTED WITH THE MUTANT MU-OPIOID RECEPTOR GENE IN SPINAL CORD

***Tao, P-L.^{1,3}, Kao, J-H.¹, Law, P-Y² and Loh, H.H.²,** ¹Graduate Institute of Life Science, National Defence Medical Center, Taiwan, ROC; ²Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN, USA and ³Center for Neuropsychiatric Research, National Health Research Institutes, Taiwan, ROC.

54. ESTABLISHMENT OF A CENTRAL POST-STROKE PAIN MODEL USING GLOBAL CEREBRAL ISCHAEMIC MICE

***Tokuyama, S.¹, Harada, S.¹, Nakamoto, K.¹,** ¹Department of Clinical Pharmacy, Kobe Gakuin University, School of Pharmaceutical Sciences, Kobe, Japan

55. EGFP-NOP MICE

Toll, L.¹, Wu, J.¹, Mercatelli, D.¹, Kieffer, B.² ¹Torrey Pines Institute for Molecular Studies, Port St. Lucie, FL, USA, ²Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France

56. SYNERGISM BETWEEN THE DELTA-OPIOID AGONIST SNC80 AND AMPHETAMINE OCCURS VIA A GLUTAMATERGIC NMDA-RECEPTOR DEPENDENT MECHANISM

Bosse, K.E.¹, Jutkiewicz, E.M.¹, Mabrouk, O.S.², Schultz, K.N.², Kennedy, R.T.², Gnegy, M.E.¹ and *Traynor, J.R.¹

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57. INVOLVEMENT OF SUPRASPINAL AND PERIPHERAL NALOXONAZINE- INSENSITIVE OPIOID RECEPTOR SITES IN THE EXPRESSION OF μ -OPIOID RECEPTOR AGONIST-INDUCED PHYSICAL DEPENDENCE

***Uzawa, N., Mori, T., Sugiyama, K., Saitou, Y., Shibasaki, M., Suzuki, T.** Dept Toxicology, Hoshi University School of Pharmacy and Pharmaceutical sciences, Tokyo 142-8501, Japan

58. SELF ADMINISTRATION OF OXYCODONE BY ADOLESCENT AND ADULT MICE DIFFERENTIALLY AFFECT STRIATAL NEUROTRANSMITTER RECEPTOR GENE EXPRESSION

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59. THE EFFECT OF μ -OPIOID RECEPTOR POLYMORPHISMS ON RECEPTOR FUNCTION

***Knapman, A., Connor, M.** The Australian School of Advanced Medicine, Macquarie University, Sydney, NSW, Australia

60. SOCIAL STRESS ENGAGES OPIOID MODULATION OF THE LOCUS COERULEUS-NOREPINEPHRINE SYSTEM AND INCREASES THE SALIENCE OF REWARD.

***Chaijale, N., Curtis A.L., Snyder, K., Bhatnagar, S., Valentino, R.J.** Childrens Hospital of Philadelphia, Philadelphia, PA, USA

61. MORPHINE MODULATES BREAST CANCER CELL METASTATIC POTENTIAL

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1. DRUG ADDICT QUALITY OF LIFE ASSOCIATED WITH SUBSTITUTION THERAPY IN INDONESIA

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Monday 4.00 – 5.00pm

Rationale: Drug addict number in Indonesia is constantly high in around 0.5% to 0.8% of population in last five years (CHRUI-NNB, 2010). Recognizably, addiction has impact on health related quality of life (HRQoL), which is very unique. To develop program for HRQoL improvement in drug users, this analysis aims to describe its level in Indonesia.

Methods: This analysis used secondary data from 2011 survey on drug abuse that selected sample of 2210 drug addicts drawn by Respondent Driven Sampling method in 17 provincial capitals in Indonesia (CHRUI-NNB, 2011). Quality of life measured was HRQoL using the generic instrument of WHO-BREF comprising of four dimensions (physic, psychology, social and environment). The data were analyzed based on joining solely substitution therapy or not. Presence of diseases (HepC, AIDS and suspected Tuberculosis) was assessed by self report. Study ethical clearance was issued by the University of Indonesia.

Findings: Drug users sampled averagely aged 20 to 39 years old, mostly were males (99.3%), and 30% were married. Only few subjects (6.9%) were joining substitution therapy. Mean total score of HRQoL of drug users in substitution therapy show higher figure (61.7) than without this therapy (58.4), and this phenomenon is alike for all dimensions. Disease occurrence may reduce HRQoL, such as score for AIDS was 56.9 in contrast to non-AIDS 59.0, and score for TB was 57.1 below 59.1 in Non-TB, yet similar score of around 58 for Hep C or not.

Conclusion: Drug addict involved in exclusively substitution therapy would give positive impact to their quality of life, while the co-morbidity might reduce HRQoL.

2. CORTICAL MOTOR DOMINANCE AND MOVEMENT-RELATED CORTICAL POTENTIALS ASSOCIATED WITH FINGER FORCE PRODUCTION

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Tuesday 4.45 – 5.45pm

The findings of movement-related cortical potentials associated with motor effectors have shown that multiple movement parameters were controlled by human body, such as timing, force, orientation, and acceleration, etc. However, some constraints subsisted during movement control and execution, such as finger interdependence or finger neuro-anatomical factors. In the past, rate of force development was used to examine the control of finger force production. The mechanism within varied rates of force development during force production associated with higher level of motor system still remained unknown. In terms of motor effectors, the controls of muscles and cortical activations have shown parsimonious adaptation in finger interdependence. The control of force is thought to be changed after specific execution of fingers on both behavioral and cortical level of analyses. This study tried to apply specific feedback to investigate the mechanism of neuro-motor adaptation related to six different rates of force development. 15 college students were required to produce several effector-related tasks associated with force control after motor learning. Force outputs and movement-related cortical potentials were collected and a three-way repeated-measured ANOVA in terms of motor learning, nominal force levels and rates of force development were used for analyses. We found that there were existing different control mechanisms within fingers in terms of different end-effectors. The results and implications of this study would further explore the behavior-brain relationships. It is suggested that the underlying mechanism of finger coordination or the complex motor system could be recognized neurophysiologically and cognitively.

The study was supported by National Science Council grants, Taiwan. NSC-99-2410-H-033-055

3. GRK5 GENETIC POLYMORPHISMS ARE ASSOCIATED WITH METHADONE MAINTENANCE TREATMENT DOSE

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Monday 4.00 – 5.00pm

Methadone maintenance treatment has been applied in clinical for heroin dependence patients. This drug has been suggested to be administered with an initial low dose and then gradually increase to a maintenance dose in consideration for the tolerance effect for each individual. In this study, we tested the hypothesis that genetic polymorphisms in human genome may harbor susceptibility gene for the use of methadone maintenance dose. A total of 366 patients under methadone treatment were recruited with records of maintenance dose, plasma methadone and metabolite concentrations, and treatment outcome profiles. The genomic DNA were genotyped using the Axiom® Genome-Wide CHB 2 Array, which was population-optimized to have a better genomic coverage of common alleles (MAF >5%) in the Han Chinese genome. Gene-based association analysis identified a candidate gene, G protein-coupled receptor kinase 5 (GRK5), showing genome-wide significance with methadone dose ($p=2.37 \times 10^{-6}$). Further fine mapping was performed through selection of 36 single nucleotide polymorphisms (SNPs) within the genetic region and in consideration the minor allele frequencies of Chinese ethnic group and the functions within the exons of GRK5. These SNPs were genotyped on the same 366 patients. The SNPs rs11819686, rs10886472, rs11198907, rs4752300, and rs1537576 of both genotype (GLM $p < 0.0038$, FDR < 0.027) and allele types (GLM $p < 0.0035$, FDR < 0.021) within the intron 1, intron 3, and intron 4 regions showed significant associations with methadone maintenance dose. The allele type of rs11198898, rs10787959, rs1268947, and rs2275036 at intron 2 were significantly associated with the adverse reaction symptom scores of constipation (GLM, $p < 0.0046$, FDR < 0.041). SNP rs17098759 was associated with the symptom score of weakness (GLM, $p = 0.0002$, FDR, $p = 0.0072$). We concluded that GRK5 may be an important candidate gene which harbors biomarkers for methadone maintenance dose and adverse reactions.

4. CHANGES IN WILLINGNESS TO SELF-MANAGE CENTRAL NERVOUS SYSTEM AMONG THE ETHNIC GROUPS OF CHITTAGONG HILL TRACTS BANGLADESH: FINDINGS FROM A POPULATION-BASED COHORT

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Tuesday 4.45 – 5.45pm

Plants form the only easily accessible healthcare alternative for the most of ethnic population in Bangladesh. The life, tradition, and culture of ethnic groups remained almost static since last several hundreds of years. The knowledge accrued by the ethnic groups through generations shows the in-depth understanding

of the forest resources. The ethnic groups' areas were surveyed through periodical tours in various seasons. Knowledgeable persons of ethnic communities were contacted and information was collected through interviews, observations, and discussions held during field surveys. The discussions revealed local name of plants, plant parts used by the ethnic communities. The plants were scientifically identified with their botanical names and author citation. Information on 24 plants was obtained to treat central nervous system in the observations. These plants included *Aconitum napellus* L., *Aegle marmelos* (L.) Corrêa, *Aloe vera* (L.) Burm.f., *Amomum aromaticum* Roxb., *Andrographis paniculata* (Burm.f.) Nees, *Azadirachta indica* A.Juss., *Bacopa monnieri* (L.) Wettst., *Coccinia grandis* (L.) Voigt, *Cordia dichotoma* G.Forst., *Glycyrrhiza glabra* L., *Holarrhena pubescens* Wall., *Luffa cylindrica* (L.) M.Roem., *Nigella sativa* L., *Olea europaea* L., *Oroxylum indicum* (L.) Kurz, *Oxalis corniculata* L., *Phyllanthus fraternus* G.L.Webster, *Plantago major* L., *Senna alata* (L.) Roxb., *Solanum anguivi* Lam., *Sphaeranthus indicus* L., *Terminalia bellirica* (Gaertn.) Roxb., *Tinospora cordifolia* (Willd.) Miers, and *Tribulus terrestris* L.. The plant materials are used singly or sometimes in combination with milk, honey, curd, water or other plant parts. The observations support the use of plants in curing central nervous system and noted that the formulations contain a number of plants not usually used by the ethnic communities in other regions of Bangladesh. Further detailed exploration and collection of plants information, chemical studies, and screening for medicinal properties will provide cost effective and reliable source of medicine for the welfare of humanity.

5. SEQUENCE OF DRUG USE IN INDONESIA

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Monday 4.00 – 5.00pm

Rationale: Indonesia has recognized problems related to drug dependence shown by its prevalence in around 0.5% to 0.8% of population in last five years (CHRUI-NNB, 2010). Identifying the drug type used one earlier than the other, or pattern of drug use sequence, would help development of secondary prevention program.

Methods: By using data collected in Indonesia surveys on drug abuse conducted in 17 provincial capitals, there were 2207 and 2210 of drug addicts drawn through Respondent Driven Sampling method in year 2008 and 2011 respectively. Sequence of drug use was measured based on the self reported age of first try of each drug type. Percentages of each order then were arranged, and it leads to depict drug use sequence pattern.

Findings: Most of drug addicts were males with age ranged from 13 to 58 years. The pattern of sequence of drug use shows no substantial difference in both survey years, with cigarette smoking as the entry point, and over 50% of them continued to using ganja or alcohol. Tranquilizer was preferred by around one-third. ATS user might continue taking opiate in small probability (around 2%). Nearly one in five alcohol or benzodiazepine users tended to forward consuming opiate. Recently ATS was more well-liked. Gender influence over the pattern of drug use sequence was not clear.

Conclusion: The drug use in Indonesia recorded ganja, alcohol and tranquilizer as the gate, with cigarette smoking prior to these drugs. Heroin remains to be consumed, hence ATS popularity increases.

6. THE INFLUENCE OF NEUROLEPTICS AND BENZODIAZEPIN THERAPY DRUGS ON THROMBOCYTES AGGREGATION IN VIVO IN PATIENT WITH OPIOID DEPENDENCE

Shamanskaya, M.G.

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Tuesday 4.45 – 5.45pm

Background: Neurotropic drugs of different groups are widely used nowadays. Besides the central action they influence other systems – immunity and hemostasis.

Aim: of influence of some neurotropic drugs on aggregation ability of thrombocytes in vivo in patient with opioid dependence.

Methods: The aggregation of thrombocytes was examined by analyzer "Biola" (Moscow) with turbidimetric method (Born's method 1962). Adrenalin, ADP (10 mcg/ mL), collagen was used as inducers. Statistical processing of results of research was conducted by using nonparametric method W-Wilcoxon, Mann Whitney.

Results: It was established that after injections of diazepam 1 hours - agonists of benzodiazepine receptors (0,5 mg/kg of weigh) refused the aggregation effect, in spite of adding inducers of aggregation (adrenalin ($p < 0,01$), collagen ($p < 0,01$), ADP ($p < 0,01$)). 1 hours after administration of the blocking agent D2- of dopamine receptors of haloperidol - (0,1 mg/kg of weigh) and 1 hours after administration of levomepromazin - (0,5 mg/kg of weigh) also restrained the aggregation in the setting of inducers of different nature: adrenalin ($p < 0,01$), ADP ($p < 0,01$), collagen ($p < 0,01$).

Conclusion: Inhibiting influence of diazepam is caused by covalent bonding of drug with glycoprotein receptors GP IIb/IIIa, which have a common RGD- consequence for bonding with fibrinogen. Another probable mechanism is connected with metabolism of arachidonic acid. In this case diazepam changes the conformation of thrombocyte membrane, which influence the activity of phospholipase C, phosphoinositid's level, formation of thromboxane A₂, brake action of mobilization Ca²⁺ and phosphorylation of protein P-47.

The haloperidol's and levomepromazin effect can be explained only by it's prevention of dissociation of α to $\beta\gamma$ - subunit of G-protein, which leads not only to brakeage of response of dopaminoreceptors and β -adrenoreceptors, but also others, including α - adrenoreceptors. Just only dissociation of subunits of G-protein can be the necessary condition of receptors' activation. As approval of this statement can be the fact of inhibiting of aggregation ability in case of adding not only adrenalin, but adding ADP and collagen, biological effects of which are connected with other receptors and occur in case of dissociation of α to $\beta\gamma$ - subunit of G-protein. If this statement is true, we amplified signalling mechanisms in blood platelet in case of agonists adrenoreceptors actions.

7. THE KINETICS OF μ -OPIOID RECEPTORS AND μ -OPIOID RECEPTORS IN LIVING CELLS

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Monday 4.00 – 5.00pm

The μ -opioid receptors (MORs) and δ -opioid receptors (DORs) are both members of the G protein-coupled receptor family. They play important roles in the regulation of pain. The internalized MORs are processed for recycling back to the cytoplasmic membrane following stimulation with the selective MOR agonist DAMGO. The internalized DORs are entered to degradation pathway in the present of its selective agonist Deltorphin I.

