Program: INRC *Annapolis Maryland July 10 – 15, 2005*

Sunday, July 10

15:00 – 18:00; 19:30 – 20:30 **Registration** **Marriott Ballroom Foyer**
19:30 – 21:30 **Opening Reception** **Marriott Ballroom**

Monday, July 11

7:00 – 8:30 **Continental breakfast** **Marriott Ballroom Foyer**

8:30 – 9:30 **P1 Plenary Lecture** **Solomon Snyder** **Marriott Ballroom**
Novel Neurotoxic and Neuroprotective Mechanisms

9:30 – 12:30 **Symposium I** **Recent advances in pain research** **Marriott Ballroom**
Chair F. Porreca, Cochair H. Fields

9:30 – 9:55 **S1 F. Porreca** MECHANISMS OF OPIOID-INDUCED NEUROPLASTICITY AND HYPERALGESIA

9:55 – 10:15 *Coffee break*

10:15 – 10:40 **S2 H.L. Fields** VIEWING MOTIVATION THROUGH AN OPIOID LENS: THE INTERSECTION OF ANALGESIA AND ADDICTION

10:40 – 11:05 **S3 G.W. Pasternak** MULTIPLE MU OPIOID RECEPTORS: INTEGRATING MOLECULAR BIOLOGY AND BEHAVIOR.

11:05 – 11:35 **S4 C. Stein** OPIOID-IMMUNE INTERACTIONS IN PAIN CONTROL

11:30 – 11:45 **S5 C. Inturrisi** DYNORPHIN-INDUCED ALLODYNIA IS PREVENTED BY A SPATIAL KNOCKOUT OF NMDA RECEPTORS IN THE LUMBAR SPINAL CORD DORSAL HORN

11:45 – 12:00 **S6 L.Y.-M. Huang** REMOTE NERVE INJECTION OF MU-OPIOID RECEPTOR ADENO-ASSOCIATED VIRAL VECTOR INCREASES ANTINOCICEPTION OF INTRATHECAL MORPHINE

12:00 – 12:15 **S7 W. Walwyn** INDUCTION OF DELTA OPIOID RECEPTOR (DOR) FUNCTION BY UP-REGULATION OF MEMBRANE DORS IN MOUSE PRIMARY AFFERENT NEURONS.

12:15 – 12:30 **S8 C. Kornetsky** A COMPARISON OF MORPHINE-INDUCED ANALGESIA IN YOUNG AND AGED RATS USING PERIPHERAL AND CENTRAL NOCICEPTIVE STIMULATION.

12:30 – 15:30 **Lunch and Poster Session I** **Historic Inns**

Pain

M1 ANALGESIC SYNERGY BETWEEN DELTA OPIOID AND 5-HT$_3$ RECEPTORS IS DEPENDENT UPON LIPID RAFTS K. Rajput, D. Paul, Dept. of Pharmacology and Experimental Therapeutics, Louisiana State University Health Science Center, New Orleans, LA USA

M2 PROPENTOFYLLINE-INDUCED ASTROCYTE MODULATION LEADS TO ALTERATIONS IN GLT-1 AND ANTI-ALLODYNIA AFTER NERVE TRANSECTION V.L.Tawfik, J.A.DeLeo. Dartmouth Medical School, Dept Pharmacology, Hanover, NH USA

M3 DOES SPINAL CO-ADMINISTRATION OF MORPHINE WITH SUB-ANALGESIC DOSE OF DAMGO INHIBIT THE DEVELOPMENT OF MORPHINE TOLERANCE? J. Xu, B. Clark, M. Diaz, H. Gutstein. Department of Anesthesiology, M.D. Anderson Cancer Center, Houston, USA

M4 REPEATED LUMBAR PUNCTURE IN RATS: A NOVEL METHOD FOR THE EXPERIMENTAL STUDY OF OPIOID TOLERANCE B. Clark, J. Xu, M. Diaz, and H. Gutstein Department of Anesthesiology M.D. Anderson Cancer Center, Houston, TX USA

M5 DOES SYSTEMIC CO-ADMINISTRATION OF MORPHINE WITH SUB-ANALGESIC DOSES OF FENTANYL INHIBIT THE DEVELOPMENT OF MORPHINE TOLERANCE? K. Barker, H. Gutstein. Department of Anesthesiology MD Anderson Cancer Center, Houston, TX USA

M6 DORSAL HORN KEPI (Kinase Enhanced PP1 Inhibitor) EXPRESSION: REGULATION BY MORPHINE and CFA TREATMENTS J. P. Gong, Q. R. Liu, G.R. Uhl Mol. Neurobiol. NIDA-IRP, NIH, DHSS Baltimore, MD USA

M7 ANTINOCICEPTIVE EFFECT OF FLUVOXAMINE ON THERMAL AND MECHANICAL NOCICEPTION AFTER PERIPHERAL NERVE INJURY IN THE MOUSE C. Nozaki, A. Saitoh, J. Kamei Dept. Pathophysiol. Ther, Sch. Pharm. Pharm. Sci., Hoshi Univ., Tokyo, Japan

M8 INVOLVEMENT OF SPINAL HISTAMINERGIC SYSTEM ON NOCICEPTIVE BEHAVIORS ELICITED BY SPERMINE IN MICE M. Yoshida (1), Y. Iwata (1), H. Watanabe (1), H. Mizoguchi (1), C. Watanabe (1), A. Yonezawa (1), T. Sakurada (2), S. Sakurada (1) (1) Dept. of Physiol. And Anat., Tohoku Pharmaceut. Univ., Sendai, Japan, (2) Dept. of Biochem., Daiichi Coll. of Pharmaceut. Sci., Fukuoka, Japan

M9 MORPHINE PRIMING POTENTIATES DELTORPHIN ANALGESIA IN CFA-TREATED RATS
L. Gendron (1), MJ. Esdaile (1), T. Stroh (1), C. Cahill (2) and A. Beaudet (1) (1) McGill Univ., Montreal, Canada, (2) Queen's Univ, Kingston, Canada

M10 LEUKOCYTE-DERIVED OPIOIDS PRODUCE ANALGESIA IN NEUROPATHIC PAIN
D. Labuz, S.A. Mousa, C. Stein, H. Machelska Anaesthesiologie, Charité, Campus Benjamin Franklin, Berlin, Germany

M11 CONCOMITANT ACTIVATION OF β-ENDORPHIN-CONTAINING NEURON SUPPRESSES THE MORPHINE-INDUCED REWARDING EFFECT UNDER A NEURO-PATHIC PAIN-LIKE STATE
K. Niikura, M. Narita, M. Narita, K. Hashimoto, Y. Yajima, T. Suzuki.Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan

M12 FUNCTIONAL CHANGES IN OPIOIDERGIC SYSTEM UNDER A NEUROPATHIC PAIN-LIKE STATE FOLLOWING CHRONIC ETHANOL CONSUMPTION IN THE RAT SPINAL CORD K. Miyoshi, M. Narita, M. Narita, T. Suzuki Dept. of Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan

M13 THE CHANGES IN BASAL MEMBRANE OF SCHWAN CELLS IN DRG EXPLAIN THE BIPHASIS BEHAVIOR OF THE PERIPHERAL NEUROPATHY IN DIABETIC RATS M. Becker (1), A. Shahar (3), Z. Nevo (2), C.G. Pick (1) (1) Dept. of Anatomy, (2) Clinic Biochemistry, Sackler Faculty of Medicine, Tel-Aviv University, (3) NVR ltd, Nes-Ziona, Israel

M14 COMPARISON OF SELECTIVE OPIOID AGONISTS IN THE PRODUCTION OF ANALGESIA FOR NEUROPATHIC PAIN DUE TO DEMYELINATION A. L. Dunne (1), H. J. Gould, III (2), D. Paul (1) (1)Dept. of Pharmacology and Experimental Therapeutics, (2)Dept. of Neurology, LSUHSC, New Orleans, LA, USA

M15 GENDER DIFFERENCES IN PAIN PROCESSING DURING PROTRACTED OPIATE ABSTINENCE M. Steinfeld, L. Kunik, L. Cohen, I. Galynker Dept. Psychiatry, Beth Israel Medical Center, New York, NY USA

M16 OLIGONUCLEOTIDES TARGETING EXONS 7, 8 AND 9 OF THE MOR-1 SPLICE VARIANT BLOCK OPIOID ANALGESIA IN THE RAT J. Matulonis (1), X-Y. Pan (2), G.W. Pasternak (2), G. Rossi (1) (1) Long Island Univ. C.W. P, Brookville, NY, USA, (2) Dept. of Neurology, Mem. Sloan Kettering Cancer Ctr., New York NY, USA

M17 LONG-TERM MAINTENANCE OF ANALGESIA WITH CHRONIC ORAL OXYCODONE TREATMENT IN FEMALE RATS L.M. Schrott, L.M. Franklin Dept. of Pharmacology, Toxicology, & Neuroscience, LSU Health Sciences Center, Shreveport, LA USA

M18 NERVE INJURY CHANGES THE LEVEL OF Ca^{++}-CHANNEL SUBUNIT: COMPARISON TO OPIOID-TOLERANT STATE M. Nakajima, M. Narita, Y. Nagumo, Y. Yajima, T. Suzuki Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo Japan

M19 OPIODS MODUATE THE TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 (TRPV1) ION CHANNEL J. Endres-Becker, P.A. Heppenstall, S. Mousa, D. Labuz, M. Schäfer, C. Stein, C. Zöllner Dept. of Anaesthesiology and Critical Care Medicine; Charité – Campus Benjamin Franklin, Berlin, Germany

M20 EFFECT OF NOP AGONISTS AND ANTAGONISTS IN A CHRONIC PAIN MODEL L. Toll, T. Khroyan, W. Polgar, J. Orduna, R. Moisa, F. Jiang, C. Olsen, N. Zaveri SRI International, Menlo Park, CA USA

M21 REPEATED STRESS INDUCES TOLERANCE TO STRESS-INDUCED ANTI-NOCICEPTION IN PREPROENKEPHALIN KNOCKOUT MICE N. Gajawada, R. Baliram, K. Lutfy Dept. of Pharm. Sci., Western Univ. of Health Sci., Pomona, CA USA

M22 ASSESSING EFFECTS OF OPIOIDS ON PAIN-SUPPRESSED BEHAVIORS E. Bilsky (1), G. Stevenson (2), T. Vanderah (3), F. Porreca (3) , S. Negus (2) (1) University of New England, Biddeford, ME, (2) McLean Hospital-Harvard Medical School, Belmont, MA, (3) University of Arizona, Tucson, AZ

M23 SALVINORIN A, A KAPPA OPIOID RECEPTOR AGONIST, IS AN ULTRASHORT ACTING ANALGESIC C.R. McCurdy (1,3,4), K.J. Sufka (2,3,4), J.E. Warnick (2), M.J. Neito (1) (1) Dept. Medicinal Chemistry, (2) Dept. Psychology, (3) Dept. Pharmacology, (4) Research Institute of Pharmaceutical Sciences, School of Pharmacy, Univ. of Mississippi, University, MS 38677 USA

Genetics and gene variants

M24 ASSOCIATION GENOME SCAN USING 155 UNRELATED COGA SUBJECTS AND >15,000 SNP MARKERS C. Johnson, G.R. Uhl, Molecular Neurobiology, NIDA-IRP, NIH, DHSS, Baltimore, MD USA

M25 LINKAGE DISEQUILIBRIUM, HAPLOTYPE AND ASSOCIATION STUDIES OF A CHROMOSOME 4 GABA RECEPTOR GENE CLUSTER: CANDIDATE GENE VARIANTS FOR ADDICTIONS T. Drgon, C. D'Addario, G.R. Uhl Molecular Neurobiology, NIDA-IRP, NIH, DHHS, Baltimore MD USA

M26 NrCAM IN OPIATE VULNERABILITY: POSITIONAL CLONING, OPIATE-REGULATION, HAPLOTYPE-SPECIFIC EXPRESSION AND ALTERED MORPHINE REWARD IN KNOCKOUT MICE H. Ishiguro (1), Q.-R. Liu1, J.-P. Gong1, F.S. Hall1, H. Ujike3, M. Morales, T. Sakurai3, M. Grumet4, G.R. Uhl 1 1 Molecular Neurobiol Br, (2) Cellular Neurosci Br, NIDA-IRP, NIH, DHSS, Baltimore, MD, USA,3 Dept. Neuropsychiatry, Okayama Univ. Med. Sch., Okayama, Japan, 4 Dept. Neurobiol., Mt. Sinai Sch. Med., New York, NY, USA

M27 NOVEL PRODYNORPHIN TRANSCRIPTS AND PROTEINS IN THE ADULT HUMAN BRAIN T. Yakovleva, A. Nikoshkov, Y.L. Hurd, I. Bazov, Z. Marinova, L. Terenius, G. Bakalkin Dept. of Clinical Neurosci., Karolinska Institutet, Stockholm, Sweden

M28 NOCICEPTIN/ORPHANIN FQ RECEPTOR (ORL1) GENE AND HEROIN ADDICTION K.S. LaForge, D. Proudnikov, S. Barral-Rodriguez, M.J. Kreek Rockefeller Univ., New York, NY USA

M29 FUNCTIONAL CHARACTERIZATION OF AN ALTERNATIVELY SPLICED VARIANTS, MMOR-1B4, OF THE MOUSE MU OPIOID RECEPTOR GENE, OPRM Y.-X. Pan, J. Xu, M. Xu, G.W. Pasternak Dept. Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY USA

M30 ASSESSMENT OF HUMAN MU OPIOID RECEPTOR SPLICE VARIANTS THROUGH MORPHINE-INDUCED ADENYLYL CYCLASE SUPERACTIVATION L. Pan, J. Xu, M.-M. Xu, Y.-X. Pan, G.W. Pasternak Dept. Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY USA

M31 EXPRESSION OF MU OPIOID RECEPTOR IN PC12 CELLS L.A. Towart, Y.-X. Pan, G.W. Pasternak Depts. Neurol. Neurosci., Cornell Univ., NY; Dept. Neurol., Mem. Sloan-Kettering Cancer Ctr., New York, NY USA

Species homologs

M32 FUNCTIONAL PHARMACOLOGY OF THE CLONED GUINEA PIG MU OPIOID RECEPTOR (GP-MOR) M. Wallisch, S.A. Smith, C. Nelson, T. Ransom, G.D. Olsen Depts. of Physiol. & Pharmacol., Pediatrics, Div. of Pul. Crit. Care Med., Sch. of Med. Oregon Health & Science Univ., Portland, OR, USA

M33 PHARMACOLOGICAL PROFILE OF A NEW MU OPIOID RECEPTOR FROM ZEBRAFISH E. Marrón Fdez de Velasco, I. Rodríguez-Martín, R.E. Rodríguez Dept. Biochem.and Mol. Biology, Inst. of Neuroscience, Univ. of Salamanca, Salamanca, Spain

M34 ZFOR4, A DELTA OPIOID RECEPTOR FROM ZEBRAFISH: AGONIST-MEDIATED INTERNALIZATION AND MAP KINASE ACTIVATION V. Gonzalez-Nuñez (1), K. Roberts (2), C.J. Evans (2), R.E. Rodríguez (1) (1) Dept. of Biochem. and Molec. Biol., Inst. Neurosci., Castilla y León. Univ., Salamanca, Spain, (2) Hatos Research Center for Neuropharmacology, NPI, UCLA, USA

Toxicity

M35 DECOY PEPTIDES THAT BIND DYNORPHIN NON-COVALENTLY PREVENT NMDA-RECEPTOR-MEDIATED NEUROTOXICITY AND ISCHEMIC BRAIN INJURY A.S. Woods, Y. Wang, T. Shippenberg NIDA IRP, NIH, Baltimore MD, USA

M36 ACUTE METHADONE INDUCED RESPIRATORY DEPRESSION IN THE NEONATAL GUINEA PIG R. Nettleton (1), T. Ransom (1), C. Nelson (1,2), S. Abraham (3) G. D. Olsen (1) (1)Dept. of Phys. & Pharm., (2)Div. Pulm. Crit. Care Med., (3) Stem Cell Center, Oregon Health & Science Univ., Portland, OR, USA

M37 EFFECTS OF PAEONIFLORIN ON NEUROTOXIN-INDUCED NEURONAL CELL DAMAGE AND EXPERIMENTAL PARKINSONISM IN MU-KNOCKOUT MICE H.Y. Tsai (1,2), Y.T. Lin (3), K.W. Chien (1), Y.F. Chen (1,2) (1) Dept. Pharmacol., China Medical Univ., Taichung, Taiwan (2) Dept. Pharmacy, China Medical Univ. Hospital, Taichung, Taiwan (3) Dept. Nursing, Jen-The Junior College of Medicine, Nursing and Management, Mialoi, Taiwan

M38 MODULATION OF METHAMPHETAMINE NEUROTOXICITY BY ENDOGENOUS K-OPIOID RECEPTOR SYSTEMS E.K. Oh, T.S. Shippenberg, V.I. Chefer Integrative Neuroscience Section, DHHS/NIH NIDA IRP, Baltimore, MD USA

M39 PROTECTION AGAINST ISCHEMA/REPERFUSION MEDIATED MYOCARDIAL CELL DEATH BY A DELTA-OPIOID AGONIST, DELTORPHIN II X. Yue (1), E. Navratilova (1), E. Varga (1, 2), J. Bahl (2), D. O'Connell (2), H. Yamamura (1,2), W. Roeske (2) (1) Dept. Med. Pharmacol., (2) Sarver Heart Center, Univ. of Arizona, AZ, USA

Receptor-receptor Interactions

M40 ALLOSTERIC INTERACTION AND SELECTIVITY OF HETERODIMERIZED DELTA AND KAPPA OPIOID RECEPTORS Z. Xie, R.G. Bhushan, D.J. Daniels, P.S. Portoghese Dept. Medicinal Chemistry, College of Pharmacy, Univ. Minnesota, Minneapolis, USA

M41 EVIDENCE FOR INTERACTIONS BETWEEN THE CENTRAL ENDOGENOUS ENDOTHELIN AND OPIOID SYSTEMS X.Y. Wang, H. Xu, R.B. Rothman IRP, NIDA, NIH, DHHS, Baltimore, MD 21224 USA

M42 MECHANISMS OF ADENOSINE A2A and DOPAMINE D2 RECEPTOR HETERO-DIMERIZATION A.S. Woods, S. Ferre, NIDA IRP, NIH, Baltimore MD USA

M43 INTERACTIONS BETWEEN MU-OPIOID RECEPTORS AND α 2A ADRENERGIC RECEPTORS PROMOTE RECEPTOR INTERNALIZATION AND DESENSITIZATION M. Tan, C.-W. Xie Dept. Psychiatry Biobehav., Univ.California-Los Angeles, CA USA

M44 THE CHEMOKINE RECEPTOR CXCR4 IS INVOLVED IN THE CXCL12/SDF-1-α ANTAGONISM OF KAPPA- OR DELTA-OPIOID RECEPTOR-INDUCED ANTI-NOCICEPTION X.H. Chen, E.B. Geller, .S. Deitz, T. J. Rogers & M. W. Adler Center for Substance Abuse Research, Temple Univ. Sch. of Med., Phila., PA USA

Addiction/Human

M45 THE β-ENDORPHIN-DERIVED PEPTIDE GLYCYL-GLUTAMINE INHIBITS NICOTINE CONDITIONED PLACE PREFERENCE AND WITHDRAWAL G. Göktalay (1,2), J. Hamilton (1), S. Cavun (1,2), M. Levendusky (1), W. Millington (1) (1) Albany College Pharmacy, Albany, NY, (2) Uludag University, Bursa, Turkey

M46 A NOVEL DEPOT FORMULATION OF BUPRENORPHINE FOR TREATMENT OF OPIOID DEPENDENCE S. Sigmon (1), G. Bigelow (2) (1) Dept. Psychiatry, Univ. Vermont, Burlington, VT, (2) Dept. Psychiatry and Behavioral Sciences, Johns Hopkins Univ., Baltimore, MD USA

M47 ROLE OF OREXIN NEURONS IN THE BRAIN REWARDING SYSTEM Y. Nagumo, M. Narita, M.Narita, M. Miyatake, Y. Yajima, T. Suzuki Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan

15:30 - 18:50 **Symposium II** **Human genetics of addiction** **Marriott Ballroom**
Chair J Pollock, Cochair MJ Kreek

15:30 – 15:55 **S9** **G.R. Uhl** HUMAN MOLECULAR GENETICS OF ADDICTION: REMARKABLE RECENT PROGRESS

15:55 – 16:20 **S10** **M.T. Tsuang** OPIATE DEPENDENCE: COMORBIDITY AND FAMILIAL VULNERABILITY

16:20 – 16:40 *Coffee break*

16:40 – 17:05 **S11** **W. Berrettini** THE MU OPIOID RECEPTOR GENE AND ADDICTIONS

17:05 – 17:30 **S12** **J. Gelernter** FIRST RESULTS FROM A GENOMEWIDE LINKAGE SCAN FOR OPIOID DEPENDENCE

17:30 – 17:55 **S13** **H.J. Edenberg** GENETICS OF ALCOHOLISM

17:55 – 18:10 **S14** **D.A. Nielsen** A TPH2 HAPLOTYPE ASSOCIATES WITH OPIOID DEPENDENCE IN AFRICAN AMERICANS

18:10 – 18:25 **S15** **K. Ikeda** A POSSIBLE GENETIC MECHANISM OF INDIVIDUAL SENSITIVITY TO OPIATES

18:25 – 18:40 **S16** **W. Sadee** A118G VARIANT AFFECTS THE mRNA AND PROTEIN EXPRESSION OF HUMAM MU OPIOID RECEPTOR

18:40 – 18:55 **S17** **C. Bryant** MORPHINE ANALGESIC TOLERANCE IN 129/S6 AND 129/P3 MICE: META-ANALYSIS OF INBRED STRAINS INDICATES THAT MOTOR PERFORMANCE GENETICALLY CORRELATES WITH TOLERANCE AND BASELINE NOCICEPTION

Tuesday, July 12

7:00 – 8:30 **Continental breakfast** **Marriott Ballroom Foyer**

8:30 – 9:30 **P3** **Founders' Lecture** **Mary Jeanne Kreek** **Marriott Ballroom**
Hypothesis to Pharmacotherapy - Endorphin System to Functional SNPs of Opioid Genes:
An INRC Odyssey 1964-2005

9:30 – 10:30 **P2** **Plenary Lecture** **Nora Volkow** **Marriott Ballroom**
View from NIDA

10:30 – 10:50 *Coffee break*

10:50 - 12:30 **Symposium III** **Imaging opioid systems** **Marriott Ballroom**
Chair V.M. Pickel Cochairs J.-K. Zubieta, J Frost

10:50 – 11:15 **S18** **V.M. Pickel** TARGETING OF MU-OPIOID AND CANNABINOID1 (CB1) RECEPTORS WITHIN THE MESOLIMBIC DOPAMINE REWARD CIRCUIT

11:15 – 11:40 **S19** **A. Beaudet** *IN VIVO* TAGGING OF CELL SURFACE DELTA OPIOID RECEPTORS

11:40 – 12:05 **S20** **J.-K. Zubieta** MU-OPIOID RECEPTOR MEDIATED NEUROTRANSMISSION AT THE INTERFACE OF REWARD AND STRESS RESPONSE MECHANISMS

12:05 – 12:20 **S21** **B.L. Kieffer** DELTA OPIOID RECEPTOR IMAGING *IN VIVO*

12:30 – 15:30 **Lunch and Poster Session II** **Historic Inns**

Anatomy, Imaging

T1 CB1 CANNABINOID RECEPTORS ARE LOCATED WITHIN DOPAMINERGIC NEURONS AND THEIR AFFERENT TERMINALS IN THE RAT VENTRAL TEGMENTAL AREA (VTA) C.D. Rios, V.M. Pickel Weill Medical College of Cornell University, Depts Neurology, Neuroscience, New York, NY USA

T2 AMPA RECEPTOR TRAFFICKING WITHIN THE VENTRAL TEGMENTAL AREA OF RATS RECEIVING CHRONIC INTERMITTENT MORPHINE ADMINISTRATION D.A. Lane (1), E.E. Colago (1), Y. Zhou (2), S. Schlussman (2), M.J. Kreek (2), V.M. Pickel (1) (1)Dept. of Neurology and Neuroscience, Weill Medical College of Cornell University, (2)Laboratory of the Biology of Addictive Diseases, Rockefeller University, New York, NY USA

T3 TARGETING DIFFERENCES OF MU AND DELTA OPIOID RECEPTORS IN CULTURED NEURONS T.A. Libby (1,2), M. Riedl (1), F. Williams (1,3), R. Elde (1,2) (1) Dept. Neurosci., (2) Grad. Prog. Neurosci., (3) Veterinary and Biomedical Sciences, Univ. Minnesota, Minneapolis, MN, USA

T4 CONDITIONAL NMDA-NR1 RECEPTOR SUBUNIT GENE DELETION IN THE AMYGDALA
M. Glass (1), V.M. Pickel (1), C. Inturrisi (2) (1) Depts. Neurol. & Neurosci., and (2) Pharmacol., Weill Med. Coll. Cornell Univ., NY, NY, USA

T5 mPKCI DISTRIBUTION AND DEVELOPMENTAL PROFILE IN MOUSE CENTRAL NERVOUS SYSTEM
Q. Liu, J.B. Wang, Dept. Pharmaceutical Sci., School of Pharmacy, Univ. Maryland, Baltimore, MD, USA

T6 SUBCELLULAR DISTRIBUTION OF M2-MUSCARINIC RECEPTORS IN RELATION TO DOPAMINERGIC NEURONS OF THE RAT VENTRAL TEGMENTAL AREA M. Garzón (1,2), V.M. Pickel (1) (1)

Dept. Neurol. & Neurosci., Weill Med Coll, Cornell Univ, New York (2)Dept. Anat., Histol. & Neurosci., Med. Coll. UAM, Madrid

T7 DELTA OPIOID RECEPTOR ACTIVATION SITES IN GABA-CONTAINING AXONS REGULATING SLEEP IN THE CAT VENTRAL ORAL PONTINE TEGMENTUM M.X. Alvira-Botero, M. Garzón Dept. Anat., Histol. & Neurosci., Med. Coll. UAM, Madrid, Spain

T8 DORSAL HORN NEUROPLASTICITY AFTER SPARED NERVE INJURY B.K. Taylor, A.B. Intondi, Y. Carl, X. Zhang, J.E. Zadina, Tulane Univ. HSC & VA, New Orleans, LA USA

Behavioral pharmacology

T9 EFFECT OF TRK-820, A KAPPA OPIOID RECEPTOR AGONIST, ON BEHAVIORAL RESPONSES TO METHAMPHETAMINE, COCAINE AND NICOTINE IN RATS K. Hasebe (1), K. Kawai (1), M. Takagi, T. Suzuki (1), K. Kawamura (1), T. Tanaka (1), M. Narita (2), H. Nagase, K. Okano (1), T. Suzuki (2) (1) Pharmaceutical Research Lab., Toray Industries Inc., Kanagawa, Japan, (2) Dept. Toxicology, Hoshi Univ., Sch. Pharm., Pharm. Sci., Tokyo, Japan, (3) Department of Medical Chemistry, Sch. Pharma. Sci. Kitasato Univ., Tokyo, Japan

T10 The ROLE OF BRAIN CORTICOTROPIN-RELEASING FACTOR RECEPTOR TYPE I IN STRESS AND OPIATE-INDUCED REINSTATEMENT OF COMDITIONED PLACE PREFERENCE IN RATS Q. Fang (1), J. Wang (2), L. Lu (3,4) (1) Dept. Pharmacology, Affiliated Hospital of Guiyang Medical College, Guiyang, China, (2) Dept. Pharmacology, New York Medical College, Valharla, NY, USA, (3) National Lab. Medical Neurobiology, Fudan Univ., Shanghai, China, (4) Behav. Neurosci. Branch, NIDA- IRP/NIH, Baltimore MD, USA

T11 THE DOPAMINE D3 RECEPTOR AND REACTIVITY TO OPIATE-ASSOCIATED STIMULI: RESOLVING THE PARADOX B. Le Foll , H. Francès, J. Diaz, P. Sokoloff INSERM U. 573, Centre Paul Broca, Paris, France

T12 MEDIATION OF COCAINE- AND STRESS-INDUCED POTENTIATION BY DESENSITIZED KAPPA OPIOID RECEPTORS H.C. Brenhouse, C. Brown, K. Siniakowicz, J.P. McLaughlin Northeastern Univ., Boston, MA USA

T13 KAPPA-OPIOID RECEPTOR INACTIVATION PRODUCES TIME-RELATED ALTERATIONS IN DOPAMINE DYNAMICS AND COCAINE RESPONSIVENESS V.I. Chefer (1), J. Pintar (2), T. Shippenberg (1) (1) Integrative Neuroscience Section, DHHS/NIH/NIDA/IRP, Baltimore, MD, USA, (2) Dept. Neuroscience and Cell Biology, CABM, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ, USA

T14 ADOLESCENT DRUG-INDUCED AGGRESSION: MODULATION BY SEROTONIN TYPE 1A RECEPTORS? K. Rasakham, L. Ricci, R. Melloni Dept. of Psych., Northeastern Univ., Boston, MA USA

T15 SITES AND EFFECTS OF DEXTROMETHORPHAN ON TREATMENT OF MORPHINE ADDICTION IN RATS P.L. Tao, Y.L. Shen, E.Y.-K. Huang, Dept. and Institute of Pharmacology, National Defense Medical Center, Taipei, Taiwan, R.O.C.

T16 ROLE OF DELTA-OPIOID RECEPTOR SUBTYPES IN ANXIETY-RELATED BEHAVIORS ON THE ELEVATED PLUS MAZE IN RATS A. Saitoh, N. Hirose, J. Kamei Dept. Pathophysiol. Ther., Sch. Pharm. Pharm. Sci., Hoshi Univ., Tokyo, Japan

T17 EFFECTS OF SNC80 ON THE EXPLORATORY BEHAVIOR OF OLFACTORY-BULBECTOMIZED MICE IN THE HOLE-BOARD TEST N. Hirose, A. Saitoh, J. Kamei Dept. Pathophysiol. Ther., Sch. Pharm. Pharm. Sci., Hoshi Univ., Tokyo, Japan

T18 OPPOSITE EFFECTS OF ACETYLCHOLINE ENHANCEMENT IN VTA AND NAC ON DRUG SEEKING ELICITED BY CUES AFTER ABSTINENCE IN A MODEL OF RELAPSE TO HEROIN IN RATS W. Zhou, F. Zhang, S. Tang, M. Lai, H. Zhu, H. Liu Ningbo Addiction Research and Treatment Center, Ningbo, China

T19 PRESENCE AND FUNCTIONAL EXPRESSION OF CB2 CANNABINOID RECEPTORS IN BRAIN THAT IS INVOLVED IN DEPRESSION AND SUBSTANCE ABUSE E.S. Onaivi (1,2), H. Ishiguro (4), J.-P. Gong (2), S. Patel (1), P. Meozzi (1), L. Myers (1), Z. Mora (1), A. Perchuk (1), P. Tagliaferro (5), C. Leonard (3), E. Gardner (3), A. Brusco (5), B. Akinshola (6), Q.-R. Liu (2), B. Hope (3), G.R. Uhl (2) (1) Dept. Biology, William Paterson University, Wayne, NJ, (2) Molec. Neurobio. Branch, (3) Behav. Neurosci. Branch, NIDA-IRP, NIH/DHHS, Baltimore, MD, (4) Institute Basic Med. Sci., Univ. Tsukuba, Japan, (5) Univ. Buenos Aires, Argentina, (6) Howard Univ., Washington DC USA

T20 AN ENDOCANNABINOID HYPOTHESIS OF DRUG REWARD E.S. Onaivi Dept. Biology, William Paterson University, Wayne, NJ, USA

T21 IMPAIRMENT OF THE DELTA-OPIOID RECEPTOR FUNCTION INDUCES ASTROGLIOGENESIS-DEPENDENT EMOTIONAL DYSFUNCTION N. Kuzumaki, M. Narita, M. Narita, T. Suzuki Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan

T22 SUPPRESSION OF PSYCHOLOGICAL DEPENDENCE ON OXYCODONE UNDER CHRONIC PAIN-LIKE STATE IN MICE T. Suzuki, M. Ozaki, A. Nakamura, M. Narita Dept. of Toxicol., Hoshi Univ. Shc. of Pharm. and Pharmaceut. Sci., Tokyo, Japan

T23 IMPLICATION OF MOR1B IN PHYSICAL DEPENDENCE ON ETHANOL K. Hoshino, M. Narita, K. Miyoshi, M. Souma, T. Suzuki. Dept. of Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan

T25 MECHANISMS OF NOCICEPTIN(14-17)-INDUCED NOCICEPTIVE BEHAVIORS IN THE MOUSE SPINAL CORD H. Watanabe (1), H. Mizoguchi (1), A. Yonezawa (1), C. Watanabe (1), T. Sakurada (2), S. Sakurada (1) (1) Dept. Physiol. and Anat., Tohoku Pharmaceut. Univ., Sendai, Japan, (2) Dept. of Biochem., Daiichi Coll. of Pharmaceut. Sci., Fukuoka, Japan

T26 ENVIRONMENTAL CUES ASSOCIATED WITH THE DIFFERENT REINFORCEMENTS OF MORPHINE INDUCE THE ACTIVATION OF VENTRAL SUBICULUM THROUGH THE SPECIFIC NEUROTRANSMITTERS IN RATS L. Kang, Z. Dai, L. Qu, L. Ma Pharmacology Research Center, Shanghai Medical College, Fuda Univ., Shanghai, People's Republic of China

T27 DEXTROMETHORPHAN POTENTIATE THE INHIBITORY EFFECTS OF ANTI-NT4 ON MORPHINE TOLERANCE H. Hatami (1), S. Oryan (1) , A. Ahmadiani (2), S. Semnanian (3) , B. Kazemi (2) (1) Dept. of Biol., Teacher Trainning Univ., (2) Neurosci. Res. Ctr., Shaheed Beheshti Univ. (3) Dept. Physiol., Tarbiat Modarres Univ., Tehran, Iran

T28 INVOLVEMENT OF ASTROCYTES IN THE DEVELOPMENT OF REWARDING EFFECTS INDUCED BY MORPHINE IN MICE M. Asato, M. Narita, M. Narita, M. Miyatake, M. Shibasaki, A. Nakamura, T. Suzuki Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan

T29 ALTERATIONS IN PROTEIN KINASE A ACTIVITY IN MOUSE BRAIN AND SPINAL CORD IS DEPENDENT ON THE LEVEL AND THE DURATION OF MORPHINE ANTINOCICEPTIVE TOLERANCE G.D. Dalton, F.L. Smith, W.L. Dewey Dept. Pharmacology and Toxicology, Virginia Commonwealth Univ. School of Medicine, Richmond, VA USA

T30 MORPHINE-INDUCED BEHAVIORAL SENSITIZATION OR CONDITIONED PLACE PREFERENCE IS UNALTERED IN PREPROENKEPHALIN KNOCKOUT MICE P. Marquez, N. Gajawada, R. Baliram, K. Lutfy Dept. of Pharm. Sci., Western Univ. of Health Sci., Pomona, CA USA

Regulation by Opiate Systems

T31 DORSAL HORN KEPI (Kinase Enhanced PP1 Inhibitor) EXPRESSION: REGULATION BY MORPHINE and CFA TREATMENTS J.-P. Gong, Q.-R. Liu, G.R. Uhl. Molec Neurobiol Branch, NIDA-IRP, NIH/DHSS, Baltimore, MD USA

T32 RODENT BDNF GENES, NOVEL PROMOTERS, NOVEL SPLICE VARIANTS AND REGULATION BY HEROIN AND COCAINE Q.-R. Liu (1), L. Lu (2), X.-G. Zhu (1), Y. Shaham (2), G.R. Uhl (1) (1) Molec. Neurobiol. Branch, (2) Behav. Neurosci. Brach, NIDA-IRP, NIH/DHSS, Baltimore, MD, USA

T33 ROLE OF SPINAL BRAIN-DERIVED NEUROTROPHIC FACTOR IN THE SUPPRESSION OF PSYCHOLOGICAL DEPENDENCE ON MORPHINE UNDER A CHRONIC PAIN-LIKE STATE IN MICE Y. Yajima, M. Narita, A. Nakamura, M. Shibasaki, M. Miyatake, M. Narita, A. Usui, C. Kaneko, T. Yamaguchi, T. Suzuki Dept. of Toxicol., Hoshi Univ. Sch. of Pharm. and Pharmaceut. Sci., Tokyo, Japan

T34 MORPHINE-INDUCED MICROGLIAL BDNF EXPRESSION THROUGH TRANSACTIVATION N. Takayama, H. Ueda Div. of Mol. Pharmacol. & Neurosci., Nagasaki Univ., Grad. Sch.Biomed. Sci., Nagasaki, Japan

T35 INVOLVEMENT OF CAMP-PKA SIGNAL PATHWAY IN REGULATION OF Na+, K+-ATPase BY MORPHINE G. Liu, Z.Q. Wu, M. Li, J. Chen, Z.Q. Chi, Dept. of Neuropharmacol, Shanghai Inst. of Materia Medica, Shanghai Insts.for Biol. Sci., Chinese Acad. of Sci, Shanghai, China

T36 CHRONIC MORPHINE TREATMENT CAUSES PROTEASOME-MEDIATED DEGRADATION OF Gβ IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS L. Moulédous, J. Neasta, S. Uttenweiler-Joseph, A. Stella, M. Matondo, M. Corbani, B. Monsarrat, J.-C. Meunier Institut de Pharmacologie et de Biologie Structurale, CNRS, Toulouse, France

T37 PROTEOMIC ANALYSIS OF THE EFFECT OF MORPHINE TREATMENT ON THE EXPRESSION PROFILE OF PSD-ASSOCIATED PROTEINS IN MOUSE HIPPOCAMPUS J.A. Morón, N. Abul-Husn, G. Dolios, R.Wang, L. Devi Depts. Pharmacology and Human Genetics, Mount Sinai Medical School, New York USA

T38 MORPHINE-INDUCED CHANGES IN PRESYNAPTIC ACTIVE ZONE PROTEINS IN THE MOUSE HIPPOCAMPUS N.S. Abul-Husn, J.A. Morón, R. Wang, L.A. Devi. Depts. Pharmacol., Biol. Chem., Human Genetics, Mount Sinai School of Medicine, New York, NY USA

T39 ROLE OF GABA-A RECEPTOR α6 SUBUNIT IN MORPHINE TOLERANCE N. Guo (1), E. Kozela (2), P. Popik (2) , L. Yu (1), J.T.A. Meij (1) (1) Dept. Cell Biol., Neurobiol. & Anat., Univ. Cincinnati, Cincinnati, OH, USA, (2) Inst. Pharmacol., Polish Acad. Sci., Kraków, Poland

T40 CHRONIC MORPHINE UPREGULATES Gα12 AND CYTOSKELETAL PROTEINS IN CHO CELLS EXPRESSING THE CLONED MU OPIOID RECEPTOR H. Xu (1), X.Y. Wang (1), D. Zimmerman (1), E.S. Boja (2), J. Wang (3), E.J. Bilsky (4), R.B. Rothman (1) (1) CPS, IRP, NIDA, NIH, DHHS, Baltimore, MD USA, (2) LBC, NHLBI, NIH, Bethesda, MD USA, (3) Univ. Maryland, Baltimore, MD, (4) Univ. of New England College of Osteopathic Medicine, Biddeford, ME, USA.

T41 DIFFERENTIAL EFFECTS OF KAPPA OPIOID AGONISTS ON G PROTEIN EXPRESSION IN CELLS EXPRESSING THE CLONED HUMAN KAPPA OPIOID RECEPTOR R.B. Rothman , X.Y. Wang, T.S. Benaderet, C.M. Dersch, H. Xu (1) CPS, IRP, NIDA, NIH, DHHS, Baltimore, MD USA

T42 LPS-STIMULATED INTERLEUKIN-6 mRNA IS REDUCED BY THE KAPPA-SELECTIVE LIGAND, U50, 488 IN A MOUSE MONOCYTE-LIKE CELL LINE A.L. Parkhill, J.M. Bidlack Dept. Pharmacology and Physiology, Univ.Rochester School of Medicine and Dentistry, Rochester, NY, USA

T43 PHA -DEPENDENT KAPPA AGONIST -INDUCED IL-7 RECEPTOR mRNA EXPRESSION IN R1.1 THYMOMA CELL LINE M. Khimich, J.M. Bidlack Dept. Pharmacology and Physiology, Univ. Rochester School of Medicine and Dentistry, Rochester, NY, USA

T44 CHANGES IN PHOSPHORYLATION STATE OF CONNEXIN43 AT ASTROCYTIC GAP JUNCTIONS IN THE MOUSE SPINAL CORD INDUCED BY CHRONIC *IN VIVO* TREATMENT WITH MORPHINE M. Suzuki, M. Narita, M. Narita, K. Niikura, A. Nakamura, T. Suzuki Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan

T45 REGULATION OF G-PROTEIN COUPLED RECEPTOR ENDOCYTOSIS BY PHOSPHOLIPASE D2 T. Koch, D. Wu, L. Yang, L.O. Brandenburg, V. Höllt Dept. Pharmacology and Toxicology, Otto-von-Guericke Univ. Magdeburg, Magdeburg, Germany

T46 CHRONIC STRESS ALTERS GENE EXPRESSION IN DYNORPHIN KNOCKOUT AND WILDTYPE MICE J.D. Lowe, P. Amieux, J. McLaughlin, C. Chavkin Dept Pharmacology, Univ. Washington, Seattle WA USA

T47 NEUROPETIDE REGULATION IN MICE CHRONICALLY TREATED BY MORPHINE F.M. Décaillot (1), F.Y. Che (2), L. Fricker (2), L.A. Devi (1). (1) Mt. Sinai School of Medicine, New York, USA, (2) Albert Einstein College of Medicine, New York, NY, USA

T48 DENDRITIC SPINE FORMATION AND LOCALIZATION OF NMDA AND AMPA RECEPTORS IN PRIMARY HIPPOCAMPAL NEURONS ARE CONTROLLED BY SIGMA-1 RECEPTORS S.-Y. Tsai, T. Hayashi, T.-P. Su Cellular Pathobiology Unit/DPS/ CNRS/IRP/NIDA /NIH/DHHS, Baltimore, MD, USA

T49 MORPHINE REGULATION OF PC1/3 AND PC2: IMPLICATION FOR THE SWITCH FROM DRUG USE TO DRUG ABUSE A. Anghel (1), K. Lutfy (1,2), Y. Liu (1), Y. Nie (1) and T.C. Friedman (1) (1)Endocrinology, Charles Drew Univ., Los Angeles, CA, (2)Pharm. Sci., Western Univ., Pomona, CA USA

T50 MU AND KAPPA OPIOIDS DIFFERENTIALLY MODULATE VENTRAL TEGMENTAL AREA OUTPUTS DEPENDING ON EFFERENT TARGET E.B. Margolis (1), H. Lock (1), V. Chefer (2), T. Shippenberg (2), G.O. Hjelmstad (1), H.L. Fields (1) (1) Ernest Gallo Clinic & Research Center, UCSF, Emeryville, CA, USA (2) Integrative Neuroscience Section, DHHS/NIH, NIDA/IRP, Baltimore, MD USA

T51 NEUROPETIDE REGULATION IN MICE CHRONICALLY TREATED BY MORPHINE
F.M. Décaillot (1), F.Y. Che (2), L. Fricker (2), L.A. Devi (1) (1) Mt. Sinai School of Medicine, New York, NY, USA, (2) Albert Einstein College of Medicine, New York, NY, USA

T52 PROTEOMIC ANALYSIS OF THE EFFECT OF MORPHINE TREATMENT ON THE EXPRESSION PROFILE OF PSD-ASSOCIATED PROTEINS IN MOUSE HIPPOCAMPUS
J.A. Morón (1), N. Abul-Husn (1), G. Dolios (2), R. Wang (2), L. Devi (1) (1) Dept. Pharmacology and Biological Chemistry, (2) Dept. Human Genetics, Mount Sinai School of Medicine, New York, NY, USA

Consequences of receptor activation and physiology
T53 KAPPA-OPIOID RECEPTOR ACTIVATION OF p38 MAP KINASE: ROLES OF RECEPTOR PHOSPHORYLATION AND ARRESTIN M.R. Bruchas, J.D. Lowe, A. Francois, C. Chavkin Dept. Pharmacol, Univ. Washington, Seattle, WA, USA

T54 ACTIVITY-STATE-SENSITIVE ANTIBODIES TO OPIOID RECEPTORS A. Gupta, F.M. Décaillot, O. Tkalych, L.A. Devi Dept. Pharmacology and Biological Chemistry, Mt Sinai Sch Medicine, New York, NY USA

T55 NOCICEPTIN RECEPTOR KNOCKOUT MICE DISPLAY ALTERED ETHANOL SENSITIVITY K. Sakoori, N.P. Murphy Neural Circuit Mechanisms Res. Group, RIKEN Brain Sci. Inst., Wakoshi, Japan

T56 MU AND KAPPA OPIOID RECEPTORS ACTIVATE ERK/MAP KINASE VIA DIFFERENT PKC ISOFORMS AND SECOND MESSENGERS IN ASTROCYTES C.J. Coscia, P.D. Haas, A.L. Clark, J.W. Hahn, A. Kiss, M.M. Belcheva Dept. Biochem and Mol.Biol., St. Louis Univ. Sch. Med., St. Louis, MO USA

T57 INVOLVEMENT OF EPIDERMAL GROWTH FACTOR RECEPTOR TRANS-ACTIVATION IN THE MORPHINE-INDUCED REWARDING EFFECT IN MICE T. Takeuchi, M. Narita, Y. Yajima, T. Suzuki Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut., Sci., Tokyo, JAPAN

T58 INDUCTION OF c-FOS AND ZIF268 IN THE CENTRAL EXTENDED AMYGDALA PARALLELS HYPERALGESIA INDUCED BY NALOXONE FOLLOWING SYSTEMIC MORPHINE IN DRUG NAÏVE RATS P.B. Osborne (1), A.S. Hamlin (1), G.P. McNally (2) (1) Pain Management Res. Inst., Univ. Sydney, Australia, (2) Dept. Psychology, UNSW, Australia

T59 SELECTIVE ACTIVATION OF ACCUMBENS PROJECTION PATHWAYS BY MORPHINE INDUCED CATALEPSY AND STEREOTYPY A.S. Hamlin (1), G.P. McNally (2), R.F. Westbrook (2), P.B. Osborne (1) (1) Pain Management Res. Inst., Univ. Sydney, Australia and (2) Dept. Psychology, UNSW, Australia

T60 DELTA-1-STIMULATION DOWN REGULATES DELTA-2-RESPONSES IN HEART S.H. Deo, S. Johnson-Davis, M.A. Barlow, D. Yoshishige, J.L. Caffrey Univ. North Texas Health Science Center, Fort Worth, TX, USA

T61 KNOCKOUT OF THE MU OPIOID RECEPTOR ENHANCES SURVIVAL OF PROGENITOR CELLS IN THE ADULT HIPPOCAMPUS G. Harburg (1), F.S. Hall (2), A. Harrist (3), I. Sora (4), G.R. Uhl (2), A. Eisch (1) (1) Dept. Psychiatry, U.T. Southwestern Med. Ctr., Dallas, TX, (2) Molec. Neurobiol. Branch, NIDA-IRP, NIH, Baltimore, MD, (3)Univ. Penn. Sch. Med., Philadelphia, PA, (4) Dept. Neurosci., Tohoku Univ. Grad. Sch. Med., Sendai, Japan

T62 ETHYNYLESTRADIOL INDUCED CHOLESTASIS CAUSES SCRATCHING IN RATS S. Inan, A. Cowan Dept. Pharmacology, Temple Univ. School of Medicine Philadelphia, PA, USA

15:30 – 18:50 **Symposium IV Prescription and non-prescription drug abuse** **Marriott Ballroom**
Chair F. Vocci, Cochair M.B. Max

15:30 – 15:55 **S22** **F. Vocci** OPIATE ABUSE PATTERNS IN THE UNITED STATES: A CHANGING SCENE

15:55 – 16:20 **S23** **J. White** BUPRENORPHINE IMPLANTS (PROBUPHINE®) FOR TREATMENT OF OPIOID DEPENDENCE: CLINICAL TRIAL RESULTS

16:20 – 16:40 *Coffee break*

16:40 – 17:05 **S24** **M. Max** RECENT DEVELOPMENTS IN THE DRUG TREATMENT OF CHRONIC PAIN

17:05 – 17:30 **S25** **W. Ling** HYPERALGESIA: PUTTING ASUNDER WHAT GOD HATH JOINED TOGETHER?

17:30 – 17:45 **S26** **D. Mash** NORIBOGAINE: A METABOLITE OF THE NATURALLY OCCURRING SUBSTANCE IBOGAINE MEDIATES THE BENEFICIAL EFFECTS OF THE DRUG ON OPIATE WITHDRAWAL AND DEPENDENCE

17:45 – 18:00 **S27** **B.A. Moore** TREATMENT OUTCOME OF NONMEDICAL PRESCRIPTION OPIATE USERS IN OFFICE-BASED BUPRENORPHINE TREATMENT: COMPARISON WITH HEROIN AND COMBINED USERS

18:00 – 18:15 **S28** **J. Prosser** NEUROPSYCHOLOGICAL CORRELATES OF PROLONGED ABSTINENCE IN OPIOID ADDICTION

18:15 – 18:30 **S29** **J. Dorsey** OPIOID AND NONOPIOID ANALGESICS: REPORTED FATAL EXPOSURES 1983-2003

18:30 – 18:45 **S30** **P. Portoghese** REDUCED ABUSE LIABILITY OF BIVALENT MU AGONIST-DELTA ANTAGONIST COMPOUNDS IN THE CONDITIONED PLACE PREFERENCE (CPP) ASSAY

18:45 – 19:00 **S31** **H.H. Loh** CHRONIC NALOXONE-INDUCED REWARDING AND CRAVING EFFECTS IN KN-S196A MICE

Wednesday, July 13

7:00 – 8:30 **Continental breakfast** **Marriott Ballroom Foyer**

8:30 – 9:30 **P4 Plenary Lecture** **Richard Huganir** **Marriott Ballroom**
Regulation of Glutamate Receptors and Brain Function

9:30 – 12:30 **Symposium V** **Opioid receptor regulation** **Marriott Ballroom**
ChairJ.B. Wang, Cochair C. Evans

9:30 – 9:55 **S32** **J.B. Wang** MECHANISMS UNDERLYING MU OPIOID RECEPTOR PHOSPHORYLATION

9:55 – 10:15 *Coffee break*

10:15 – 10:40 **S33** **G. Hendersen** ROLE OF PROTEIN KINASE C IN MU-OPIOID RECEPTOR DESENSITIZATION AND MORPHINE TOLERANCE *IN VITRO*

10:40 – 11:05 **S34** **L.M. Bohn** β-ARRESTIN-2 and μ OPIOID RECEPTOR REGULATION IN MICE

11:05 – 11:30 **S35** **V. Höllt** MU OPIOID RECEPTOR INTERNALIZATION AND DESENSITIZATION

11:30 – 11:45 **S36** **L.-Y. Liu-Chen** COMPARTMENTALIZATION OF THE KAPPA OPIATE RECEPTOR IN LIPID RAFTS ATTENUATES AGONIST-INDUCED ACTIVATION

11:45 – 12:00 **S37** **E. Navratilova** PEPTIDE AND NON-PEPTIDE AGONISTS USE DIFFERENT MECHANISMS TO REGULATE THE HUMAN DELTA-OPIOID RECEPTOR

12:00 – 12:15 **S38** **D.E. Selley** ACUTE ADAPTATION OF MU OPIOID RECEPTORS TO MORPHINE OCCUPANCY

12:15 – 12:30 **S39** **J.R. Traynor** ENDOGENOUS RGS PROTEINS DIFERENTIALLY MODULATE FULL AND PARTIAL MU OPIOID AGONISTS AT ADENYLYL CYCLASE

12:30 – 14:00 **Lunch** **Marriott**

12:30 – 14:00 **Executive committee meeting** **Marriott First Floor Meeting Room**

14:00 - 15:30 **NIDA Update & Grant Writing Workshop for Young Investigators**
L. Miner, R. Liu, M. Green (*NIDA*) with E Unterwald and others

Afternoon and evening otherwise free

Thursday, July 14

7:00 – 8:30 **Continental breakfast** **Marriott Ballroom Foyer**

8:30 – 9:30 **P5 Plenary Lecture** **A.G. Phillips** **Marriott Ballroom**
Memory and addiction: Double duty for corticolimbic circuits

9:30 – 12:30 **Symposium VI** **New opioid compounds** **Marriott Ballroom**

Chair W. Schmidt Cochair I. Carroll

9:30 - 9:55 **S40 W. Schmidt** EXCITING TIMES FOR NOVEL OPIOIDS, 2005

9:55 – 10:15 *Coffee break*

10:15 – 10:40 **S41 I. Carroll** SELECTIVE KAPPA OPIOID RECEPTOR ANTAGONIST JDTIC BLOCKS STRESS-INDUCED REINSTATEMENT OF COCAINE REINFORCED RESPONDING AND HAS ANTIDEPRESSANT-LIKE EFFECTS IN RATS

10:40 – 11:05 **S42 B.L. Roth** NOVEL KOR LIGANDS REVEAL MODE OF BINDING OF SALVINORIN A

11:05 -11:20 **S43 H. Umeuchi** NALFURAFINE HYDROCHLORIDE (TRK-820): A POSSIBLE NEW ANTIPURITIC AGENT

11:20 – 11:35 **S44 H. Schmidhammer** NOVEL, HIGHLY POTENT OPIOID AGONISTS AND ANTAGONISTS IN THE MORPHINAN SERIES

11:35 -11:50 **S45 C. Mitch** DISCOVERY OF NEW OPIOID RECEPTOR ANTAGONISTS

11:50 – 12:05 **S46 E.E. Codd** THE NOVEL DELTA OPIOID RWJ-394674 IS BIOTRANSFORMED TO THE POTENT MU OPIOID, RWJ-413216

12:05 – 12:20 **S47 R. Polt** BIOUSIAN GLYCOPEPTIDES [ENDORPHIN ANALOGUES] PENETRATE THE BBB

12:30 – 15:30 **Lunch and Poster Session III** **Historic Inns**

Structure-Activity Relationships

Th1 A STUDY WITH CHIMERIC PEPTIDES OF Met-ENKEPHALIN and FMRFa: EFFECT OF HALOGENATION AND C-TERMINAL MODIFICATION K. Hanif, K. Gupta, S. Gupta, S. Manikandan, S. Pasha Institute of Genomics and Integratuve Biology, Delhi, India

Th2 GLYCOSYLATION OF ENKEPHALINS PROMOTES PENETRATION OF THE BBB L. Yeomans-Maldonado (1), C.M. Keyari (1), R.D. Egleton (2), E.J. Bilsky (3) R. Polt (1) (1) Carl S. Marvel Labs, Dept. Chemistry, Univ. of Arizona, Tucson, AZ (2) Dept. Pharmacology, AZ Health Sciences Center, Tucson, AZ (3) Dept. Pharmacology, Univ. of New England, Biddeford, ME USA

Th3 β-ENDORPHIN BIOTRANSFORMATION IN THE RAT STRIATUM: EVIDENCE FOR THE EXTRACELLULAR ACTIVITY OF INSULIN-DEGRADING ENZYME B. Reed, B.T. Chait, M. J. Kreek Rockefeller Univ., New York, NY USA

Th4 FROM A SPECIFIC ANTAGONIST TO POTENT AGONISTS – BIOLOGICAL AND PHARMACO-LOGICAL ACTIVITIES OF NOVEL DERIVATIVES OF CYPRODIME M. Spetea, F. Schüllner, R.C. Moisa , I.P. Berzetei-Gurske, M.D. Aceto, L.S. Harris, A. Coop, H. Schmidhammer Dept. of Pharmaceut. Chem., Univ. of Innsbruck, Austria, SRI International, Biosci. Div., Menlo Park, Dept. Pharmacol. Toxicol., Virginia Commonwealth Univ., Richmond, USA, Dept. Pharmaceut. Sci., Univ. Maryland, Sch. Pharmacy, Baltimore, MD, USA

Th5 THE ANTINOCICEPTION INDUCED BY TAPS: INVOLVEMENT IN THE ENDOGENOUS KAPPA-OPIOID PEPTIDE R. Urushiyama (1), K. Ito (1), H. Watanabe (1), A. Yonezawa (1), H. Mizoguchi (1), C. Watanabe (1), T. Fujimura (2), K. Murayama (2), T. Sakurada (3), S. Sakurada (1) (1) Dept. of Physiol. and Anat., Tohoku Pharmaceut. Univ., Sendai, Japan, (2) Div. of Biochem. Anal. Ctr. Lab. of Med. Sci., Juntendo Univ. School of Med., Tokyo, Japan, (3)

Dept. of Biochem., Daiichi Coll. of Pharmaceut. Sci., Fukuoka, Japan

Th6 ANTINOCICEPTIVE PROPERTY OF A NOVEL DERMORPHIN TETRAPEPTIDE ANALOG AMIDINO-TAPA K. Moriyama, K. Ohwada, H. Mizoguchi, C. Watanabe, A. Yonezawa, S. Sakurada Dept. of Physiol. and Anat., Tohoku Pharmaceut. Univ., Sendai, Japan

Th7 FUNCTIONAL SIGNIFICANCE OF A METHYL GROUP: MODULATING KAPPA OPIOID ANTAGONIST ACTIVITY THROUGH CONFORMATIONAL RIGIDITY S. Runyon, H. Navarro, F.I. Carroll Research Triangle Institute, Research Triangle Park, NC USA

Th8 DIFFERENTIAL ANTINOCICEPTIVE EFFECTS OF [DMT1]ENDOMORPHIN-1 and [DMT1] ENDOMORPHIN-2 IN MICE Y. Jinsmaa (1), Y. Fujita (2), K. Shiotani (2), A. Miyazaki (3), T. Li (2), Y. Tsuda (2,3,4), Y. Okada (2,3,4), A. Ambo (5), Y. Sasaki (5), E. Marczak (1), S.D. Bryant (1), L.H. Lazarus (1) (1) Med. Chem. Group, Lab of Pharmacol. Chem., Natl. Inst of Environ. Health Sci, Res.Triangle Park, NC, USA, (2) Grad. Sch. Food Med. Sci, (3) Faculty Pharm. Sci, Dept. Med. Chem. and (4) High Tech. Res. Center, Kobe Gakuin Univ., Kobe, Japan, (5) Dept. Biochem., Tohoku Pharm.Univ., Sendai, Japan

Th9 SYNTHESIS OF N-PHENETHYL PARA-E- AND PARA-F-OXIDE-BRIDGED PHENYLMORPHANS J. Zezula, L.B. Singer, A.K. Przybyl, J. Deschamps, D. Parrish, A.E. Jacobson, K.C. Rice Lab. Medicinal Chemistry, NIDDK, NIH, DHHS, Bethesda, MD, (2) Lab. for the Structure of Matter, Naval Research Lab., Wash DC USA

Th10 A PORTRAIT OF POTENT MU- AND DELTA-OPIOID RECEPTOR LIGANDS L.H. Lazarus (1), S.D. Bryant (1), Y. Jinsmaa (1), E. Marczak (1), Y. Okada (2), Y Tsuda (2), Y. Fujita (2), T Li (2), K. Shiotani (2), A. Miyazaki (2), A. Ambo (3), Y. Sasaki (3), G. Balboni (4), S. Salvadori (5) (1)Med. Chem., LPC, NIEHS, RTP, NC, (2) Fac. Pharm. Sci., Kobe Gakuin Univ., Kobe, Japan, (3)Tokoku Pharm. Univ., Sendai, Japan, (4) Dept. Toxicol., Univ. Cagliary, Cagliary, Italy, (5) Dept. Pharm. Sci., Univ. Ferrara, Ferrara, Italy

Th11 SYNTHESIS AND EVALUATION OF NOVEL ENANTIOMERIC N-Phenylethyl-5-phenylmorphans A.-C. Hiebel , G. De Martino, R.B. Rothman, C.M. Dersch, J. Deschamps, A.E. Jacobson, K.C. Rice Lab. Med. Chem., NIDDK, NIH, Bethesda, MD Clin. Psychopharm. Sec., NIDA, NIH, Baltimore, MD Lab. Struct. Matter, Naval Res. Lab., Washington, D.C. USA

Th12 SPIROCYCLIC INDANES AS LIGANDS FOR THE NOP (ORL-1) RECEPTOR R.R. Goehring, X. Zhou, J.-C. Huang, L.J. Barnett, Q. Sun, S.F. Victory, K.J. Valenzano, W.S. Miller, S. Shan, D.J. Kyle Discovery Research, Purdue Pharma, L.P., Cranbury, NJ, USA

Th13 THE NEW DELTA OPIOID ANTAGONIST, TYR-TIC–(2S,3R)βMEPHE-PHE-OH REVEALS DISTINCT DELTA SITES IN RAT AND MOUSE BRAIN M. Szucs (1), G.Toth (1), I. Kertesz (1), L. Bakota (2), K. Gulya (2), J. Pintar (3), E. Birkas (1) (1) Biological Research Center, Szeged, (2) Dept. Cell Biol., SZTE, Szeged, Hungary, (3) UMDNJ, Piscataway, NJ, USA

Th14 FURTHER STUDY OF THE STRUCTURE-ACTIVITY RELATIONSHIPS OF NEOCLERODANE DITERPENES AT OPIOID RECEPTORS T. Prisinzano (1), W. Harding (1), K. Tidgewell (1), M. Schmidt (1), C.M. Dersch (2), R.B. Rothman (2) (1) Division of Medicinal and Natural Products Chemistry, University of Iowa, Iowa City, IA, (2) Clinical Psychopharmacology Section, IRP, NIDA, NIH, DHHS, Baltimore, MD USA

Th15 XENOPUS LAEVIS PRODYNORPHIN SEQUENCE REVEALS NOVEL FAMILY MEMBERS OF THE OPIOID PEPTIDES S. Benyhe (1), P. Pattee (2), S.R. Nagalla (2), E-A. Ilie (1), G. Toth (1), A. Borsodi (1) (1) Inst. Biochem., Biol. Res. Ctr., Hungarian Acad. Sci., Szeged, Hungary (2) Center for Biomarker Discovery, Oregon Health and Sci. Univ., Portland, Oregon USA

Th16 MORPHINE-6-GLUCURONIDE INDUCES CONDITIONED PLACE PREFERENCE WHEREAS MORPHINE-3-GLUCURONIDE INDUCES CONDITION PLACE AVERSION IN MICE V. Olsen, M. Handal, Å.