Previous study reports that DORs form hetero-oligomers with MORs and DOR agonist could induce co-internalization and co-degradation of two receptors. Therefore, the behavior of the receptor hetero-oligomers are different from MORs and DORs existed alone. However, little is known about the kinetics and interaction of these receptors in living cells. In the present study, we use total internal reflection fluorescence (TIRF) microscope to observe the dynamics of DORs and MORs on the cytoplasmic membrane in living cells. We have successfully constructed a live-cell imaging system for MORs and DORs, and calculated the kinetics of these receptors in different conditions. In the presence of their selective agonist, the movement of the receptors were slowed down in a time-dependent manner. The diffusion coefficient of DORs was decreased more rapidly than that of MORs. Using confocal microscopy, we further found that the internalized MORs frequently appeared in the tubular endosomes, but DORs were more inclined to present in vesicular endosomes. Our research provides a approach to study the kinetics feature of the receptors on the membrane and help us to understand the interaction of opioid receptors.

8. CROSSTALK BETWEEN DELTA OPIOID RECEPTOR AND NERVE GROWTH FACTOR SIGNALING MODULATES NEUROPROTECTION AND DIFFERENTIATION IN RODENT CELL MODELS

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Tuesday 4.45 – 5.45pm

Both opioid signaling and neurotrophic factor signaling have played an important role in neuroprotection and differentiation in the nervous system. Little is known about whether these two signaling pathways crosstalk to each other to affect neuroprotection and differentiation. Previously, we found that nerve growth factor (NGF) could induce expression of the delta opioid receptor gene (Oprl, dor) mainly through PI3K/Akt/NF- κ B signaling in PC12h cells. In this study, using two NGF-responsive rodent cell model systems, PC12h cells and F11 cells, we found the delta opioid neuropeptide [D-Ala², D-Leu⁵]enkephalin (DADLE)-mediated neuroprotective effect could be blocked by pharmacological reagents, the delta opioid antagonist naltrindole, PI3 kinase inhibitor LY294002 and MAPK inhibitor PD 98059, Trk kinase inhibitor K252a, respectively. Western blot analysis revealed that DADLE activated both the PI3K/Akt and MAPK pathways in the two cell lines. siRNA Oprl gene knockdown experiment showed that the upregulation of NGF mRNA level was inhibited with concomitant inhibition of the survival effects of DADLE in both cell models. siRNA Oprl gene knockdown also attenuated the DADLE-mediated neurite outgrowth in PC12h cells as well as phosphorylation of MAPK and Akt in PC12h and F11, respectively. These data together strongly suggest that delta opioid peptide DADLE acts through the NGF-induced functional G protein-coupled delta opioid receptor to provide its neuroprotective and differentiating effects at least in part by regulating survival and differentiating MAPK and PI3K/Akt signaling pathways in rodent neuronal cells.

9.A NEW POTENT μ -OPIOID RECEPTOR SELECTIVE AGONIST INSPIRED BY TETRAPEPTIDES FROM A MARINE-DERIVED PENICILLIUM PRODUCES ANALGESIA WITHOUT INDUCING RECEPTOR ENDOCYTOSIS

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Monday 4.00 – 5.00pm

The therapeutic potential of opioid receptor agonists is often limited by their high propensity to cause tolerance after chronic administration. One of the possible mechanisms responsible for the development of tolerance is the agonist-dependent modulation of receptor internalization. Based on our discovery of a D-amino acid rich tetrapeptide from *Penicillium bilaii*, we have developed a structurally novel μ -opioid receptor selective (binding $K_i = 1.5$ nM (μ), 300 nM (δ) and 1000 nM (κ)) agonist (Bilaid C2) that fails to produce receptor internalization in contrast to other peptidic agonists endomorphins 1 & 2, Met-enkephalin and DAMGO. Bilaid C2 produced analgesia comparable to that of morphine when administered intrathecally (3 μ g) but failed to produce antinociception subcutaneously (s.c.) at doses as high as 100 mg/kg, indicating poor blood-brain-barrier (BBB) penetration. With the aim of improving BBB permeability, we have made further chemical modifications to Bilaid C2 including the introduction of a saccharide, a helical penetrating sequence, as well as enzymatically and/or chemically reversible moieties to mask the N-terminus. A number of these analogues produced analgesic effects through s.c. administration at doses comparable to that of morphine. These compounds are potential candidates for the development of s.c. injectable analgesics that may have altered tolerance and dependence inducing properties.

10.A DELTA-OPIOID RECEPTOR-STAT5B-Gi/Go PATHWAY LEADS TO NEURITE OUTGROWTH AND NEURONAL DIFFERENTIATION

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Tuesday 4.45 – 5.45pm

Opioid receptors are coupled to Gi/Go proteins and recent findings have shown that can form a multicomponent signalling complex, consisting of members of G protein and the Signal Transducer and Activator of Transcription STAT5B. We thus wondered whether activation of the opioid receptors could direct differentiation and neurite outgrowth through a molecular pathway involving STAT5B and other signaling intermediates. We demonstrate that prolonged δ -opioid receptor (δ -OR) activation with opioid agonists induces STAT5B phosphorylation in Neuro-2A cells. Moreover, DSLET-activation of δ -OR triggers neurite outgrowth and neuronal survival; these effects are blocked by

pertussis toxin treatment, and after expression of a dominant negative mutant of STAT5B (DN-STAT5B), suggesting that the signalling pathway participating in this mechanism involves Gi/o proteins and p-STAT5B. Additional studies have shown that the while DSLET exposure of neuroblastoma cells induces a marked increase of specific differentiation marker proteins, overexpression of the DN-STAT5B attenuated significantly these effects. Taken together, our findings demonstrate that δ -OR activation leads to a number of neurotropic events via a $G_{\alpha i/o}$ -linked and STAT5B-dependent manner.

This work was supported by the GSRT and the EU grant "Normolife" (LSHC-CT2006-037733).

11. REPEATED MORPHINE ADMINISTRATION DECREASES ACTIVATION OF EXTRASYNAPTIC GABAA RECEPTORS IN THE PERIAQUEDUCTAL GRAY AREA (PAG)

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Monday 4.00 – 5.00pm

Tonic inhibition of PAG output neurons by GABA is important for the normal control of descending pain regulation. Opioids inhibit GABA release in the PAG resulting in activation of PAG output neurons and the descending antinociception pathway that inhibits nociceptive impulses in the spinal cord. Extrasynaptic GABAA receptors are known to regulate the gain of inhibitory systems and firing thresholds of neurons. However, it is not known if tonic GABA release in the PAG activates extrasynaptic GABAA receptors or if these receptors are modulated following repeated administration of morphine. Male Sprague-Dawley rats were pretreated twice a day with saline or morphine (5 mg/kg, s.c.) for 3 days. This pretreatment caused a shift to the right for DAMGO inhibition of miniature GABAergic inhibitory postsynaptic currents (mIPSCs). Superfusion of the GABAA antagonist, bicuculline (10 μ M), inhibited mIPSCs and blocked a resting current indicating the presence of both synaptic and extrasynaptic GABAA-mediated currents in the PAG. Bicuculline reduced the holding current by 18 ± 4 pA ($n = 10$) in saline pretreated rats but this effect was significantly reduced in rats pretreated with morphine (2 ± 1 pA, $n = 6$, $t(14) = 3.46$, $p < 0.05$). The bicuculline-induced currents reversed at the expected chloride equilibrium potential in chloride (-24 ± 3 mV) and gluconate-substituted (-84 ± 2 mV) intracellular solutions suggesting that GABAA receptors elicit a tonic current that is inhibited by bicuculline. Tonic currents are often mediated by extrasynaptic GABAA receptors containing the delta-subunit. The GABAA agonist THIP selectively activates GABAA receptors containing delta subunits. THIP (10 μ M) increased the BIC-blocked currents by 5-fold (84 ± 22 pA, $n = 8$) in opioid-naïve slices and morphine treated slices (77 ± 24 pA, $n = 6$). Western blot analyses for GABAA-delta subunits did not show a significant difference between total GABAA-delta subunits in the PAG of saline and morphine-pretreated rats. These results suggest that GABAA-delta receptors are not internalized but that activation of extrasynaptic GABAA receptors is decreased in morphine tolerant rats. Supported by NIH R01DA027625 (SLI).

12. SPONTANEOUS GABA RELEASE DECREASES WITH DEVELOPMENT IN THE ROSTRAL VENTROMEDIAL MEDULLA (RVM)

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Tuesday 4.45 – 5.45pm

Recent studies have shown evidence that electrical stimulation of the RVM produces pain facilitation in young animals but biphasic pain facilitation and inhibition in adults. This developmental change appears to be dependent on endogenous opioid activity prior to postnatal day 21. Opioid inhibition of GABA release onto RVM neurons output neurons to the spinal cord is thought to be the mechanism of opioid-induced antinociception in the RVM but cellular studies have primarily studied opioid responses in young RVM slices (< 18 days postnatal). In this study, basal spontaneous GABA release in the presence of tetrodotoxin (TTX 1 μ M) was compared in young (0.07 ± 0.03 Hz; N = 5; postnatal days 8-18) and adult rats (>35 days; N = 3; 0.03 ± 0.03 Hz, Mann-Whitney U (6, 30), $p < 0.05$). Interestingly, removal of TTX had little effect on spontaneous GABA release in adult rat slices (0.08 ± 0.05 Hz; N = 7) but significantly increased spontaneous GABA sIPSCs in young slices (0.4 ± 0.1 Hz, N = 3; Mann-Whitney U (21, 24), $p < 0.05$). This result indicates that spontaneous firing of GABAergic neurons intrinsic to the RVM may change during development. Replacing the KCl-based intracellular pipette solution with CsCl significantly enhanced the rate in young slices (1.7 ± 0.3 Hz; N = 4). Opioid inhibition of evoked GABAergic IPSCs was not significantly different with age (met-enkephalin (ME 10 μ M) inhibition: young, $59 \pm 7\%$; N = 5 compared to adult, $52 \pm 12\%$; N = 4). The ability of ME to inhibit mIPSCs was also similar in young ($47 \pm 12\%$, N = 7) compared to adult slices ($47 \pm 6\%$, N = 13). However, there was a trend toward larger ME (10 μ M) activation of GIRK currents in adults (52 ± 15 pA, N = 6) compared to young rats (26 ± 12 pA, N = 4). Further experiments will determine whether the decreased GABAergic tone in adult rats contributes to the observed developmental differences in behavioral responses to electrical stimulation of the RVM. Supported by NIH R01DA027625 (SLI).

13. POSTSYNAPTIC BUT NOT PRESYNAPTIC MU OPIOID RECEPTORS IN THE PREBÖTZINGER COMPLEX DESENSITIZE.

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Monday 4.00 – 5.00pm

The most significant adverse consequence of the use of opiates is respiratory depression which can lead to death. Opioid agonists suppress respiration by activating mu-opioid receptors (MOPr) in the ventral respiratory column and more specifically within glutamatergic neurons of the preBötzing complex (preBötC) which is believed to be the dominant rhythm generator. We sought to determine if MOPrs within the preBötC undergo agonist-dependent desensitization using whole-cell patch-clamp electrophysiology in mouse brain slices. The preBötC contains a heterogeneous population of neurons, but cells coexpressing MOPrs and neurokinin-1 receptors (NK1) are thought to generate the inspiratory rhythm. 67% of neurons responded to the MOPr agonist Met-Enkephalin (30 μ M) which was measured either as an outward current in cells voltage clamped to -60mV ($33.5 \text{ pA} \pm 4.4 \text{ pA}$) or as a hyperpolarization in cells current clamped to a membrane potential of -45mV ($8.2 \text{ mV} \pm 2.8 \text{ mV}$). Of the Met-

Enkephalin responding cells, 57.1% showed an inward current in response to the NK1 agonist [Sar⁹,Met(O₂)I¹]-Substance P (500nM). We also measured GABA-B receptor responses as these receptors couple to the inwardly rectifying potassium channels that presumably underlie the MOPr response. The GABA-B agonist baclofen (20 μ M) produced an outward current in 81% of the Met-Enkephalin responding cells. Although the MOPr currents were small, we were able to measure significant desensitization ($57.1\% \pm 6.2\%$) after 10 minutes of Met-Enkephalin application. Because the postsynaptic Met-Enkephalin responses were small and only occurred in a subset of preBötC neurons, we examined if presynaptic MOPrs within the preBötC might produce a more robust inhibition of glutamatergic release since preBötC neurons synapse onto neurons in both ipsi- and contralateral preBötC. Met-Enkephalin (30 μ M) produced a $57.2\% \pm 7.8\%$ inhibition of the amplitude of evoked excitatory postsynaptic currents, however this inhibition remained stable over a 10 minute application. Thus, postsynaptic MOPrs in the preBötC undergo agonist-dependent desensitization in the subset of cells that express them, whereas presynaptic MOPrs may produce a more robust and homogenous response that does not undergo rapid agonist-induced desensitization. Further studies will examine whether tolerance develops to the MOPr effects in the preBötC after in vivo treatment with opiates. (Funded by MRC)

14. STUDIES ON DETERMINATION OF BIOLOGICAL COMPOSITION OF SEA-WEEDS COLLECTED FROM THE SUNDARBANS WITHIN BANGLADESH

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Tuesday 4.45 – 5.45pm

The Sundarbans is the largest single block of tidal halophytic mangrove forest in the world. The Sundarbans is a UNESCO world heritage site covering parts of Bangladesh and Indian state of West Bengal. Two-third of the Sundarbans is in Bangladesh and the remaining third in West Bengal of India. Three selected sea-weeds namely Laminaria spp., Fucus spp., and Sargassum spp. were collected during October 2012 to March 2013 from the Sundarbans within Bangladesh in order to find out their systematic status, and chemical & biochemical composition. High abundance of Laminaria spp., and Fucus spp. were found in January, February, and March 2013 respectively during the period of investigations. The maximum, and the minimum percentage of protein were noted to be 61.21 in Laminaria spp. in the month of October 2012, and 2.75 in Laminaria spp. in March 2013 where as the lowest percentage of carbohydrate was observed as 11.25 in Laminaria spp. in October 2012. Another significant constituent in sea-weeds for example: ash was recorded as 9% in Fucus spp. in March 2013, and 3.25% in Laminaria spp. in the month of October 2012 as highest, and lowest percentage respectively. The mean percentage of highest contents of Calcium (Ca) recorded in Sargassum spp. was 4.11 ± 0.25 in March 2013, and the lowest in Laminaria spp. was 1.49 ± 0.27 in February 2013. The height Iodine (I) contents observed in Fucus spp. was 0.113 ± 0.025 in February 2013, and the lowest in Sargassum spp. was 0.015 ± 0.005 in February 2013, and the minimum contents were 175.25 ppm in Laminaria spp. January 2013. These investigations were conducted to have a nutritional data for Laminaria spp., Fucus spp., and Sargassum spp. in order to popularize their consumption, and utilization in Bangladesh.

15. PHOSPHOINOSITIDE 3-KINASE γ INTEGRATES CYCLIC AMP AND AKT

SIGNALING OF THE μ -OPIOID RECEPTOR
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Monday 4.00 – 5.00pm

The μ -opioid receptor (MOR) has been characterized as main mediator of opioid signaling in neuronal cells. Gi subunits of heterotrimeric G proteins and their inhibitory effects on cAMP were shown to convey pain depressant signaling effects of MOR. Recent investigations revealed PI3K γ and Akt as additional elements of MOR signaling. PI3K γ is mainly expressed in cells of the immune system. Only recent investigations describe an expression in the neuronal system. The present study reveals PI3K γ as a connecting point of pronociceptive cAMP- and antinociceptive PI3K/Akt –signaling pathways in neuronal cells.

The human neuroblastoma cell line SK-N-LO and dorsal root ganglia from mice have been used to characterize MOR signaling reactions. In both cellular systems cAMP level was manipulated by inhibition of cAMP phosphodiesterases and stimulation of adenylate cyclases and corresponding effects on MOR dependent PI3K/Akt signaling have been analyzed. In order to investigate the special role of PI3K γ in MOR signaling SK-N-LO cells expression of PI3K γ was stably down regulated using shRNA approach.

Stimulation of SK-N-LO cells with morphine resulted in activation of Akt and Erk1/2. PI3K γ down-regulated cells showed a significantly reduced Akt phosphorylation. Moreover, forskolin pretreatment of the cells resulted in inhibition of morphine induced Akt activation. Inhibitory effects of cAMP and protein kinase A on MOR induced stimulation of PI3K γ and Akt suggest a conjunction of both signaling pathways.

Together our data disclose PI3K γ as a mediator of the inhibitory action of cAMP and PKA on Akt implicating this PI3K species as a knot point of pronociceptive cAMP/PKA signaling and antinociceptive signaling reactions of PI3K/Akt in neuronal cells.

Keywords: μ -opioid receptor, PI3K γ , cAMP, dorsal root ganglia, pain, nociception

16. CORRELATION OF ENDOCYTOSIS WITH KINETICS OF PHOSPHORYLATION AND β -ARRESTIN 2 ASSOCIATION IN μ -OPIOID RECEPTORS

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Tuesday 4.45 – 5.45pm

Attempts to develop opioid analgesics with reduced ability to produce tolerance and dependence after chronic exposure, have recently focused on ligand-direct signalling or biased agonism, whereby different agonists at a G protein-coupled receptor (GPCR) can activate distinct downstream signaling pathways. We have used AtT20 cells stably expressing FLAG-tagged MOR to compare the ability of a number of μ -opioid receptor ligands to promote phosphorylation, β arrestin-2 association and endocytosis. We selected a range of agonists including Endomorphin2, morphine and Bilaid-C2, which have moderate G-protein coupling efficacy but with a different profile in producing endocytosis from robust to very low respectively; and Met-enkephalin or DAMGO with high G-protein coupling efficacy and endocytosis capacity. The results show a good correlation between agonist-induced phosphorylation of Ser375 and internalization. After 3 min agonist exposure, Ser375 phosphorylation was maximal for DAMGO, Met-enkephalin and endomorphin-2, modest for morphine and very weak for Bilaid-C2. In contrast, we observed a different pattern in dephosphorylation rate of Ser375 between these agonists. The fastest rate of dephosphorylation was observed with non-internalizing agonist Bilaid-C2. The rank order of Ser375 dephosphorylation rate was Bilaid-C2 > morphine > Met-enkephalin \geq DAMGO >> Endomorphin-2. We used BRET assay to assess MOR- β arrestin2 interaction after agonist application. The data indicate that agonist-induced β arrestin2 association correlates well with Ser375 phosphorylation. DAMGO, Met-enkephalin and Endomorphin-2 produced rapid increases in BRET ratio, whereas morphine produced smaller increase in the BRET ratio and Bilaid-C2 induced very little β arrestin2 recruitment. Interestingly, the rank order of rate of Ser375 dephosphorylation was the same as β arrestin2 dissociation. These results demonstrate that agonists such as endomorphin-2 with high bias to produce internalization display relatively a slow rate of dephosphorylation and arrestin dissociation.

17. NANOPARTICULATE MORPHINE CONJUGATES FOR THE TREATMENT OF INFLAMMATORY PAIN

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Monday 4.00 – 5.00pm

Inflammation, accompanied by tissue acidosis, is an essential component of many painful syndromes, including arthritis, fibromyalgia, endometriosis, cystitis, cancer, trauma, wounds and postoperative pain. Conventional opioid analgesics are limited by side effects (e.g. sedation, apnoea, addiction, tolerance, constipation). The inhibition of inflammatory pain can be achieved by activating opioid receptors on peripheral sensory nerve terminals. Nanocarriers have been developed to selectively activate those receptors and prevent the distribution of opioids to healthy (noninflamed) tissues such as brain or enteric nerve plexus. Here we examine a new nanoparticulate pH-sensitive morphine polyglycerol conjugate (PGM) in comparison to conventional morphine sulphate (MS). We hypothesized that morphine is released only at acidic pH in a rat model of inflammatory pain. Following intravenous (i.v.) or intraplantar (i.pl.) injection into the inflamed paw, an analgesic effect (as measured by elevated paw pressure thresholds; PPT) was only detected in the inflamed paw and was completely abolished by i.pl. injection of the quaternary opioid antagonist naloxone methiodide (NLXM). The highest dose of i.pl. MS increased PPT in the contralateral noninflamed paw indicating central effects. Moreover, i.v. MS increased PPT dose-dependently in both hindpaws and was not completely reversible by i.pl. NLXM, suggesting the involvement of central opioid receptors. Microdialysis experiments revealed the presence of free morphine in inflamed paws after i.pl. injection of MS or PGM. Small amounts of free morphine were also detectable in the contralateral noninflamed paw 1h after i.pl. MS. These results suggest that such new “controlled release” formulations of potent conventional opioid analgesics could improve pharmacological therapies avoiding central effects.

18. OPIOID RECEPTOR COUPLING TO K⁺ CHANNELS IN PERIPHERAL SENSORY NEURONS: RELEVANCE FOR ANALGESIC EFFICACY AND SPECIES DIFFERENCES

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Tuesday 4.45 – 5.45pm

Opioid treatment of pain is limited by adverse side effects in the central nervous system (CNS). A better understanding of the mechanisms underlying opioid analgesia mediated outside of the CNS would help to develop opioids acting at peripheral sites of tissue injury without centrally mediated unwanted effects. In the CNS, activation of opioid receptors inhibits voltage-gated calcium (Ca²⁺)-channels and activates G protein-coupled inwardly rectifying potassium (GIRK) channels through binding of G-protein G_{βγ} subunits. Both events reduce membrane excitability. However, studies on GIRK channel expression and function in peripheral neurons are scarce and have produced conflicting results. Here

we report that GIRK channels in sensory dorsal root ganglion (DRG) neurons are crucially involved in the generation of opioid antinociception.

We found very low mRNA and protein expression of different GIRK subunits in DRG of naïve mice. However, expression of mRNA and protein of GIRK-1 and -2 was prominent in DRG of naïve rats. We detected GIRK currents after mu-opioid receptor activation by DAMGO in rat but not in mouse DRG neurons. We generated transgenic mice expressing a Flag-GIRK2 construct selectively in DRG neurons by use of a Nav1.8 promoter. DAMGO evoked GIRK currents in DRG neurons isolated from these Nav1.8-GIRK2 mice but not from wildtype littermates. We assessed how expression of GIRK2 affects nociceptive behavior by measuring hindpaw withdrawal in animals with painful hindpaw inflammation. Local injection of DAMGO abolished the thermal and mechanical hypersensitivity in Nav1.8-GIRK2 mice but not in wildtype littermates.

Our data show that GIRK channels in DRG neurons are crucial for generation of peripheral opioid antinociception and that these molecular mechanisms may explain species differences. These findings should be considered in drug development and in basic and clinical pain research.

19. TOLL-LIKE RECEPTOR 4 CHANGES OPIOID LIGAND BINDING IN BRAIN HOMOGENATES

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Monday 4.00 – 5.00pm

In the infancy of opioid research, attention was focused directly toward the stereoselective receptors that were discovered to be critical for opioid analgesic responses. However opioids can paradoxically increase pain sensitivity in humans and rodents leading to hyperalgesia and tolerance. Work by the Hutchinson and Watkins group has demonstrated that genetic removal of immune receptors such as toll-like receptor 4 (TLR4) significantly reduces the development of hyperalgesia and tolerance whilst simultaneously increasing anti-nociception. It is thought that opioids bind non-stereoselectively to TLR4 leading to a neuroinflammatory response within the CNS, compromising opioid-induced analgesia and contributing to various unwanted actions. The existing data on the kinetics of non-stereoselective opioid binding is limited and requires further investigation. Wildtype (WT) and TLR4KO mouse brains were harvested as required and homogenised using the gentleMACS™ Octo Dissociator. Homogenates were diluted to 10mg/mL and [³H](-)naloxone added to 1.63nM. 1mL aliquots were vacuum filtered and washed with 5mL of 0.9% PBS at required time points. [³H](-)Naloxone dissociation was initiated by adding 1μM of cold drug. Data was analysed using non-linear regressions. Our pilot data has demonstrated a significant change in (-)naloxone association rate kinetics in TLR4KO (1.33x10⁻⁹ sec n=6) mouse brain homogenates compared with WT mouse brain homogenates (-2.28x10⁻⁹ sec n=6). This was represented by faster (-)naloxone association in the genetic absence of TLR4 compared to wildtype conditions (P<0.0005). However, unexpectedly we did not observe a change (P>0.5) in total [³H](-)naloxone binding between the WT (12982 DPM +/- 847 n=3) and TLR4KO (13895 DPM +/- 1169 n=3) brain homogenates. These results further suggest an involvement of TLR4 in opioid pharmacology, but underlie the complexity of this TLR4 opioid interaction.

20. BIASED SIGNALLING OF ENDOGENOUS OPIOIDS AT THE MU RECEPTOR

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Tuesday 4.45 – 5.45pm

The endogenous opioid system, with multiple ligands targeting the same receptor, may represent a natural example of functional selectivity or signalling bias, where different ligands binding to the same receptor generate different receptor conformations linked to distinct signaling pathways and a particular physiological response. Understanding these mechanisms can provide essential information on how opioid receptors regulate nociception and other physiological functions, including gastrointestinal motility, and may lead to development of pain therapies that preserve potent analgesia while minimising side effects, such as constipation. This study aims to elucidate cellular and molecular mechanisms elicited by endogenous opioids both in the central and enteric nervous systems, and to investigate the existence of functional selectivity in the endogenous opioid system.

Quantification of signalling bias of a range of endogenous opioids at the Mu opioid receptor (MOR) has been performed in a simple cellular model, to obtain unique ligand activity profiles or “fingerprints” for these ligands. The ability of each ligand to activate multiple signal transduction pathways was measured; including G protein activation, inhibition of adenylyl cyclase (AC), activation of extracellular regulated kinases (ERKs), recruitment of β -Arrestins and receptor internalisation. The bias between each of these pathways was quantified by the application of a novel analytical method based on the operational model of agonism. We found a number of endogenous opioids that exhibit signalling bias at the MOR. As signalling through GPCRs is highly dependent upon the cellular system, the ligand activity profiles of these endogenous opioids were then validated in more physiological relevant cellular systems. Specifically, quantification of signalling bias has been performed in a neuronal cell line, and currently we are generating ligand additional activity profiles for biased endogenous opioids in primary dorsal root ganglia (DRG) and enteric neurons. This work will establish whether signalling bias by endogenous opiates exists in primary DRG and enteric neurons.

Future work will examine the physiological outcome of biased signalling by endogenous opioids by using animal models for pain and gastrointestinal motility. These experiments will enable links between distinct signalling profiles and specific physiological responses in the intestine and spine to be established, providing new information essential for the design opioids with effective analgesia and less gastrointestinal side effects.

21. DEVELOPMENT OF MIXED EFFICACY MOR/DOR LIGANDS AND MODIFICATION TO IMPROVE BLOOD/BRAIN BARRIER PENETRATION

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Monday 4.00 – 5.00pm

It has been demonstrated that the co-administration of a mu opioid receptor (MOR) agonist with a delta opioid receptor (DOR) antagonist produces the expected MOR mediated analgesia, but displays reduced negative side effects, in particular a reduced level of tolerance and dependence, features that limit the clinical use of opioid analgesics. However, combining two drugs with different selectivities and efficacies has considerable disadvantages related to potential diverse pharmacokinetic properties of the two chemical entities. Therefore we, and others, have taken a multifunctional approach by combining both MOR agonism and DOR antagonism in a single molecule. We have successfully generated a series of mixed efficacy cyclic peptides using our previously published homology models of the opioid receptors to guide ligand design. These peptides when tested *in vitro* displayed low nanomolar affinity for MOR and DOR with moderate selectivity over the kappa opioid receptor (KOR). This series of ligands also displayed MOR efficacy similar to morphine and reduced DOR activation, with some compounds showing a complete lack of efficacy at DOR. As low bioavailability is typical of peptides, we installed a C-terminal β -glucoserine, in which the side chain hydroxyl of serine is covalently O-linked to a β -glucose, onto the mixed efficacy peptide scaffold with the most appropriate *in vitro* characteristics. This is a means of improving blood-brain barrier penetration and increasing the metabolic stability. These β -glucoserine derivatives were assessed for *in vivo* activity and shown to produce dose-dependent antinociception after peripheral administration. This demonstrates that although peptides are not usually thought of as “druggable” molecules, these ligands are viable tools for exploring therapeutic peptide analgesics as well as potentially developing molecules with reduced dependence liability. Supported by RO1 DA-03910.

22. A NOVEL MECHANISM FOR RESTRAINT STRESS-INDUCED COCAINE RELAPSE: OREXIN-INDUCED ENDOCANNABINOID RETROGRADE DISINHIBITION IN THE VENTRAL TEGMENTAL AREA

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Tuesday 4.45 – 5.45pm

Activation of hypothalamic orexin neurons that project to the ventral tegmental area (VTA) is associated with the reinstatement of reward seeking, while its mechanism(s) remain unclear. Here, we reveal a novel mechanism for stress-induced cocaine seeking involving orexin-induced endocannabinoid (eCB) signaling in VTA dopamine neurons. Orexin A inhibited GABAergic transmission in dopamine neurons of VTA slices via a presynaptic mechanism. This effect was antagonized by OX1 (SB 334867) and CB1 (AM 251), but not OX2 (Compound 29), antagonists, mimicked by a CB1 agonist (WIN 55,212-2) and prevented by internally applied GDP-βS (a G-protein inhibitor) and a phospholipase C inhibitor (U73122). JZL 184, an inhibitor of monoacylglycerol lipase that hydrolyses 2-arachidonoylglycerol (2-AG), an eCB, potentiated and prolonged the effect of orexin A in VTA slices. Consistently, the effect of orexin A was prevented by tetrahydrolipstatin, an inhibitor of diacylglycerol lipase, a 2-AG synthesizing enzyme. In a conditioned place preference (CPP) test in mice, a 30 min-restraint stress, which increased the number of c-Fos-containing orexin neurons in the hypothalamus and orexin A levels in the VTA, significantly reinstated extinguished cocaine CPP. This acute stress-induced cocaine reinstatement was prevented by SB 334867 or AM 251, and was abolished in CB1 receptor-knockout mice. It was also prevented by a centrally applied CRF antagonist. These results suggest that acute restraint stress activates hypothalamic orexin neurons to release orexins, which then activate postsynaptic OX1 receptors on VTA dopaminergic neurons, through a Gq-protein coupled phospholipase C and diacylglycerol lipase enzymatic cascade, generating 2-AG. This eCB then retrogradely inhibits GABA release through presynaptic CB1 receptors, leading to disinhibition of VTA dopamine neurons and cocaine seeking behavior.