Ripel, F. Boix, J. Mørland Norwegian Institute of Public Health Division of Forensic Toxicology and Drug Abuse, Oslo, Norway

Th17 NALFURAFINE CAUSES SPECIES-SPECIFIC DIURESIS THROUGH KAPPA OPIOID RECEPTORS S. Inan (1), D.Y.W. Lee (2), L.Y. Liu-Chen (1), Z. Ma (2), B. Cohen (2), A. Cowan (1) (1) Dept. Pharmacology, Temple Univ. School of Medicine, Philadelphia, PA, (2) McLean Hospital, Harvard Medical School, Belmont, MA, USA

Th18 A STUDY WITH CHIMERIC PEPTIDES OF Met-enkephalin and FMRFa: EFFECT OF HALOGENATION AND C-TERMINAL MODIFICATION K. Hanif, K. Gupta, S. Gupta, S. Manikandan, S.Pasha Institute of Genomics and Integratuve Biology, Delhi, India

Th19 LY255582 INHIBITS BASAL, MORPHINE- AND FEEDING-INDUCED DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS SHELL (NAs) M.A. Statnick, A.E. Sahr, B.J. Eastwood, C.H. Mitch Lilly Research Laboratories, Indianapolis, IN USA

Th20 THE DEVELOPMENT OF TOLERANCE TO FENTANYL DERIVATIVES IS RELATED TO THEIR POTENCY AND ANALGESIC DURATION T. Wen, J.E. Pintar Dept. of Neurosci, UMDNJ USA

Th21 DEVELOPMENT OF OPIOID GLYCOPEPTIDES AS TREATMENTS FOR ACUTE AND CHRONIC PAIN J. Lowery (1), R. Paolino (1), L. Yeomans (2), J. Bidlack (3), R. Polt (2), E. Bilsky (1) (1) Univ. New England, Biddeford, ME; (2) Univ.Arizona, Tucson, AZ; (3) Univ.Rochester Medical Center, Rochester, NY USA

Th22 *IN VITRO* AND *IN VIVO* CHARACTERIZATION OF OPIOID INVERSE AGONISTS AND NEUTRAL ANTAGONISTS E. Bilsky (1), D. Wang (2), W. Sadée (2), (1) Univ. New England, Biddeford, ME; (2) Ohio State Univ., Columbus, OH USA

Th23 A NOVEL MU OPIOID AGONIST BASED ON SALVINORIN A K.J. Tidgewell (1), R.A. Moyer (3), L.M. Bohn (3), W.W. Harding (1), C. Dersch (2), R.B. Rothman (2), T.E. Prisinzano (1) (1) College of Pharmacy, Univ. Iowa, (2) IRP, NIDA, NIH, DHHS, Baltimore, MD, (3) Depts. Pharmacology & Psychiatry, Ohio State Univ.USA

Th24 PHARMACOKINETICS AND BEHAVIORAL EFFECTS OF SALVINORIN A, A NATURALLY OCCURRING KAPPA-OPIOID HALLUCINOGEN, IN NON HUMAN PRIMATES E.R. Butelman (1), M.D. Schmidt (2), M.S. Schmidt (2), W.W. Harding (2), K. Tidgewell (2), D.J. Murry (2), M.J. Kreek (1), T.E. Prisinzano (2) (1) Rockefeller Univ., New York NY, USA, (2) Univ. Iowa, Iowa City IA, USA

Th25 CHARACTERIZATION OF A PUTATIVE NOVEL ENDOGENOUS PEPTIDE LIGAND FOR THE MU OPIOID RECEPTOR B. Anton, M. Matus, H. Gompfh, J.C. Calva, A. Salazar, R. Arreola, L. Parra-Gamez R. Acevedo, P. De los Heros, B. Peng, S.L. Cruz, G. Gamba, C. Allen, J. Pintar, P. Leff Instituto Nacional Psiquiatria-RF Mexico, CROET Oregon Hlth. & Sci. Univ. Portland, OR, USA, Fac. Med., Dpto. Anatomia, UNAM, IIB & INCMNSZ, Mexico, UMDNJ-RBJ Med. Sch, Piscataway, NJ, USA, Dpto. Farmacol. Cinvestav, Mexico

Th26 PLASMON WAVEGUIDE RESONANCE (PWR) SPECTROSCOPY, A NOVEL AND SENSITIVE TOOL TO EXAMINE LIGAND BINDING TO THE HUMAN CANNABINOID RECEPTOR T. Georgieva (1), E. Varga (1), M. Eaton (1), Z. Salamon (2), V.J. Hruby (2), W.R. Roeske (1), G. Tollin (2), H.I. Yamamura (1); Depts Medical Pharmacology (1), Biochemistry (2), Univ. Arizona, Tucson, AZ, USA

Memory

Th27 DELTA OPIOID RECEPTORS MODULATE MEMORY PROCESSES: EVIDENCE FROM BEHAVIORAL ANALYSIS OF KNOCK-OUT MICE G. Scherrer (1), S. Grappi (2), F. Hartmann (1), A. Matifas (1), C. Gambarana (2) and B.L. Kieffer (1) (1) IGBMC, CNRS/INSERM/ULP, UMR7104, Illkirch, France (2) Dept. Neuroscience, Pharmacology Unit, Univ. Siena, Siena, Italy

Th28 EFFECTS OF KAPPA-OPIOID RECEPTOR AGONIST ON THE DEFICITS OF LEARNING AND MEMORY IN MICE T. Mamiya, J. Inagaki, T. Asanuma, M. Ukai Dept. Chem. Pharmacol., Fac. Pharm., Meijo Univ., Nagoya, Japan

Th29 A UNIQUE PATTERN OF BEHAVIORAL EFFECTS OF BIG DYNORPHIN, A PRODYNORPHIN DERIVED PEPTIDE IS MEDIATED THROUGH NMDA RECEPTORS A. Kuzmin1,2, N. Madjid 1, S.O. Ogren1, L. Terenius2, G. Bakalkin2 Depts. 1Neurosci.and 2Clinical Neurosci., Karolinska Institutet, Stockholm, Sweden

Th30 PERSISTENT DISRUPTION OF AN ESTABLISHED MORPHINE CONDITIONED PLACE PREFERENCE FOLLOWING RECONDITIONING M.H. Milekic, S.D. Brown, C. Castellini, C.M. Alberini Dept. Neuroscience, Mt. Sinai School of Medicine, New York, NY USA

Regulation of Opiate Systems

Th31 MOR EXPRESSION IS INDUCED IN DENTATE GYRUS GRANULE CELLS AFTER FOCAL CEREBRAL ISCHEMIA AND STIMULATION OF ENTORHINAL AFFERENTS R. Stumm, H. Rüthrich, V. Höllt. Inst. Pharmacol./Toxicol., Otto-von-Guericke-Univ. Magdeburg, Germany

Th32 COMPARISON OF MU-OPIOID RECEPTOR DESENSITIZATION IN RAT LOCUS COERULEUS NEURONS AND IN HEK293 CELLS E.A. Johnson, E. Kelly, G. Henderson, C.P. Bailey Dept. Pharmacology, Univ. Bristol, UK

Th33 MU-OPIOID RECEPTOR (MOR) DESENSITIZATION AND TRAFFICKING BY MORPHINE AND 6-MONOACETYL-MORPHINE C.P. Bailey, E. Braksator, S.J. Mundell, E. Johnson, E. Kelly, G. Henderson Dept. Pharmacology, Univ. Bristol, UK

Th34 FEEDBACK REGULATION OF OPIOID RECEPTOR ENDOCYTOSIS BY ADENYLYL CYCLASES H. Ammer, A.I. Giesen, R. Schulz Institute of Pharmacology, Toxicology and Pharmacy, University of Munich, Germany

Th35 DOWNREGULATION OF KOP-R IN BRAINS OF RATS WITHDRAWN FOR 14 DAYS FROM AN ESCALATING DOSE "BINGE" COCAINE ADMINISTRATION PARADIGM
A. Bailey, R. Gianotti, A. Ho, M.J. Kreek Lab. Biology of Addictive Diseases, Rockefeller Univ., New York, NY, USA

Th36 MECHANISMS OF MU OPIOID RECEPTOR TRANSCRIPTIONAL REGULATION BY INTERLEUKIN-4
J. Kraus, C. Börner, S. Kolbitz, V. Höllt Dept. Pharmacology, Magdeburg University, Germany

Th37 DIFFERENTIAL EXPRESSION OF THE KAPPA OPIOID RECEPTOR ON MONOCYTES/ MACROPHAGES C.M. Tipton, J.M. Bidlack Dept. Pharm/Physiol, Univ. Rochester, Rochester, NY, USA

Th38 THE EXPRESSION OF PRODYNORPHIN GENE IS TRANSCRIPTIONALLY INHIBITED BY LIPOPOLYSACCHARIDE TREATMENT IN U-937 MONOCYTE/MACROPHAGE CELLS B. Sun, J.M. Bidlack Dept. Pharmacology and Physiology, Univ. Rochester, School of Medicine, Rochester, NY, USA

Th39 ACTIVATION OF MOUSE MICROGLIAL CELL LINE BY IFN-γ AND LPS LEADS TO DOWN-REGULATION OF DOR mRNA S. Sumagin, J.M. Bidlack Dept. Pharmocology and Physiology Univ. Rochester School of Medicine and Dentistry, Rochester, NY, USA

Th40 MODULATION OF BASAL MU OPIOID RECEPTOR (MOR) ACTIVITY IN MORPHINE-DEPENDENT MICE D. Wang (1), E.J. Bilsky (2), W. Sadée (1) (1) Dept. Pharmacol., Ohio State Univ., Columbus, Ohio, (2) Dept. of Pharmacol., Univ. New England, Biddeford, ME, USA

Th41 MORPHINE-INDUCED GASTROINTESTINAL TRANSIT IN β-ARRESTIN2 KNOCK OUT MICE K.M. Raehal, L.M. Bohn Depts. Pharm & Psychiatry, Ohio State Univ. College of Medicine, Columbus, OH USA

Th42 CANNABINOIDS INDUCE MU OPIOID RECEPTOR TRANSCRIPTS IN JURKAT T CELLS

C. Börner, J. Kraus, V. Höllt Dept. of Pharmacology and Toxicology, Univ. Magdeburg, Germany

Th43 THE DELTA OPIOID RECEPTOR (DOR) IN NG108-15 CELLS AND EXPRESSED IN CHO CELLS LOCALIZES IN LIPID RAFTS P. Huang, W. Xu, S.-I. Yoon, C. Chen, P.L.-G. Chong, L.-Y. Liu-Chen Depts. Pharmacol. and Biochem., Ctr. Subs. Abuse Res., Temple Univ. Med. Sch., Philadelphia, PA USA

Th44 THE MU-OPIOID RECEPTOR IN HEK 293 CELLS IS MODULATED BY CO-EXPRESSED METABOTROPIC GLUTAMATE RECEPTOR 5 H. Schröder, T. Koch, S. Schulz, V. Höllt, Dept. Pharmacology and Toxicology, Otto-von-Guericke Univ. Magdeburg, Magdeburg, Germany

Th45 POTENTIATION OF DELTA OPIOID RECEPTOR BINDING BY ACTIVATION OF 5HT3 RECEPTORS D. Paul, L. Minor Dept. Pharmacology, LSU Health Sciences Center, New Orleans, LA USA

Th46 NALTREXONE UP-REGULATES DELTA OPIOID RECEPTOR BINDING WITHOUT INCREASING MATURE RECEPTOR PROTEIN K.M. Wannemacher (1), P.N. Yadav (2), M. Doligosa (1), R.D. Howells (1,2) (1) Dept. Biochem. Mol. Biol., UMDNJ-Grad. Sch. Biomed Sci. (2) UMDNJ-NJ Med. Sch., Newark, NJ, USA

Th47 THE HISTONE DEACETYLASE INHIBITOR, TRICHOSTATIN A, STIMULATES EXPRESSION OF THE DELTA OPIOID RECEPTOR IN HEK 293 CELLS P.N. Yadav (1), K.M. Wannemacher (2), M. Balan (2), R.D. Howells (1,2) (1) Dept. Biochem. & Mol. Biol., UMDNJ-New Jersey Medical School, (2) UMDNJ-Graduate School of Biomedical Sciences, Newark, NJ, USA

Th48 DOES FILAMIN REGULATE OPIOID RECEPTORS VIA THE ACTIN CYTO-SKELETON? I. Onoprishvili (1), M.L. Andria (1) E.J. Simon (1,2) Depts. (1) Psychiatry, (2) Pharmacology, NYU School of Medicine NY, NY **USA**

Th49 ROLE OF β-ARRESTIN 1 IN HUMAN DELTA OPIOID RECEPTOR REGULATION B. Aguila, L. Coulbault, E. Rippoll, N. Marie, A. Hasbi, P. Jauzac, S. Allouche UPRES EA 3919 Biologie moléculaire et cellulaire de la signalisation, Univ. Caen, France

Th50 SERINE 363 PHOSPHORYLATION OF THE DELTA OPIOID RECEPTOR MEASURED BY A PHOSPHOSPECIFIC ANTIBODY M. Pfeiffer, S. Schulz, R. Stumm, V. Höllt Dept. Pharmacology, Univ. Magdeburg, Germany

Th51 DIFFERENTIAL REGULATION OF HUMAN DELTA OPIOID RECEPTOR BY AGONISTS B. Aguila, N. Marie, A. Hasbi, P. Jauzac, S. Allouche UPRES EA 3919, Biologie moléculaire et cellulaire de la signalisation, Univ. Caen, France

Th52 LACK OF EVIDENCE FOR AGONIST-SPECIFIC CONFORMATIONS OF THE MU-OPIOID RECEPTOR USING PERTUSIS TOXIN INSENSITIVE G PROTEINS M.J. Clark, C.A. Furman, T.D. Gilson, J.R. Traynor Dept. Pharmacology, Univ. Michigan, Ann Arbor, MI, USA

Th53 UP-REGULATION OF THE MU OPIOID RECEPTOR BY LIPOPOLYSACCHARIDE IN TPA-DIFFERENTIATED HL-60 CELLS J. Beltran, A. Pallur, S.L. Chang Dept. Biol., Seton Hall Univ., S. Orange, NJ, USA

Th54 ROLE OF THE T394 MUTANT IN AGONIST-INDUCED MU OPIOID RECEPTOR INTERNALIZATION E. Barbier, J.B. Wang Dept. Pharmaceutical Sci., School of Pharmacy, Univ. Maryland, Baltimore, MD, USA

Th55 ENKEPHALIN REGULATES INCREASED IN CONSTITUTIVELY ACTIVE MU RECEPTORS DURING OPIATE WITHDRAWAL J. Shoblock, N. Maidment NPI, UCLA, Los Angeles, CA USA

Th56 DELTA OPIOID RECEPTOR FUNCTION IN MIDBRAIN NEURONS AFTER CHRONIC MORPHINE S.P. Hack, E.E. Bagley, B.C.H. Chieng, M.J. Christie Pain Management Research Institute, Univ. Sydney, Australia

Th57 NANDROLONE DECANOATE AFFECTS THE ENKEPHALIN SYSTEM IN THE FEMALE RAT BRAIN
K. Magnusson (1) M. Hallberg (1) A.-S. Lindqvist (2) C. Fahlke (2) F. Nyberg (1) (1) Dept. Pharm. Biosci., Div. Biol. Res. on Drug Dependence, Uppsala Univ., Uppsala, Sweden, (2) Dept. Psychol., Gôteborg Univ., Gôteborg, Sweden

Th58 MORPHINE PROMOTES PHOSPHORYLATION OF THE DELTA OPIOID RECEPTOR AT SERINE 363
D. Stropova, E. Navratilova, M.C. Eaton, E.V. Varga, T.W. Vanderah, W.R. Roeske, H.I. Yamamura Univ. Arizona, Tucson, AZ, USA

Th59 MATURATION OF HUMAN κ OPIOID RECEPTORS (hKOR) EXPRESSED IN CHO CELLS
J.-G. Li, C. Chen, L.-Y. Liu-Chen Dept. Pharmacology, Temple Univ. Sch. Med., Philadelphia, PA, USA

Th60 CHRONIC ORPHANIN FQ/NOCICEPTIN (OFQ/N) INDUCES MU RECEPTOR INTERNALIZATION V.I. Ramirez , D.M. Sherry, K.M. Standifer Univ. Houston, Houston, TX USA

Th61 RGS4 BINDS DIRECTLY TO THE C-TERMINAL TAILS OF THE MU-AND DELTA-OPIOID RECEPTORS TO MODULATE G PROTEIN SIGNALING L. Leontiadis (1), H.E. Hamm (2) Z. Georgoussi (1) (1) Lab. Cell. Signal. and Mol. Pharmacol., Inst. Biol., EKEFE "Demokritos", Athens, Greece, (2) Dept. Pharmacol.,Vanderbilt Univ. Sch. Medicine, Nashville, TN, USA

Interactions, other substances

Th62 CANNABINOID–OPIOID RECEPTOR INTERACTIONS IN NEURITE OUTGROWTH IN NEURO 2A CELLS I. Gomes (1), C. Rios (2), R. Iyengar (1), L.A. Devi (1) (1)Dept. Pharmacology and Biological Chemistry, Mount Sinai School of Medicine, New York, NY, (2) Weill College of Medicine, Cornell Univ., New York, NY

Th63 NEURONAL PATTERS OF EXPRESSION OF CB2 CANNABINOID RECEPTOR IMMUNO-REACTIVITY IN THE CENTRAL NERVOUS SYSTEM J.-P. Gong (1), E. Onaivi (2), G.R. Uhl (1) Molec Neurobiol Br, NIDA-IRP, NIH/DHSS, Baltimore, MD, (2) Dept. Biol., William Paterson Univ., Wayne, NJ USA

Th64 DIFFERENTIAL LONG-TERM NEUROADAPTATIONS OF GLUTAMATE RECEPTORS IN THE BASOLATERAl AND CENTRAL AMYGDALA AFTER WITHDRAWAL FROM COCAINE SELF-ADMINITRATION IN RATS L. Lu , J. Dempsey, Y. Shaham, B. Hope Behav. Neurosci. Branch, NIDA-IRP, NIH/DHHS, Baltimore, MD USA

Th65 EFFECT OF AC5 KNOCKOUT IN COCAINE-INDUCED INCREASES IN STRIATAL DOPAMINE LEVELS IN MICE Y. Zhang (1), S.D. Schlussman (1), A. Ho (1), Y. Ishikawa (2), M.J. Kreek (1) (1) Lab. Biology of Addictive Diseases, Rockefeller Univ., New York, NY, USA, Depts. Physiology and Medicine, Yokohama City Univ. School of Medicine, Yokohama, Japan.

Th66 PSYCHOSTIMULANT SUPERSENSITIVITY IN THE PROTEIN KINASE-C INTERACTING PROTEIN (PKCI) KNOCKOUT MICE E. Barbier, O. Egbulefu, S. Gerani-Diznab, J.B. Wang Dept. of Pharmaceutical Sci., School of Pharmacy, Univ. Maryland, Baltimore, MD, USA

Th67 DOPAMINE RECEPTOR INTERACTIONS WITH ARRESTINS IN NEOSTRIATAL NEURONS T.A. Macey1, C. Chavkin1, K.A. Neve2 1Dept. Pharmacol, UW, Seattle, WA, USA 2 Dept. BEHN, OHSU, Portland, OR, USA

Th68 MICROARRAY STUDY OF COCAINE EFFECTS IN HUMAN PRIMARY MICROGLIAL CELLS V. Yuferov (1), D. Nielsen (1), S. Hu (2), P. Peterson (2), M.J. Kreek (1) (1) Rockefeller Univ., New York, NY, (2) Univ. Minn. Med. Sch., Minneapolis, MN USA

Th69 OPIOID OR DOPAMINE D1 RECEPTOR BLOCKADE ENHANCES HYPOTHALAMIC VASOPRESSIN GENE EXPRESSION AFTER ACUTE BINGE COCAINE Y. Zhou, V. Yuferov, J. Adomako-Mensah, A. Ho, M.J. Kreek Rockefeller Univ., New York, NY USA

Th70 COCAINE-INDUCED BEHAVIORAL SENSITIZATION IN MICE LACKING ORL-1 RECEPTORS R. Baliram, N. Gajawada, P. Kotha, K. Lutfy Dept. Pharm. Sci., Western Univ. Health Sci., Pomona, CA USA

Th71 COCAINE-INDUCED BEHAVIORAL SENSITIZATION IN OPIOID PEPTIDE KNOCKOUT MICE J. Borse, R. Baliram, N. Gajawada, K. Lutfy Dept. Pharm. Sci., Western Univ. Health Sci., Pomona, CA

Th 72 DEMONSTRATION OF LIGAND-SPECIFIC DELTA OPIOID RECEPTOR CONFORMATIONS BY PLASMON WAVEGUIDE RESONANCE SPECTROSCOPY Eva Varga, Isabel Alves, Zdzislaw Salamon, Teodora Georgieva, Victor Hruby, William Roeske, Gordon Tollin, Henry Yamamura; Departments of Medical Pharmacology, Chemistry; Biochemistry University of Arizona, Tucson, Ariz, USA

15:30 – 16:15 **Business Meeting** **Marriott Ballroom**

16:15 – 18:15 **Symposium VII** **Hot topics** **Marriott Ballroom**
Chair G.R. Uhl, Cochair J.M. Bidlack

16:15 – 16:30 **S48** **P. Leff** CLONING AND FUNCTIONAL CHARACTERIZATION OF A NOVEL OPIOID PEPTIDE SYSTEM FOR THE MU OPIOID RECEPTOR

16:30 – 16:45 **S49** **Z. Wang** LACK OF OPIOID TOLERANCE AND DEPENDENCE IN S286ACaMKII MUTANT MICE

16:45 – 17:00 **S50** **M.J. Christie** OPIOID WITHDRAWAL BUT NOT TOLERANCE IN SINGLE MIDBRAIN NEURONS FROM β-ARRESTIN-2 KNOCKOUT MICE

17:00 – 17:15 *Coffee Break*

17:15 – 17:30 **S51** **J.E. Pintar** INHIBITION OF FOOD INTAKE BY THE OPIOID ANTAGONIST LY255582 IS LOST IN TRIPLE OPIOID RECEPTOR KNOCKOUT MICE.

17:30 – 17:45 **S52** **M. Narita** INVOLVEMENT OF THE NEURONAL MIGRATING REGULATOR, REELIN, IN THE DEVELOPMENT OF TOLERANCE TO MORPHINE-INDUCED ANTI-NOCICEPTION

17:45 – 18:00 **S53** **J Mathews** ATTENUATION OF MORPHINE TOLERANCE THROUGH A NOVEL G β/γ MECHANISM

20:00 – 23:00 **Banquet** **Marriott Ballroom**
L. Fricker, entertainment organizer

Friday, July 15

7:00 – 8:30 **Continental breakfast** **Marriott Ballroom Foyer**

8:30 – 9:30 **P6** **Plenary Lecture** **M. Caron** **Marriott Ballroom**
Animal models of psychostimulant actions: implications of new GPCR signaling paradigms

9:30 – 12:30 **Symposium VIII** **Psychostimulants, opioids and monoamine interactions** **Marriott Ballroom**
Chair T. Shippenberg, Cochairs I Sora, C Chavkin

9:30 – 9:55 **S54** **T.S. Shippenberg** REGULATION OF MONOAMINE TRANSPORTER FUNCTION AND CELL SURFACE EXPRESSION BY K-OPIOID SYSTEMS: IMPLICATIONS FOR ADDICTION TREATMENT

9:55 – 10:15 *Coffee break*

10:15 – 10:40 **S55** **I. Sora** EXCLUSIVE EXPRESSION OF μ-OPIOID RECEPTORS IN NORADRENERGIC NEURONS REVERSES THE DECREMENTS IN STRESS RESPONSES NOTED IN μ -OPIOID RECEPTOR KNOCKOUT MICE

10:40 – 11:05 **S56** **M. Morari** NOCICEPTIN/ORPHANIN FQ RECEPTOR ANTAGONISTS AS A NOVEL APPROACH FOR THERAPY OF PARKINSON'S DISEASE

11:05 – 11:20 **S57** **J. McLaughlin** PRIOR KAPPA OPIOID RECEPTOR (KOR) ACTIVATION MEDIATES THE STRESS-INDUCED POTENTIATION OF THE COCAINE CONDITIONED PLACE PREFERENCE (CPP) RESPONSE.

11:20 – 11:35 **S58** **E.M. Unterwald** DELTA OPIOID RECEPTOR DESENSITIZATION DURING COCAINE WITHDRAWAL IS ASSOCIATED WITH INCREASED ANXIETY-LIKE BEHAVIORS.

11:35 – 11:50 **S59** **C. Du** OPTICAL BRAIN MONITORING OF COCAINE-INDUCED CEREBROVASCULAR AND INTRACELLULAR CALCIUM EFFECTS IN THE LIVING RAT

11:50 – 12:10 **S60** **F.S. Hall** ROLES FOR DOPAMINE (DAT), SEROTONIN (SERT) AND VESICULAR MONOAMINE 2 (VMAT2) TRANSPORTERS IN D-AMPHETAMINE-CONDITIONED PLACE PREFERENCE

12:10 – 12:25 **S61** **J.M. Brown** DELETION OF THE ppNOCICEPTIN GENE ATTENUATES MPTP -, BUT NOT METHAMPHETAMINE-, INDUCED DOPAMINE DAMAGE

12:30 **Adjourn**

Extended Program and Abstracts

Sunday, July 10

15:00 – 18:00; 19:30 – 20:30 **Registration** **Marriott Ballroom Foyer**
19:30 – 21:30 **Opening Reception** **Marriott Ballroom**

Monday, July 11

7:00 – 8:30 **Continental breakfast** **Marriott Ballroom Foyer**

8:30 – 9:30 **P1 Plenary Lecture Solomon Snyder Marriott Ballroom**
Novel Neurotoxic and Neuroprotective Mechanisms

Solomon H. Snyder, Department of Neuroscience, Johns Hopkins University, School of Medicine, 725 North Wolfe Street Baltimore, MD USA We have elucidated a novel apoptotic cascade whereby diverse forms of cell stress activate inducible NO synthase (iNOS). The generated NO nitrosylates glyceraldehyde-3-phosphate dehydrogenase (GAPDH) abolishing catalytic activity and conferring the ability to bind to Siah. Siah is an ubiquitin-3-ligase which possesses a nuclear localization signal (lacking in GAPDH) and causes the translocation of the two proteins to the nucleus. Within the nucleus GAPDH stabilizes the rapidly turning over Siah. Siah elicits apoptosis by degrading several nuclear proteins. Evidence for this cascade includes the physiologic binding of nitrosylated GAPDH to Siah and the abolition of nuclear translocation of GAPDH and of cell death with mutation of a single amino acid in GAPDH that prevents it's binding to Siah. The monoamine oxidase inhibitor Deprenyl is neuroprotective by binding GAPDH and preventing GAPDH-Siah interactions. Neurotoxicity elicited by mutant Huntingtin, the protein that causes Huntington's disease, involves nuclear translocation of its N-terminal fragment. GAPDH binds to this fragment and, with Siah, elicits nuclear translocation and toxicity. The high energy inositol pyrophosphates such as IP7 and IP8 and generated by a family of IP6 kinase enzymes. One of these, IP6 kinase-2 (IP6K-2) is exclusively nuclear and mediates cell death. Over-expression of IP6K2 markedly augments sensitivity to cytotoxic agents. Deletion by RNA interference of IP6K-2 substantially reduces cytotoxicity. Apoptotic stimuli elicit translocation of IP6K-2 from the nucleus to mitochondria which appears to trigger mitochondrial "sickening." We recently showed that IP7 phosphorylates proteins and speculate that selective phosphorylation of outer membrane mitochondrial proteins may underlie the toxic actions of IP6K-2. We recently demonstrated a bilirubin-bilirubin biliverdin reductase amplification cycle whereby nM concentrations of bilirubin are comparable to glutathione cycle (GSH) as physiologic cytoprotectants. The hydrophilic GSH protects soluble proteins while bilirubin prevents lipid peroxidation of membranes.