23. SPINAL ACTIONS OF TRIPTANS AND OPIOIDS IN AN INFLAMMATORY PAIN STATE

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Monday 4.00 – 5.00pm

Triptans, such as sumatriptan, are 5-HT_{1B/D} agonists which are usually associated with the treatment of migraine. There is, however, evidence that triptans act within the spinal cord to reduce non-cranial inflammation induced somatic pain (Nikai et al. 2008 Pain 139:533-40). In the present study, we compared the spinal actions of opioids and triptans in a rat model of hindpaw inflammation and examined their cellular actions within the

lumbar spinal cord. All experiments were approved by the Royal North Shore Hospital ACEC, and were performed on untreated Sprague-Dawley rats (naïve animals), or on rats 3 days after intraplantar injection of complete Freund's adjuvant (CFA animals). Intrathecal injection of sumatriptan, the 5-HT_{1A} agonist R(+)-8-OH-DPAT and DAMGO all reduced hindpaw mechanical allodynia in CFA animals. By contrast, DAMGO and R(+)-8-OH-DPAT, but not sumatriptan, reduced acute thermal (and to a lesser extent mechanical) pain in naïve animals. In lumbar spinal slices from naïve and CFA animals, sumatriptan, R(+)-8-OH-DPAT and DAMGO all reduced the amplitude of afferent evoked excitatory postsynaptic currents (EPSCs) in lamina I/II neurons. The inhibition of evoked EPSCs produced by sumatriptan (55 ± 14 % versus 16 ± 7 %) and DAMGO (75 ± 4 % versus 55 ± 5 %) was, however, greater in slices from CFA compared to naïve animals. Furthermore, the 5-HT_{1B} agonist CP93129 inhibited evoked EPSCs in slices from CFA, but not naïve animals (60 ± 9 % versus 1 ± 5 % inhibition). The 5-HT_{1D} agonist PNU109291 had no effect on evoked EPSCs in lumbar slices either animal group. These findings suggest that inflammation induces a functional upregulation of opioid and 5-HT_{1B} receptor systems in spinal lumbar pain pathways. Supported by Australian NH&MRC (632546).

24. DESIGN, SYNTHESIS AND PHARMACOLOGY OF OPIOID DELTA AGONISTS WITH A NOVEL FUNDAMENTAL OXAZATRICYCLODECANE STRUCTURE

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Tuesday 4.45 – 5.45pm

The δ opioid receptor is expected to be an attractive drug target without morphine-like side effects, but there is still no δ receptor selective agonist launched as a therapeutic agent. For the past 20 years, although quite a lot of research for the synthesis and pharmacology of δ receptor agonists have been reported, most of them are SNC-80 analogues which possess a diarylmethylpiperazine or a similar structure.

We have recently reported the synthesis of the morphinan derivatives with a novel oxazatricyclodecane skeleton^[1,2], which is a new structural class of δ receptor agonists. Representative compound SYK-89 showed high affinity and moderate selectivity for the δ receptor. However, this compound exhibited low agonistic activity in cAMP functional assay and only a weak analgesic effect in mice acetic acid writhing test.

In the present study, we postulated that the low agonistic activities of the oxazatricyclodecane derivatives would mainly result from the existence of a possible accessory site, which is an extra structural part and interferes with a suitable conformational change of the δ receptor bound to a δ agonist (induced-fit). Based on this hypothesis, we designed and synthesized derivatives without the possible accessory site, dioxamethylene moiety. As we expected, these derivatives showed high affinities and full agonistic activities for the δ receptor. Further optimization of these structures gave the improvement of analgesic activities. In our ongoing studies, elucidation of the structure-activity relationships of these oxazatricyclodecane derivatives will provide new therapeutic agents.

[1] Hayashida, K.; Fujii, H.; Hirayama, S.; Nemoto, T.; Nagase, H. *Tetrahedron*, 2011, 67, 6682-6688.

[2] 8th AFMC International Medicinal Chemistry Symposium, Dec. 2011, Japan

25. THE ROLE OF DIFFERENT PHOSPHORYLATION SITES IN RAPID DESENSITIZATION OF THE μ -OPIOID RECEPTOR

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Monday 4.00 – 5.00pm

Tolerance and addiction to opioids are serious clinical and social problems that result in part from the loss of μ -opioid receptor (MOR) function when activated by different opioids. Phosphorylation of specific residues in the C-terminal domain of MOR is thought to be a key step in desensitization, endocytosis and loss of MOR function. Sequential phosphorylation of S375 along with the flanking residues T370, T376 and T379 are required for endocytosis (Just et al., Mol Pharmacol (2013) 83:633-639) but their role in rapid desensitization of MOR is unknown. The aim of this study was to determine the influence of C-terminal phosphorylation sites on rapid desensitization of MOR. Wild type mMOR, 3S/T-A (S363A, S370A, S375A), 6 S/T-A (S363A, S370A, S375A, T376A, T379A, T383A) and 11S/T-A (T354A, S355A, S356A, T357A, S363A, S370A, S375A, T376A, T379A, T383A, T394A) were expressed stably in AtT20 cells. Using perforated patch-clamp recording we examined the effects of MOR activation, desensitization and re-sensitization using activation of GIRK channels by a submaximal concentration of met-enkephalin (10 nM) to measure receptor activity and somatostatin (100 nM) coupling to native SSTR2 (or sst2 according to IUPHAR) to determine heterologous desensitization. MOR desensitization and resensitization produced by 5 min exposure to met-enkephalin (10 μ M) at 37°C did not differ from wild type for 3S/T-A or 6S/T-A but desensitization was abolished in the 11S/T-A mutant. Desensitization, when detected, was largely homologous. Because 3S/T-A suppresses, and 6S/T-A abolishes MOR endocytosis, these findings suggest that homologous desensitization can occur independently of the phosphorylation and arrestin-dependent mechanisms that drive endocytosis. However, C-terminal phosphorylation sites are necessary for desensitization because mutation of all C-terminal sites (11S/T-A) abolishes desensitization.

26. DESENSITIZATION OF THE μ -OPIOID RECEPTOR IN INTACT ATT20 CELLS: IS C-TERMINAL PHOSPHORYLATION ESSENTIAL?

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Tuesday 4.45 – 5.45pm

Internalization of the μ -opioid receptor (MOR) is not essential for receptor desensitization and resensitization, and inhibiting internalization may even increase resensitization speed. Phosphorylation of the MOR c-terminal is important for internalization, however, the relationship between receptor phosphorylation and desensitization is not as well established. It has also been suggested that protein kinase C-mediated phosphorylation of MOR is associated with desensitization produced by morphine but not DAMGO. We investigated acute desensitization of mouse MOR (MOR-WT) and mutant MOR in which important phosphorylation sites were deleted in intact AtT-20 cells. Heterologous desensitization was assessed using somatostatin receptor responses.

MOR-WT and 3 c-terminus phosphorylation site mutants: AAA-point mutations of S363, T370, S375; TPD - all c-terminal Ser/Thr

to Ala and TSST – TSST motif and T394 backmutant of TPD were stably transfected in AtT20 cells. MOR activation of GIRK was measured using FLIPR membrane potential dye in a FlexStation 3. The degree of desensitization was quantified using saturating agonist concentrations added after the desensitizing stimulus.

DAMGO and morphine hyperpolarized AtT20-MOR cells.

Treatment with the PKC agonist PMA or the unspecific protein kinase inhibitor staurosporine did not affect agonist potency or desensitization. Morphine hyperpolarized AtT-20-MOR cells and those expressing the AAA, TPD and TSST variants with EC50s (nM) of 16 \pm 5, 31 \pm 5, 14 \pm 2 and 13 \pm 2 respectively. After 30 minutes treatment with morphine (1 μ M), the response to a subsequent addition of 10 μ M morphine was reduced by 76 \pm 2% (WT), 56 \pm 4% (AAA), 62 \pm 3% (TPD) and 69 \pm 4% (TSST). The response to somatostatin (1 μ M) 30 minutes after morphine (1 μ M) was inhibited by 65 \pm 1% (WT), 17 \pm 3% (AAA), 30 \pm 5% (TPD) and 47 \pm 6% (TSST). Qualitatively similar data was obtained for DAMGO. The time course of the development of homologous and heterologous desensitization was similar.

Mutations of key phosphorylation sites in the C-terminal region of mouse MOR did not dramatically affect agonist potency, and morphine induced desensitization of MOR persists even after complete deletion of phosphorylation sites on c-terminal. This suggests that other regions of the receptor may be involved in acute agonist-induced inhibition of signalling. Interestingly there is a significant difference in the degree of heterologous desensitization of somatostatin responses following activation of some of the MOR phosphomutants, suggesting distinct mechanisms at play.

27. CAMKII α CONTROLS THE BIOGENESIS OF LET-7 MICRORNAS IN OPIOID TOLERANCE

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Monday 4.00 – 5.00pm

Emerging evidence suggests that microRNAs (miRNAs) – mediated cellular adaptations are critical for drug addiction. We previously reported that let-7 family miRNAs contribute to the development of opioid tolerance by targeting the μ opioid receptor. Chronic morphine treatment induced a marked increase of let-7 expression, which functionally correlated with the development of opioid tolerance.

The aim of this study was to understand the mechanisms how let-7 is regulated by opioids. We first determined the transcription status of let-7 and found that the expression of primary let-7 (pri-let-7) remained unchanged in SH-SY5Y cells that were treated with morphine (1 μ M, for 48 h). In agreement with the *in vitro* observation, morphine pellet implantation (one 75 mg morphine pellet/mouse, s.c.) did not alter the level of pri-let-7 in mouse brain front cortex region. These findings suggested that the robust elevation of let-7 occurred at the post-transcriptional level. Of interest, in the presence of KN93, inhibitor of Ca²⁺/calmodulin-dependent protein kinase II (CaMKII), chronic morphine treatment failed to generate let-7 up-regulation in SH-SY5Y cells. We further determined whether inactivation of CaMKII α by T286A point mutation would affect let-7 expression and opioid tolerance. Indeed, antinociceptive tolerance was absent in CaMKII α ^{T286A} mutant mice. Meanwhile, the level of let-7 in CaMKII α ^{T286A} mutant mice was much lower than that in wild-type mice, and was resistant to chronic morphine stimulation. Taken together, these data suggested that the activity of CaMKII α was required for the biogenesis of let-7 in opioid tolerance.

28. DISRUPTION OF THE ACTIN CYTOSKELETON DELAYS RECOVERY OF MU OPIOID RECEPTOR FROM DESENSITIZATION IN LOCUS COERULEUS NEURONS

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Tuesday 4.45 – 5.45pm

The actin cytoskeleton acts to support cellular structure, but it also plays a role in GPCR signaling and regulation. The network of actin filaments beneath plasma membrane has been shown to confine mu opioid receptors (MOR) into a compartment and restrains global lateral diffusion properties. The present study used latrunculin B (Lat B) to disrupt actin polymerization in HEK293 cells or mouse locus coeruleus neurons (LC). The effect of Lat B on lateral diffusion of Flag-tagged MOR (Flag-MOR) in HEK293 cells was studied by line-scanning fluorescence correlation spectroscopy. In Lat B treated cells, the diffusion rate of Flag-MOR was faster than control. The effect of Lat B on [Met5]enkephalin (ME)-induced receptor internalization was studied in brain slices of locus coeruleus neurons from transgenic mice expressing Flag-MOR. In the Lat B treated cells the amount of internalized Flag-MOR and the endosome size was increased compared to control following treatment with ME (30 µM, 10 min). The desensitization measured by the decline in the hyperpolarization induced by ME (30 µM) was not altered but there was only a partial recovery from desensitization following the wash. The impaired recovery from desensitization correlated with a reduction in the recycling of receptors back to the plasma membrane. Experiments using LC slices obtained from mutant S375A knock-in mice showed that the desensitization and the recovery were not altered by Lat B. This study suggests that the actin cytoskeleton plays a key role in the recovery from desensitization by regulating the receptor trafficking. The study is supported by NIH grant DA08163.

29. PHOSPHORYLATION OF MU OPIOID RECEPTOR REDUCED MORPHINE ANALGESIA IN INFLAMMATORY PAIN STATE

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Monday 4.00 – 5.00pm

An inflammatory pain is a chronic pain induced by algesic or inflammatory substances. It is well established that morphine is effective against the thermal hyperalgesia during inflammatory pain state. However, we found that morphine is ineffective against the mechanical allodynia on the inflammatory pain state. In the present study, the changes in the spinal mu-opioid receptors, which is involved in the morphine analgesia, is investigated in the inflammatory pain state. To develop the inflammatory pain, complete Freund's adjuvant (CFA) was injected i.pl to the hind-paw of male ddY mice. The protein level of mu-opioid receptors was quantified by western blot. Mechanical pain threshold and analgesic effect of morphine were measured by von Frey filament test. The remarkable mechanical allodynia was observed after the CFA injection on inflamed side but not non-inflamed side. The

analgesic effect of morphine injected s.c. was markedly decreased bilaterally after the CFA injection. However, the protein levels of mu opioid receptor were significantly decreased in the DRG only on inflamed side after CFA injection. The present results suggest that the reduction of morphine analgesia in inflammatory pain state on inflamed side reflect the down regulation of mu opioid receptor, whereas another mechanism is also involved in the reduction of morphine analgesia on non-inflamed side in inflammatory pain state. Interestingly, the reduced morphine analgesia after CFA injection was completely reversed on non-inflamed side, and partially reversed on inflamed side by intrathecal pretreatment with protein kinase C inhibitor, which was injected at the same time of development of inflammation by CFA injection. In conclusion, the reduction of morphine analgesia on inflamed side may mainly reflect the down regulation of mu opioid receptor, whereas the reduction of morphine analgesia on non-inflamed side may mainly reflect the inactivation of mu opioid receptor by phosphorylation.

30. THE DELTA-OPIOID RECEPTOR CONTRIBUTES TO MORPHINE TOLERANCE IN AN ANIMAL MODEL OF CHRONIC INFLAMMATORY PAIN

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Tuesday 4.45 – 5.45pm

Opioids are well known for their robust analgesic effects. Although they can activate 3 receptor subtypes (mu, delta, and kappa opioid receptors; MOPRs, DOPRs, KOPRs), in vivo, commercial opioids preferentially target MOPRs. Chronic activation of MOPR is however accompanied by various unwanted effects such as analgesic tolerance. Among other mechanisms, interactions between MOPR and DOPR are thought to play an important role in morphine-induced behavioral adaptations. Here, we investigated the role and the regulation of DOPR and the MOPR/DOPR heteromer formation during the development of morphine-tolerance in an animal model of chronic inflammatory pain. We found that prolong morphine treatment, in the setting of chronic pain, induced an increase in DOPR expression at the postsynaptic density fraction in the dorsal horn of the spinal cord. In addition, our results showed an increase in MOPR/DOPR heteromer abundance in spinal cord dorsal horn and periaqueductal gray homogenates. Finally, using behavioral approaches, we observed that blockade of DOPR with the selective antagonist naltrindole (s.c. or i.t.) attenuates the development of morphine tolerance in a dose-dependent manner. Altogether, our results suggest that targeting the DOPR provides a valuable strategy to attenuate the analgesic tolerance that develops after repeated morphine administration in the setting of chronic inflammatory pain. Further studies are needed to understand cellular and molecular mechanisms involved in this process.

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31. ENDOTOXIN ENHANCEMENT OF MORPHINE-INDUCED CONDITIONED PLACE PREFERENCE

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Monday 4.00 – 5.00pm

Because of its potent analgesic effects, morphine is widely used in clinical pain management. However, it has a high potential for abuse and addiction. In this study, we examined the effects of the endotoxin, lipopolysaccharide (LPS), on the sensitivity to morphine in adult rats. Adult Fischer 344 rats were given two intraperitoneal (i.p.) injections of either LPS (250 µg/kg) or saline (vehicle), followed by an injection of either a higher dose of LPS (2 mg/kg) or saline after 24 h. After 72 h, the rats were given an i.p. injection of either morphine (3.5 mg/kg) or saline every other day during the 6 d of the conditioning phase of a conditioned place preference (CPP) experiment. During the post-conditioning phase, the rats were assessed for their drug preference. In the groups given LPS, there was a stronger preference for morphine compared to the group that was given only saline, and there was a sustained response to morphine as evidenced by greater than 20 d of extinction. In a parallel study, tail-flick latencies were measured to evaluate the analgesic effects of morphine following exposure to LPS. The rats were pretreated with LPS (2 mg/kg) or saline, followed by treatment with cumulative doses of morphine ranging from 0.5 – 10 mg/Kg. Tail-flick latencies in response to morphine were significantly longer in the rats given LPS compared to those given saline. Collectively, our data indicate that systemic infections can increase the sensitivity to morphine both physiologically and behaviorally (partially supported by K02 DA016149 & RC2 AA019415 to SL Chang).

32. ADOLESCENT DRINKING AFFECTS ENDOGENOUS OPIOIDS IN HIPPOCAMPUS AND AMYGDALA INDEPENDENT OF REARING CONDITION

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Tuesday 4.45 – 5.45pm

Early onset of alcohol drinking predicts increased risk for alcohol use disorders (AUD) later in life but the mechanisms are not understood. A link between alcohol and endogenous opioids are well described and it is known that endogenous opioids are affected by early-life rearing conditions as evidenced by distinct changes in the opioid networks in young and adult rats. However, the effects of adolescent drinking on opioids are poorly described and little is known whether these effects are dependent on early-life experiences. To optimize and individualize prevention and treatment strategies for AUD, a better understanding of the link between the early-life environmental factors, ethanol drinking and endogenous opioids is of importance. The aim was to study interactions between rearing conditions and adolescent drinking on the opioid peptides dynorphin B (DYNB), met-enkephalin-Arg6Phe7 (MEAP) and beta-endorphin (BEND).

A maternal separation (MS) model was used to simulate different rearing conditions. The pups were separated 360 min daily from the dam during the first three postnatal weeks. Pups subjected to 15 min MS were controls. After weaning, on postnatal day 34, all rats were individually housed and given a free choice between 20% ethanol and water, or water only, for 12 weeks. Ethanol was available 24h on Mondays, Wednesdays and Fridays.

A main effect of ethanol was found with higher immunoreactive (ir) levels of DYNB in the hippocampus and higher ir MEAP levels in the amygdala. A main effect of rearing condition was found in the pituitary gland and periaqueductal grey with lower ir BEND levels in the MS360 rats. No interaction between rearing condition and ethanol was observed. Previous studies have reported differences in basal and ethanol-induced effects in adult rats subjected to MS15 and MS360. This study shows that adolescent drinking attenuate these differences. It is possible that the individual housing had a larger impact on opioid levels than the rearing environment and therefore masked the previously seen differences in opioid peptides. Since DYNB impairs learning and MEAP reduces anxiety-like behaviour it is of particular interest to note that voluntary adolescent drinking targets hippocampal DYNB and MEAP in the amygdala.

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33. SINGLE HOUSING DURING ADOLESCENCE CAUSE ALTERATIONS IN BASAL LEVELS OF ENDOGENOUS OPIOID PEPTIDES

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Monday 4.00 – 5.00pm

The endogenous opioid system (EOS) exert a variety of effects and one of its most noteworthy functions is the regulation of natural rewards like food, sex and social interactions. Disturbance of natural reinforcers can cause basal alterations in the EOS and consequently contribute to an intrinsic vulnerability for different neurobiological disorders, e.g. addiction and mood disorders. Early in life, social interactions are important for the normal development of the EOS. In this study, the aim was to investigate the effects of social isolation (i.e. single housing with the possibility to hear and smell other animals) during adolescence on basal levels of endogenous opioids.

On postnatal day 22 (PND22) Sprague-Dawley pups were weaned and randomly assigned into three different experimental groups; long social isolation (LSI), short social isolation (SSI) and controls. At PND29 the rats were sacrificed by decapitation. The animals in the LSI group were individually housed from PND22 until PND29. The SSI rats were group housed until 30 minutes prior to decapitation when they were singly housed in a separate cage. The controls were group housed until decapitation. The brains were dissected and trunk blood collected. Tissue levels of three opioid peptides, Met-enkephalin-Arg6Phe7 (MEAP), dynorphin B (DYNB) and nociceptin/orphanin FQ (N/OFQ), as well as serum corticosterone were analysed using radioimmunoassays.

The LSI rats had statistically significant lower immunoreactive (ir) MEAP levels compared to controls in hypothalamus, frontal cortex, medial prefrontal cortex, striatum, amygdala, substantia nigra and periaqueductal gray. Elevated levels of ir N/OFQ were seen in both SSI and LSI compared to controls. None of the isolation groups differed in ir DYNB levels compared to control. The ir corticosterone in SSI rats were increased compared to controls.

These results show that disturbance of social interactions during adolescence induce changes in endogenous opioids and confirm the importance of social behaviour for development during this sensitive time window. A deranged EOS may have long-term consequences for later susceptibility to addiction and other disorders where the EOS is implied in pathogenesis.

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34. REDUCED MICRO-RNA 200B-429 CLUSTER EXPRESSION IN THE NUCLEUS ACCUMBENS IS INVOLVED IN THE POST-TRANSCRIPTIONAL MODULATION UNDER NEUROPATHIC PAIN

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Tuesday 4.45 – 5.45pm

Neuropathic pain is the most difficult type of pain to control, and patients lose their motivation for the purposive pursuit with a decrease in their quality of life. It has been widely recognized that the ascending anatomical dopamine projection from the ventral tegmental area (VTA) to the nucleus accumbens (N.Acc.) is mostly related to motivational functions and the reinforcing effects of opioids. On the other hand, microRNAs (miRNAs) are small, noncoding RNA molecules that direct the post-transcriptional suppression of gene expression, and play an important role in regulating synaptic plasticity. MiRNAs have been shown to respond to various cellular stressors to play a role on psychiatric disorders, such as schizophrenia and autism. Thus, miRNAs are thought to play a role in functions of the central nervous systems. In this study, we found that sciatic nerve ligation induced a drastic decrease in the expression of miR200b and miR429 in N.Acc. neurons. The expression of DNA methyltransferase 3a (DNMT3a), which is the one of the predicted targets of miR200b/429, was significantly increased in the N.Acc. at 7 days after sciatic nerve ligation. Next, we microinjected the miR200b/429-expression lentivirus into the N.Acc. to evaluate pain behaviors by sciatic nerve ligation. We found that its microinjection partially but significantly reduced the thermal hyperalgesia and mechanical allodynia by sciatic nerve ligation. The results of these analysis provide new insight into a post-transcriptional modification at the brain that is accompanied by a dramatic decrease in miR200b and miR429 along with the dysfunction of "mesolimbic motivation/valuation circuitry" under a neuropathic pain-like state. These phenomena may result in a decrease in DNMT3a in neurons of the N.Acc. under neuropathic pain, which leads to the long-term transcription-silencing of several genes.

35. THE TRANSLOCATION OF THE δ -OPIOID RECEPTOR ON CHOLINERGIC INTERNEURONS IN THE STRIATUM

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Monday 4.00 – 5.00pm

In many neuronal populations under baseline conditions the δ -opioid receptor (DOR) is constitutively retained in intracellular compartments with only low levels of DOR present at the plasma membrane. Under certain conditions intracellular DOR may be inserted into the cell membrane, increasing the levels of functional DOR and enhancing the effects of DOR agonists. In the striatum, DOR is known to be highly expressed on cholinergic interneurons (CIs), whose inhibitory function is believed to play a vital role in the regulation of the striatum. In these neurons, DOR follows the distribution pattern described above – with neurons containing non-functional, intracellular pools of the receptor in addition to expressing some receptor at the membrane. Research in our lab has demonstrated that, in the NACsh of DOR-eGFP mice, following Pavlovian conditioning DOR has translocated from the

cytoplasm to the perisomatic membrane of CIs. The translocation and activity of DOR in the NACsh is not required for Pavlovian learning or the expression of Pavlovian behaviours but for the expression of outcome-specific Pavlovian-instrumental transfer (PIT). However, while this demonstrates a behavioural trigger for DOR insertion, the molecular mechanisms of this translocation event remain unknown. The present study investigates whether the neurotransmitter substance P (SP) may drive insertion of DOR into the perisomatic membrane of CIs in the striatum. In the past, SP has been controversially linked to DOR translocation in small dorsal root ganglion neurons via a proposed interaction between DOR and SP leading to their cotrafficking. This mechanism has been challenged by later studies. Here, we propose an alternative interaction between the two molecules – namely that SP signalling triggers the translocation of DOR. SP (300nm) was administered to the striatum of DOR-eGFP mice both in vivo and in vitro. Immunohistochemical staining for GFP and choline acetyltransferase (ChAT) was performed on treated sections and DOR-eGFP receptor at the membrane of CIs was quantified. Application of SP to the dorsal medial striatum (DMS) significantly increased the amount of DOR-eGFP ($p < 0.01$) at the perisomatic membrane of CIs in the region. This demonstrates a novel mechanism of DOR translocation which may contribute to the behavioural effects of opioids in the striatum.

36. POMC NEURONS IN THE HYPOTHALAMUS MODULATE PERIPHERAL IMMUNE FUNCTION

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Tuesday 4.45 – 5.45pm

The pathophysiological state of cancer cachexia frequently affects advanced cancer patients. The close association between chronic illness such as cancer and the nutritional deterioration does not only impair the quality of life but also increases the risk for morbidity and mortality. On the other hand, β -endorphin, an endogenous opioid polypeptide primarily produced by the hypothalamus, is known to have the ability to inhibit stress hormone production and produces analgesia, and enhances innate immune function. β -Endorphin is a cleavage product of pro-opiomelanocortin (POMC), which is also the precursors hormone for ACTH and melanocyte stimulating hormone (α -MSH). We investigated possible changes in the expression of POMC in the hypothalamus under the cancer cachexia caused by intraperitoneal inoculation of pancreatic cancer cells. At 3 weeks after tumor inoculation, when mice exhibited slight weight loss, POMC expression in the hypothalamus was significantly decreased compared to that in control mice. Under these conditions, decrease in the macrophage colony-stimulating factor (M-CSF) was found in the serum from the peritoneal metastasis model mice. Furthermore, we found that the M-CSF was decreased in the serum from the POMC knockout mouse. Next, we used POMC-cre mouse with AAV for POMC neuron specific photostimulation. The suppression of POMC neurons in the hypothalamus resulted in decreased M-CSF in the mouse serum. These findings suggest that POMC neurons engage to activate peripheral immunity and anti-inflammatory cytokines.

37. INVOLVEMENT OF DELTA-OPIOIDERGIC SYSTEM IN ADULT NEUROGENESIS

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Monday 4.00 – 5.00pm

Opioid analgesics and endogenous opioid peptides have a wide range of physiologic and behavioral effects on pain perception, mood, motor control and autonomic function. The delta-opioidergic system has been recognized as a neurotransmitter system that could be directly involved in emotionality and immunity. Recently, we reported that a long-term blockade of delta-opioid receptor function using the selective delta-opioid receptor antagonist naltrindole (NTI) induced astrogliosis. These findings support the concept that the delta-opioid receptor plays an important role in cell proliferation, gliogenesis and neurogenesis. Neural stem cells capable of producing new neurons and astrocytes are maintained in a self-replicating state by cytokines and neurotrophic factors. In the present study, we investigated the role of the delta-opioidergic system in adult neurogenesis. We found a dramatic increase of neural progenitor cells in the hippocampal dentate gyrus of mice that were chronically treated with the selective delta-opioid receptor agonist. In addition, we showed that enriched environment-induced hippocampal neurogenesis was inhibited by treatment with NTI. These findings suggest that the endogenous delta-opioidergic system could facilitate hippocampal neurogenesis. We are currently investigating the possible involvement of delta-opioid systems in the migration of immature neuronal cells in the brain.

38. DIFFERENTIAL EFFECTS OF MORPHINE AND METHAMPHETAMINE ON THE ACTIVATION OF MESOLIMBIC AND NIGROSTRIATAL DOPAMINERGIC SYSTEMS.

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Tuesday 4.45 – 5.45pm

Methamphetamine and morphine induce several behavioral changes mediated through the activation of dopaminergic system; high doses of methamphetamine induce a decrease of locomotor activity accompanied by stereotyped behavior in rats and mice, whereas high doses of morphine induce hyperlocomotion in mice and hypolocomotion in rats. We hypothesized here that distinct phenotype of behavior induced by methamphetamine and morphine would result from differential activation for the mesolimbic and nigrostriatal dopaminergic systems. Therefore, we firstly measured dopamine release from nucleus accumbens and striatum simultaneously induced by methamphetamine or morphine using in vivo microdialysis in rats. In the present study, methamphetamine potently increased the release of dopamine from both nucleus accumbens and striatum, whereas morphine increased the release of dopamine from only nucleus accumbens. It is known that inhibition of GABA release into the ventral tegmental area, which is the cell body of the mesolimbic

dopaminergic system, is critically involved in the release of dopamine from the nucleus accumbens by morphine. We next examined the release of GABA into the ventral tegmental area and substantial nigra compacta, the cell body of nigrostriatal dopaminergic system by morphine using microdialysis. As we expected, inhibition of GABA release induced by morphine was only observed in the ventral tegmental area, but not in the substantial nigra compacta. Furthermore, the ratio between μ -opioid receptors vs. GAD1/2 mRNA was significantly higher in the ventral tegmental area than those in the substantial nigra compacta. These differences may influence the differential releasing pattern of dopamine from the nucleus accumbens and striatum by morphine. Furthermore, differential effects of morphine and methamphetamine on the activation of mesolimbic and nigrostriatal dopaminergic systems result in the induction of differential phenotype of behavior in rodents.

39. INVOLVEMENT OF SIGMA-I RECEPTOR CHAPERONE ON THE EXPRESSION OF WITHDRAWAL SIGNS IN MORPHINE-DEPENDENT MICE

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Monday 4.00 – 5.00pm

It is known that withdrawal syndromes after the cessation of μ -opioid receptor agonists remains an obstacle in the clinical treatment of pain, and free radical formation is taking a part in the expression of withdrawal signs in morphine-dependent mice. Recent findings suggest that sigma-1 receptor chaperone has a prominent role to maintain the homeostasis against several stress including oxidative stress by quenching effect for free radical. Therefore, the present study was designed to investigate the involvement of sigma-1 receptor chaperone as well as free radical on the expression of withdrawal signs in morphine-dependent mice. Morphine was injected by slow release emulsion, and withdrawal signs were precipitated by the injection of naloxone 1 day after the slow release emulsion administration of morphine. Withdrawal signs precipitated by naloxone in morphine-dependent mice, especially body-weight loss and diarrhea, were almost completely suppressed by the sigma-1 receptor antagonist NE100 and the free radical scavenger fullerene. Furthermore, we also found that protein level of sigma-1 receptor chaperone were potently upregulated in the ascending colon. These results suggest that sigma-1 receptor chaperone as well as formation of free radical plays a significant role in the expression of withdrawal signs in morphine-dependent mice. Previous finding showed that 5-HT plays a role in the diarrhea during the withdrawal state in morphine-dependent rats. Therefore, we next examined the interaction between 5-HT and free radical or sigma-1 receptor chaperone. In the present study, 5-HT-induced diarrhea was suppressed by fullerene, however NE100 did not affect 5-HT-induced diarrhea. These findings indicate that free radical scavenger and sigma-1 receptor antagonist could independently suppress withdrawal diarrhea in morphine-dependent rodents.