9:30 – 12:30 **Symposium I Recent advances in pain research Marriott Ballroom**
Chair F. Porreca, Cochair H. Fields

9:30 – 9:55 **S1 F. Porreca MECHANISMS OF OPIOID-INDUCED NEUROPLASTICITY AND HYPERALGESIA** ***T. King, A. Vardanyan, F. Porreca, Department of Pharmacology, University of Arizona, Tucson, AZ 85724, USA*** Recent studies have shown that sustained opioid administration produces broad, pronociceptive neuroplastic adaptations which are accompanied by behavioral hyperalgesia. We have characterized the effects of sustained morphine administration in rats on the characteristics of primary afferent fibers and the spinal dorsal horn. Infusion of morphine by osmotic minipump elicited an upregulation of CGRP and substance P in the spinal dorsal horn as well as an enhanced, capsaicin-evoked release of these transmitters. Morphine administration elicited upregulation of spinal NK-1 receptors. Stimulus-evoked release of substance P produced NK-1 receptor internalization in the superficial dorsal horn of saline-treated rats, but in both superficial and deep lamina of the dorsal horn of morphine treated rats. Similarly, sustained morphine exposure elicited significantly greater capsaicin-evoked plasma extravasation and hindpaw flinching when compared with control animals. The morphine-induced increased expression of substance P was characterized by increased numbers of substance P positive small, but not large, diameter cells. The numbers of "peptide rich" dorsal root ganglion cells, characterized by lack of IB4 binding, were increased significantly by morphine exposure. Additionally, however, the proportion of normally "peptide poor", IB4 positive cells (which do not express substance P) showed a six-fold increase in expression of substance P after morphine treatment representing a morphine-induced "phenotypic switch". Sustained opioid-administration produced

an increase in pp-38 MAPK in cells in the DRG. Lesions of NK-1 receptors in the spinal dorsal horn with a substance P-saporin conjugate resulted in a block of opioid-induced hyperalgesia and NK-1 receptor knock-out mice did not demonstrate opioid-induced hyperalgesia. These pronociceptive, neuroplastic adaptations produced by opioids are reminiscent of states of inflammatory pain. The consequence of these pronociceptive adaptations on backgrounds of chronic pain are largely unknown but are likely to be significant.

9:55 – 10:15 *Coffee break*

10:15 – 10:40 **S2 H.L. Fields VIEWING MOTIVATION THROUGH AN OPIOID LENS: THE INTERSECTION OF ANALGESIA AND ADDICTION** ***H.L. Fields, Department of Neurology and Ernest Gallo Clinic and Research Center, University of California San Francisco, Emeryville, CA USA*** Because of their powerful analgesic potency, opioids have achieved broad acceptance as an essential treatment for pain. However, they also have significant addiction liability and are widely abused. The most powerful analgesics and the most addicting opioids are agonists at the mu opioid receptor. Despite decades of attempts, there is currently no opioid medication that retains strong analgesic activity without also producing reward. At the level of systems neuroscience, opioid sensitive mesolimbic circuits that mediate reward can engage descending opioid sensitive brainstem analgesia producing circuits. However, there is currently no simple conceptual model that would encompass the interaction of analgesia and reward in a unified and biologically meaningful manner. I propose that a full understanding of the relation and interaction of reward and analgesia requires viewing pain primarily as a motivational state that contributes to behavioral decision making. Thus, noxious stimuli engage robust action systems and when this motivational demand occurs in a conflict situation; e.g. in the presence of a predator or food; a decision is required; one option is to respond to the noxious stimulus, the other is to inhibit the response to the noxious stimulus in order to evade the predator or to consume the food. A key factor in the decision process is assessing the value of the different options. One major function of opioid mediated motivational systems is to encode the reward value (or anticipated reward value) of a particular goal, such as a palatable food (Kelley AE et al, Physiol. Behav 76, 2002). In fact, we have shown that Nucleus Accumbens (NAc) neurons encode reward value and are activated by reward predictive cues. NAc mu agonist injections produce both analgesia and feeding (Altier & Stewart, JPET 285, 1998). We propose that the release of endogenous mu opioids within the reward circuit concurrently enhances consumption of palatable foods and suppresses responses to noxious stimuli. In this case, the release of the endogenous opioid and the resulting analgesia reflects the decision to consume food and inhibit conflicting behaviors.

10:40 – 11:05 **S3 G.W. Pasternak MULTIPLE MU OPIOID RECEPTORS: INTEGRATING MOLECULAR BIOLOGY AND BEHAVIOR** ***G.W. Pasternak, Laboratory of Molecular Neuropharmacology, Memorial Sloan-Kettering Cancer Center, New York, NY USA*** Although most opioids used clinically are mu-selective, based upon their receptor binding selectivities, clinicians have long appreciated differences among them. Patients unable to tolerate one mu drug due to nausea/vomiting, for example, may take a different one without a problem. Even the relative analgesic activity of mu drugs varies from patient to patient. Similar findings have been seen in preclinical studies. Our initial proposal of multiple mu opioid receptors 25 years ago has now been confirmed at the molecular level. MOR-1 undergoes extensive splicing, with at least 25 mouse, 10 human and 6 rat variants. The 3' variants maintain identical amino acid sequences from the N-terminus through all seven transmembrane domains, differing only at the tip of the C-terminus. Since the TM's define the binding pocket, it is not surprising that all these variants display high affinity and mu selectivity in receptor binding assays. However, the variants diverge functionally, showing differences in both potency and efficacy among both opioids and variants. Differences in their distributions among regions and even within a cell further suggest that these variants may play a role in the diverse pharmacology of mu opioids.

11:05 – 11:35 **S4 C. Stein OPIOID-IMMUNE INTERACTIONS IN PAIN CONTROL** ***C. Stein, Dept. of Anesthesiology and Critical Care Medicine, Charite – Campus Benjamin Franklin, Freie Universität, Berlin, Germany*** This presentation will discuss the contribution of immune cells to the inhibition of pain and some functional consequences of prolonged opioid administration on the immune system. Opioid peptides produced by immune cells can interact with the peripheral nervous system. A prerequisite is inflammation, accompanied by hyperalgesia. Opioid receptors are present on peripheral terminals of sensory neurons and are upregulated in inflammation. Interactions between these receptors and opioid peptides result in local analgesia, both in animals and in humans. We have investigated the production and release of β-endorphin (END) from immune cells, as well as mechanisms governing

the migration of opioid-containing cells to peripheral injured tissue. Different fragments of proopiomelanocortin (POMC) mRNA were amplified and the content and release of END upon stimulation of immune cell suspensions by corticotropin releasing factor (CRF) and cytokines were investigated. We also examined the identity of opioid-containing cells by immunofluorescence and flow cytometry, and the involvement of chemokines and adhesion molecules (L-, P- and E-selectins, VLA-4, CD18, ICAM-1) in the recruitment of these cells to inflamed tissue. Lymphocytes contain Exon 1-3, Exon 2-3 and Exon 3 of POMC mRNA. END is secreted in a Ca++ dependent manner. In the early stages of inflammation opioids are contained in 50 – 70 % of inflammatory cells (mostly neutrophils), in later stages mostly in monocytes. The recruitment of these cells is dependent on chemokines, L- and P-selectins, CD18 and ICAM-1 but not on VLA-4. END activates peripheral opioid receptors and produces analgesia by inhibiting the excitability of sensory nerves and/or the release of excitatory neuropeptides. Targeting of immune cells containing opioids to injured tissues may be a novel concept of pain control. On the other hand, *in vitro* studies have raised concerns about possible immunosuppressive effects of exogenous opioids. However, no substantial clinical evidence has emerged to support these concerns. Support: D. Forschungsgemeinschaft

11:30 – 11:45 **S5 C. Inturrisi DYNORPHIN-INDUCED ALLODYNIA IS PREVENTED BY A SPATIAL KNOCKOUT OF NMDA RECEPTORS IN THE LUMBAR SPINAL CORD DORSAL HORN** ***S. South, M. Ohata, D. Hegarty, Q. Xu, C. Inturrisi. Dept. of Pharmacol., Weill Med. College of Cornell Univ., New York, NY, USA*** A single intrathecal (IT) injection of dynorphin A (1-17) (DYN) produces allodynia in mice that is blocked by an NMDA receptor antagonist. To confirm and extend this observation, we used a spatial-temporal knockout (KO) of the NR1 subunit of the NMDA receptor (NR1 KO) (South et al., 2003). Mechanical allodynia (von Frey), cold allodynia and thermal hyperalgesia were measured prior to and 2 and 5 days after IT DYN. DYN produced mechanical allodynia but not thermal hyperalgesia or cold allodynia in both the Control and the NR1 KO mice. However, while the allodynia was bilateral in the Controls, it was observed only with the contralateral paw in the NR1 KO mice. Thus, a spatial KO of the NMDA receptor, confined to one side of the SCDH, provided protection on that side from DYN-induced allodynia. These results demonstrate conclusively that postsynaptic NMDA receptors, at the level of the SCDH, are required for development of the mechanical allodynia induced by IT DYN. They may also offer some insight into the mechanism by which endogenous DYN mediates the allodynia that occurs following injury. Support: DA001457, DA007274 and DA000198.

11:45 – 12:00 **S6 L.Y.-M. Huang REMOTE NERVE INJECTION OF MU-OPIOID RECEPTOR ADENO-ASSOCIATED VIRAL VECTOR INCREASES ANTINOCICEPTION OF INTRATHECAL MORPHINE** ***L.Y.-M. Huang, Y. Gu, G. Li Dept. of Neuroscience and Cell Biology, Univ Texas Medical Branch, Galveston, TX, USA*** We injected a recombinant adeno-associated viral vector (rAAV) containing the mu-opioid receptor (MOR) gene into the sciatic nerve of adult rats and examined changes in the antinociceptive effects of intrathecal morphine. Four weeks after the introduction of rAAV-MOR, the expression of MOR in dorsal root ganglia (DRGs) was examined. Transduced MORs were found in all types (i.e., small, medium and large) of DRG neurons. Western analyses indicated that MOR expression was increased by 1.7-fold. The upregulation persisted for more than six months. The effects of intrathecal morphine on paw withdrawal latencies (PWLs) to heat were studied in rats inflamed with Complete Freund's Adjuvant (CFA). Compared with rats injected with rAAV-EGFP (enhanced green fluorescence protein), the antinociceptive potency of intrathecal morphine in rAAV-μOR rats was significantly increased and the effective dose (ED50) for morphine was 5.4 fold lower. With minimum tissue damage and a large persistent increase in the opioid potency, remote nerve injection of rAAV-MOR to upregulate MOR would be a useful therapeutic strategy for the treatment of pain.

12:00 – 12:15 **S7 W. Walwyn INDUCTION OF DELTA OPIOID RECEPTOR (DOR) FUNCTION BY UP-REGULATION OF MEMBRANE DORS IN MOUSE PRIMARY AFFERENT NEURONS** ***W. Walwyn (1), N. Maidment (1), C. Evans (1) M. Sanders (2) B. Kieffer (3) T. Hales (4). (1) Dept. Psych. & Biobehav. Sciences & (2) Psychology, UCLA, LA (3) IGBMC, CNRS/INSERM/ULP, Ilkirck, Fr (4) Dept. Pharmacol. & Phys. GWU, DC USA*** USAThe role of DORs in analgesia is controversial. We examined DORcoupling to Ca2+ channels in mouse DRG neurons under basal conditions and after DOR up-regulation. DAMGO, DADLE, U50,488 (1 uM), and baclofen (50 uM) inhibited Ca2+ currents, the DOR ligands DPDPE and DELT II (1 uM) did not. The effect of DADLE (1 uM) was blocked by the mu opioid receptor (MOR) antagonist CTAP (300 nM) but not by TIPP, a DOR antagonist (300 nM). Despite a lack of functional DORs, flow cytometry revealed that DRG neurons express cell surface DORs. Deletion of the MORgene in MOR -/- mice and 18 h incubation of DRG neurons with CTAP followed by brief (10')

DPDPE exposure upregulated cell surface DORs by 149 ± 9% and 139 ± 5%, respectively. DPDPE and DELT II (1 uM) inhibited Ca2+ currents in both cases. Our observations suggest that DORs in primary afferent fibers have little functional significance in opioid analgesia under basal conditions in which MORs predominate. However up-regulation of cell surface DORs induces their coupling to Ca++ channels.

12:15 – 12:30 **S8 C. Kornetsky A COMPARISON OF MORPHINE-INDUCED ANALGESIA IN YOUNG AND AGED RATS USING PERIPHERAL AND CENTRAL NOCICEPTIVE STIMULATION.** ***C. Kornetsky, C. Knapp, S. Crosby Departments of Psychiatry, and Pharmacology, Boston Univ. Sch. Med. Boston, MA USA*** Psychophysical methods may provide a means for detecting differences between aged and young rats in their sensitivity to nociceptive stimuli. In this study these methods were used to determine thresholds for nociceptive stimuli delivered to both brain and the tail after either saline or morphine administration to aged and young rats. The analgesic effects of morphine were also assessed using latency of tail-flick response. Age related differences in response to morphine injection were not detected using the tail-flick latency measure. Aged animals were less sensitive to morphine-induced analgesia when thresholds for either centrally or peripherally applied nociceptive stimuli were determined. Baseline thresholds were significantly greater in aged rats for stimuli delivered to the tail but not those delivered to the brain. These results suggest 1) spinal reflex to nociceptive stimuli may be altered by aging, limiting the usefulness of latency of response in assessing aged-related changes in analgesic response and 2) and aged rats are less sensitive to the analgesic effects of morphine. (Support: K05 DA00099 and R21 DA13678)

12:30 – 15:30 **Lunch and Poster Session I** **Historic Inns**

Pain

M1 ANALGESIC SYNERGY BETWEEN DELTA OPIOID AND 5-HT$_3$ RECEPTORS IS DEPENDENT UPON LIPID RAFTS ***K. Rajput, D. Paul, Dept. of Pharmacology and Experimental Therapeutics, Louisiana State University Health Science Center, New Orleans, LA USA*** Delta opioids interact with α-2 adrenergic and 5HT$_3$ agonists to produce analgesic synergy. In the present study we used Methyl-β-Cyclodextrin, a cholesterol binding agent, to evaluate the role of lipid rafts in these interactions in male CD-1 mice. The drugs were given by intrathecal injections and nociception was evaluated by tail-flick assay. Methyl-β-Cyclodextrin produce no significant shift in single drug dose response curves as compared to drugs prepared in water. We found that there was a significant analgesic synergy when the two individual combinations, DPDPE (delta agonist) and 2-methyl-5HT (5HT$_3$ agonist); DDPDE and Clonidine (α-2 agonist), prepared in water were given. But the combination of DPDPE and 2-methyl-5-HT prepared in 10mM Methyl-β-Cyclodextrin produced additive interaction. In contrast, DPDPE and clonidine prepared in 10mM Methyl-β-Cyclodextrin produced analgesic synergy. These results are evidence that the delta-5HT$_3$ interaction is dependent upon lipid rafts but delta-α-2 interaction is not. *Support: HEF grants BoR 1999-04 and BoR 2001-022 from the Louisiana Board of Regents to D.P.*

M2 PROPENTOFYLLINE-INDUCED ASTROCYTE MODULATION LEADS TO ALTERATIONS IN GLT-1 AND ANTI-ALLODYNIA AFTER NERVE TRANSECTION ***V.L. Tawfik, J.A. DeLeo. Dartmouth Medical School, Dept Pharmacology, Hanover, NH USA*** We have previously shown that attenuation of glial activation with the methylxanthine, propentofylline (PPF), reduces mechanical allodynia after nerve transection. Currently, we determined how PPF-induced glial modulation contributes to sensitization. Rats received daily PPF (10 μg, i.t.) starting 1 hour prior to L5 nerve transection. Real time RT-PCR analysis of lumbar spinal cord revealed increased mRNA for GLT-1 in PPF-treated animals at days 4 and 12 compared with L5 saline controls. Spinal cord immunohistochemistry revealed decreased GLT-1 in the ipsilateral dorsal horn. PPF reversed this alteration and increased co-localization between GLT-1 and the astrocyte marker GFAP. Western blot analysis showed that PPF significantly induced GLT-1 protein while suppressing GLAST at day 12. Furthermore, 3 day treatment of cultured astrocytes with 1000 μg PPF resulted in a change from a polygonal morphology to a stellate, process-bearing phenotype similar to that observed after cAMP induction. These findings suggest that alterations in glutamate transporters may contribute to PPF-induced anti-allodynia through a mechanism involving a return of astrocytes to a homeostatic phenotype.

M3 DOES SPINAL CO-ADMINISTRATION OF MORPHINE WITH SUB-ANALGESIC DOSE OF DAMGO INHIBIT THE DEVELOPMENT OF MORPHINE TOLERANCE? ***J. Xu, B. Clark, M. Diaz, H. Gutstein. Department of Anesthesiology, M.D. Anderson Cancer Center, Houston, TX USA*** A recent study showed that co-administration of morphine with sub-analgesic doses of DAMGO inhibited the development of morphine tolerance.

These studies used an intrathecal catheter to deliver drugs. Catheters can induce an inflammatory response, and have been shown to decrease the potency of morphine. Catheters could also affect the development of opioid tolerance. Therefore, we developed a method for repeated lumbar puncture (LP) to eliminate these potential complications. We then determined whether co-administration of an analgesic dose of morphine (0.1 nmole) with a subanalgesic dose of DAMGO (100 fmole) would inhibit the development of opioid tolerance as previously described. Sprague-Dawley rats underwent LP under isoflurane anesthesia and were injected with either morphine and DAMGO or morphine alone daily for 5 days. We found that the dose required to elicit robust analgesia using LP was roughly 100 times less than that required when using a cervically placed intrathecal cather. We also found that DAMGO did not inhibit the development of morphine tolerance. Rather, DAMGO blocked the analgesic effect of morphine. Our results differ markedly from previous findings. This could be due to the different administration technique used. These findings could lead to a better understanding of how opioids cause analgesia. .

M4 REPEATED LUMBAR PUNCTURE IN RATS: A NOVEL METHOD FOR THE EXPERIMENTAL STUDY OF OPIOID TOLERANCE ***B. Clark, J. Xu, M. Diaz, and H. Gutstein Department of Anesthesiology M.D. Anderson Cancer Center, Houston, TX USA*** Spinal opioid administration in rats has been a vital tool for studying the pharmacological effects of opioids. Intrathecal catheterization, via the cervical or lumbar routes, has been the predominant method used to deliver opioids spinally. However, these methods have undesirable complications, such as significant morbidity and mortality. Also, catheter placement causes an inflammatory response that can alter the potency of opioids and could affect the development of opioid tolerance. To address these issues, we developed a novel method of repeated lumbar puncture (LP) in rats to study the effects of chronic spinal opioid administration. Male Sprague-Dawley rats received daily injections of 0.1 nmole morphine (which is a 100-fold less than the dose reported to produce an equivalent amount of analgesia after cervical catheterization) under brief isoflurane anesthesia. Analgesia was assessed by tail flick latency 45 minutes after injection. Animals developed tolerance over 5 days without apparent morbidity, indicating that repeated LP is an effective technique for studying mechanisms of spinal opioid analgesia and tolerance development.

M5 DOES SYSTEMIC CO-ADMINISTRATION OF MORPHINE WITH SUB-ANALGESIC DOSES OF FENTANYL INHIBIT THE DEVELOPMENT OF MORPHINE TOLERANCE? ***K. Barker, H. Gutstein. Department of Anesthesiology MD Anderson Cancer Center, Houston, TX USA*** Mechanisms underlying opioid tolerance are poorly understood. Opioid tolerance was thought to develop because receptors internalized, terminating signaling. However, morphine does not cause internalization, yet tolerance develops. A new theory suggests that tolerance is due to sustained opioid signaling. In support of this theory, a recent study showed that sub-analgesic doses of DAMGO co-administered with morphine caused receptor internalization and inhibited the development of morphine tolerance. These studies were designed to determine whether this occurs with systemic drug administration. Rats were injected daily for 4 days with 2.5mg/kg morphine S.C. alone or with sub-analgesic doses (100ng/kg to 10ag/kg) of fentanyl. Other rats received 40μg/kg fentanyl alone or with sub-analgesic doses of morphine. On day 5, the primary analgesic was given alone. We found that fentanyl co-administered with morphine did not inhibit tolerance development. Surprisingly, analgesia was inhibited. The same result was obtained when sub-analgesic morphine was co-administered with fentanyl. These findings may lead to a better understanding of how opioids cause analgesia.

M6 DORSAL HORN KEPI (Kinase Enhanced PP1 Inhibitor) EXPRESSION: REGULATION BY MORPHINE and CFA TREATMENTS ***J. P. Gong, Q. R. Liu, G.R. Uhl Mol. Neurobiol. NIDA-IRP, NIH, DHSS Baltimore, MD USA*** KEPI is a recently-characterized PKC-activated protein phosphatase 1 (PP1) inhibitor that was cloned based on its regulation by morphine in rat striatum. The multifocal CNS distribution of immunoreactive KEPI (iKEPI) includes dense fiber and terminal immunostaining in spinal cord dorsal horn laminae I-II, observed using recently-developed polyclonal antibodies raised to KEPI peptide sequences conjugated to hemocyanin that satisfy several criteria for specificity of immuno-reactivity. Primary spinal cord cultures display iKEPI in neuronal processes, cell membranes, and cytoplasmic regions. Dorsal root ganglion perikarya and afferent/efferent fibers also display dense KEPI immunoreactivity (iKEPI). Dorsal horn iKEPI is enhanced in animals sacrificed following acute systemic injections of morphine. By contrast, iKEPI is downregulated in dorsal horns ipsilateral to unilateral hindpaw intraplantar injections of complete Freund's adjuvant (CFA). KEPI may thus be involved in the spinal cord adaptations that follow opiate administration or noxious inflammatory stimuli, dorsal horn PKC-dependent dephosphorylation by PP1 may thus change with morphine and with inflammation, and these biochemical events are good candidates to play roles in the dorsal horn adaptations that follow such treatments. Support NIDA-IRP.

M7 ANTINOCICEPTIVE EFFECT OF FLUVOXAMINE ON THERMAL AND MECHANICAL NOCICEPTION AFTER PERIPHERAL NERVE INJURY IN THE MOUSE ***C. Nozaki, A. Saitoh, J. Kamei Dept. Pathophysiol. Ther, Sch. Pharm. Pharm. Sci., Hoshi Univ., Tokyo, Japan*** Effects of fluvoxamine, a selective serotonin reuptake inhibitor, on thermal hyperalgesia and mechanical allodynia in neuropathic pain model mice induced by sciatic nerve ligation (SNL) were examined. The experiments were conducted 2 or 6 weeks after the unilateral SNL. Ipsilateral thermal hyperalgesia and mechanical allodynia were observed both 2 and 6 weeks after SNL. Fluvoxamine (10 mg/kg, s.c.) had no effect on SNL-induced thermal hyperalgesia and mechanical allodynia in mice 2 weeks after the sciatic nerve ligation. However, same dose of fluvoxamine significantly reduced the mechanical allodynia but not thermal hyperalgesia in SNL mice 6weeks after the surgery. The antinociceptive effect of fluvoxamine on SNL-induced mechanical allodynia in mice 6 weeks after the surgery was abolished by pretreatment with naloxone (1 mg/kg, i.p.). These results suggest that anti- nociceptive effect of fluvoxamine in chronic state SNL-induced neuropathic pain may be partially related to opioidergic activity.

M8 INVOLVEMENT OF SPINAL HISTAMINERGIC SYSTEM ON NOCICEPTIVE BEHAVIORS ELICITED BY SPERMINE IN MICE ***M. Yoshida (1), Y. Iwata (1), H. Watanabe (1), H. Mizoguchi (1), C. Watanabe (1), A. Yonezawa (1), T. Sakurada (2), S. Sakurada (1) (1) Dept. of Physiol. and Anat., Tohoku Pharmaceut. Univ., Sendai, Japan, (2) Dept. of Biochem., Daiichi Coll. of Pharmaceut. Sci., Fukuoka, Japan*** Previous study has demonstrated that intrathecal (i.t.) administration of spermine produced nociceptive behavioral responses (scratching, biting, and/or licking) that were mediated through the activation of the polyamine recognition site on NMDA receptor without affecting substance P system [Pain, 86, 55-61 (2001)]. In the present study, we found that the nociceptive behaviors induced by spermine (10 pmol) were inhibited dose-dependently by i.t. co-administration of agmatine and arcaine, the selective antagonists for the polyamine recognition site. Interestingly, these nociceptive behaviors were also attenuated by i.t. co-administration of the histamine H1 receptor antagonists, but not by the H2 receptor antagonists. The pretreatment i.t. with the antiserum against histamine resulted in a significant reduction of the response to spermine. These results indicate that i.t.-administered spermine may promote the release of histamine through the polyamine recognition site on NMDA receptor located on the histaminergic neuron, and then released histamine activates the histamine H1 receptor to produce the nociceptive behaviors.

M9 MORPHINE PRIMING POTENTIATES DELTORPHIN ANALGESIA IN CFA-TREATED RATS ***L. Gendron (1), MJ. Esdaile (1), T. Stroh (1), C. Cahill (2) and A. Beaudet (1) (1) McGill Univ., Montreal, Canada, (2) Queen's Univ, Kingston, Canada*** We have shown that prolonged morphine (MS) treatment and chronic inflammatory pain both enhance the density of delta opioid receptors (DOR) at the plasma membrane of lumbar spinal cord neurons. In this study, we sought to determine whether pre-treatment with MS of animals with chronic inflammatory pain would further increase the bio-availability of DOR and thereby potentiate DOR-mediated analgesia. We found that chronic inflammatory pain (CFA injection) induced a bilateral increase in the binding and internalization of a fluorescent DOR agonist (fluo-deltorphin) in dorsal horn neurons as compared to controls, which was accompanied by enhanced analgesic effects of deltorphin (DLT). Treatment of CFA-injected rats with MS further enhanced DLT-induced anti-hyperalgesia as compared to rats treated with CFA alone. Surprisingly, this increased effectiveness of DLT was not paralleled by an increase in the binding and internalization of fluo-DLT in dorsal horn neurons. In any event, the present data demonstrate that treatment with MS potentiates analgesic effects of DOR agonists in CFA-injected rats.

M10 LEUKOCYTE-DERIVED OPIOIDS PRODUCE ANALGESIA IN NEUROPATHIC PAIN ***D. Labuz, S.A. Mousa, C. Stein, H. Machelska Anaesthesiologie, Charité, Campus Benjamin Franklin, Berlin, Germany*** We previously showed that leukocyte-derived opioids decrease inflammatory pain in response to corticotropin releasing factor (CRF) injection. Neuropathic pain results from nerve injury that can lead to inflammation (neuritis) activating the immune system. Here we assess the contribution of leukocyte-derived β-endorphin (END) to CRF analgesia in the chronic constriction injury (CCI) neuropathic pain model in mice. CCI resulted in mechanical allodynia (von Frey test). END-containing leukocytes accumulated at the injured nerve at 2 and 14 days after CCI. CRF injected at the CCI site blocked mechanical allodynia at both stages. The CRF anti-allodynic effect was reversed by naloxone methiodide, anti-END and anti-intercellular adhesion molecule-1 (ICAM-1) antibodies. Together, CRF analgesia apparently results from activation of peripheral opioid receptors by END derived from leukocytes which use ICAM-1 to accumulate at the injured nerve. Our studies challenge the current opinion that leukocytes act exclusively as generators of neuropathic pain. Thus, when opioids derived from such cells come into play, neuropathic pain may be diminished not only at the

beginning but also at later stages of the injury.

M11 CONCOMITANT ACTIVATION OF β-ENDORPHIN-CONTAINING NEURON SUPPRESSES THE MORPHINE-INDUCED REWARDING EFFECT UNDER A NEURO-PATHIC PAIN-LIKE STATE ***K. Niikura, M. Narita, M. Narita, K. Hashimoto, Y. Yajima, T. Suzuki.Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan*** The present study was undertaken to investigate the mechanism of the suppression of the morphine-induced rewarding effect a neuropathic pain-like state. Here we found that the suppression of the mu agonist-induced place preference with nerve ligation was reversed by pre-microinjection of a specific antibody toβ-endorphin (β-EP) into the ventral tegmental area (VTA). Using the fluoro-gold (FG) microinjection into the VTA, numerous FG-labeled cells were detected in the parascicular nucleus, lateral preoptic nucleus, dorsolateral hypothalamus and zona incerta of rat with nerve ligation. Subpopulations of β-EP-positive cells were co-labeled by either FG or delta-fosB, an excitatory neuronal maker, in rats with nerve ligation. These data provide direct evidence that the β-EP-containing neuron projecting to the VTA from the pain processing regions may be continuously activated by nerve ligation, resulting in the long-lasting down-regulation of mu-receptors. This phenomenon could lead to the suppression of the morphine-induced rewarding effect under a neuropathic pain-like state.

M12 FUNCTIONAL CHANGES IN OPIOIDERGIC SYSTEM UNDER A NEUROPATHIC PAIN-LIKE STATE FOLLOWING CHRONIC ETHANOL CONSUMPTION IN THE RAT SPINAL CORD ***K. Miyoshi, M. Narita, M. Narita, T. Suzuki Dept. of Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan*** Chronic ethanol consumption produces a painful neuropathy. However, little is known about the molecular mechanism of chronic ethanol-induced neuropathic pain. In the present study, mechanical hyperalgesia was observed during ethanol consumption and even after ethanol withdrawal in rats, and it lasted for 14 weeks. The morphine-induced antinociception and the increased [^{35}S] GTPγS binding to membranes of the spinal cord induced by a mu-opioid receptor agonist DAMGO were significantly decreased under ethanol-dependent neuropathic pain-like state. Immuno-histochemical study showed the increase in phosphorylated-mu-opioid receptor immuno-reactivities in the superficial spinal dorsal horn of chronic ethanol fed-rats. Furthermore, double immuno- staining revealed that phosphorylated-cPKC was increased and co-localized with mu-opioid receptors in the superficial spinal dorsal horn of ethanol-fed rats. These findings provide further evidence for a substantial role of spinal cPKC-dependent mu-opioid receptor dysfunction in the development or/and maintenance of ethanol-dependent neuropathic pain-like state in rats.

M13 THE CHANGES IN BASAL MEMBRANE OF SCHWAN CELLS IN DRG EXPLAIN THE BIPHASIS BEHAVIOR OF PERIPHERAL NEUROPATHY IN DIABETIC RATS ***M. Becker (1), A. Shahar (3), Z. Nevo (2), C.G. Pick (1) (1) Dept. of Anatomy, (2) Clinic Biochemistry, Sackler Faculty of Medicine, Tel-Aviv University, (3) NVR ltd, Nes-Ziona, Israel*** The present study was aimed to explain the behavioral profile of sensitivity to pain as reflected by histological examinations in diabetes. The diabetes was induced by single injection of the STZ. The pain perception was 3 and 10 weeks after STZ injection using hot plate and tail flick tests. The bi-phasic phenomenon, of the pain response (hypersensitivity at 3d week and hyposensitivity at 10th week) was observed in both behavioral tests and was resemble to bi-phasic pattern described previously in humans. Parallel electrophysiological monitoring showed a continuous decrease in motor nerve conduction velocity. Thickening of basal membrane in blood vessels and in the glomerular is the hallmark of diabetes in general. Heparan sulfate and laminin was examined in Schwann cells basal membrane of L5 DRG by specific antibody labeling. The significant decrease in heparan sulfate and increase in laminin densities in diabetic rats were noticed. The abnormality in Schwann cells basal membrane content noticed in STZ-rats may underline an sensory neurones damage, leading to painful diabetic peripheral neuropathy monitored by reduction in nerve conduction velocity.