40. OPIOIDERGIC MECHANISM IS LOCALIZED IN THE DOWNSTREAM OF $\alpha 7$ NICOTINIC ACETYLCHOLINE RECEPTOR, BUT NOT $\alpha 4\beta 2$, IN THE CENTRAL NERVOUS SYSTEM

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Tuesday 4.45 – 5.45pm

Nicotine is a neuronal stimulant and produces various pharmacological effects through the activation of nicotinic acetylcholine receptors (nAChRs) in the central nervous system; for example, antinociception and serum corticosterone (SCS) increase. Nicotine also has a physical dependence liability. And there is a growing evidence suggesting that nicotine effects, at least in part, elicit through the activation of opioidergic system. On the other hand, it is well-known that two nAChR subtypes, i.e., $\alpha 7$ and $\alpha 4\beta 2$ nAChRs, are expressed in the central nervous system. The aim of this experiment is to clarify the nAChR subtype, which was coupled with opioidergic system. Nicotine-induced antinociception was evaluated by tail-pinch test, and SCS level was quantified by fluorometrical assay, using ICR male mice. For the estimation of nicotine withdrawal, we evaluated SCS increase as an indicator, because we have reported that the magnitude of SCS increase is an excellent quantitative indicator of morphine withdrawal. Nicotine-induced antinociception is reduced by opioid receptor antagonist naloxone, while nicotine-induced SCS increase is not suppressed by naloxone, indicating that opioidergic system might participate in the nicotine-induced antinociception, but not SCS increase. Nicotine-induced antinociception was reduced by both $\alpha 4\beta 2$ nAChR antagonist (dihydro- β -erythroidine; DH- β -E) and $\alpha 7$ nAChR antagonist (methyllycaconitine; MLA), while nicotine-induced SCS increase was suppressed by DH- β -E, but not MLA. Furthermore, naloxone (0.01-5 mg/kg) elicited SCS elevation in mice receiving repeated nicotine (3 mg/kg, twice a day for 7 days) in a dose-dependent manner, and the naloxone-induced SCS increase was correlated with the doses (1-5 mg/kg, twice a day for 9 days) and the days (3 mg/kg, twice a day for 3-9 days) of repeated nicotine. When opioid antagonist, naltrexone (1-10 mg/kg), was administered together with repeated nicotine (5 mg/kg, s.c., twice a day for 5 days), naloxone-induced SCS increase was significantly suppressed, indicating that opioidergic system might participate in the development of physical dependence on nicotine. Concomitant administration of MLA with repeated nicotine, but not DH- β -E, suppressed the SCS increase by naloxone. These results suggest that opioidergic mechanism might be located on the downstream of $\alpha 7$ nAChR, but not $\alpha 4\beta 2$, in the central nervous system.

41. NOVEL KAPPA OPIOID RECEPTOR ACTIVATING SALVINORIN A ANALOGUES MOM SAL B AND EOM SAL B ATTENUATE DRUG-SEEKING BEHAVIOURS IN THE RAT AND MODULATE DOPAMINE TRANSPORTER FUNCTION

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Monday 4.00 – 5.00pm

Activation of kappa-opioid receptors (KOPr) by Salvinorin A (Sal A) attenuates cocaine prime-induced drug-seeking in rats with fewer side effects compared to traditional arylacetamide KOPr agonists. In the present study, we investigate the behavioural

anti-addiction effects and side effect profile of two potent, longer acting, Sal A analogues, 2-methoxy-methyl Salvinorin B (MOM Sal B), and ethoxymethyl salvinorin B (EOM Sal B) and evaluate their ability to modulate the function of the dopamine transporter (DAT). Our aim is to identify novel anti-addiction compounds with reduced side effects and identify their mechanism of action. Both MOM Sal B and EOM Sal B significantly attenuate cocaine prime-induced drug-seeking behaviour in cocaine self-administering rats at a dose of 0.3 mg/kg. However, increased immobility and decreased swimming in the forced swim test indicates depressive side effects similar to those seen in the parent compound, Sal A. MOM Sal B rapidly increases DAT function in tissue taken from the rat striatum, nucleus accumbens and medial prefrontal cortex using rotating disk electrode voltametry techniques. The dopamine uptake kinetics in the nucleus accumbens showed a significant increase in V_{max} (1592 ± 176) with no change in K_m (1.6 ± 0.4) compared to controls (V_{max} (1026 ± 181) and K_m (1.5 ± 0.6) ($p < 0.05$)), indicating an increase in the number of functional transporters with no change in dopamine binding affinity. The effects on DAT function were reversed by pre-incubation with KOPr antagonist nor-BNI. Both MOM Sal B ($p < 0.01$) and EOM Sal B ($p < 0.05$) rapidly phosphorylate ERK in vitro in a KOPr-dependent manner using phospho specific antibodies and Western blotting techniques. Inhibition of ERK activation by U0126, a MEK inhibitor also prevented the KOPr mediated increases in DAT function. This study shows two novel potent Sal A analogues that have behavioural anti-addiction effects and modulate DAT function in a KOPr and ERK dependent manner.

Supported by Health Research Council of New Zealand, Neurological Foundation of New Zealand, and Wellington Medical Research Foundation.

42. MICROGLIAL ACTIVATION PRECEDES ANTI-OPIOID SYSTEM IN MORPHINE ANALGESIC TOLERANCE

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Tuesday 4.45 – 5.45pm

It is well known that the analgesic tolerance and dependence caused by chronic morphine treatment are associated with neural plasticity at the cellular, synaptic and neural circuit levels in the central nervous system. However, little is known of the plasticity mechanisms in terms of neural circuit. Our previous studies demonstrated that NMDA receptor and brain-derived neurotrophic factor (BDNF) play key roles in the development of morphine analgesic tolerance. In addition to these mechanisms, we firstly reported that morphine induces the activation of cultured microglia in terms of morphological change and gene expression of BDNF, which is associated with NMDA. In the present study, we attempted to clarify whether all these three components are all involved, if they work, which precedes other machineries. When mice are given with chronic morphine for 6 days, microglial activation was only observed at the early stage. The injection of minocycline to inhibit microglial activation or liposome-encapsulated chondronate to disrupt activated microglia abolished morphine analgesic tolerance. Although, the up-regulation of BDNF and NMDA receptor subunit, NR2A in western blot were observed in the PAG, which is the major brain region involved in morphine analgesia, the treatment with minocycline and the injection of BDNF antibody abolished BDNF and NR2A expression in PAG. Thus, these results suggest that microglial activation, BDNF release and NMDA receptor play pivotal roles in morphine analgesic tolerance via anti-opioid machineries.

43. EFFECTIVENESS OF AMIDINO-TAPA AGAINST MORPHINE-RESISTANT NEUROPATHIC PAIN

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Monday 4.00 – 5.00pm

Amidino-TAPA is a peptidic analgesic, which shows high affinity and selectivity for μ -opioid receptors and produces extremely potent and longer lasting antinociception. Unlike traditional narcotic analgesics, the spinal antinociception of amidino-TAPA is mediated through the activation of distinct μ -opioid receptors, MOR-1J, MOR-1K and MOR-1L, which are amidino-TAPA-sensitive but morphine-insensitive splice variants. Most notable character of amidino-TAPA is its effectiveness against morphine-resistant neuropathic pain. In the present study, the antinociceptive mechanism of amidino-TAPA against the neuropathic pain was investigated.

The neuropathic pain model was developed in mice according to the method by Seltzer and his colleagues with minor modification. The antinociceptive effects of morphine and amidino-TAPA were measured using the von Frey filament at 7 days after the nerve ligation. As reported well, the spinal antinociception of morphine was markedly suppressed in ipsilateral paw in compared in contralateral paw. In contrast, amidino-TAPA showed same magnitude of spinal antinociception in both paws. In mice the μ -opioid receptor splice variants containing exon-1 of MOR-1 gene had been knocked down, the spinal antinociception induced by amidino-TAPA was attenuated in both paws with the same magnitude. In contrast, the spinal antinociception induced by amidino-TAPA was more strongly attenuated in ipsilateral paw than in contralateral paw under the condition that MOR-1J, MOR-1K or MOR-1L had been knocked down. On the other hand, the spinal antinociception of morphine in sham-operated mouse was attenuated under the condition which exon-1 containing splice variants, but not MOR-1J, MOR-1K or MOR-1L, had been knocked down. After the sciatic nerve ligation, mRNA expression of exon-1-containing splice variants, but not MOR-1K or MOR-1L, were markedly reduced in DRG of ipsilateral side in compared in DRG of contralateral side. On the contrary, mRNA expression of any splice variants in spinal cord was not affected after the sciatic nerve ligation.

In conclusion, the reduction of spinal antinociception of morphine after sciatic nerve ligation may be caused by the reduction of the morphine-sensitive splice variants in DRG. On the contrary, the spinal antinociception of amidino-TAPA is maintained after the sciatic nerve ligation, since amidino-TAPA-sensitive splice variants in DRG are maintained.

44. VOLUNTARY ALCOHOL INTAKE AND CBI AND MOP RECEPTOR DENSITY IN OUTBRED WISTAR RATS

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Tuesday 4.45 – 5.45pm

Alcohol use disorders (AUD) are heterogeneous with regard to etiology, liability for addiction and response to treatment. Individual vulnerability is dependent on a complex interaction between different neurotransmitter systems. The endocannabinoid and opioid systems are implicated in AUD. Antagonists to the CBI receptor, e.g. rimonabant, as well as the MOP receptor, e.g. naltrexone, decreases voluntary alcohol intake, and alcohol consumption modulates receptor density and receptor-mediated signalling.

The aim of the present study was to investigate a possible association between behavioural characteristics, voluntary alcohol intake and alcohol-induced effects on CBI and MOP receptor density.

Forty adult male outbred Wistar rats were tested in the open field and the multivariate concentric square field™ tests prior to alcohol access. The rats were randomly assigned into water or alcohol groups. The animals had access to 20% ethanol solution using a two-bottle binge-like free-choice paradigm, with 24 hour access for three consecutive days per week for seven weeks. Ten to 11 days after the final alcohol session the animals were decapitated, the brains were immediately frozen and later sectioned into 12 mm coronal sections. The CBI ligand [3H] SRI141176 and the MOP ligand [3H] DAMGO were used in an autoradiography method followed by densitometric measurements.

During the last week of access to alcohol the average (\pm SEM) ethanol intake was 3.3 ± 0.2 g/kg. The alcohol-drinking rats had up-regulated CBI receptor density in cingulate and motor cortex and also up-regulated MOP receptors in the posteromedial cortical amygdaloid nucleus relative to water-drinking rats. In the central and medial amygdaloid nucleus, alcohol consumption resulted in down-regulation of CBI receptor density. MOP receptor density was down-regulated in the globus pallidus, lateral septal nucleus and the nucleus accumbens compared to the water-drinking controls. These differences were revealed after the 11-day time interval between the last alcohol session and decapitation. The results reveal long-lasting alterations in areas of relevance for risk-taking behaviour, reinforcement and addiction processes.

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45. INVOLVEMENT OF THE LONG-CHAIN FATTY ACID RECEPTOR GPR40 IN CFA-INDUCED INFLAMMATORY PAIN MODEL MICE

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Monday 4.00 – 5.00pm

GPR40 has been reported to be activated by long-chain fatty acids, such as docosahexaenoic acid (DHA). However, reports studying functional role of GPR40 in the brain are lacking. The present study focused on the relationship between pain regulation and GPR40, investigating the functional roles of brain GPR40 during chronic pain caused using a Complete Freund's Adjuvant (CFA)-induced inflammatory pain mouse model. Long-lasting hyperplasia of paw, persistent mechanical allodynia and thermal hyperalgesia were elicited in CFA-treated mice. Under these conditions, GPR40 protein expression in the hypothalamus and medulla oblongata was increased at 7 days after CFA injection, in comparison with the saline control group, but not at 1 or 3 days. GPR40 was colocalized with NeuN, a neuron marker, but not with GFAP, an astrocyte marker. GFAP protein expression was markedly increased in the hypothalamus at 1 day after CFA injection. This increase was significantly inhibited by the intracerebroventricular injection of flavopiridol (5 nmol), a cyclin-dependent kinase inhibitor, depending on the decreases in both the increment of GPR40 protein expression and the induction of mechanical allodynia at 7 days after CFA injection. The i.c.v. injection of DHA (50 µg) and GW9508 (1.0 µg), a GPR40-selective agonist, significantly reduced mechanical allodynia at 7 days after CFA injection. These effects were inhibited by i.c.v. pretreatment with GW1100 (10 µg), a GPR40 antagonist. These findings suggested that brain GPR40 might have had an important role in this pain control system. Furthermore, astrocytes might have contributed to the observed regulation of GPR40 protein expression.

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46. G PROTEIN-GATED INWARDLY RECTIFYING POTASSIUM (GIRK) CHANNELS PLAY A PRIMARY ROLE IN THE ANTINOCICEPTIVE EFFECT OF OXYCODONE, BUT NOT MORPHINE AND FENTANYL, AT SUPRASPINAL SITES.

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Tuesday 4.45 – 5.45pm

Oxycodone, morphine, and fentanyl are mu-opioid receptor (MOR) agonists prescribed to control moderate to severe pain. Previous studies suggested that these opioids exhibit different analgesic profiles. We therefore hypothesized that distinct mechanisms mediate the differential effects of these opioids and investigated the role of G protein-gated inwardly rectifying potassium (GIRK) channels in their supra-spinal and spinal antinociceptive effects. The antinociceptive effects of oxycodone with both i.c.v. (0.3-10 nmol)

and i.t. (1-30 nmol) administration were markedly and similarly attenuated by tertiapin-Q (30 pmol, i.c.v. and i.t.), a selective GIRK channel inhibitor, in mice tail-flick test. In contrast, i.c.v. morphine (0.1-3 nmol) and fentanyl (0.03-1.7 nmol) administration was insensitive to GIRK channel inhibition, while i.t. morphine (0.1-3 nmol) and fentanyl (0.03-1.7 nmol) administration was markedly attenuated. Using bone cancer pain model, we further examined the antinociceptive effect of these opioids. Antinociceptive effect of subcutaneous administration of oxycodone (5.6 mg/kg), but not that of morphine (30 mg/kg) and fentanyl (0.1 mg/kg), was strongly attenuated in the presence of tertiapin-Q (30 pmol, i.c.v.), indicating the importance of GIRK channel function in the antinociceptive effect in an opioid-dependent manner. These results demonstrated that GIRK channels play a primary role in the antinociceptive effects of oxycodone, but not morphine and fentanyl, at supraspinal sites, and suggested that supraspinal GIRK channels are responsible for the unique analgesic profile of oxycodone.