M14 COMPARISON OF SELECTIVE OPIOID AGONISTS IN THE PRODUCTION OF ANALGESIA FOR NEUROPATHIC PAIN DUE TO DEMYELINATION ***A. L. Dunne (1), H. J. Gould, III (2), D. Paul (1) (1)Dept. of Pharmacology and Experimental Therapeutics, (2)Dept. of Neurology, LSUHSC, New Orleans, LA, USA*** Demyelination of peripheral neurons is associated with pain that has neuropathic qualities. Intraneural injection of doxorubicin causes demyelination of neurons by killing Schwann cells. Rats showed no significant thermal hyperalgesia regardless of the dose of doxorubicin injected into the sciatic nerve, but rats treated with 1.0µg showed significant allodynia throughout the testing period. In preliminary pharmacologic characterization, morphine produced significant analgesia, but only at a dose that caused profound sedation. We administered a selective mu (DAMGO), delta (DPDPE), and kappa (U50488H) agonist, as well as the ORL-1 agonist nociceptin intrathecally to specifically

determine which opioid receptor subtypes are involved in the analgesia produced by morphine. Only intrathecal DPDPE and U50488H produced significant analgesia as assessed with an electro-von Frey apparatus. Nociceptin produced variable analgesia. These results are evidence that the analgesia that morphine produces in this model of neuropathic pain is primarily due to its action at the delta opioid receptors, not mu opioid receptors.

M15 GENDER DIFFERENCES IN PAIN PROCESSING DURING PROTRACTED OPIATE ABSTINENCE ***M. Steinfeld, L. Kunik, L. Cohen, I. Galynker Dept. Psychiatry, Beth Israel Medical Center, New York, NY USA*** Background: Detoxified opiate abusers have demonstrated impairment in brain regions that mediate the emotional aspects of pain and divided attention. Recent literature has demonstrated gender differences in pain processing. This ongoing study is assessing the impact of gender on pain processing, divided attention, and the relationship between the two in opiate abusers. Methods: The TSA-II NeuroSensory Analyzer was used to assess pain processing. Performance on the Stroop CW test during painful vs. non-painful conditions was compared in 23 Methadone-Withdrawn (MW) abstinent opiate addicts, and 23 healthy controls. Results: Surprisingly, Stroop performance improved under painful vs. non-painful conditions. The mean improvement in Stroop performance under painful conditions for MW subjects was 12.70 + 12.02, and for Controls was 2.24 + 11.13. There was a marginally significant group effect ($F(1,45)= 3.78$, $p=.059$), and a significant sex effect ($F(1,45)= 6.24$, $p= .016$), but the interaction between the two was not significant. Nonetheless, the impact on Stroop performance under painful conditions was significantly higher in male opiate abusers than in male controls ($t(25)= 2.07$, $p= .049$). Conclusion: There may be a significant gender difference in the relationship between pain processing and attention in former opiate abusers in protracted abstinence.

M16 OLIGONUCLEOTIDES TARGETING EXONS 7, 8 AND 9 OF THE MOR-1 SPLICE VARIANT BLOCK OPIOID ANALGESIA IN THE RAT ***J. Matulonis (1), X-Y. Pan (2), G.W. Pasternak (2), G. Rossi (1) (1) Long Island Univ. C.W. P, Brookville, NY, USA, (2) Dept. of Neurology, Mem. Sloan Kettering Cancer Ctr., New York NY, USA*** Antisense-mapping approaches have revealed fascinating differences among mu opioids, particularly between morphine and M6G. Recently, we identified 3 new spliced variants, rMOR-1C1, rMOR-1C2 and rMOR-1D, of the rat mu opioid receptor gene, Oprm. All variants contained the same exons 1, 2 and 3 of the original rMOR-1 followed by different combinations of the new downstream exons (exons 7, 8 and 9) in place of exon 4. All variants showed little differences in mu opioid selectivity in receptor binding assay, but displayed marked differences in agonist-induced G protein coupling in [^{35}S]GTPγS binding assay. In the present study, we examined the behavioral role of the newly spliced variants using an antisense oligonucleotide approach in morphine and M6G analgesia in rats. We examined 3 different opiate-sensitive sites in the brain (PAG, RVM, LC). Antisense was injected into one of 3 sites. The three antisense probes targeting exons 7, 8, and 9 significantly blocked the analgesic actions of morphine and M6G in the PAG, LC, and most dramatically in the RVM. Mismatch oligodeoxynucleotides were inactive and similar to that of the controls. These results suggest that exons 7, 8, and 9 play an important role in both morphine and M6G mediated analgesic actions in rat. Suppor: LIU/CWP Grant (GCR) and NIH grants, DA00220 (GWP) and DA13997 (YXP).

M17 LONG-TERM MAINTENANCE OF ANALGESIA WITH CHRONIC ORAL OXYCODONE TREATMENT IN FEMALE RATS ***L.M. Schrott, L.M. Franklin Dept. of Pharmacology, Toxicology, & Neuroscience, LSU Health Sciences Center, Shreveport, LA USA*** Despite its use for chronic pain treatment in humans, little is known about the effects of chronic oxycodone treatment in rats. Using an oral dosing paradigm, we determined if tolerance and dependence developed to chronic oxycodone. Adult female Sprague-Dawley rats (n=6 per group) were adapted to an oral gavage procedure and baseline hotplate (52°C) latency measured. The rats were then weighed and treated daily with the water vehicle or oxycodone HCl and were assessed for hotplate latency 3 times a week. The initial oxycodone dose was 10 mg/kg/day and analgesia was maintained for 8 days. By day 10, tolerance was evident and the dose was increased by 1 mg/kg/day for the next 7 days. Analgesic action was restored and the rats were maintained at 17 mg/kg/day for next 8 days. The dose of oxycodone was then increased 0.5 mg/kg/day to a final dose of 25 mg/kg/day. Analgesia was maintained through the final gavage on day 40. There was no change in the hot plate latency in the water treated group throughout the testing. Oxycodone did decrease weight gain by almost 25% compared to the water-treated rats after 40 days of treatment, but was otherwise fairly well-tolerated.

M18 NERVE INJURY CHANGES THE LEVEL OF Ca^{++}-CHANNEL SUBUNIT: COMPARISON TO OPIOID-TOLERANT STATE ***M. Nakajima, M. Narita, Y. Nagumo, Y. Yajima, T. Suzuki Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo Japan*** Voltage-dependent calcium channels are a diverse family of proteins that have a variety of biological functions, including within the cell. The calcium channel α2delta-1 subunit is

a structural subunit important for functional calcium channel assembly. In the present study, we investigated the increase in mRNA levels of α2delta-1 subunit in the dorsal root ganglion (DRG), but not in the spinal cord, under the neuropathic pain-like state caused by sciatic nerve ligation in mice. Chronic treatment of mu- and kappa- agonists induced the development of antinociceptive tolerance without any changes in the expression of α2delta-1 subunit in the spinal cord. These findings suggest that no change in α2delta-1 subunit expression in the spinal cord was noted during either neuropathic pain-like state or opioid-tolerant state, whereas its increase in mRNA levels of α2delta-1 subunit in the DRG may be, at least in part, implicated in the development of a neuropathic pain-like state.

M19 OPIOIDS MODULATE THE TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 (TRPV1) ION CHANNEL ***J. Endres-Becker, P.A. Heppenstall, S. Mousa, D. Labuz, M. Schäfer, C. Stein, C. Zöllner Dept. of Anaesthesiology and Critical Care Medicine; Charité – Campus Benjamin Franklin, Berlin, Germany*** Allodynia and hyperalgesia to thermal and mechanical stimuli often occur after tissue injury, inflammation or nerve lesions. The prominent role of the transient receptor potential vanilloid 1 (TRPV1) ion channel in thermal hyperalgesia has been illustrated in many experimental studies. Opioid receptors couple with multiple subtypes of voltage-dependent Ca2+ channels and inhibit excitation and sensitization of primary afferent neurons ("nociceptors"). However, it has not been examined whether opioids can modulate TRPV1. The present study provides evidence that an important mechanism underlying opioid modulation of nociceptors is regulation of TRPV1. Patch-clamp studies showed a significant reduction of TRPV1 activity in sensory neurons after opioid treatment. *In vitro* studies using immunohistochemistry showed colocalisation of TRPV1 and MOR on sensory neurons. *In vivo* behavioral studies demonstrate that intraplantar injection of morphine can significantly reduce capsaicin-induced flinching/lifting behaviours. This occurs through local activation of μ-opioid receptors in the injected hindpaw.

M20 EFFECT OF NOP AGONISTS AND ANTAGONISTS IN A CHRONIC PAIN MODEL ***L. Toll, T. Khroyan, W. Polgar, J. Orduna, R. Moisa, F. Jiang, C. Olsen, N. Zaveri SRI International, Menlo Park, CA USA*** Nociceptin/Orphanin FQ (N/OFQ), a member of the opioid peptide family, binds to the receptor NOP, but unlike opioid peptides, has a complex pharmacology. N/OFQ is hyperalgesic when administered ICV but analgesic in the spinal cord. The effects of N/OFQ in chronic pain are also complicated and depend on the assay and delivery of the peptide. The chronic constriction injury model of chronic pain produces allodynia of the affected foot that can be quantified with von Frey hair. Systemic administration of our small-molecule NOP ligands showed that neither the selective agonist SR14150 nor the selective antagonist SR14148 produced a significant change in the allodynic response. The non-selective mu /NOP partial agonist SR16435, however, exhibited a significant anti-allodynic response that was blocked by naloxone, indicating that the activity was due to the compound's mu component. Interestingly, this anti-allodynic action was greatly potentiated by the NOP antagonist SR14148. These studies indicate that while NOP ligands may not have beneficial effects in chronic pain states per se, NOP antagonists may be used to potentiate the analgesic response or lower the dose of a classical opiate. Support: DA14026 (NZ)

M21 REPEATED STRESS INDUCES TOLERANCE TO STRESS-INDUCED ANTI-NOCICEPTION IN PREPROENKEPHALIN KNOCKOUT MICE ***N. Gajawada, R. Baliram, K. Lutfy Dept. of Pharm. Sci., Western Univ. of Health Sci.,Pomona, CA USA*** The role of the endogenous opioid peptides in stress-induced antinociception (SIA) has been previously established. However, it is not fully understood which opioid peptide is involved in tolerance that develops to SIA after repeated stress. Thus, the present study was designed to determine whether repeated stress would produce tolerance to SIA and cross-tolerance to morphine in mice lacking enkephalin. Preproenkephalin knockout (PENK KO) mice were repeatedly (once daily for 7 days) exposed to stress (swim in 32^{o}C water for 3 min) and, 24 h after the last swim, tested for SIA using the tail flick test. The same mice were then tested for morphine-induced antinociception on the next day. Acute stress produced antinociception in naïve mice, a response that was blunted in repeatedly stressed mice, showing that chronic stress produced tolerance to SIA in PENK KO mice. In contrast, repeated stress did not alter the antinociceptive effect of morphine in PENK KO mice. Taken together, our results suggest that enkephalins may be involved in cross-tolerance between stress and morphine but not in tolerance to SIA. Support: NIDA DA 16682 (KL)

M22 ASSESSING EFFECTS OF OPIOIDS ON PAIN-SUPPRESSED BEHAVIORS ***E. Bilsky (1), G. Stevenson (2), T. Vanderah (3), F. Porreca (3) , S. Negus (2) (1) University of New England, Biddeford, ME, (2) McLean Hospital-Harvard Medical School, Belmont, MA, (3) University of Arizona, Tucson, AZ USA*** Most preclinical antinociceptive assays measure pain-evoked behaviors, and potential analgesics are tested for their ability to reduce

these behaviors. Clinical and veterinary medicine relies heavily on measures of pain-suppressed behaviors. We are developing assays that measure pain-evoked and pain-suppressed behaviors. Acetic acid (i.p.) produced concentration- and time-dependent increases in writhing (pain-evoked behavior) and decreases in LMA and feeding (pain suppressed behaviors). Morphine decreased writhing at doses that did not restore LMA to baseline levels. Higher doses of morphine brought LMA levels up to (and past) baseline levels of activity. There was some overlap of these doses with doses that stimulated activity in control animals. We are investigating the effects of opioids using protocols where they do not stimulate LMA in controls. We are also assessing morphine effects in a rat osteoarthritis model (tactile allodynia, hind-limb weight bearing and LMA). These studies may help improve the predicative reliability of preclinical antinociceptive assays.

M23 SALVINORIN A, A KAPPA OPIOID RECEPTOR AGONIST, IS AN ULTRASHORT ACTING ANALGESIC ***C.R. McCurdy (1,3,4), K.J. Sufka (2,3,4), J.E. Warnick (2), M.J. Neito (1) (1) Dept. Medicinal Chemistry, (2) Dept. Psychology, (3) Dept. Pharmacology, (4) Research Institute of Pharmaceutical Sciences, School of Pharmacy, Univ. of Mississippi, University, MS USA*** Salvinorin A is a structurally unique, naturally occurring, non-nitrogenous, kappa opioid receptor (KOR) agonist derived from the leaves of Salvia divinorium. Salvia divinorium extracts and leaves have long been used for their psychoactive effects by traditional healers in the Oaxaca, Mexico. KOR agonists have long been known to cause dysphoric and psychoactive effects when administered to humans. The traditional usage of Salvia extracts has also included relief of pain. Given the role of KORs in analgesic processes, we set out to determine whether salvinorin A has antinociceptive activity in two murine nociceptive assays. Dose (0-8 mg/kg) and time course effects (up to 45 min) of salvinorin A anti-nociception were evaluated on the hot-plate (54˚C) and acetic acid (0.6%) abdominal constriction assays. Finally, pretreatment with the KOR antagonist norBNI fully blocked salvinorin A-induced analgesia. These findings demonstrate that salvinorin A produces a KOR mediated analgesic effect that is similar to that produced by typical KOR agonists but with a very short duration of action.

Genetics and gene variants

M24 ASSOCIATION GENOME SCAN USING 155 UNRELATED COGA SUBJECTS AND >15,000 SNP MARKERS ***C. Johnson, G.R. Uhl, Molecular Neurobiology, NIDA-IRP, NIH, DHSS, Baltimore, MD USA*** Strong genetic contributions to substance abuse vulnerability are documented in classical genetic studies. The power of previously-reported association genome scans has been limited by modest marker densities. GAW data from the collaborative study on the genetics of alcoholism (COGA) now allows us to assess association results for Affymetrix "10k" array and Illumina genotypes obtained from unrelated Caucasian individuals with alcohol dependence diagnoses and those who are free from alcoholism. We examine the extreme 5%-iles of markers with greatest allele frequency differences between abusers and controls. The most positive markers identify the alcohol dehydrogenase (ADH) locus and eleven other regions previously linked to alcoholism in at least one previous genome scanning study. These results support the possibility that careful evaluation of association in the unrelated members of samples collected for linkage may benefit from the high marker densities provided by SNP methods. These data provide continued support for the idea that common allelic variants contribute to human vulnerability to abuse of addictive substances. Support NIDA-IRP. Thanks to GAW and COGA investigators.

M25 LINKAGE DISEQUILIBRIUM, HAPLOTYPE AND ASSOCIATION STUDIES OF A CHROMOSOME 4 GABA RECEPTOR GENE CLUSTER: CANDIDATE GENEVARIANTS FOR ADDICTIONS ***T. Drgon, C. D'Addario, G.R. Uhl Molecular Neurobiology, NIDA-IRP, NIH, DHHS, Baltimore MD USA*** Strong genetic contributions to individual differences in vulnerability to addictions are well supported by classical genetic studies. Linkage and association genome scans for addiction vulnerability have provided converging evidence for several chromosomal regions that are likely to harbor allelic variants that contribute to such vulnerability. We and others have delineated such a candidate addiction-associated chromosome 4p12 "rSA3" region in convergent data from association genome scanning studies for polysubstance abuse (Uhl and others 2001), linkage based studies for alcoholism (Long and others 1998; Reich and others 1998), association-based studies for alcoholism and association-based studies for individual differences in electroencephalographic (EEG) spectralpower phenotypes (Edenberg and others 2004; Porjesz and others 2002). The rSA3 region contains interesting candidate genes that include genes that encode the α 2, α 4, β 1 and γ 1GABAA receptor subunits (Covault and others 2004; Edenberg and others 2004; Lappalainen andothers 2005). We now report assessment of single nucleotide polymorphism (SNP) genotypes in this region in three samples of substance abusers and controls. These results delineate the haplotypes and patterns of linkage disequilibrium in this

region, focus attention on the GABRA2 gene and identify varying associations between GABRA2 genotypes and addiction phenotypes. While the results fail to support large roles for chromosome 4p12 GABA receptor gene cluster variants in human addiction vulnerability, they are consistent with the possibility that such variants could play smaller roles.

M26 NrCAM IN OPIATE VULERABILITY: POSITIONAL CLONING, OPIATE-REGULATION, HAPLOTYPE-SPECIFIC EXPRESSION AND ALTERED MORPHINE REWARD IN KNOCKOUT MICE ***H. Ishiguro (1), Q.-R. Liu (1), J.-P. Gong (1), F.S. Hall (1), H. Ujike (3), M. Morales, T. Sakurai (3), M. Grumet (4), G.R. Uhl (1) (1) Molecular Neurobiology Branch, (2) Cellular Neuroscience Branch, NIDA-IRP, NIH, DHSS, Baltimore, MD, USA, (3) Dept. Neuropsychiatry, Okayama Univ. Med. Sch., Okayama, Japan, (4) Dept. Neurobiol., Mt. Sinai Sch. Med., New York, NY, USA*** Several lines of evidences support roles for the cell adhesion molecule NrCAM in addictions. Differential display identifies NrCAM as a morphine- regulated gene. NrCAM is expressed in neurons linked to reward and memory. Fine mapping within a chromosome 7 region that contains previously-linked and -associated genomic markers identifies NrCAM haplotypes that are associated with substance abuse vulnerabilities in studies of four samples of abusers and controls. The NrCAM gene is alternatively spliced and generates transcripts from multiple promoters. NrCAM displays haplotype-specific gene expression in human postmortem brain samples. Heterozygous and homozygous knockout mice display reduced opiate-conditioned place preferences. These observations support NrCAM as a positionally-cloned and drug-regulated gene whose variants are likely to change expression and alter substance abuse vulnerability in human addictions and animal models of drug reward.

M27 NOVEL PRODYNORPHIN TRANSCRIPTS AND PROTEINS IN THE ADULT HUMAN BRAIN ***T. Yakovleva, A. Nikoshkov, Y.L. Hurd, I. Bazov, Z. Marinova, L. Terenius, G. Bakalkin Dept. of Clinical Neurosci., Karolinska Institutet, Stockholm, Sweden*** Transcription from multiple promoters and alternative mRNA splicing constitute the basis for cell-specific gene expression and mRNA and protein diversity. Here we characterize prodynorphin gene (PDYN) transcripts and proteins in the adult human brain and study their functions in model cell lines. Seven PDYN mRNAs were identified. Two transcripts, FL1 and FL2 encode the full-length (FL) PDYN. The dominant, FL1 transcript shows high expression in limbic-related structures, the nucleus accumbens and amygdala. The second, FL2 transcript is expressed in few brain structures such as the claustrum and hypothalamus. FL-PDYN was identified in the brain as the dominant PDYN protein product. Three novel PDYNs expressed from spliced or truncated PDYN transcripts either lack a central segment but are still processed into dynorphins, or are translated into N-terminally truncated proteins. One truncated PDYN is located in the cell nucleus suggesting a novel non-opioid function for this protein. The complexity of PDYN expression and diversity of its protein products may be relevant for diverse levels of plasticity in adaptive responses for the dynorphin system.

M28 NOCICEPTIN/ORPHANIN FQ RECEPTOR (ORL1) GENE AND HEROIN ADDICTION ***K.S. LaForge, D. Proudnikov, S. Barral-Rodriguez, M.J. Kreek The Rockefeller Univ., New York, NY USA*** We previously reported elucidation of the structure of the human ORL-1 gene (OPRL1) and the identification of nine single nucleotide polymorphisms (SNPs) in the gene. Two SNPs (the synonymous C510T in exon III and IVS III C67T in intron III) had overall allelic frequencies >10%. In the current study, we genotyped additional subjects using TaqMan assays for the C510T and IVS III C67T SNPs. Study subjects were unrelated individuals recruited in New York City, either long-term heroin addicts who were in, or qualified for, methadone maintenance treatment (n=261), or control subjects with no history of drug or alcohol dependence (n=169). Statistical analyses were limited to the three largest ethnic/cultural groups (Caucasians, African Americans, and Hispanics) with data stratified by group. No deviations from Hardy Weinberg Equilibrium were observed. In control subjects, we found a difference in allele frequencies among ethnic groups for the C510T SNP (LR Chi-Sq=8.78, p=0.012), with a range from 0.06 to 0.19. No differences in genotype, allele, or haplotype frequencies between case or control groups were found at a significance level of $p<0.05$. Support: DA00049, DA05130, DA12848, and RR00102

M29 FUNCTIONAL CHARACTERIZATION OF AN ALTERNATIVELY SPLICED VARIANT, MMOR-1B4, OF THE MOUSE MU OPIOID RECEPTOR GENE, OPRM ***Y.-X. Pan, J. Xu, M. Xu, G.W. Pasternak Dept. Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY USA*** Our previous studies identified sixteen alternatively spliced variants in the mouse mu opioid receptor gene, Oprm. Of sixteen splice variants, ten variants are carboxyl terminal variants. These C-terminal variants differ from each other only at their intracellular tips of the carboxyl termini. All the C-terminal variants displayed high affinity for mu opioids in receptor binding assays, with the

exception of mMOR-1B4. In present studies, we have further characterized mMOR-1B4 with [^{3}H]diprenorphine binding and [^{35}S]GTPγS binding assays. The results indicated that mMOR-1B4 binding was mu-selective. However, agonists displayed far lower binding affinities to mMOR-1B4, with the exception of etorphine, etonitazene, [DMT1]DALDA and buprenorphine. mMOR-1B4 responded to a number of agonists in [^{35}S] GTPγS binding assays, although the maximum level of stimulation was lower than that seen with the other carboxyl terminal variants. Current studies further explore the hypothesis that the terminal 39 amino acids are responsible for the distinct binding properties, possible due to their association with intracellular or membrane factors that facilitates the formation of an antagonist receptor conformation. Support: DA13997 (Y.-X. P.) and DA02615 and DA00220 (G.W.P.).

M30 ASSESSMENT OF HUMAN MU OPIOID RECEPTOR SPLICE VARIANTS THROUGH MORPHINE-INDUCED ADENYLYL CYCLASE SUPERACTIVATION ***L. Pan, J. Xu, M.-M. Xu, Y.-X. Pan, G.W. Pasternak Dept. Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY USA*** In previous studies we have identified and characterized six new alternatively spliced variants of the human mu opioid receptor gene, Oprm. These variants showed little difference in mu opioid binding selectivity, but significant differences in agonist-induced G-protein coupling and adenylyl cyclase activation. In these studies, we further investigated the functional significance of human MOR splice variants through morphine-induced adenylyl cyclase (AC) superactivation. Results showed that morphine induced AC superactivation for all variants in a time- and dose-dependent manner. The results also showed significant differences in half-time, potency (EC50) and efficacy (% of maximum stimulation) among variants. After morphine chronic treatment, these variants also showed different patterns of cAMP levels in a time-course study. Further exploring mechanisms behind differential AC superactivation among these human MOR splice variants will provide important insights to understanding highly individual response to morphine among humans. Support: DA13997 (YX P), DA02615, DA00220 (GWP)

M31 EXPRESSION OF MU OPIOID RECEPTOR IN PC12 CELLS ***L.A. Towart, Y.-X. Pan, G.W. Pasternak Depts. Neurol. Neurosci., Cornell Univ., NY; Dept. Neurol., Mem. Sloan-Kettering Cancer Ctr., New York, NY USA*** Rat pheochromocytoma PC12 cells are triggered by NGF to differentiate toward neuronal-like cells and have been long used *in vitro* to study neuronal differentiation. Evidence indicates that there are a variety of endogenous opioid systems present in different strains of PC12 cells, and these opioid systems are reported to be involved to a certain degree with neuronal function (Chang & Mok, 2001). Inoue and Hatanaka demonstrated that NGF increased the expression of enkephalin specific binding sites on PC12 cells, and the specific binding sites were characterized pharmacologically as the DOR. Furthermore, a NGF dose-dependent, time-related increase of DOR transcripts in PC12 cells has been reported. The presence of other opioid receptor transcripts in PC12 cells has not been reported by other laboratories. In the present study, we investigate the expression of MOR-1 and its splice variants in PC12 cells. MOR-1 mRNA expression was detected by RT-PCR and confirmed by sequence analysis. In addition to MOR-1 itself, we have observed considerable splicing of the Oprm gene in PC12 cells. While some of the splice variants conform to those previously reported by our laboratory and others, other variants appear to be unique.

Species

M32 FUNCTIONAL PHARMACOLOGY OF THE CLONED GUINEA PIG MU OPIOID RECEPTOR (GP-MOR) ***M. Wallisch (1), S.A. Smith (2), C. Nelson (1,3), T. Ransom (1), G.D. Olsen (1) (1) Dept. of Physiol. & Pharmacol., (2) Dept. of Pediatrics, (3) Div. of Pul. Crit. Care Med., Sch. of Med. Oregon Health & Science Univ., Portland, OR, USA*** The guinea pig is a powerful animal model to study opioid induced respiratory depression of the neonate after chronic in utero exposure to the opioid analgesics methadone and morphine. Our goal is to focus on the molecular pharmacology of the mu opioid receptor in the respiratory control areas of the brainstem. Here we report the characterization of our recently cloned gp-MOR (Smith et al., 2004) when stably expressed in Chinese hamster ovarian (CHO) cells, and compare the guinea pig to the homologous human and rat mu opioid receptors. The functionality of our newly established cell line is assayed using saturation and competition ligand binding and opioid-stimulated GTP-γ-S binding. We also show the development of tolerance in the MOR expressing CHO cells when cultured in the presence of methadone and morphine for 0-48h. Thus we present data showing our cloned gp MOR possesses functional characteristics that are similar to the human MOR. Support: DA007912

M33 PHARMACOLOGICAL PROFILE OF A NEW MU OPIOID RECEPTOR FROM ZEBRAFISH ***E. Marrón Fdez de Velasco, I. Rodríguez-Martín, R.E. Rodríguez Dept. Biochem. and Mol. Biology, Inst. of Neuroscience, Univ. of Salamanca, Salamanca, Spain*** The zebrafish has been proposed as a candidate organism for

the study of interactions between the genome and environment and it can be considered as a valid model to unravel the molecular basis of drug tolerance and dependence. We have previously reported the molecular characterization and expression of ZFOR2 a putative mu opioid receptor from zebrafish that has a peptide sequence that is 72% homologous to the mammalian mu opioid receptor. Here we present the pharmacological characterization of ZFOR2. When expressed in HEK-293 cells, ZFOR2 binds the non-selective opioid antagonist [3H]-Diprenorphine with high affinity (kD=0.85nM, Bmax=9389fmol/mg prot.). We also obtained good affinity for the mu agonist [3H]-DAMGO (kD=4.7nM, Bmax=1135fmol/mg prot.), and for [3H]-Bremazocine (KD=1.89nM, Bmax=12268fmol/mg prot.). The binding of [3H]-Diprenorphine is displaced by several compounds: Bremazocine>Naloxone> DynorphinA> Morphine >Endomorphin1>BW377U86 >CTOP>DAMGO> Endomorphin2>Met-enkephali> Fentanil>Leu Enkephalin >DSLET >DPDPE. Also, [^{35}S] GTPγS binding assays show activation of G-proteins by DAMGO, Dynorphin A, Morphine, Endomorphins, Enkephalins, BW377U86 and DSLET.

M34 ZFOR4, A DELTA OPIOID RECEPTOR FROM ZEBRAFISH: AGONIST-MEDIATED INTERNALIZATION AND MAP KINASE ACTIVATION ***V. Gonzalez-Nuñez (1), K. Roberts (2), C.J. Evans (2), R.E. Rodríguez (1) (1) Dept. of Biochem. & Molec. Biol., Inst. Neurosci., Castilla y León. Univ., Salamanca, Spain, (2) Hatos Research Center for Neuropharmacology, NPI, UCLA,LA, CA USA*** We previously reported the pharmacological characterization of ZFOR4, a zebrafish opioid receptor homologous to the mammalian delta opioid receptor. In this work we have determined the biochemical processes that take place after the activation of ZFOR4 by endogenous zebrafish opioid peptide ligands (predicted from zebrafish pro-enkephalin) and by several synthetic agonists. Etorphine and enkephalins mediate the rapid internalization of ZFOR4 in a dose- and time-dependent manner, although the internalization triggered by etorphine is much faster and drastically diminishes the number of surface receptors after both acute and chronic treatment. We have observed that the ligand-induced internalization of ZFOR4 is independent of the Gi/o proteins. These ligands as well as morphine, an agonist that does not elicit ZFOR4 internalization, are able to stimulate MAPK activation. Following more prolonged treatment with etorphine we have also observed that the basal level of ERK1/2 phosphorylation is reduced, suggesting signalling desensitization, either as a result of internalization or of the signalling machinery.

Toxicity

M35 DECOY PEPTIDES THAT BIND DYNORPHIN NON-COVALENTLY PREVENT NMDA-RECEPTOR-MEDIATED NEUROTOXICITY AND ISCHEMIC BRAIN INJURY ***A.S. Woods, Y. Wang, T. Shippenberg NIDA IRP, NIH, Baltimore MD, USA*** Prodynorphin -derived peptides elicit various pathological effects including neurological dysfunction and cell death. These actions are reduced by N-methyl-D-aspartate receptor (NMDAR) but not opioid receptor antagonists suggesting NMDAR -mediation. Here we show that a conserved epitope of the NR1 subunit of the NMDAR binds dynorphin peptides (DYNp) non-covalently. Synthetic peptides containing this epitope form stable complexes with DYNp and prevent the potentiation of NMDAR- gated currents produced by DYNp. They attenuate DYNp - evoked cell death in brain and spinal cord and prevent, as well as reverse, DYNp - induced paralysis and allodynia. They protect against ischemic brain injury and, when administered after ischemic insult, improve motor function. These data reveal a novel mechanism whereby prodynorphin-derived peptides facilitate NMDAR function and produce neurotoxicity. Furthermore, they suggest that synthetic peptides that bind DYNp, thus preventing their interaction with NMDAR, may be novel therapeutic agents for the treatment of brain and spinal cord injury.

M36 ACUTE METHADONE INDUCED RESPIRATORY DEPRESSION IN THE NEONATAL GUINEA PIG ***R. Nettleton (1), T. Ransom (1), C. Nelson (1,2), S. Abraham (3) G. D. Olsen (1) (1)Dept. of Phys. & Pharm., (2)Div. Pulm. Crit. Care Med., (3) Stem Cell Center, Oregon Health & Science Univ., Portland, OR, USA*** Methadone is an opioid with analgesic properties and the ability to depress the respiratory response to increased arterial PCO2. It is currently the only drug approved for the treatment of heroin addicted pregnant women. Infants born to women on methadone maintenance display life threatening withdrawal symptoms and have a higher incidence of sudden infant death syndrome. In this study we determine the acute respiratory response of neonatal guinea pigs to methadone while breathing room air and during a 5% CO2 challenge. Guinea pigs aged 3, 7, or 14 days were given a single subcutaneous dose of methadone (5 or 10mg/kg) or saline. Respiratory parameters including frequency, tidal volume, and minute ventilation were determined along with metabolic parameters including O2 consumption and CO2 production. Results show a time, dose, and age dependent depression of respiratory and metabolic parameters. In addition, the baseline respiratory responses of 7-day-old guinea pigs were developmentally immature during a 5% CO2 challenge as compared to room air responses. Support: DA007912

M37 EFFECTS OF PAEONIFLORIN ON NEUROTOXIN-INDUCED NEURONAL CELL DAMAGE AND EXPERIMENTAL PARKINSONISM IN MU-KNOCKOUT MICE ***H.Y. Tsai (1,2), Y.T. Lin (3), K.W. Chien (1), Y.F. Chen (1,2) (1) Dept. Pharmacol., China Medical Univ., Taichung, Taiwan (2) Dept. Pharmacy, China Medical Univ. Hospital, Taichung, Taiwan (3) Dept. Nursing, Jen-The Junior College of Medicine, Nursing and Management, Mialoi, Taiwan*** Paeoniflorine, a major component from paeoniae radix, has several pharmacological effects including antiallergic, antinociceptive, anti-spasmodic and anti-inflammatory action. We previously reported that paeoniflorin showed protective effects on neuronal damage. Neurotoxins, such as 6-OHDA and MPTP, are highly selective to dopaminergic neurons of the substantia nigra pars compacta (SNc) and induce animal models of Parkinsonism. The latter compound is converted by MAOB to the MPP+ ion which is toxic to neurons by interfering with mitochondrial metabolism. Mu opioid receptor density in the globus pallidus (GP) is decreased as a result of a nigrostriatal lesion, the extent to which the change correlates with the expression of parkinsonian signs remains to be defined. It has been shown that the binding of D2 dopamine receptor in muopioid receptor knockout (MOR KO) was significantly higher than that of the wild type in the caudate putamen, whereas D1 dopamine receptor remains no changes. In this study, MOR KO mice will be used to study the effect of paeoniflorin on dopamine metabolism in MPTP-indcued experimental Parkinsonism and neurotoxin-induced neuronal cell damage. From our results, we found that after MPTP-treatment, striatum dopamine concentration was decreased two folds compared with that of wild type. Paeoniflorin co-administered with MPTP, increased dopamoine turnover rate. Moreover, paeoniflorin could protect neuronal cell from neurotoxin- induced damage.

M38 MODULATION OF METHAMPHETAMINE NEUROTOXICITY BY ENDOGENOUS K-OPIOID RECEPTOR SYSTEMS ***E.K. Oh, T.S. Shippenberg, V.I. Chefer Integrative Neuroscience Section, DHHS/NIH NIDA IRP, Baltimore, MD USA*** Synthetic κ-opioid receptor (KOPr) agonists attenuate alterations in dopamine (DA) neurotransmission that occur after neurotoxic doses of methamphetamine (MET). To examine the neuroprotective role of endogenous KOPr systems we examined the effects of nor-binaltorphimine (BNI) upon basal and stimulated DA release in dorsal striatum. Mice received MET injections (10 mg/kg X 4, every 2 hrs) 24 hrs after BNI administration (20 mg/kg). Microdialysis was conducted 7 days later. Locomotor activity and body temperature were also assessed. MET-pretreated animals had lower basal, KCl- , MET- , and amphetamine (intra-striatal)-evoked DA levels relative to controls. BNI treatment further decreased these parameters. BNI treatment did not affect DA levels in control mice. Striatal tissue content of DA was reduced in MET-pretreated mice, and BNI treated mice showed a trend toward a further reduction in tissue DA levels. Our results indicate that selective KOPr blockade exacerbates the effects of a neurotoxic dose regimen of MET. These results are consistent with the existence of a tonically active KOPr system that opposes the effects of psychostimulants on DA neurotransmission.

M39 PROTECTION AGAINST ISCHEMA/REPERFUSION MEDIATED MYOCARDIAL CELL DEATH BY A DELTA-OPIOID AGONIST, DELTORPHIN II ***X. Yue (1), E. Navratilova (1), E. Varga (1, 2), J. Bahl (2), D. O'Connell (2), H. Yamamura (1,2), W. Roeske (2) (1) Dept. Med. Pharmacol., (2) Sarver Heart Center, Univ. of Arizona, AZ, USA*** We have used an immortalized myocardial cell line, HL-1, to establish a cellular model for opioid protection against ischemia/reperfusion mediated myocardial damage. We found that HL-1 cells express physiologically sufficient number of delta-opioid receptors (Bmax = 150 fmol/mg). SNC 80 stimulated [^{35}S] GTPγS binding to HL-1 cell membranes with an EC50 of 239 nM, indicating that these delta-opioid receptors are functionally active. Treatment with a delta-opioid agonist (deltorphin II) protected HL-1 cells against ischemia /reperfusion mediated death in a dose dependent manner. Pre-administration of delta-opioid receptor antagonist naltrindole (10nM) partially reversed the protective effect. Interestingly however, at higher doses opioid antagonists exhibited a pronounced toxic effect in HL-1 cardiomyocytes. The HL-1 cell line could be a useful tool to study the effects of opioids on cardiomyocytes and to identify novel drugs for the prevention and treatment of heart attacks.

Receptor-receptorInteractions

M40 ALLOSTERIC INTERACTION AND SELECTIVITY OF HETERODIMERIZED DELTA AND KAPPA OPIOID RECEPTORS ***Z. Xie, R.G. Bhushan, D.J. Daniels, P.S. Portoghese Dept. Medicinal Chemistry, College of Pharmacy, Univ, Minneapolis MN, USA*** Previous studies have suggested that heterodimerized delta and kappa opioid receptors function cooperatively and exhibit delta-1 and kappa-2 selectivity. In the present study, we employed delta and kappa selective ligands to provide additional evidence for these properties. In HEK293 cells that coexpressed delta and kappa opioid receptors, [^{3}H]naltrindole binding was enhanced 27-fold in the presence of norBNI, and [^{3}H]norBNI binding was enhanced 36-fold in the presence of naltrindole relative to the mixed cells that singly

expressed delta or kappa opioid receptors. These results strongly suggest that delta-kappa heterodimers are allosterically coupled. Additional support for the delta-1/kappa-2 phenotype selectivity of delta-kappa heterodimers was obtained from studies on the activation of ERK1/2 by studying the interaction between the selective delta-1/kappa-2 bivalent antagonist, KDN-21, and selective agonists in HEK293 cells containing coexpressed delta and kappa receptors. The finding that KDN-21 inhibited the activation of ERK1/2 by DPDPE (delta-1) and bremazocine (kappa-2), but not deltorphin II (delta-2) and U69593 (kappa-1) supports the idea that phenotypic delta-1 and kappa-2 receptors are heterodimeric delta-kappa receptors. We propose that the delta-2 and kappa-1 phenotypes are homodimers.

M41 EVIDENCE FOR INTERACTIONS BETWEEN THE CENTRAL ENDOGENOUS ENDOTHELIN AND OPIOID SYSTEMS ***X.Y. Wang, H. Xu, R.B. Rothman IRP, NIDA, NIH, DHHS, Baltimore, MD, USA*** We tested the hypothesis that the central endothelin (ET) and opioid systems functionally interact. Chronic i.c.v. administration of anti-ET IgG had no significant effect on expression of mu and delta receptors, but increased kappa receptor expression by 55 % in the caudate. Anti-ET IgG also decreased the Emax for DAMGO- but not for SNC80-stimulated [^{35}S]GTP-γ-S binding. Chronic i.c.v. administration of anti-β-endorphin IgG down-regulated ET-A receptor expression in the caudate (51%), and had no effect on μ, δ, κ opioid, and ET-B receptors. Anti-β-endorphin IgG also increased caudate ET-1 levels by 32%, and increased the efficacy of DAMGO-stimulated [^{35}S]GTP-γ-S binding in the caudate without altering its potency. These results suggest that β-endorphin in the CSF coordinately regulates ET-1 levels and the ET-A receptor, and that ET in the CSF negatively regulates kappa opioid receptors in rat caudate. These findings provide further support for the hypothesis that CSF neuropeptides have regulatory effects and additionally demonstrate that the central ET and opioid system functionally interact.

M42 MECHANISMS OF ADENOSINE A2A and DOPAMINE D2 RECEPTOR HETERO-DIMERIZATION ***A.S. Woods, S. Ferre, NIDA IRP, NIH, Baltimore MD USA*** Electrostatic interactions between a basic epitope of the D2 receptor containing adjacent arginine residues and an acidic epitope of the A2A receptor containing a phosphorylated serine are involved in receptor heteromerization. In the present study we demonstrate that this arginine-phosphate electrostatic interaction possesses a "covalent-like" stability. Hence, these bonds can withstand fragmentation by mass spectrometric collision-induced dissociation at energies similar to those that fragment covalent bonds and they demonstrate an extremely low dissociation constant by plasmon resonance. The present work also highlights the importance of phosphorylation-dephosphorylation events in the modulation of this electrostatic attraction. Phosphorylation of the acidic epitope, a casein kinase one consensus site, makes it available to interact with the basic epitope. On the other hand, phosphorylation of serine and/or threonine residues adjacent to the basic epitope, a protein kinase A consensus site, slows down the attraction between the receptor epitopes. Although analyzed here in the frame of receptor heteromerization, the arginine-phosphate electrostatic interaction most likely represents a general mechanism in protein-protein interactions.

M43 INTERACTIONS BETWEEN MU-OPIOID RECEPTORS AND α 2A ADRENERGIC RECEPTORS PROMOTE RECEPTOR INTERNALIZATION AND DESENSITIZATION ***M. Tan, C.-W. Xie Dept. Psychiatry Biobehav., Univ.California-Los Angeles, CA USA*** Studies in heterologous cells show that mu opioid and α 2A-adrenergic receptors form physical association with each other upon acute activation of either receptor, which leads to enhanced receptor signaling. We further examined whether interactions between these two receptors affect receptor desensitization and trafficking in neurons during chronic agonist exposure. Both the mu agonist DAMGO and α 2 agonist clonidine acutely inhibited voltage-gated Ca2+ currents in cultured dorsal root ganglia (DRG) neurons. Their inhibitory actions were significantly reduced following prolonged incubation with either agonist alone. This heterologous desensitization was not prevented by inhibitors for PI 3-kinase, MAP kinase, protein kinase C or A. Immunocytochemical analyses demonstrated colocalization of mu and α 2A receptors in DRG neurons. Incubation with either clonidine or DAMGO for 2 hr induced significant internalization of both receptors, reversible by yohimbine or CTOP. Our results suggest that interactions between mu and α 2A receptors may promote receptor desensitization and internalization in neurons.

M44 THE CHEMOKINE RECEPTOR CXCR4 IS INVOLVED IN THE CXCL12/SDF-1-α ANTAGONISM OF KAPPA- OR DELTA-OPIOID RECEPTOR-INDUCED ANTI-NOCICEPTION ***X.H. Chen, E.B. Geller, M.S. Deitz T. J. Rogers & M. W. Adler Center for Substance Abuse Research, Temple Univ. Sch. of Med., Phila., PA USA*** We have recently reported that CXCL12/SDF-1-α (SDF-1) blocks the antinociception induced by the mu agonist

DAMGO via CXCR4 receptors expressed in the brain, and that cross-desensitization occurs between the mu, delta or kappa opioid receptors and the CXCR4 receptor for SDF-1 in antinociception. In the present study, we asked whether blocking the chemokine receptor CXCR4 would affect the desensitization by SDF-1 on antinociception induced by injection of kappa or delta opioid agonists into the periaqueductal grey of rats. The cold-water tail-flick test was used as an antinociceptive index. The results showed that SDF-1 (100 ng), 30 min before injection of the opioid agonists, can reduce the antinociception induced by the kappa agonist dynorphin (20 μg) or delta agonist DPDPE (100 ng), but this antinociception is not reduced when the SDF-1 antagonist AMD 3100 is given 15 min before the injection of the chemokine. These results confirm that CXCR4 receptors mediate the SDF-1 block of the antinociception induced by kappa or delta, as well as mu, agonists. Support: DA 06650, 13429, 16544, 14230

Addiction/Human

M45 THE β-ENDORPHIN-DERIVED PEPTIDE GLYCYL-GLUTAMINE INHIBITS NICOTINE CONDITIONED PLACE PREFERENCE AND WITHDRAWAL ***G. Göktalay (1,2), J. Hamilton (1), S. Cavun (1,2), M. Levendusky (1), W. Millington (1) (1) Albany College Pharmacy, Albany, NY, (2) Uludag University, Bursa, Turkey*** Glycyl-glutamine (Gly-Gln) is an inhibitory dipeptide synthesized from β-endorphin. Previously, we showed that Gly-Gln prevents acquisition of a conditioned place preference (CPP) to morphine. In this study, we tested whether Gly-Gln's inhibitory activity extends to other rewarding drugs, specifically nicotine. Rats were conditioned with nicotine (0.6 mg/kg, sc) for 4 days and tested on day 5. Gly-Gln (100 nmol, icv) prevented acquisition of nicotine CPP when administered daily immediately before nicotine and dose-dependently (3-100 nmol) inhibited expression of nicotine CPP when administered 2 min before testing on day 5. Gly-Gln had no effect on animals that did not receive nicotine. Gly-D-Gln was ineffective. To study withdrawal, rats were treated with nicotine (9 mg/kg/day) for 7 days and conditioned place aversion (CPA) was induced with a single dose of mecamylamine (1 mg/kg, sc). Gly-Gln (100 nmol) prevented acquisition of mecamylamine, but not U-50488, CPA. Gly-Gln thus inhibits the rewarding effects of nicotine and attenuates cognitive aspects of withdrawal in nicotine addicted rats. Support: DA018029

M46 A NOVEL DEPOT FORMULATION OF BUPRENORPHINE FOR TREATMENT OF OPIOID DEPENDENCE ***S. Sigmon (1), G. Bigelow (2) (1) Dept. Psychiatry, Univ. Vermont, Burlington, VT, (2) Dept. Psychiatry and Behavioral Sciences, Johns Hopkins Univ., Baltimore, MD USA*** A novel depot formulation of buprenorphine, using a sustained-release microcapsule technology (Biotek, Inc., Woburn, MA), may offer effective opioid dependence treatment while minimizing risks of patient nonadherence and illicit diversion. We describe two studies examining the safety and pharmacokinetics of buprenorphine depot in opioid-dependent volunteers, as well as its efficacy in suppressing opioid withdrawal, attenuating the effects of exogenous opioid challenge, and providing clinical detoxification. A single s.c. dose produced elevations of buprenorphine plasma levels for six weeks. Depot buprenorphine was safe, well-tolerated, provided relief from opioid withdrawal and produced substantial opioid blockade. These results suggest that depot buprenorphine offers substantial promise for enhancing the delivery of effective opioid dependence treatment. We will present data from these two trials and as-yet-unpublished data on this product's extended biodelivery and effects combining data from both trials, which comprise all existing biodelivery and pharmacodynamic data for depot buprenorphine to date in humans.

M47 ROLE OF OREXIN NEURONS IN THE BRAIN REWARDING SYSTEM ***Y. Nagumo, M. Narita, M.Narita, M. Miyatake, Y. Yajima, T. Suzuki Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan*** In the present study, we investigated the role of orexinergic systems in the rewarding effect induced by the mu-opioid receptor agonist morphine in rodents. Extensive co-expression of tyrosine hydroxylase (TH) with orexin receptors was observed in the mouse ventral tegmental area (VTA). Both orexin A and orexin B produced a phospholipase C-dependent increase in intracellular Ca2+ release through Gq11α and G β-γ subunits, respectively, in TH-positive cells. In *in vivo* behavioral assay, the levels of dopamine and its major metabolites in the nucleus accumbens (N.Acc.) were markedly increased by the microinjection of orexins into the rat VTA. The morphine-induced place preference and dopamine release in the N.Acc.observed in wild-type mice were significantly suppressed by a deletion of prepro-orexin gene. These findings provide novel evidence that orexinergic systems are directly implicated in the rewarding effect induced by mu-opioids through activation of the mesolimbic dopamine pathway in rodents.

15:30 - 18:50 **Symposium II Human genetics of addiction** **Marriott Ballroom**
Chair J Pollock, Cochair MJ Kreek

15:30 – 15:55 **S9 G.R. Uhl HUMAN MOLECULAR GENETICS OF ADDICTION: REMARKABLE RECENT PROGRESS** ***G.R Uhl, Molecular Neurobiology, NIDA-IRP, NIH, DHSS, Baltimore MD USA*** Strong evidence supports substantial genetic contributions to vulnerability to addictions that include addictions to opiates. Association and linkage-based "top down" approaches are identifying loci that contain variants likely to contribute to human vulnerability to addictions to opiates and other addictive substances. I will present results from: 1) the several hundred million person/genotypes that we have analyzed in three successive waves to seek allelic variants that are associated with polysubstance abuse vulnerability in individuals of two distinct ethnicities, 2) the ways in which the results from these association studies converge with those that we have obtained in analyzing samples from alcoholic and methamphetamine-abusing populations, and 3) the ways in which these results converge with data from linkage-based genome scans for other addictions. Evidence from fine-mapping and other studies that supports the cell adhesion molecule NrCAM as regulated by opiates, containing allelic variants that predispose to addictions in several human samples, and exerting striking influences on opiate reward when its levels of expression are modified in knockout mice will be presented. The ways in which all of these data, taken together, are remarkably consistent with polygenic genetic architecture for addictions and with "common disease/common allele" working hypotheses about the nature of the underlying genetic influences we be discussed, along with the idea that we may have already identified markers for a significant fraction of the genetic variation that contributes to human addiction vulnerability, based on evidence that includes the convergence of more recent results with the results obtained in previously-reported studies.

15:55 – 16:20 **S10 M.T. Tsuang OPIATE DEPENDENCE: COMORBIDITY AND FAMILIAL VULNERABILITY** ***M. T. Tsuang, M. Lyons Institute of Behavioral Genomics, Department of Psychiatry, University of California, San Diego, CA, Department of Psychology, Boston University, Boston, MA USA*** Previous research on opiate dependence using the Vietnam Era Twin Registry has shown a significant genetic influence on opiate dependence and demonstrated that, compared to any other illicit drug, more of the genetic influence on opiate dependence is specific to opiate dependence. This previous research explicated the nature of the relationship of opiate dependence with other illicit drugs, but leaves important unanswered questions about the nature of the relationship of opiate dependence with dependence on licit substances and mood disorders. We studied 3362 male twin pairs from the Vietnam Era Twin Registry. Twins were interviewed using the Diagnostic Interview Schedule for DSM-III-OR. The prevalence of opiate dependence was 1%. We examined the comorbidity between opiate dependence and a number of other disorders. There was significant comorbidity between opiate dependence and alcohol dependence, nicotine dependence, and antisocial personality disorder, but not bipolar disorder, major depression, and dysthymia. We then examined the relationship between opiate dependence in one twin and the presence of each of the significantly comorbid disorders in the co-twin (cross-twin cross-trait analyses). To avoid the potentially confounding influence of opiate dependence on the comorbid disorders, we examined only co-twins of opiate dependent subjects who themselves were not opiate dependent. Opiate dependence in one twin was significantly associated with an elevated risk of alcohol dependence (OR=3.3), antisocial personality disorder (OR=11.3), and tobacco dependence (OR=8.2) in the co-twin, even in the absence of opiate dependence in the co-twin. We repeated these cross-twin cross-trait analyses stratified by zygosity. If the relationship between opiate dependence and another disorder is significantly stronger across MZ pairs than DZ pairs, then a significant genetic influence on the relationship is implicated. We did not observe any significant MZ versus DZ differences, but because of the low prevalence of opiate dependence, we had very modest statistical power for these comparisons. Our results demonstrate a significant shared familial vulnerability of opiate dependence with alcohol and nicotine dependence and antisocial personality disorder, but we could not distinguish between the family environment and genetic factors as the basis of the shared vulnerability. Unlike our population-based sample, a clinical sample might provide a greater number of cases, but for studying comorbidity, a clinical sample is problematic because of the possible effects of Birkson's bias (i.e., individuals with two disorders may be more likely to come to clinical attention, even if there is no actual association between the disorders).

16:20 – 16:40 *Coffee break*

16:40 – 17:05 **S11 W. Berrettini THE MU OPIOID RECEPTOR GENE AND ADDICTIONS** ***W.H. Berrettini Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA USA*** In QTL studies of C57BL/6J and DBA/2J mice, the proximal region of murine chromosome 10, containing the mu opioid receptor gene (OPRM1), is known to contain

sequences that influence multiple opioid-related phenotypes, such as voluntary oral morphine consumption, morphine analgesia and density of mu receptors. These data are consonant with mu receptor null mutant experiments, showing that the mu opioid receptor is essential for the rewarding and analgesic effects of morphine.We have created reciprocal congenics for the interval of proximal chromosome 10 containing the OPRM1 locus, and phenotypic characterization reveals capture of the chromosome 10 locus for voluntary morphine oral consumption. Although we have not been able to establish linkage disequilibrium between opioid dependence (OD) and the human OPRM1 locus, others have reported this. We have demonstrated that, at a functional variant in the human OPRM1 gene, A118G, the G allele predicts response to naltrexone among alcohol dependent individuals, and the G allele predicts abstinence during transdermal nicotine therapy for nicotine dependence. These diverse results suggest that the OPRM1 gene plays a multifaceted role in addition genetics and pharmacogenetics. Support: NIDA P60 DA 5186 and NCI P50 84718

17:05 – 17:30 **S12 J. Gelernter FIRST RESULTS FROM A GENOMEWIDE LINKAGE SCAN FOR OPIOID DEPENDENCE** ***J. Gelernter (1), C. Panhuysen (2), R. Weiss (3), K. Brady (4), V. Hesselbrock (5), B. Rounsaville (1), J. Poling (1), M. Wilcox (2), L. Farrer (2), H.R. Kranzler (5) (1)Yale Univ. School of Medicine, Dept. of Psychiatry, and VA CT Healthcare Center, (2) Boston University School of Medicine, Dept. of Medicine (Genetics Program), (3) McLean Hospital, Belmont, MA and the Dept. of Psychiatry, Harvard Medical School, Boston, MA, (4) Medical Univ. of South Carolina, Dept. of Psychiatry, Charleston, SC, (5) Univ. of CT Health Center, Dept. of Psychiatry, Farmington, CT USA*** Risk for opioid dependence (OD), as for other forms of substance dependence, is genetically influenced. In order to identify the chromosomal regions of genes that increase risk for opioid dependence, we completed a genetic linkage study based on affected sibling pairs. Assessment was via the SSADDA, a detailed and highly reliable diagnostic instrument. We used cluster analysis to identify subgroups of subjects, to decrease genetic heterogeneity. We genotyped more than 400 short tandem repeat markers throughout the genome. Our results showed several "suggestive" linkages for DSM-IV OD. We observed stronger evidence of linkage with cluster-defined traits, suggesting that they may aid in defining more genetically homogeneous OD-related syndromes.

17:30 – 17:55 **S13 H.J. Edenberg GENETICS OF ALCOHOLISM** ***H.J. Edenberg Biochemistry & Molecular Biology, Indiana Univ. School of Medicine, Indianapolis, IN USA*** Alcohol dependence (alcoholism) is a complex genetic disease, with both environment and genetics contributing to vulnerability. There is substantial variability in the disease, and therefore it is likely that there is variability in the genetic contributions toward vulnerability. The Collaborative Study on the Genetics of Alcoholism (COGA) was designed to discover genes that affect risk for alcoholism and genes affecting related phenotypes. COGA is a family-based study, in which probands were recruited from treatment facilities. COGA initially used a whole-genome survey to identify regions linked to alcohol dependence and related endophenotypes. We are now successfully identifying individual genes within those regions that are associated with alcohol dependence and related phenotypes, by intensive SNP genotyping of candidate genes and analysis of linkage disequilibrium. I will describe our strategy for gene identification, and present recent results.

17:55 – 18:10 **S14 D.A. Nielsen A TPH2 HAPLOTYPE ASSOCIATES WITH OPIOID DEPENDENCE IN AFRICAN AMERICANS** ***D.A. Nielsen (1), D. Proudnikov (1), S. Barral (2), S. Kellogg (1), J. Ott (2), M.J. Kreek (1) (1) Lab. of the Biol. of Add. Diseases, (2) Lab. of Stat. Genetics, Rockefeller Univ., New York, NY USA*** The TPH2 gene, which codes for the rate-limiting enzyme in the biosynthesis of serotonin, a neurotransmitter involved in impulse control, was sequenced in subjects from our on-going studies of addictive diseases (n=161-187). We identified 48 variants. Two rare variants code for amino acid changes. Six SNPs were genotyped in consecutively ascertained subjects (2/95-9/99; n=643). Opioid dependent patients (n=266) met Federal guidelines for MMTP. Controls (n=170) were non-drug users. In the control group, 3 SNPs significantly differed in allele frequencies (p=0.001, 0.001, 0.0003) between the 3 major ethnicities. Haplotype analysis revealed an point-wise association of a specific haplotype with opioid dependence in African Americans (p=0.011, OR=6.8). This result indicates that a variant(s) in or near TPH2 may be involved in vulnerability to develop opioid dependence in African Americans. Support: NIDA DA-P60-05130, DA-00049, DA-12848, M01-RR00102 (MJK), MH44292 (JO)

18:10 – 18:25 **S15 K. Ikeda A POSSIBLE GENETIC MECHANISM OF INDIVIDUAL SENSITIVITY TO OPIATES** ***K. Ikeda (1), S. Ide (1,2), S. Kasai (1), G.R. Uhl (3), I. Sora (1,4) (1) Div. Psychobiol., Tokyo Inst. Psychiatry, Tokyo, Japan (2) Lab. Neuropharmacol., Hiroshima International Univ.,Hiroshima, Japan (3) Mol. Neurobiol. Branch, NIDA-IRP, NIH/DHHS, Baltimore, MD, USA, (4) Div. Psychobiol., Tohoku Univ. Grad. Sch. Med. Sendai, Japan*** Significant individual differences in opiate sensitivity can hamper effective pain treatments and

increase risks of drug abuse. To reveal the mechanisms underlying the individual differences, we first investigated genetic mechanisms of mouse interstrain differences in opioid sensitivity. We found that a 5.3 kb nucleotides insertion in the 3' noncoding region of the mu-opioid receptor (MOR) gene caused reduced morphine-induced analgesia in CXBK mice. Next, 3'-rapid amplification of cDNA ends-PCR (3'RACE-PCR) analyses and reverse transcription PCR analyses revealed that 10180 and 13632 nucleotides are transcribed from an exon of the C57BL/6 mouse and human MOR genes, respectively, as the 3' untranslated region (UTR) of the MOR-1 mRNA, the main transcript of the gene. Furthermore, we identified more than 50 genetic polymorphisms in the 3'UTR of the human MOR gene. Individual differences in opioid sensitivity might be predicted by differences in these polymorphisms as in the case of CXBK mice.

18:25 – 18:40 **S16 W. Sadee *A118G* VARIANT AFFECTS THE mRNA AND PROTEIN EXPRESSION OF HUMAM MU OPIOID RECEPTOR** ***Y. Zhang, D. Wang, A.D. Johnson, A.C. Papp, W. Sadée Program in Pharmacogenomics, Department of Pharmacology, The Ohio State University, Columbus, OH, USA*** As a primary target for opioid drugs and peptides, the mu opioid receptor (OPRM1) plays a key role in pain perception and addiction. Genetic variants of *OPRM1* have been implicated in predisposition to drug addiction, in particular the single nucleotide polymorphism *A118G*, leading to an Asn40Asp substitution. We have measured allele-specific mRNA expression of *OPRM1* in human autopsy brain tissues, using *A118G* as a marker. In 8 heterozygous samples measured, the *A118* mRNA allele was 1.5- to 2.5-fold more abundant than the *G118* allele. Transfection into CHO cells of a cDNA representing only the coding region of *OPRM1,* carrying *A*, *G*, *C*, or *T* in position 118, resulted in 1.5-fold lower mRNA levels only for *OPRM1-G118*, and more than tenfold lower OPRM1 protein levels, measured by Western blotting and receptor binding assay. After inhibition of transcription with actinomycin D, we failed to reveal differences in mRNA stability between *A118* and *G118* alleles, indicating a defect in transcription or mRNA maturation. These results indicate that *OPRM1-G118* is a functional variant with profound effects on both mRNA and protein yield.

18:40 – 18:55 **S17 C.D Bryant MORPHINE ANALGESIC TOLERANCE IN 129/S6 AND 129/P3 MICE: META-ANALYSIS OF INBRED STRAINS INDICATES THAT MOTOR PERFORMANCE GENETICALLY CORRELATES WITH TOLERANCE AND BASELINE NOCICEPTION** ***C.D. Bryant (1,2), K.W. Roberts (2), C.J. Evans (1,2). (1) UCLA Interdepartmental Program in Neuroscience, (2) UCLA Department of Psychiatry, Los Angeles, CA USA*** The 129/S6 and 129/P3 inbred mouse strains reportedly do not exhibit tolerance to morphine analgesia. Understanding the mechanism for this trait could have major implications for treating pain. We attempted to replicate this finding with a standard morphine tolerance regimen in our laboratory. Mice were administered escalating doses of morphine once daily for 6 days and given a challenge dose on day 7. The results indicate that analgesic tolerance developed in both substrains in both the hot plate and tail immersion assays. Additionally, co-administration of the non-competitive NMDA receptor antagonist MK-801 with morphine effectively attenuated the development of tolerance in the 129/S6 strain in the hot plate assay. Last, meta-analysis of several inbred mouse strains indicates that rotarod performance (a motor task) correlates highly with tolerance susceptibility and baseline nociception. We propose that the apparent lack of tolerance is due to a less intense motor response that has been overlooked in these mice.