47. HOUSING CONDITIONS AFFECT BASAL AND ALCOHOL-INDUCED OPIOID LEVELS IN ADOLESCENT RATS

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Monday 4.00 – 5.00pm

The opioid system is involved in alcohol-induced reward and addiction but our knowledge of alcohol-induced effects on opioids before and after adolescent drinking is sparse. Many of the animal models of voluntary intake are based on single housing, but for a social animal such as a rat this is stressful, even more so in adolescent animals. In the current study we therefore address the issue of housing conditions, age and time of exposure to alcohol and their combined effects on opioid peptide levels in the rat brain.

Three groups of rats were used: 4 and 10 week old rats exposed once to alcohol and a third group of 4 week old rats exposed to alcohol for six weeks in a voluntary binge drinking model with sessions three times a week. All alcohol-drinking animals were single housed. Control groups were age-matched water drinkers that were single or group housed. Brain structures and the pituitary gland were dissected and the immunoreactive levels of the opioid peptides dynorphin B (DYNB), met-enkephalin-Arg6Phe7 (MEAP) and beta-endorphin (BEND) were analysed using radioimmunoassay.

Not surprisingly, extensive age differences were found. Housing conditions were important for MEAP and DYNB in all ages, but the structures affected differed between ages. Interactions between housing condition and age were found to affect DYNB in the medial prefrontal cortex, striatum and hippocampus. Several alcohol-induced effects were found, but there were few differences between long-term drinking and an acute session. However, MEAP in the hypothalamus, medial prefrontal cortex and hippocampus responded oppositely to acute or long-term exposure.

The results show that housing conditions affect opioid levels to a large extent and that these effects can be different depending on age and duration. These effects can in turn affect the alcohol-induced changes. Housing conditions can therefore be an important confounding factor, but is often neglected in the literature. In light of the importance of social interactions during adolescence, affordable models, in which both social conditions and alcohol-induced effects can be studied, need to be developed and evaluated.

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48. THE INTERACTION OF SEVERAL ANTIDEPRESSANT DRUGS WITH ACUTE AND CHRONIC METHADONE IN MICE AND POSSIBLE CLINICAL IMPLICATIONS

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Tuesday 4.45 – 5.45pm

Background: Depression is highly prevalent among chronic-pain patients and methadone maintenance treatment (MMT) patients. Controversy regarding effective antidepressant treatment for these patients persists.

Objective: Assessing the two models in mice, using the hotplate assay.

Methods: In the model of depressed pain patients, the impact of low, sub-threshold doses of 6 antidepressants with different mechanisms of action (fluvoxamine, escitalopram, reboxetine, venlafaxine, desipramine, clomipramine) on the antinociceptive properties of a single (acute) dose of methadone. In the model of depressed MMT patients, the impact of low, sub-threshold doses of 3 antidepressant drugs with different mechanisms of action (escitalopram, desipramine, clomipramine) on the antinociceptive properties of chronic treatment with methadone.

Results: Following injection, acute methadone elicited analgesia in a dose-dependent manner. Fluvoxamine and desipramine, each at a sub-threshold dose induced a synergistic effect with methadone. Escitalopram, reboxetine, venlafaxine and clomipramine given separately at a sub threshold dose induced no interaction. Following two weeks of methadone administration (through an implanted mini-pump, i.p.) injection of escitalopram did not elicit any analgesic effect, desipramine augmented the analgesic effect of methadone, while clomipramine reduced it notably.

Conclusion: Possible clinical implications are that while escitalopram, reboxetine, venlafaxine and clomipramine do not affect acute methadone's antinociception in mice and are safe to be given together with methadone, fluvoxamine and desipramine notably augment methadone induced antinociceptive effects and should be avoided due to the risk of inducing opiate-overdose. Possible clinical implications of chronic methadone remain to be determined as tricyclic antidepressants' cardiac effects may prevent their use in MMT patients.

49. THE PERIAQUEDUCTAL GRAY CONTRIBUTES TO OXYCODONE, BUT NOT METHADONE ANTINOCICEPTION IN THE RAT

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Monday 4.00 – 5.00pm

Studies examining ligand-biased signaling demonstrate that opioid efficacy and tolerance can vary greatly depending on the opioid (Madia et al., 2009). Given that opioids such as morphine inhibit pain, in part, by binding to mu-opioid (MOP) receptors in the periaqueductal gray (PAG), microinjection of opioids directly into the PAG provides an opportunity to link ligand-biased signaling at the MOP receptor to antinociception. Previous research has shown that administration of morphine, fentanyl, DAMGO, dermorphin, or β -endorphin into the PAG produces antinociception (Bobeck et al., 2009; Bodnar et al., 1988; Macey et al., 2010). Although it is assumed that microinjection of any MOP receptor agonist into the PAG would produce antinociception, this hypothesis has not been tested. The present study tested this hypothesis by measuring antinociception and tolerance to PAG microinjection of the commonly used MOP receptor agonists' oxycodone and methadone. Male Sprague-Dawley rats were implanted with a guide cannula aimed at the ventrolateral PAG and allowed 1 week to recover before behavioral testing. Microinjection of $1/4$ log cumulative doses of oxycodone into the PAG produced a dose-dependent inhibition of nociception ($ED_{50} = 191 \mu\text{g}$) measured with the hot plate test. Analysis of the time course for this antinociception revealed a peak effect at 5 min and duration of approximately 30 min. Twice daily injections of oxycodone into the PAG for 2 days did not alter the oxycodone dose-response curve measured on Day 3, indicating a lack of tolerance to repeated oxycodone injections. In contrast to oxycodone, there was no dose (1.0, 5.6, 10, 56, 100 $\mu\text{g}/0.4 \mu\text{L}$) at which microinjection of methadone into the PAG produced antinociception. These data demonstrate that the antinociception produced by activation of MOP receptors in the ventrolateral PAG are engaged by some, but not all MOP receptor agonists. Moreover, previous studies showing tolerance to morphine or fentanyl microinjections into the ventrolateral PAG (Bobeck et al., 2012) are in direct contrast to oxycodone; which does not appear to produce tolerance with repeated injections into the PAG.

This study was supported by NIH grant DA015498.

50. INCREASED REGIONAL GRAY MATTER VOLUME IN μ -OPIOID RECEPTOR KNOCKOUT MICE AS DETERMINED BY MRI-VOXEL-BASED MORPHOMETRY

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Tuesday 4.45 – 5.45pm

μ -opioid receptor knockout (MOR-KO) mice has revealed the central role of MORs in various behavioral functions including not only analgesic effects but responses to stress, reward, tolerance compared with wild type (WT) mice. However, it remains to be completely elucidated how μ -opioid receptors expressed in neural circuits are related with particular behavioral functions in these mice, and one possibility is that their reduced MOR expression leads to changes in neural circuitry. In this study, we have examined the brains of MOR-KO mice to determine the volumetric changes associated with MOR deletion. We scanned whole brain of 45 MOR-KO and 45 WT mice at 12 weeks of age using high resolution, three-dimensional magnetic resonance T2 imaging MRI for automated voxel-based morphometry (VBM) analysis. Our findings were as follows: (1) MOR-KO mice had increased intracranial gray matter volume compared to WT mice; (2) significant regional volumetric changes were detected in periaqueductal gray (PAG), hypothalamus, olfactory bulb and cerebellum; (3) the greatest brain volume differences were located approximately in ventrolateral PAG, arcuate nucleus of hypothalamus, glomerular layer of olfactory bulb, copula of pyramis, paramedian lobule of cerebellum, and cerebellar lobule. These findings suggest that factors other than elimination of MOR alone may account for some of the behavioral differences observed in these mice, and that levels of MOR expression may influence a broader range of neural function in humans. Although the precise relationship between volumetric changes and MOR deletion could not be determined based on this study alone, our findings suggest that morphological changes detected in MOR-KO mice by MRI-VBM methods may relate to some of the behavioral differences observed in MOR-KO mice.

51. PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL REGULATION OF IL-6 EXPRESSION BY NOCICEPTIN/ORPHANIN FQ (N/OFQ) PEPTIDE RECEPTOR

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Monday 4.00 – 5.00pm

The neuropeptide, N/OFQ, has immunomodulatory actions in addition to modulating nociception, anxiety and many other responses. Expression of Interleukin-6 (IL-6), a cytokine linked to inflammation and pain, is modulated by N/OFQ-N/OFQ peptide (NOP) receptor signaling at the mRNA and protein levels. To better understand how N/OFQ modulates IL-6, IL-6 mRNA levels in U937 human monocyte cells were assessed by quantitative PCR (qPCR). Time course studies revealed that N/OFQ initially increased IL-6 mRNA at 15 min, but decreased IL-6 mRNA over the next 4 hr. Pretreatment with a NOP receptor antagonist blocked the N/OFQ-induced increase in IL-6 mRNA at 15 min, with no effect on the subsequent reduction in IL-6 at 4 hr. N/OFQ-induced up-regulation of IL-6 mRNA also was blocked by inhibitors of NF κ B and Akt signaling, but not by inhibitors of PKC, p38 or ERK MAP kinase; suggesting potential mechanisms by which N/OFQ increases IL-6. We previously reported that Single Prolonged Stress (SPS), a PTSD model, produced hyperalgesia and allodynia, and increased N/OFQ levels in serum, CSF and brain. To explore potential relationships between N/OFQ, cytokines and PTSD-related pain, we assessed IL-6 mRNA and protein levels in the spinal cord dorsal horn of male Sprague-Dawley rats exposed to SPS and matching controls by qPCR, multiplex cytokine analysis and immunolabeling. SPS increased IL-6 mRNA in spinal cord and brain between days 14-28 days, but IL-6 levels in sera were unchanged. Preliminary studies indicate that treatment of SPS rats with a NOP receptor antagonist from day 7-21 blocked IL-6 protein up-regulation in spinal cord at day 21 and reversed hyperalgesia and allodynia, suggesting that N/OFQ mediates IL-6 up-regulation during chronic stress and that this up-regulation may contribute to allodynia and hyperalgesia in the PTSD model.

Animal protocol was approved by IACUC and the US Army Medical Research and Materiel Command Animal Care and Use Review Office, complied with the Animal Welfare Act and adhered to the principles described in the Guide for Care and Use of Laboratory Animals.

52. INVOLVEMENT OF μ - AND δ -OPIOID RECEPTOR FUNCTION IN THE REWARDING EFFECT OF (\pm)-PENTAZOCINE

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Tuesday 4.45 – 5.45pm

Most opioid receptor agonists have abuse potential, and the rewarding effects of opioids can be reduced in the presence of pain. While each of the enantiomers of pentazocine has a differential pharmacological profile, (\pm)-pentazocine has been used clinically for the treatment of pain. However, little information is available regarding underlying mechanisms of rewarding effects of (\pm)-pentazocine, and whether the (\pm)-pentazocine-induced rewarding effects can be suppressed under pain. Therefore, the present study was performed to investigate the effects of pain on the acquisition of the rewarding effects of (\pm)-pentazocine, and to examine the mechanism of the rewarding effects of (\pm)-pentazocine using the conditioned place preference paradigm. (\pm)-Pentazocine and (-)-pentazocine, but not (+)-pentazocine, produced significant rewarding effects. Even though the rewarding effects induced by (\pm)-pentazocine were significantly suppressed under pain induced by formalin, a high dose of (\pm)-pentazocine produced significant rewarding effects under pain. In the normal condition, (\pm)-pentazocine-induced rewarding effects were blocked by a low dose of naloxone, whereas the rewarding effects induced by high doses of pentazocine under pain were suppressed by naltrindole (a δ -opioid receptor antagonist). Interestingly, (\pm)-pentazocine did not significantly affect dopamine levels in the nucleus accumbens. These findings suggest that the rewarding effects of (-)-pentazocine may contribute to the abuse potential of (\pm)-pentazocine through μ - as well as δ -opioid receptors, which depend on the pain situation, without robust activation of the mesolimbic dopaminergic system.

53. NALTREXONE ON TREATMENT OF NEUROPATHIC PAIN IN MICE LOCALLY TRANSFECTED WITH THE MUTANT MU-OPIOID RECEPTOR GENE IN SPINAL CORD

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Monday 4.00 – 5.00pm

The opioid antagonists, such as naloxone and naltrexone, exhibited agonistic properties at the mutated μ -opioid receptor, MORS196ACSTA, in which the conserved Ser196, Thr327, and Cys330 were mutated to Ala, Ala, and Ser, respectively. In our previous study, systemic naloxone (10 mg/kg, s.c.) elicited antinociceptive effect (determined by tail-flick test) without the induction of tolerance, dependence, and rewarding effect in mice 2 weeks after intrathecal administration of dsAAV2-MORS196ACSTA-EGFP. In the present study, we further investigated whether this antinociceptive paradigm could be effective in mice with neuropathic pain. The spinal nerve ligation (SNL) surgery was performed on mice 3 or 4 weeks after intrathecal injection of the lentivirus which carried the gene of MORS196ACSTA-EGFP. The von Frey tests were used to detect the anti-allodynic effects of systemic morphine or naltrexone

before and after gene transfection. After 14 days of naltrexone or morphine treatment (10 mg/kg, s.c., q.d.) from day 1 or day 6 after surgery, the natural withdrawal signs were counted at 22 and 46 hours after the last drug injection. Our data have shown that the ipsilateral paw withdrawal pressure was significantly decreased one day after SNL surgery and persisted at least for 19 days. Naltrexone (10 mg/kg, s.c.) or morphine (10 mg/kg, s.c.) elicited significant anti-allodynia effects. The paw withdrawal pressure after naltrexone or morphine treatment was significantly increased when compared to saline treatment. The SNL-induced allodynia was improved gradually and almost back to normal after chronic naltrexone or morphine treatment on day 19. However, chronic treatment of morphine (10 mg/kg, s.c., q.d.) but not naltrexone (10 mg/kg, s.c., q.d.) induced natural withdrawal signs. These data imply that systemic injection of naltrexone may have therapeutic potential for chronic neuropathic pain without the development of dependence after the gene therapy with the expression of MORS196ACSTA in the spinal cord. (Supported by a grant from National Science Council, Taiwan, ROC).

54. ESTABLISHMENT OF A CENTRAL POST-STROKE PAIN MODEL USING GLOBAL CEREBRAL ISCHAEMIC MICE

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Tuesday 4.45 – 5.45pm

Stroke is the leading cause of disability in the world. Central post-stroke pain (CPSP), an intractable secondary disease, is a serious problem that occurs following cerebral stroke. However, the detailed mechanisms underlying CPSP and standard treatments for it are not well established. Therefore, we examined the nociceptive threshold and alterations in the current stimulus threshold of primary afferent neurons in bilateral carotid artery occlusion (BCAO) and middle cerebral artery occlusion (MCAO) mice. Male ddY mice were subjected to 30 min of BCAO or 2h of MCAO. The development of mechanical and thermal hyperalgesia and changes in current stimulus threshold in the hind paws were measured after BCAO or MCAO using the von Frey test, plantar test and a Neurometer, respectively. The threshold for mechanical and thermal hyperalgesia in both hind paws was significantly decreased on day 3 after BCAO as compared with pre-BCAO treatment. Furthermore, the sensitivity of C and A β fibers (at stimulation of 5 and 2000 Hz, respectively) was increased on day 3 after BCAO as compared with pre-BCAO treatment, while that of A δ fibers was not altered. On the other hand, in MCAO mice, the sensitivity of A δ and A β fibers (at 2000 and 250 Hz stimulation, respectively) was significantly increased on day 3 after MCAO, while that of C fibers (at 5 Hz stimulation) was unaltered. In addition, the paw withdrawal threshold of the left hind paw as measured by the von Frey filament test was significantly decreased on day 1 and day 3 after MCAO when compared with day 0, while that in the right hind paw was not different. Our data show the development of bilateral hyperalgesia in these both models. Potentially, in BCAO model, C and A β fiber-specific hypersensitization after stroke may have contributed to these symptoms. In MCAO model, myelinated a fiber-specific hypersensitization after stroke may have contributed to these symptoms.