Tuesday, July 12

7:00 – 8:30 **Continental breakfast** **Marriott Ballroom Foyer**

8:30 – 9:30 **P3** **Founders' Lecture** **Mary Jeanne Kreek Marriott Ballroom**
Hypothesis to Pharmacotherapy - Endorphin System to Functional SNPs of Opioid Genes:
An INRC Odyssey 1964-2005

9:30 – 10:30 **P2** **Plenary Lecture** **Nora Volkow** **Marriott Ballroom**
View from NIDA

10:30 – 10:50 *Coffee break*

10:50 - 12:30 **Symposium III** **Imaging opioid systems** **Marriott Ballroom**

Chair V.M. Pickel Cochairs J.-K. Zubieta, J Frost

10:50 – 11:15 **S18 V.M. Pickel TARGETING OF MU-OPIOID AND CANNABINOID1 (CB1) RECEPTORS WITHIN THE MESOLIMBIC DOPAMINE REWARD CIRCUIT** ***V.M. Pickel Dept. of Neurology and Neuroscience, Weill Medical College of Cornell University, New York, NY USA*** Activation of either mu-opioid receptors (MOR) or CB1 receptors can produce rewarding effects through mechanisms involving dopaminergic neurons in the ventral tegmental area (VTA), which project to the shell of the nucleus accumbens (Acb) and other limbic forebrain regions. We used electron microscopic immunolabeling to establish the subcellular distributions of the MOR and CB1 receptors in relation to each other and to the dopaminergic neurons or their targets, which express dopamine D2 receptors (D2R). In the VTA, the MOR were rarely detected in dopaminergic neurons, but were located in many of their inhibitory-type inputs. CB1 and D2 receptors also were present in afferent terminals to dopaminergic neurons in the VTA, although each receptor subtype was more commonly seen on the surface or associated with endomembranes of dopaminergic dendrites in this region. In the Acb, CB1 immunolabeling was seen in somatic, dendritic and axonal terminals that also contained either MOR or D2 receptors. Within the Acb somatodendritic profiles, CB1 and D2 receptors had partially overlapping endomembrane distributions, consistent with their known capacity to form heterodimers in striatal neurons. Moreover, CB1-labeled axon terminals were presynaptic to dendrites expressing either MOR or D2 receptors, whose activation could affect the generation endocannabinoid retrograde signaling molecules. Our results provide ultrastructural evidence that MOR are strategically positioned for mediation of indirect activation of mesolimbic dopaminergic neurons in the VTA and inhibition of the physiological responses of Acb spiny neurons, some of which are also influenced by activation of CB1 and/or D2 receptors. In addition, these observations establish a cellular substrate for direct and possibly interactive effects of CB1 and D2 receptors within the mesolimbic dopaminergic neurons and their Acb targets. The results provide strong evidence for cross-talk between receptor systems that are important for understanding and treating drug addiction. Support: DA05130 and DA004600

11:15 – 11:40 **S19 A. Beaudet *IN VIVO* TAGGING OF CELL SURFACE DELTA OPIOID RECEPTORS** ***A. Beaudet (1), L. Gendron (1), M.J. Esdaile (1), T. Stroh (1), J.P. Vincent (2), C. Cahill (3) (1) McGill Univ., Montreal, Canada, (2) IPMC, Univ of Nice, France, (3) Queen's Univ, Kingston, Canada*** Current high resolution receptor imaging techniques are mainly applicable to cell cultures and do not easily lend themselves to animal studies. On the other hand, receptor localization techniques, such as autoradiography and immunohistochemistry, are ideally suited for animal studies, but do not allow us to distinguish functional from non functional (or intracellular) receptors. In order to provide dynamic imaging of pharmacologically available delta opioid receptors (DOR) in rat spinal cord, we developed an *in vivo* internalization assay based on intrathecal (i.t.) injection and subsequent internalization of a fluorescent DOR agonist, fluo-deltorphin (fluo-DLT). Using this technique, we compared the distribution and density of fluorescently labeled cells in the lumbar spinal cord and dorsal root ganglia (DRG) of normal rats and rats subjected to chronic inflammatory pain following injection of CFA into the hindpaw. We found that CFA treatment induced a selective increase in the binding and internalization of fluo-DLT in a subset of small- and medium-sized DRG neurons as well as in neurons of the dorsal horn of the lumbar spinal cord. This increase was accompanied by enhanced analgesic effects of i.t. deltorphin (DLT), as compared to controls. However, complementary experiments carried out in MOR-KO mice or in rats injected with capsaisin in the hindpaw demonstrated that whereas the increase in DOR availability in the spinal cord was dependent on the integrity of mu opioid receptors (MOR), and hence was presumably due to the release of MOR-acting endogenous opioids, the increase in the binding and internalization of DOR in DRGs was due to enhanced neuronal activity. These results demonstrate that the methodological approach proposed here allows for both the identification of cellular targets of exogenously administered opioids and the quantification of the state of responsiveness of DOR on those cellular targets in different experimental conditions.

11:40 – 12:05 **S20 J.-K. Zubieta MU-OPIOID RECEPTOR MEDIATED NEUROTRANSMISSION AT THE INTERFACE OF REWARD AND STRESS RESPONSE MECHANISMS** ***J.-K. Zubieta, Depts. of Psychiatry and Radiology and Mental Health Research Institute, University of Michigan, Ann Arbor, MI USA*** Dopaminergic circuits have been clearly implicated in the effects of drugs of abuse, and more recently, in the interface between reward responses and the effects of stressors. In the indirect striatopallidal pathway, the mild stress of novel environments has also been shown to recruit enkephalinergic cell populations. These cells form part of the broader endogenous opioid system, itself a stress-responsive system furthermore implicated in the direct or indirect action of a number of drugs of abuse. Work from our laboratory has been examined the function of the endogenous opioid system in response to both emotional and stress challenges directly in human subjects with external imaging techniques

(positron emission tomography and selective mu-opioid receptor radiotracers). This work has demonstrated the activation of mu-opioid receptor mediated neurotransmission in response to experimental stress models (moderate levels of sustained pain, a physical and emotional stressor) in reward-related regions (e.g., nucleus accumbens, ventral pallidum, amygdala) directly in human subjects. Furthermore, that substantial variability was encountered in the capacity to activate this neurotransmitter system between subjects. Further experiments show that variability to be partially accounted for by the effects of sex and gonadal steroids, as well as by frequent genetic polymorphisms. The latter include genetic polymorphisms implicated in the risk for substance abuse, such as those affecting the function of the enzymes catechol-o-methyl transferase and monoamino oxidase-A, as well as the affinity of the mu-opioid receptor for the endogenous opioid β-endorphin. An additional element examined in our studies is whether the endogenous opioid system also responds to cognitive stimuli of positive characteristics (the expectation of pain relief while undergoing the experimental pain stressor). These studies showed that this neurotransmitter system is indeed activated by positive cognitive expectations. The data so far collected indicates that the endogenous opioid system plays a substantial role in the neurobiological interface between environmental challenges (e.g., stress) and the responses of the organism. Furthermore, that its function is substantially modulated by genetic, sex and positive cognitive influences, of direct relevance for the understanding of the neurobiology of interactions between stress and reward mechanisms. Support: R01 DA 069612 and R01 AT 001415.

12:05 – 12:20 **S21 B.L. Kieffer DELTA OPIOID RECEPTOR IMAGING *IN VIVO* *G. Scherrer, P. Tryoen, D. Filliol, A. Matifas, A. Dierich, J.-L. Vonesch, B.L. Kieffer IGBMC, CNRS/INSERM/ULP, 1 Rue Laurent Fries BP 10142, 67404 Illkirch Cedex France*** Genetically encoded fluorescent proteins are unique reporters for monitoring dynamic cellular processes. Enhanced-GFP (eGFP) has been widely used as a fusion partner to study G protein-coupled receptor cellular trafficking. We have examined functional properties of a C-terminal eGFP-delta opioid receptor fusion in a stable HEK 293 cell line. Receptor binding, signaling, internalization and down-regulation were unaffected by the eGFP fusion. To explore delta receptor functional neuroanatomy *in vivo*, we have generated knock-in mice expressing the fluorescent receptor. To this aim we have introduced eGFP into the mouse delta receptor gene by homologous recombination. The targeting construct was designed to replace the stop codon in exon 3 by the in-frame eGFP cDNA, leading to a C-terminal eGFP-delta receptor fusion *in vivo*. We have obtained animals harbouring the mutant allele. In these mutant mice, the fluorescent delta receptor is expressed. Here we will present our analysis of the knock-in mice using various fluorescent imaging techniques. These animals represent a novel promising tool to tackle delta receptor pharmacology and trafficking *in vivo.*

12:30 – 15:30 **Lunch and Poster Session II** **Historic Inns**

Anatomy, Imaging

T1 CB1 CANNABINOID RECEPTORS ARE LOCATED WITHIN DOPAMINERGIC NEURONS AND THEIR AFFERENT TERMINALS IN THE RAT VENTRAL TEGMENTAL AREA (VTA) ***C.D. Rios, V.M. Pickel Weill Medical College of Cornell University, Department of Neurology and Neuroscience, New York, NY USA*** Marijuana activation of CB1 cannabinoid (CB1) receptors evokes motivational and reward associated behaviors, in part, through modulation of mesocorticolimbic dopaminergic (DA) neurons in the ventral tegmental area (VTA). To determine the relevant functional sites of cannabinoid action, we examined the electron microscopic immunolabeling for CB1 receptors and the catecholamine synthesizing enzyme, tyrosine hydroxylase (TH), which identifies DA neurons. CB1 receptors were distributed in TH labeled dendrites and their afferent terminals or apposed glia. The terminals showed synaptic junctions typical of excitatory or inhibitory inputs. The dendritic CB1 receptors were mainly localized to endomembranes but was also seen on the plasma membrane, the functional site for receptor activation. Our results provide the first ultrastructural evidence that VTA CB1 receptors have strategic positions that enable cannabinoids to affect DA neuronal activity through both direct and indirect (pre-synaptic and glial) mechanisms. Support: DA04600, NS007384-10

T2 AMPA RECEPTOR TRAFFICKING WITHIN THE VENTRAL TEGMENTAL AREA OF RATS RECEIVING CHRONIC INTERMITTENT MORPHINE ADMINISTRATION ***D.A. Lane (1), E.E. Colago (1), Y. Zhou (2), S. Schlussman (2), M.J. Kreek (2), V.M. Pickel (1) (1)Dept. of Neurology and Neuroscience, Weill Medical College of Cornell University, (2)Laboratory of the Biology of Addictive Diseases, Rockefeller University, New York, NY USA*** Opiates and other addictive drugs produce profound changes in AMPA receptor mediated glutamatergic transmission in the ventral tegmental area (VTA), a brain region containing mesocorticolimbic

dopaminergic neurons associated with reward and reinforcement. We used electron microscopic immunolabeling to determine whether chronic intermittent administration of escalating doses of morphine produced changes in the subcellular distribution of the Glu R1 subunit of the AMPA receptor in the VTA. The morphine treatment group showed an overall increase in total dendritic Glu R1 labeling mainly in dopaminergic dendrites as compared to controls. Despite this increase, there was a decrease in the dendritic plasmalemmal Glu R1 distribution in morphine-treated rats. Our results provide ultrastructural evidence that chronic intermittent morphine evokes changes in both the expression and surface trafficking of AMPA receptor subunits in dopaminergic neurons of the VTA. Support: DA05130, DA004600

T3 TARGETING DIFFERENCES OF MU AND DELTA OPIOID RECEPTORS IN CULTURED NEURONS ***T.A. Libby (1,2), M. Riedl (1), F. Williams (1,3), R. Elde (1,2) (1) Dept. Neurosci., (2) Grad. Prog. Neurosci., (3) Veterinary and Biomedical Sciences, Univ. Minnesota, Minneapolis, MN, USA*** Differences in subcellular distribution of mu (MOR1) and delta (DOR1) opioid receptors have been shown by immunolocalization using light and electron microscopy. We investigated targeting differences and possible determinants in neurons cultured from multiple brain regions. Neurons from frontal cortex and nucleus accumbens of neonatal rats, and hippocampus and ventral tegmental area (VTA) of fetal rats, were cultured for 7 days. On day 6, cultures were transfected with fluorescently-tagged MOR1 (YMOR) or DOR1 (RDOR) or tail-swap chimerae. Fixed cultures were immunostained for compartmental markers MAP2 or synaptotagmin/synaptophysin (syt/syp). All four constructs were seen in MAP2-ir compartment in 100% of expressing neurons. In frontal cortical and VTA cultures, RDOR was targeted to syt/syp-ir compartment significantly more frequently than was YMOR. Targeting of the chimera with the DOR1 C-tail matched that of RDOR, and the chimera with the MOR1 C-tail targeted as YMOR did. In hippocampal and accumbens cultures, targeting trended toward that seen in frontal cortex and VTA, but was not statistically significant. We conclude that presynaptic targeting differences between MOR1 and DOR1 are seen in certain brain regions, are in part determined by signal(s) in the C-tail, and may be effectively studied in frontal cortical or VTA cultures.

T4 CONDITIONAL NMDA-NR1 RECEPTOR SUBUNIT GENE DELETION IN THE AMYGDALA ***M. Glass (1), V.M. Pickel (1), C. Inturrisi (2) (1) Depts. Neurol. & Neurosci., and (2) Pharmacol., Weill Med. Coll. Cornell Univ., NY, NY, USA*** The central nucleus of the amygdala (CeA) may be a crucial substrate for neural plasticity associated with opiate abuse. The NMDA receptor plays a significant role in opioid plasticity, however advances in our understanding of NMDA receptor subunit gene expression in specific brain regions has been elusive due to the ubiquity and lethality of essential NMDA-NR1 (NR1) receptor subunit mutations by constitutive knockout methodology. Using Cre-loxP technology, we provide the first evidence for conditional NR1 knockout limited to the CeA of adult mice. Recombinant adeno-associated viral vectors containing a green fluorescent protein reporter (GFP) and Cre recombinase were stereotaxically delivered into the CeA of floxed NR1 mice to produce a robust deletion of NR1 gene expression within a 1-1.5 mm rostro-caudal extent of the target region. Using dual labeling immunohisto-chemistry for GFP and the neural marker NeuN, recombination was shown to occur specifically in neurons. The viability of conditional CeA NR1 gene deletion thus provides a promising tool to further examine the role of amygdala NMDA receptors in neural and behavioral processes associated with opiate addiction. Support: DA016735 (MG), DA001457 (CI), DA000198 (CI)

T5 mPKCI DISTRIBUTION AND DEVELOPMENTAL PROFILE IN MOUSE CENTRAL NERVOUS SYSTEM ***Q. Liu, J.B. Wang, Dept. Pharmaceutical Sci., School of Pharmacy, Univ. Maryland, Baltimore, MD, USA*** Previous evidence of the expression of mPKCI in mouse brain from northern and western blots provided some support for PKCI's potentially roles with MOR and/or other neurotransmitter receptors in CNS. However, the known light microscopic distribution of mPKCI and the cellular sites for mPKCI and MOR interaction have not been established. In the present study, we examine the distribution and developmental profile of PKCI in central nervous system (CNS) of mouse. Western blot analyses indicated that the PKCI protein was widely expressed at CNS. The expression of PKCI is at a relatively higher level in accumbens nucleus, anterior commissure, cortex of frontal pole, ventral pallidum, cerebellum and spinal cord, but moderate level in striatum, cingulate parietal cortex and brain stem. The PKCI protein was detected from embryonic tissues as early as 14 days of postconception. Preliminary study of immunohistochemical staining on brain tissue sections indicated that the PKCI protein was present mainly in neurons. These results will not only provide the supporting evidence of mPKCI interaction with MOR, but will also shed light on other potential functions of mPKCI in CNS beyond the opioid receptors.

T6 SUBCELLULAR DISTRIBUTION OF M2-MUSCARINIC RECEPTORS IN RELATION TO DOPAMINERGIC NEURONS OF THE RAT VENTRAL TEGMENTAL AREA ***M. Garzón (1,2), V.M. Pickel***

(1) (1) Dept. Neurol. & Neurosci., Weill Med Coll, Cornell Univ, New York (2) Dept. Anat., Histol. & Neurosci., Med. Coll. UAM, Madrid Muscarinic receptors activation in the ventral tegmental area (VTA) can potently affect goal directed behaviors and reward through enhanced mesocorticolimbic dopaminergic transmission. To determine the relevant functional sites for muscarinic-2 receptor (M2R)-mediated activation of these dopaminergic neurons, we examined the electron microscopic immunocytochemical dual labeling of M2R and dopamine transporter (DAT) in the rat VTA. The M2R was localized to endo- and plasma-membranes of somatodendric profiles, few of which contained DAT. M2R also was localized to axonal plasma membranes having access to extracellular ligands. The labeled axon terminals formed either inhibitory or excitatory-type synapses, the latter of which resemble those of cholinergic terminals. Their targets included M2R or DAT-labeled dendrites. These results provide ultrastructural evidence that M2R principally affects the activity of VTA dopaminergic neurons through indirect mechanisms, including autoregulation of acetylcholine release and changes in the physiological activity or release of other, largely inhibitory transmitters. The findings are directly relevant to understanding the neural substrates of drug addiction. Support: DA04600 (VMP) MECD PR2002-0413 (MG)

T7 DELTA OPIOID RECEPTOR ACTIVATION SITES IN GABA-CONTAINING AXONS REGULATING SLEEP IN THE CAT VENTRAL ORAL PONTINE TEGMENTUM ***M.X. Alvira-Botero, M. Garzón Dept. Anat., Histol. & Neurosci., Med. Coll. UAM, Madrid, Spain*** GABAergic stimulation of the ventral oral pontine reticular nucleus (vRPO) potently suppresses REM sleep; in contrast, morphine-vRPO microinjections increase REM sleep. To determine the functional sites for activation of delta opioid receptors (DOR) in relation to REM-suppressing GABA releasing sites in the cat vRPO, we used electron microscopic immunocytochemical dual labeling for DOR and GABA. Over 33% of GABA-containing axon terminals showed DOR-immunogold particles localized in the cytoplasm and on the plasma membrane. DOR-immunolabeled GABAergic terminals formed symmetric synapses with DOR-labeled (n=285) or unlabeled (n=119) dendrites. These data provide ultrastructural evidence that DOR activation in vRPO can modulate the release of GABA, whose inhibitory postsynaptic actions are also subject to DOR modulation. Moreover, DOR also was detected in some GABA dendrites receiving multiple contacts, suggesting that DOR in vRPO may also regulate postsynaptic responses of GABA neurons to other transmitters. These results are relevant to understanding the brainstem opioid-mediated control of sleep and behavioral state. Support: CAM (GR/SAL/0188/ 2004), MECD (BFI2003-00809)

T8 DORSAL HORN NEUROPLASTICITY AFTER SPARED NERVE INJURY ***B.K. Taylor, A.B. Intondi, Y. Carl, X. Zhang, J.E. Zadina, Tulane Univ. HSC & VA, New Orleans, LA USA*** We studied changes in spinal Substance P (SP), calcitonin gene related peptide (CGRP), endomorphin-2 (EM2), thiamine monophosphatase (TMP), neuropeptide Y (NPY) and Fos in relation to mechanical and cold hypersensitivity after spared nerve injury (spared sural, transected tibial & peroneal nerves). Two wk after SNI, EM2, SP and CGRP immunoreactivity decreased in the medial but not lateral region of L4 dorsal horn by 86±6%, 85±1% and 71±8%. Six mo after SNI, when mechanical hyperalgesia and cold allodynia had subsided, peptide staining largely reappeared, with ipsilateral vs. contralateral differences < 11%. NPY staining increased in the medial but not lateral region (133±20%), and had not recovered after 6 mo. SNI increased stimulation-induced Fos expression in I-II and deeper laminae of the ipsilateral DH. We conclude that SNI upregulates NPY and Fos expression and downregulates CGRP, SP and EM2 in the medial DH. The results support the concept that temporal changes in EM2- SP- and CGRP in specific dorsal horn subdivisions correlate with mechanical hyperalgesia and cold allodynia, but not mechanical allodynia. Support: DA10356, NS43383 (BKT), VA, ONR and LA HEF (JEZ)

Behavioral pharmacology

T9 EFFECT OF TRK-820, A KAPPA OPIOID RECEPTOR AGONIST, ON BEHAVIORAL RESPONSES TO METHAMPHETAMINE, COCAINE AND NICOTINE IN RATS ***K. Hasebe (1), K. Kawai (1), M. Takagi, T. Suzuki (1), K. Kawamura (1), T. Tanaka (1), M. Narita (2), H. Nagase, K. Okano (1), T. Suzuki (2) (1) Pharmaceutical Research Lab., Toray Industries Inc., Kanagawa, Japan, (2) Dept. Toxicology, Hoshi Univ., Sch. Pharm., Pharm. Sci., Tokyo, Japan, (3) Department of Medical Chemistry, Sch. Pharma. Sci. Kitasato Univ., Tokyo, Japan*** We synthesized the kappa opioid receptor agonist, TRK-820, that showed high selectivity and strong agonistic activity. It has been reported that kappa opioid receptor agonists decrease the rewarding effect, discriminative stimulus and hyper locomotion induced by abused drugs. In order to clarify the effect of TRK-820 on the behavioral responses induced by abused drugs, we examined the therapeutic and preventive effects of TRK-820 on the rewarding of methamphetamine and cocaine and the nicotine-withdrawal aversion. TRK-820 (17 μg/kg, s.c.) or vehicle was given

twice daily for 6 days to the rats that established the rewarding effect of methamphetamine. The treatment with TRK-820 showed a significant suppression of the maintenance of rewarding effect induced by methamphetamine as compared to vehicle treatment. The systemic (10 μg/kg, s.c.) or intra-accumbal (10 ng/site) treatment with TRK-820 showed a significant suppression of the formation of rewarding effect induced by methamphetamine as compared to vehicle treatment. By *in vivo* microdialysis methods, dopamine-releasing response caused by systemic methamphetamine application was inhibited by the pretreatment with TRK-820 (10 μg/kg, s.c.), which was antagonized by norbinaltorphimine (nor-BNI), an opioid kappa receptor antagonist. In DDS test, the discriminative effect of cocaine was also attenuated by the pretreatment with TRK-820 (20 μg/kg, s.c.) and the inhibitory effect of TRK-820 was reversed by the pretreatment with nor-BNI. It is of interest to note that the pretreatment with TRK-820 (30 μg/kg, s.c.) significantly decreases the nicotinic receptor antagonist mecamylamine-precipitated nicotine-withdrawal aversion in rats chronically treated with it. It is therefore likely that TRK-820 is useful for treating the dependence on psychostimulants and the nicotine withdrawal,

T10 THE ROLE OF BRAIN CORTICOTROPIN-RELEASING FACTOR RECEPTOR TYPE I IN STRESS AND OPIATE-INDUCED REINSTATEMENT OF CONDITIONED PLACE PREFERENCE IN RATS ***Q. Fang (1), J. Wang (2), L. Lu (3,4) (1) Dept. Pharmacology, Affiliated Hospital of Guiyang Medical College, Guiyang, China, (2) Dept. Pharmacology, New York Medical College, Valharla, NY, USA, (3) National Lab. Medical Neurobiology, Fudan Univ., Shanghai, China, (4) Behav. Neurosci. Branch, NIDA- IRP/NIH, Baltimore MD, USA*** Drug addiction is characterized of high rate for relapse to drug taking. Based on evidence that brain corticotropin-releasing factor (CRF) systems mediate the behavioral and physiological effects of abused drugs, it has been demonstrated that CRF receptor subtype 1, but not subtype 2, plays a key role in the reinstatement of stress or drug- induced relapse to drug seeking. However, the brain area that is involved in the mediation of CRF1 receptor in drug relapse is unknown. Using the reinstatement model of the conditioned place preference (CPP) paradigm, we examined the role of CRF1 receptors in relapse to drug seeking in different brain areas, including the bed nucleus of the stria terminalis (BNST), the amygdala and the nuclear accumbens. Rats were alternately given morphine (10 mg/kg, s.c.) and saline for 8 days to acquire the CPP. The morphine CPP disappeared following a 2-week extinction with saline-paired training. Then rats were tested for reinstatement of morphine CPP induced by a single injection of morphine (3 mg/kg, s.c.) or by 15 min of intermittent footshock; both footshock and morphine priming reinstated the CPP. To investigate the role of CRF1 receptors in different brain areas on reinstatement, CP-154,526, a CRF1 antagonist, was infused into the BNST, the amygdala and nucleus accumbens before testing for reinstatement. Infusion of CP-154,526 into the BNST significantly attenuated stress-induced reinstatement of morphine CPP, while infusions of CP-154,526 into the amygdala or nucleus accumbens had no effect. However, infusion of CP-154,526 into the amygdala or nucleus accumbens significantly blocked or attenuated drug-induced reinstatement of morphine CPP, while infusion into BNST had no effect. The present study demonstrates that the CRF1 receptors in different brain areas play different roles in stress and drug-induced relapse to drug seeking behavior. These findings also suggest that CRF1 antagonist may be of use in the treatment of drug relapse

T11 THE DOPAMINE D3 RECEPTOR AND REACTIVITY TO OPIATE-ASSOCIATED STIMULI: RESOLVING THE PARADOX ***B. Le Foll , H. Francès, J. Diaz, P. Sokoloff INSERM U. 573, Centre Paul Broca, Paris, France*** The role of the dopamine D3 receptor (D3R) in reactivity to opiates-associated stimuli is unclear. D3R antagonists block reactivity to opiate-associated stimuli, whereas D3R-deficient mice are hyper-reactive to presentation of these stimuli. To elucidate this paradox, we have evaluated the dopaminergic system and the reactivity to opiates-associated stimuli of D3R+/+ and D3R-/- mice. Reactivity to opiate-associated stimuli was influenced by the dose of morphine used. BP 897 (a D3R-selective partial agonist) inhibited the expression of morphine place preference, an effect that was abolished in D3R-/- mice. D3R-/- mice displayed decreased tyrosine hydroxylase, increased dopamine transporter mRNAs and increased dopamine reuptake in striatum. Since elevated basal extracellular dopamine levels have been previously found in D3R-deficient mice, these changes may reflect adaptive changes that tend to attenuate the hyperdopaminergia of D3R-/- mice. These results suggest that blocking the D3R blocks morphine conditioning. We propose that the paradoxical hyperactivity of D3R-/- mice to presentation of drug-associated stimuli is related to their hyper-dopaminergia phenotype.

T12 MEDIATION OF COCAINE- AND STRESS-INDUCED POTENTIATION BY DESENSITIZED KAPPA OPIOID RECEPTORS ***H.C. Brenhouse, C. Brown, K. Siniakowicz, J.P. McLaughlin Northeastern Univ., Boston, MA*** Potentiation of cocaine-induced locomotion and conditioned place preference (CPP) by social defeat stress (SDS)

may be mediated by the kappa-opioid receptor (KOR). We demonstrate here that repeated SDS increased characteristic immobility postures and doubled warm-water tail-withdrawal latency (TWL) after a 20-min exposure. The KOR-selective antagonist, nor-BNI attenuated both behaviors. SDS also potentiated both cocaine-induced locomotion and CPP in a nor-BNI sensitive manner. Pretreatment with U50,488 activated, then desensitized, KOR as demonstrated by abolition of SDS-induced increases in TWL. Notably, mice with desensitized KOR during exposure to SDS still display increased cocaine-induced locomotion compared to unstressed animals, indicating the involvement of desensitized KOR signaling. MAP kinase inhibitors were used to examine the signal transduction mechanism producing SDS-induced potentiation of cocaine locomotion and reward. Overall, the results demonstrate common mechanisms between SDS and chronic cocaine through the mediating effects of dynorphin and KOR, possibly through an untraditional signal transduction cascade. Support: NIDA DA16415 (JPM)

T13 KAPPA-OPIOID RECEPTOR INACTIVATION PRODUCES TIME-RELATED ALTERATIONS IN DOPAMINE DYNAMICS AND COCAINE RESPONSIVENESS ***V.I. Chefer1, J. Pintar2, T. Shippenberg1 1 Integrat Nsci Sect, DHHS/NIH/NIDA/IRP, Baltimore, MD, USA, (2) Dept. Neuroscience and Cell Biology, CABM, UMDNJ-RW Johnson Medical School, Piscataway, NJ, USA*** Basal dopamine (DA) release and uptake are enhanced in KOPr-1 knockout mice. These mice exhibit an augmented locomotor response to acute cocaine equal in magnitude to wildtypes (WT) administered a behavioral sensitizing cocaine treatment. To determine whether this phenotype is due to KOPr loss or is a developmental compensation, we examined the effects of the long-acting KOPr antagonist nor-binaltorphimine (norBNI) on basal and cocaine-evoked DA dynamics in the n. accumbens of WT. NorBNI administration increased basal DA dynamics. However, the effects varied with the duration of KOR-1 blockade. Basal DA release was increased whereas DA uptake was unaltered after 1 hr blockade. After 24 hrs, both release and uptake were increased. The behavioral and DA response to cocaine were enhanced at both time points. These data highlight the plasticity of mesoaccumbal DA neurons and suggest that loss of KOPr and resultant disinhibition of DA neurons trigger short-and long-term adaptations that maintain normal DA levels despite enhanced release.

T14 ADOLESCENT DRUG-INDUCED AGGRESSION: MODULATION BY SEROTONIN TYPE 1A RECEPTORS? ***K. Rasakham, L. Ricci, R. Melloni Dept. of Psych., Northeastern Univ., Boston, MA USA*** Repeated exposure to drugs of abuse, e.g., cocaine and anabolic steroids, during adolescence stimulates offensive aggression in male Syrian hamsters. Pretreatment with a selective 5HT1A agonist dose-dependently abolished cocaine-induced aggression (ED50=0.1 mg/kg). Thus, we hypothesized that drug-induced aggression may be due to the loss of 5HT1A receptors. To test this hypothesis in an anabolic steroid model we examined receptor expression after adolescent exposure to anabolic steroids using immunohistochemistry and western blot analysis. Anabolic steroids decreased 5HT1A expression by 30% in the anterior hypothalamus and 62% in the lateral hypothalamus, brain regions critical for aggression control. However, anabolic steroid treatment did not alter the number of 5HT1A-immunopositive neurons. These data demonstrate that anabolic steroids alter the production/synthesis of 5HT1A in a brain-region specific manner. These data suggest that disruption in 5HT1A expression may be an underlying factor in drug-induced aggression. Support: RO1 DA10547 (RHM).