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55. EGFP-NOP MICEToll, L.¹, Wu, J.¹, Mercatelli, D.¹, Kieffer, B.²¹ Torrey Pines Institute for Molecular Studies, Port St. Lucie, FL, USA² Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France**Monday 4.00 – 5.00pm**

The NOP receptor is the fourth member of the opioid receptor family, and the cognate receptor for nociceptin/orphanin FQ, now called N/OFQ. NOP receptors and N/OFQ are found throughout the brain, in the spinal cord, dorsal root ganglia, and a variety of peripheral tissues. To better characterize the localization and internalization properties of the NOP receptor, we created mice where the NOP receptor is replaced by an active EGFP-NOP receptor fusion protein, similar to what has been previously reported for an EGFP-delta opioid receptor. Binding studies with [³H]N/OFQ have determined that homozygous and heterozygous EGFP-NOP receptor containing mice have a similar receptor number in brain to wild type litter mates, and that N/OFQ and a variety of selective and non-selective ligands have similar binding affinities to the mutant and w.t. receptor. EGFP-NOP receptor are functional in the knock-in mice, as determined by [³⁵S]GTP γ S binding, with NOP agonists having similar potency in the knock-in and w.t. mice. The anatomic distribution of the EGFP-NOP receptor was studied by immunohistochemical localization using EGFP antibodies. Receptor localization was similar to what has been reported from in vitro autoradiographic studies. Studies examining N/OFQ-mediated receptor internalization in primary hippocampal cultures are ongoing. Direct visualization of the NOP receptor will be a useful tool in understanding receptor localization, receptor trafficking, and the co-localization of NOP and mu opioid receptors.

56. SYNERGISM BETWEEN THE DELTA-OPIOID AGONIST SNC80 AND AMPHETAMINE OCCURS VIA A GLUTAMATERGIC NMDA-RECEPTOR DEPENDENT MECHANISMBosse, K.E.¹, Jutkiewicz, E.M.¹, Mabrouk, O.S.², Schultz, K.N.², Kennedy, R.T.², Gnegy, M.E.¹ and *Traynor, J.R.¹¹ Department of Pharmacology and (2) Department of Chemistry, University of Michigan Ann Arbor, MI, 48109, USA.**Tuesday 4.45 – 5.45pm**

Delta opioid receptor (DOR) agonists have stimulant-like properties. Thus, DOR agonists increase locomotor activity, produce conditioned place preference in rodents, and generalize to the discriminative stimulus effects of stimulants in rats and monkeys. Despite these properties, the nonpeptidic DOR agonist SNC80 is not self-administered. Furthermore, SNC80 fails to promote dopamine efflux from rat striatum and does not increase extracellular dopamine levels in the caudate putamen or nucleus accumbens following systemic administration. Thus, SNC80 likely modulates the dopaminergic system indirectly through other mechanisms or signaling pathways. Glutamate is known to cause the release of dopamine through a Ca²⁺-sensitive mechanism that involves activation of N-methyl-D-aspartate (NMDA) receptors. Here we examined the hypothesis that SNC80 acts on the glutamatergic system to enhance both amphetamine-stimulated dopamine efflux and amphetamine-stimulated locomotor activity. In rat striatal slices SNC80 increased extracellular glutamate levels and decreased GABA levels. Inhibition of NMDA signaling with the selective antagonist MK801 blocked the SNC80-mediated

enhancement of both amphetamine-induced dopamine efflux and locomotor activity. Addition of exogenous glutamate potentiated amphetamine-stimulated dopamine efflux in a Mg²⁺- and MK801-sensitive manner. In the absence of Mg²⁺ SNC80 alone was able to cause dopamine efflux and also produced a greater enhancement of amphetamine-evoked dopamine efflux. Since the majority of DORs are located on inhibitory GABA terminals, we hypothesized that DOR activation inhibits GABA release, thus disinhibiting glutamate efflux to ultimately promote amphetamine-stimulated dopamine release. Perfusion of rat striatal slices with the GABAB receptor antagonist 2-hydroxysaclofen increased amphetamine-stimulated dopamine efflux to a similar extent as SNC80. Overall the results suggest that in the striatum, DOR activation by SNC80 enhances glutamate neurotransmission, most likely through GABA disinhibition, to regulate amphetamine-stimulated dopamine efflux and amphetamine-induced behaviors, primarily through activation of NMDA receptors. Supported by R01 MH083754, R01 DA11697 and F31 DA019728.

57. INVOLVEMENT OF SUPRASPINAL AND PERIPHERAL NALOXONAZINE- INSENSITIVE OPIOID RECEPTOR SITES IN THE EXPRESSION OF μ -OPIOID RECEPTOR AGONIST-INDUCED PHYSICAL DEPENDENCE

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Monday 4.00 – 5.00pm

Withdrawal signs after the cessation of μ -opioid receptor agonists remains an obstacle in the clinical treatment of pain. There is limited information available on the mechanisms that underlie the expression of the withdrawal signs of opioids, and especially regarding the involvement of μ -opioid receptor subtypes and the location of the responsible opioid receptors. Therefore, the present study was designed to determine the mechanism of the expression of withdrawal signs in μ -opioid receptor agonist-dependent mice. Morphine-, oxycodone- and fentanyl-dependent mice showed a marked loss of body-weight and other withdrawal signs after a naloxone challenge. Interestingly, the phenotype of the withdrawal signs for morphine and oxycodone was different from that of fentanyl. Furthermore, pretreatment with naloxonazine did not significantly alter the withdrawal signs precipitated by naloxone in these μ -opioid receptor agonist-dependent mice, whereas the peripherally limited opioid receptor antagonist naloxone methiodide significantly increased the loss of body-weight accompanied by diarrhea, indicating that a peripheral naloxonazine-insensitive site for opioid receptors, as an adaptation mechanism, plays an important role in the expression of at least the loss of body-weight. On the other hand, i.c.v. treatment with naloxone methiodide potently induced a jumping behavior and trembling in morphine-dependent mice. These results indicate that the prolonged activation of supraspinal μ -opioid receptors plays a role in most of the physical dependence induced by μ -opioid receptor agonists in mice. Thus, the withdrawal signs observed after the cessation of μ -opioid receptor agonists are distinctly regulated through supraspinal and peripheral naloxonazine-insensitive sites of μ -opioid receptors.

58. SELF ADMINISTRATION OF OXYCODONE BY ADOLESCENT AND ADULT MICE DIFFERENTIALLY AFFECT STRIATAL NEUROTRANSMITTER RECEPTOR GENE EXPRESSION

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Tuesday 4.45 – 5.45pm

Rationale: Illicit use of prescription opioid analgesics (e.g., oxycodone) in adolescence is a pressing public health issue. Our goal was to determine whether oxycodone self-administration differentially affects striatal neurotransmitter receptor gene expression in the dorsal striatum of adolescent compared to adult C57BL/6j mice.

Methods: Groups of adolescent mice (4 weeks old, n=12) and of adult mice (11 weeks old, n=11) underwent surgery during which a catheter was implanted into their jugular veins. Mice were placed in self-administration chambers for acquisition of oxycodone self-administration or were yoked saline control after recovering from surgery. Mice self-administered oxycodone (0.25mg/kg/infusion) 2 hrs/day for 14 consecutive days. Mice were sacrificed within one hour after the last self-administration session and the dorsal striatum was isolated for mRNA analysis. Gene expression was analyzed with real time PCR using a commercially available PCR array.

Results: We found that adolescent mice self-administered less oxycodone than adult mice over the 14 days. Adolescents and adults differed in monoamine oxidase A (Maoa) and neuropeptide Y receptor 5 mRNA in the absence of oxycodone exposure. 14-day oxycodone self administration increased Maoa mRNA levels compared to controls in both adults and adolescents. Gastrin releasing peptide receptor mRNA showed a significant Drug x Age interaction. More genes in the dorsal striatum of adolescents changed in response to oxycodone self administration compared to controls than in adult mice.

Summary: This study demonstrates for the first time that repeated oxycodone self-administration differentially altered neurotransmitter receptors gene expression in the dorsal striatum of adolescent versus adult mice.

Support: This work was supported by NIH-NIDA 1R01DA029147-01A1 (YZ) and NIH-NIDA DA05130 (MJK)

59. THE EFFECT OF μ-OPIOID RECEPTOR POLYMORPHISMS ON RECEPTOR FUNCTION

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Monday 4.00 – 5.00pm

There is significant variation in individual response to opioid drugs, one cause of which is likely to be polymorphisms on the opioid receptors themselves. The μ-opioid receptor (MOR) is the primary site of action for most analgesic opioids. Previous studies have identified a number of naturally occurring single nucleotide polymorphisms (SNPs) in the coding region of the MOR. The A118G SNP variant, present in various populations at allelic frequencies ranging from 10 – 40%, has been associated with differences in the requirement for post-operative opioid analgesics, and for drug dependence, although these results are not consistent. In vitro, MOR-A118G has been reported to exhibit an altered signalling profile compared with MOR including different β-endorphin potency and alterations in N-type Ca²⁺ channel inhibition, however these findings are also not consistent. Few studies have examined the effect of MOR polymorphisms on receptor function, or potential ligand functional selectivity. We assessed the relative potency and efficacy of a range of opioid ligands in Chinese hamster ovary (CHO) cells stably transfected with human wild type MOR and five naturally occurring MOR variants, including A118G. Receptor expression levels were similar for all mutants examined. MOR mediated adenylyl cyclase (AC) inhibition was measured using a novel, fluorescence based membrane potential assay. Treatment of CHO cells with the AC activator forskolin (FSK) hyperpolarized CHO-MOR cells with a pEC₅₀ of 7.3±0.1 to a maximum of 52±1.7% from baseline. The hyperpolarization induced by FSK (300nM) was inhibited in a dose-dependent manner by the addition of a range of MOR agonists, including DAMGO, morphine, β-endorphin and buprenorphine (n ≥ 5). In MOR-A118G cells the maximal buprenorphine inhibition of AC was reduced to 12 ± 2% (compared with 30±4% in MOR-WT, P < 0.05), without a change in potency, while responses to morphine and B-end were unaffected. Cells expressing MOR-C17T showed significantly reduced E_{max} and pEC₅₀ for morphine but not DAMGO. By contrast, MOR-C253A cells showed significantly increased E_{max} for most ligands, with no change in potency. CHOMOR-C541T was confirmed as non-functional. The differences in inhibition of AC observed between MOR variants suggest that MOR SNPs may contribute to individual variability in the response to opioid analgesics, and we are exploring this at a range of signalling pathways.

60. SOCIAL STRESS ENGAGES OPIOID MODULATION OF THE LOCUS COERULEUS-NOREPINEPHRINE SYSTEM AND INCREASES THE SALIENCE OF REWARD.

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Tuesday 4.45 – 5.45pm

The locus coeruleus-norepinephrine (LC-NE) system is a major stress response system through which stress affects arousal and cognitive function. The LC is co-regulated by stress neuromediators and endogenous opioids. Because social stress (SS) is a common stressor for humans, this study characterized the enduring impact of repeated SS on LC activity. Specifically, we determined whether SS alters the LC-NE system two (Day 7) and ten days (Day 15) after the last SS and evaluated whether these changes in LC-NE system translate to changes in cognitive function. Rats were implanted with a multiwire bundle into the LC and exposed to five sessions of the resident-intruder model of social defeat. On Day 7, LC discharge rate was decreased in stressed rats compared to controls (Day 1: 2.21 ± 0.1 Hz, Day 7: 1.35 ± 0.1 Hz for stress rats and Day 1: 2.23 ± 0.1 Hz, Day 7: 2.20 ± 0.1 Hz, for control rats; $n < 40$). By Day 15, LC rates were comparable between groups. Systemic administration of the opiate antagonist naloxone, robustly increased LC rate selectively in stressed rats when administered on Day 7 (1.18 ± 0.1 Hz pre-naloxone, 2.24 ± 0.1 Hz post-naloxone) or Day 15 (1.87 ± 0.1 Hz pre-naloxone, 4.77 ± 0.2 Hz post-naloxone). This cellular evidence of opiate withdrawal was accompanied by behavioral signs of mild opiate withdrawal. To evaluate whether SS-induced changes in LC activity affect cognitive flexibility, rats were tested in an attention set shifting task (AST) and LC activity was recorded. Stress rats performed better in intradimensional set shifting (IDS): 36.7 ± 4 and 23.8 ± 4 trials to criterion for control and stress rats, respectively ($p < 0.05$). Recordings during IDS showed that LC neurons of stressed rats were reward, but not decision responsive. During reversal learning although overall performance was comparable between groups, stressed rats made less perseverative errors. LC neurons of stressed rats selectively showed decision- and reward-related activation. LC activity also increased for stressed rats only during incorrect trials between decision and recognition of the absence of reward. These results suggest that SS engages endogenous opioid modulation of LC activity and induces a state of opiate dependence. Additionally, SS strengthens the relationship between LC activity and goal-directed behavior. Together these effects of SS on the LC system can increase vulnerability to opiate abuse by promoting the positive and negative reinforcing effects. Supported by T32-NS007413, 58077 LSDRP, MH40008, DA09082.

61. MORPHINE MODULATES BREAST CANCER CELL METASTATIC POTENTIAL

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Appropriate pain management during and after cancer surgery may play a role in prevention of tumor recurrence and metastasis. Opioids are proven to be highly effective perioperative analgesics and are widely used in cancer surgery patients. Using a mouse syngeneic model of breast cancer, we studied the effect of morphine on tumor growth and dissemination to lungs. I.P. injection of morphine (10mg/kg) to mice ($n=8$) every 12h for 3 consecutive days, caused a reduction in breast tumor growth and tumor cell dissemination to the lungs as measured 18 days after tumor inoculation. Morphine treatment also caused a reduction in circulating proteolytic enzymes of extracellular matrix (ECM), matrix metalloproteinase-9 (MMP-9) and urokinase-like plasminogen activator (uPA) measured by gelatin and casein-plasminogen zymography, respectively. Furthermore, we tested the effect of morphine on co-cultures of breast cancer cells with stromal cells, either endothelial cells or macrophages. In co-cultures of 4T1 breast cancer cells with murine RAW264.7 macrophages or murine H5V endothelial cells, the level of matrix proteases produced by cells was increased, and so was the level of matrix protease inhibitors TIMPs. Interestingly, morphine treatment of these co-cultures reduced the level of MMP-9 and increased its endogenous inhibitor, TIMP-1, thereby altering the overall proteolytic profile. Morphine affected co-cultures but not cells grown individually. This suggests that anti-tumor effects of morphine are mediated through modulation of paracrine communication between cancer cells and infiltrating cells.

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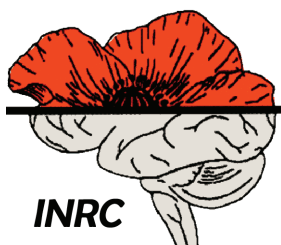
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