T15 SITES AND EFFECTS OF DEXTROMETHORPHAN ON TREATMENT OF MORPHINE ADDICTION IN RATS ***P.L. Tao, Y.L. Shen, E.Y.-K. Huang, Dept. and Institute of Pharmacology, National Defense Medical Center, Taipei, Taiwan, R.O.C***. Our previous studies have shown that dextromethorphan (DM; an antitussive drug with NMDA antagonist property) administered via i.p. is effective to treat morphine addiction in rats. However the site(s) and mechanism(s) involved are still unclear. In this study, we further investigated the anatomical brain nuclei implicated in these effects by bilateral microinjection of DM into VTA or NAc of the rats which have been treated with morphine chronically. Conditioned place preference (CPP) test was used to examine the rewarding as well as craving effect, and locomotor activity was measured to reveal the behavioral sensitization induced by chronic morphine. We found that morphine-induced craving was attenuated by local injection of DM (0.1 nmol) into either VTA or NAc. However morphine-induced behavioral sensitization was only attenuated by local injection of DM into VTA but not NAc. The dopamine turnover rate in NAc or dorsal striatum was increased by chronic morphine and reversed by DM treatment at NAc. These results imply that VTA and NAc are the brain nuclei involved in the action of DM to treat morphine addiction.

T16 ROLE OF DELTA-OPIOID RECEPTOR SUBTYPES IN ANXIETY-RELATED BEHAVIORS ON THE ELEVATED PLUS MAZE IN RATS ***Saitoh, N. Hirose, J. Kamei Dept. Pathophysiol. Ther., Sch. Pharm. Pharm.***

Sci., Hoshi Univ., Tokyo, Japan The present study was designed to examine the possible involvement of delta-opioid receptor subtypes in the anxiety-related behavior in the elevated plus-maze test. Male Lewis 6-week-old rats were used. The total numbers of visits to the closed and open arms, and the cumulative time spent and visits in the open arms were determined. Naltrindole, a delta-opioid receptor antagonist, induced a significant decrease in the percentages of time spent and visits in the open arms. Naltriben, a delta2-opioid receptor antagonist, but not 7-benzylidenenaltrexone, a delta1-opioid receptor antagonist, also exhibited the similar anxiety-related behaviors on the elevated plus maze. Furthermore, after exposure to the elevated plus-maze, the maximal increase of the plasma corticosterone levels in naltrindole-treated rats was clearly higher than that in vehicle-treated rat. Based on these results, we suggest that endogenous delta2-opioid receptor-mediated systems are involved in the regulation of anxiety and might play a physiologically important role in the regulation of adrenocortical activity.

T17 EFFECTS OF SNC80 ON THE EXPLORATORY BEHAVIOR OF OLFACTORY-BULBECTOMIZED MICE IN THE HOLE-BOARD TEST ***N. Hirose, A. Saitoh, J. Kamei Dept. Pathophysiol. Ther., Sch. Pharm. Pharm. Sci., Hoshi Univ., Tokyo, Japan*** Effects of SNC80 on the exploratory behavior on olfactory bulbectomized (OB) mice in the hole-board test. The olfactory bulbs were removed by suction. Postoperatively, animals were housed single caged for 14 days. Changes in the emotional state of mice were evaluated in terms of changes in exploratory activity, i.e. locomotor activity and head-dipping. The number of head-dipping in sham operated mice was significantly less than that in naive mice. However, the number of head-dipping in OB mice was significantly greater than that in naive mice. When OB mice were pretreated with diazepam at a dose that did not produce sedation, the number of head-dipping in OB mice was decreased to the level that observed in naive mice. Thus, we suggested that the increased head-dipping behavior in OB mice may reflect the anxiogenic and/or hyperemotional state. Furthermore, SNC80, a delta-opioid receptor agonist also significantly decreased the number of head-dipping in OB mice. Based on these results, we suggest that the activation of delta-opioid receptors may normalize the olfactory bulbectomy-induced emotional abnormality in mice.

T18 OPPOSITE EFFECTS OF ACETYLCHOLINE ENHANCEMENT IN VTA AND NAC ON DRUG SEEKING ELICITED BY CUES AFTER ABSTINENCE IN A MODEL OF RELAPSE TO HEROIN IN RATS ***W. Zhou, F. Zhang, S. Tang, M. Lai, H. Zhu, H. Liu Ningbo Addiction Research and Treatment Center, Ningbo, China*** The involvement of cholinergic neurons underlying reinforcement and reinstatement has not been systemically demonstrated. We utilized a heroin self-administration (SA) and relapse model to study the role of Ach in the heroin-seeking after withdrawal in rats. Nose-poke responding by male SD rats was reinforced with heroin (0.05 mg/kg per infusion, 4 h session daily) under PR schedule. Acute treatment of neostigmine (Neo), an inhibitor of AchE at large dose altered the primary reinforcement effects of heroin in SA tests. Neo was directly infused i.p. prior to heroin SA for 10 days, the acquisition of heroin SA was not different, but the drug seeking responding decreased after withdrawal. Micro-injection of Neo into either the VTA or the NAc did not disturb the heroin SA behavior. Infusion of Neo just prior to the testing produced a dose-dependent disruption of heroin-seeking behavior elicited by drug paired cues during reinstatement tests. Micro-infusion of Neo into the VTA produced the high responding elicited by cues. In contrast, micro-infusion of Neo into the NAc inhibited the conditioned-cued reinstatement of heroin-seeking behavior. These results indicate a crucial role for cholinergic innervation in the VTA-NAcc pathway during the expression of stimulus-reward associations that mediate cue-induced heroin-seeking behavior. Support: NBRPC 2003CB515404

T19 PRESENCE AND FUNCTIONAL EXPRESSION OF CB2 CANNABINOID RECEPTORS IN BRAIN THAT IS INVOLVED IN DEPRESSION AND SUBSTANCE ABUSE ***E.S. Onaivi (1,2), H. Ishiguro (4), J.-P. Gong (2), S. Patel (1), P. Meozzi (1), L. Myers (1), Z. Mora (1), A. Perchuk (1), P. Tagliaferro (5), C. Leonard (3), E. Gardner (3), A. Brusco (5), B. Akinshola (6), Q.-R. Liu (2), B. Hope (3), G.R. Uhl (2) (1) Dept. Biology, William Paterson University, Wayne, NJ, (2) Molec. Neurobio. Branch, (3) Behav. Neurosci. Branch, NIDA-IRP, NIH/DHHS, Baltimore, MD, (4) Institute Basic Med. Sci., Univ. Tsukuba, Japan, (5) Univ. Buenos Aires, Argentina, (6) Howard Univ., Washington DC USA*** There are two well-characterized cannabinoid receptors (CBrs), CB1 and CB2 that mediate the effects of cannabinoids and marijuana. In mice the effects of direct CB2 antisense olignucleotide injection into the brain and i.p treatment with JWH015 in motor function and plus-maze tests were evaluated. We used RT-PCR, immunoblotting, immunohistochemistry, and hippocampal cultures to determine the expression of CB2 CBrs in rat brain and in mice brain exposed to chronic mild stress (CMS) or those treated with cocaine or heroin. JWH015 reduced mouse locomotor activities while direct CB2 antisense oligonucleotide microinjection induced anxiolysis. In the CMS animals the expression of CB2 CBrs was enhanced and was modified

in the brains of cocaine and heroin treated rats. Abundant iCB2 in neuronal and glial processes were detected in brain. Contrary to the prevailing view that CB2 CBrs is restricted to peripheral tissues and predominantly in immune cells, we demonstrate that CB2 CBrs and their gene transcripts are widely distributed in the brain. The presence and functional expression of CB2 CBrs in the brain may be exploited as new target in the treatment of depression and substance abuse.

T20 AN ENDOCANNABINOID HYPOTHESIS OF DRUG REWARD ***E.S. Onaivi Dept. Biology, William Paterson University, Wayne, NJ, USA*** Pharmacological treatment of drug and alcohol dependency has largely been disappointing and new therapeutic targets and hypotheses are needed. Thus, an endocannabinoid hypothesis of drug reward is postulated. C57Bl/6 mice were evaluated in the plus-maze following abrupt cessation from chronic treatment with cocaine, diazepam, ethanol, methanandamide. In a separate group the ability of rimonabant, to block withdrawal aversions from alcohol and abused drugs was determined. The interaction between vanilloid and cannabinoid system was performed using selected agonists and antagonists. CB1 receptor antagonism reduced behavioral aversions following withdrawal from alcohol, cocaine, and diazepam. Treatment with capsaicin or WIN55212-2 induced aversions to the open arms plus-maze. The aversons induced with capsaicin, was dependent on gender and strain, and enhanced by pretreatment with WIN55212-2. Capsazepine reduced aversions, while rimonabant, produced dose dependent variable effects. Both capsazepine and rimonabant blocked the aversions induced by WIN55212-2 and capsaicin, indicating a cross-talk between cannabinoid and vanilloid systems. Cannabinoids appear to be involved in adding to the rewarding effects of addictive substances including, nicotine, opiates, alcohol, cocaine and BDZs. These results suggest that the EPCS may be important natural regulatory mechanism for reward.

T21 IMPAIRMENT OF THE DELTA-OPIOID RECEPTOR FUNCTION INDUCES ASTROGLIOGENESIS-DEPENDENT EMOTIONAL DYSFUNCTION ***N. Kuzumaki, M. Narita, M. Narita, T. Suzuki Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan*** Here we found that chronic *in vivo* treatment with a selective delta-opioid receptor antagonist naltrindole (NTI) produced a significant decrease in time spent on the lit compartment using the light-dark test and in the numbers of entries into the open arms of the elevated plus-maze in mice, providing further evidence for an implication of delta-opioid receptors in anxiety. Furthermore, we show a dramatic increase of reactive astrocytes in the cingulate cortex by repeated *in vivo* treatment with NTI, whereas *in vitro* treatment with NTI enhanced astrocyte differentiation from neural stem cell obtained from the forebrain of mice. It is of interest to note that microinjection of either astrocyte-conditioned medium, astrocyte or activated-astrocyte obtained from the cultured astrocyte of the newborn mouse cortex into the cingulate cortex of adult mice produced a significant expression of the anxiety-like behaviors. These findings raise the possibility that the long-lasting blockade of delta-opioid receptors promotes astrocyte differentiation in the mouse cingulate cortex. This phenomenon may in turn lead to aggravated anxiety.

T22 SUPPRESSION OF PSYCHOLOGICAL DEPENDENCE ON OXYCODONE UNDER CHRONIC PAIN-LIKE STATE IN MICE ***T. Suzuki, M. Ozaki, A. Nakamura, M. Narita Dept. of Toxicol., Hoshi Univ. Shc. of Pharm. and Pharmaceut. Sci., Tokyo, Japan*** Recent clinical studies have demonstrated that when opioids are used to control pain in cancer patients, psychological dependence is not a major concern. In the previous study, we demonstrated that the morphine-induced rewarding effect was markedly suppressed under chronic pain-like state in rodents. The present study was undertaken to ascertain the modulation of oxycodone-induced rewarding effect under chronic pain-like state in mice. In normal state, oxycodone (1.3 mg/kg, s.c.) produced a profound antinociception and rewarding effect in a dose-dependent manner. Under inflammatory and neuropathic pain-like states, oxycodone-induced rewarding effect was dramatically suppressed, whereas either s.c (1.3 mg/kg).- or i.c.v. (17 nmol/mouse)-administered oxycodone-induced antinociception was not changed. These findings provide further evidence of the clinical usefulness for oxycodone in patients suffering from severe pain.

T23 IMPLICATION OF MOR1B IN PHYSICAL DEPENDENCE ON ETHANOL ***K. Hoshino, M. Narita, K. Miyoshi, M. Souma, T. Suzuki. Dept. of Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan*** Several kinds of evidences suggest that the opioid antagonist naltrexone may be a safe and effective adjunct to treatment in alcohol-dependent subjects, particularly in preventing alcohol relapse. We previously reported that CXBK recombinant-inbred mice display a significant reduction in the expression of MOR1B mRNA in the brain as compared to that in their progenitor C57BL/6 mice. In the present study, we investigated whether MOR1B could be implicated in the development of physical dependence on ethanol using CXBK mice. To evaluate the role of MOR1B in modulating

acute effect of ethanol, the ethanol induced-sleeping time was measured. The ethanol-induced sleeping time in CXBK mice was longer than that in C57BL/6 mice. To develop physical dependence on ethanol in mice, CXBK and C57BL/6 mice were treated with liquid diet containing 5.0 % ethanol for 5 days. There was no significant difference in ethanol intake during the treatment between CXBK and C57BL/6 mice, whereas CXBK mice revealed less alcohol withdrawal symptoms than that found in C57BL/6 mice. These results suggest that MOR1B may be involved in the expression of alcohol withdrawal symptoms.

T25 MECHANISMS OF NOCICEPTIN(14-17)-INDUCED NOCICEPTIVE BEHAVIORS IN THE MOUSE SPINAL CORD ***H. Watanabe (1), H. Mizoguchi (1), A. Yonezawa (1), C. Watanabe (1), T. Sakurada (2), S. Sakurada (1) (1) Dept. Physiol. and Anat., Tohoku Pharmaceut. Univ., Sendai, Japan, (2) Dept. of Biochem., Daiichi Coll. of Pharmaceut. Sci., Fukuoka, Japan*** Nociceptin has been isolated from mammalian brain as a ligand for the opioid receptor like-1 (ORL-1) receptor, and mediates both nociceptive and antinociceptive effects. Recent studies reported that nociceptin metabolites showed some bioactivities in the central nervous system. In the present study, we found that intrathecal treatment with nociceptin (14-17), an C-terminal tetrapeptide of nociceptin, elicited nociceptin-like nociceptive behaviors consisting of scratching, biting, and licking, which were eliminated by ORL-1 receptor antagonists. These nociceptive behaviors were suppressed by intrathecal co-administration of tachykinin NK1 receptor antagonists, but not by NK2 receptor antagonists. Furthermore, intrathecal co-administration of histamine H1 receptor antagonists attenuated nociceptin (14-17)-induced nociceptive behaviors. These findings indicate that H1 receptors and NK1 receptors in addition to ORL-1 receptors are involved in nociceptin (14-17)-induced nociceptive behaviors in the mouse spinal site.

T26 ENVIRONMENTAL CUES ASSOCIATED WITH THE DIFFERENT REINFORCEMENTS OF MORPHINE INDUCE THE ACTIVATION OF VENTRAL SUBICULUM THROUGH THE SPECIFIC NEUROTRANSMITTERS IN ***RATS L. Kang, Z. Dai, L. Qu, L. Ma Pharmacology Research Center, Shanghai Medical College, Fuda Univ., Shanghai, People's Republic of China*** Drug-associated environmental cues are important reasons to induce craving, support compulsive drug seeking, and induce relapse. Accumulating evidences suggests that ventral subiculum, the main output way of hippocampus, may play an important role in the mechanism for the drug seeking behavior induced by drug-associated environmental cues. In this study, we used the microdialysis and HPLC method in the conditioned place preference and aversion rat models, which are the relevant paradigms for study the mechanism for the environmental cues and reinforcements of drugs. Our results demonstrated that environmental cues associated with the positive and negative reinforcements of morphine could induce the decrease in the extracellular levels of GABA (11%) and the increase in the ones of glutamate (230%) in the ventral subiculum of rats respectively. These data suggest that the environmental cues associated with the different reinforcements of opiates all could induce the activation of the ventral subiculum through specific neurotransmitters. This is a possible neurobiological mechanism for drug seeking behavior induced by drug-associated environmental cues.

T27 DEXTROMETHORPHAN POTENTIATE THE INHIBITORY EFFECTS OF ANTI-NT4 ON MORPHINE TOLERANCE ***H. Hatami (1), S. Oryan (1) , A. Ahmadiani (2), S. Semnanian (3) , B. Kazemi (2) (1) Dept. of Biol., Teacher Trainning Univ., (2) Neurosci. Res. Ctr., Shaheed Beheshti Univ. (3) Dept. Physiol., Tarbiat Modarres Univ., Tehran, Iran*** Opioids are the most efficacious and commonly used analgesics in the management of pain, however their chronic administration results in the development of tolerance, limiting their clinical usefulness in pain management .It has been proposed that opioid tolerance is a model of neuronal plasticity similar to learning and memory . Recent evidence suggests that neurotrophins may be involved in synaptic development and plasticity. Observations suggest that NT-4 is required for the synaptic plasticity mediating both tolerance and memory. Also evidence suggests that NMDA receptors are involved in the neural plasticity underlying the development of opiate tolerance. NMDA receptor antagonists can attenuate the development of morphine tolerance. So we used dextromethorphan and Anti-NT4 concomitantly to investigate their effects on morphine tolerance.All experiment were performed using adult male wistar rats. Tolerance induced by injecting morphine once per day for 4 days. Animals were treated with Anti-NT4 10 minutes before morphine. Dextromethorphan were administerated 30 minutes before Anti-NT4 to investigate its role on morphine tolerance. Administration of Anti-NT4 abolished morphine tolerance after induction. Also dextromethorphan do potentiated the inhibitory effects of Anti-NT4 on morphine tolerance.

T28 INVOLVEMENT OF ASTROCYTES IN THE DEVELOPMENT OF THE REWARDING EFFECTS INDUCED BY MORPHINE IN MICE ***M. Asato, M. Narita, M. Narita, M. Miyatake, M. Shibasaki, A. Nakamura,***

T. Suzuki Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan Long-term exposure to morphine (MRP) induces neuronal plasticity. Recently, accumulating evidence suggests that astrocytes may actively participate in synaptic plasticity. Treatment with MRP into the cortical neuron/glia cocultures for 3 days caused the activation of astrocytes as detected by a stellation of morphology and an increase in levels of glial fibrillary acid protein (GFAP), and this event was blocked by cotreatment with a glial modulator propentofillyine. Furthermore, the immunoreactivity for GFAP in the cingulate cortex of mice was clearly increased by repeated treatment with MRP, which was associated with the development of MRP-induced rewarding effect. In the behavioral study, pretreatment with propentofillyine attenuated the MRP-induced rewarding effect in mice. These findings suggest that astrocytes could, at least in part, regulate the development of MRP-induced rewarding effects.

T29 ALTERATIONS IN PROTEIN KINASE A ACTIVITY IN MOUSE BRAIN AND SPINAL CORD IS DEPENDENT ON THE LEVEL AND THE DURATION OF MORPHINE ANTINOCICEPTIVE TOLERANCE ***G.D. Dalton, F.L. Smith, W.L. Dewey Dept. Pharmacology and Toxicology, Virginia Commonwealth Univ. School of Medicine, Richmond, VA USA*** Chronic treatment with morphine produces problematic side-effects including tolerance and physical dependence. We tested the hypothesis that Protein Kinase A (PKA) plays a role in the expression of morphine antinociceptive tolerance (MAT). Male Swiss Webster mice were treated chronically with morphine and the warm-water tail-flick test was used to assess antinociception. Two models of morphine antinociceptive tolerance were used in these studies: (1) a 3-day model in which mice expressed a 10-fold or a 60-fold level of MAT and (2) a 15-day model in which mice expressed a 10-fold or a 21-fold level of MAT. Cytosolic PKA activity was increased in lumbar spinal cord (LSC) of 15-day morphine-tolerant mice that expressed a 21-fold level of MAT, while cytosolic PKA activity was increased in thalamus of 3-day morphine-tolerant mice that expressed a 60-fold level of MAT. The intracerebroventricular injection (i.c.v.) of two peptide fragments of native Protein Kinase A inhibitor (PKI) peptide, PKI-(6-22)-amide and PKI-(Myr-14-22)-amide, significantly reversed MAT in all mice. In conclusion, these studies provide evidence that PKA plays a role in morphine tolerance. Future studies will investigate the involvement of the thalamus and LSC in the expression of morphine antinociceptive tolerance.

T30 MORPHINE-INDUCED BEHAVIORAL SENSITIZATION OR CONDITIONED PLACE PREFERENCE IS UNALTERED IN PREPROENKEPHALIN KNOCKOUT MICE ***P. Marquez, N. Gajawada, R. Baliram, K. Lutfy Dept. of Pharm. Sci., Western Univ. of Health Sci., Pomona, CA USA*** Preproenkephalin knockout mice fail to display tolerance to the antinociceptive effect of morphine, raising the possibility that enkephalin is important in morphine tolerance. However, little is known about the role of enkephalin in the rewarding and addictive properties of morphine. Thus, using preproenkephalin knockout mice, the present study was designed to determine the role of enkephalin in morphine-induced behavioral sensitization and conditioned place preference (CPP). Our results show that both wild type and knockout mice displayed similar locomotor sensitization after repeated morphine administration. Likewise, morphine-induced CPP was not altered in these mice. However, as shown previously, morphine tolerance was altered in preproenkephalin knockout mice. Taken together, our results suggest that enkephalin may be important for the development of tolerance but not CPP or behavioral sensitization induced by morphine. Support: DA 16682 (KL), MIDARP grant DA 017298 (TCF/KL)

Regulation by Opiate Systems

T31 DORSAL HORN KEPI (Kinase Enhanced PP1 Inhibitor) EXPRESSION: REGULATION BY MORPHINE and CFA TREATMENTS ***J.-P. Gong, Q.-R. Liu, G.R. Uhl Molec. Neurobio. Branch, NIDA-IRP, NIH/DHSS, Baltimore, MD USA*** KEPI is a recently-characterized PKC-activated protein phosphatase 1 (PP1) inhibitor that was cloned based on its regulation by morphine in rat striatum. The multifocal CNS distribution of immunoreactive KEPI (iKEPI) includes dense fiber and terminal immunostaining in spinal cord dorsal horn laminae I-II, observed using recently-developed polyclonal antibodies raised to KEPI peptide sequences conjugated to hemocyanin that satisfy several criteria for specificity of immunoreactivity. Primary spinal cord cultures display iKEPI in neuronal processes, cell membranes, and cytoplasmic regions. . Dorsal root ganglion perikarya and afferent/efferent fibers also display dense KEPI immunoreactivity (iKEPI). Dorsal horn iKEPI is enhanced in animals sacrificed following acute systemic injections of morphine. By contrast, iKEPI is downregulated in dorsal horns ipsilateral to unilateral hindpaw intraplantar injections of complete Freund's adjuvant (CFA). KEPI may thus be involved in the spinal cord adaptations that follow opiate administration or noxious inflammatory stimuli, dorsal horn PKC-dependent dephosphorylation by PP1 may thus change with morphine and with inflammation, and these biochemical events are good candidates to play roles in the dorsal horn adaptations that follow such treatments. Support: NIDA-IRP

T32 RODENT BDNF GENES, NOVEL PROMOTERS, NOVEL SPLICE VARIANTS AND REGULATION BY HEROIN AND COCAINE ***Q.-R. Liu (1), L. Lu (2), X.-G. Zhu (1), Y. Shaham (2), G.R. Uhl (1) (1) Mole. Neurobio. Branch, (2) Behav. Neurosci. Brach, NIDA-IRP, NIH/DHSS, Baltimore, MD USA*** Molecular and genetic studies in humans and rodents have identified brain-derived neurotrophic factor (BDNF) and its regulation as candidates for involvement in addictions. Understanding BDNF's genomic structure and regulation is thus of interest. Recently, we have reported that 1) the human BDNF gene contains seven 5' exons that can each be spliced independently to the major BDNF coding exon to form diverse two-part BDNF transcripts, and 2) a human alternatively spliced natural antisense transcript termed "BDNFOS" is transcribed from the strand opposite that of BDNF so that its fifth exon is complementary to BDNF's protein coding exon VIII. To better understand BDNF genomic structure and differential regulation, we now describe rodent BDNF genes and transcripts. Transcripts initiated from six promoter sites are spliced so that six distinct initial exons are each spliced to a major coding exon. An additional three-part transcript is composed of two 5' exons spliced to the major coding exon. Interestingly, we failed to find evidence for any antisense, opposite-strand BDNFOS transcript in either mouse or rat. These rodent splice variants display specific patterns of differential expression in different brain regions and peripheral tissues. Acute heroin and acute cocaine administration increase striatal expression of a specific BDNF4 splice variant by 2.5- and 5 fold, respectively. In contrast, chronic treatments with either cocaine or heroin fail to significantly alter expression of any striatal BDNF splice variant. These data support roles for specific BDNF promoter regions and regulatory sequences in stimulant-induced alterations in BDNF expression and in the alterations that changed BDNF expression is likely to confer in the brain. Support: NIDA-IRP

T33 ROLE OF SPINAL BRAIN-DERIVED NEUROTROPHIC FACTOR IN THE SUPPRESSION OF PSYCHOLOGICAL DEPENDENCE ON MORPHINE UNDER A CHRONIC PAIN-LIKE STATE IN MICE ***Y. Yajima, M. Narita, A. Nakamura, M. Shibasaki, M. Miyatake, M. Narita, A. Usui, C. Kaneko, T. Yamaguchi, T. Suzuki Dept. of Toxicol., Hoshi Univ. Sch. of Pharm. and Pharmaceut. Sci., Tokyo, Japan*** In the previous study, we found that activation of spinal protein kinase C (PKC) could produce hyperalgesia and suppress the rewarding effect induced by morphine in mice. It is well known that brain-derived neurotrophic factor (BDNF) causes the activation of PKC. Therefore, we investigated here whether spinal BDNF-mediated nociceptive pathway could suppress the rewarding effect of morphine in mice. The hyperalgesia and allodynia after sciatic nerve ligation were eliminated by repeated i.t. treatment with BDNF antibody in mice. In addition, a single i.t. injection of BDNF induced a persistent pain-like state in normal mice. Under these conditions, the rewarding effect of morphine was markedly suppressed by a single i.t. injection of BDNF. This suppression was reversed by i.t. pretreatment with the selective PKC inhibitor Ro-32-0432. These findings suggest that BDNF/PKC-mediated nociceptive pathway in the spinal cord may play a critical role in the suppression of morphine-induced rewarding effect under a chronic pain-like state in mice.

T34 MORPINE-INDUCED MICROGLIAL BDNF EXPRESSION THROUGH TRANSACTIVATION ***N. Takayama, H. Ueda Div. of Mol. Pharmacol. & Neurosci., Nagasaki Univ., Grad. Sch. of Biomed. Sci., Nagasaki, Japan*** In primary culture of microglia obtained from embryonic rat brain, the addition of morphine at $10^{-7\sim-6}$ M concentration-dependently increased the gene expression of BDNF as early as 1 hr. Morphine-induced BDNF expression and ERK1/2 phosphorylation were abolishable by naloxone, wortmannin, PD98059, genistein and 1,10-phenanthroline, a metallo-protease inhibitor. The addition of conditioned medium factors derived from the culture of morphine-treated microglia to the fresh microglia in the presence of naloxone also increased the phosphorylation of ERK1/2. All these findings suggest that morphine induces significant changes in BDNF gene expression at relatively high concentrations through ERK1/2 phosphorylation induced by unknown growth factors generated through a MOR-mediated metalloprotease activation.

T35 INVOLVEMENT OF CAMP-PKA SIGNAL PATHWAY IN REGULATION OF Na+, K+-ATPase BY MORPHINE ***G. Liu, Z.Q. Wu, M. Li, J. Chen, Z.Q. Chi, Dept. of Neuropharmacol, Shanghai Inst. of Materia Medica, Shanghai Insts. for Biol. Sci., Chinese Acad. of Sci, Shanghai, China*** This study examined effects of the acute and chronic morphine treatment on hippocampal and striatal Na+,K+-ATPase activities and the underlying mechanisms in mice. Acute morphine treatment dose-dependently stimulated Na+ K+-ATPase activity in both brain regions, which was sensitive to the opioid antagonist, naltrexone and Gi/o protein inhibitor, pertussis toxin (PTX). This action could be significantly suppressed by co-administration of adenylyl cyclase stimulator forskolin or cAMP analogue dibutyryl-cAMP (db-cAMP), and fully abolished by a protein phosphatase 1 (PP1) and PP2A inhibitor,

okadaic acid (OKA) at doses selective against PP2A. However, the stimulation of Na+,K+-ATPase by morphine could mimicked by H-89, a selective cAMP-dependent kinase (PKA) inhibitor. Contrary to the acute morphine treatment, chronic morphine treatment significantly inhibited Na+,K+-ATPase activity. An additional decrease in Na+,K+-ATPase activity was observed by naloxone precipitation or by concomitant use of OKA at dose selective against PP2A. H-89 could significantly reverse this inhibition. Neither acute nor chronic morphine treatment changed the abundances of α catalytic isoforms of Na+,K+-ATPase. But the basal phosphorylation level of Na+,K+-ATPase was significantly increased by chronic morphine treatment, and was remarkably attenuated by acute morphine treatment. It can be concluded that the changes in phosphorylation levels was involved in the modulations of Na+,K+-ATPase by morphine, and cAMP- PKA signal pathway may contribute to the alteration in the levels of Na+,K+-ATPase phosphorylation.

T36 CHRONIC MORPHINE TREATMENT CAUSES PROTEASOME-MEDIATED DEGRADATION OF Gβ IN HUMAN NEUROBLASTOMA SH-SY5Y CELLS ***L. Moulédous, J. Neasta, S. Uttenweiler-Joseph, A. Stella, M. Matondo, M. Corbani, B. Monsarrat, J.-C. Meunier Institut de Pharmacologie et de Biologie Structurale, CNRS, Toulouse, France*** Differential proteomic analysis of membrane rafts isolated from untreated and chronically morphine-treated neuroblastoma (SHSY-5Y) cells revealed chronic morphine exposure to have reliably induced a 30 to 40% decrease in the abundance of G protein subunits αi2, αi3, β1 and β2, and prohibitin. Down-regulation of Gβ, but not of the other proteins, is highly correlated with the degree of adenylate cyclase sensitization, a hallmark of opiate dependence, elicited by chronic morphine exposure in these cells. Chronic morphine-induced down-regulation of Gβ and adenylate cyclase sensitization were blocked by MG-115 or lactacystin. Thus, sustained activation of the MOP receptor by morphine in neuroblastoma SH-SY5Y cells appears to promote proteasomal degradation of Gβ to sensitize adenylate cyclase. Together, our data suggest that chronically administered opiates may elicit dependence by altering the neuronal balance of heterotrimeric G proteins and adenylate cyclases, with the ubiquitin-proteasome pathway playing a pivotal role.

T37 PROTEOMIC ANALYSIS OF THE EFFECT OF MORPHINE TREATMENT ON THE EXPRESSION PROFILE OF PSD-ASSOCIATED PROTEINS IN MOUSE IPPOCAMPUS ***J.A. Morón (1), N. Abul-Husn (1), G. Dolios (2), R.Wang (2), L. Devi (1) (1) Dept. Pharmacology, (2) Dept. Human Genetics, Mount Sinai Medical School, New York USA*** The postsynaptic density (PSD) contains proteins involved in cell signaling that are thought to play a role in opiate addiction and dependence. However, not many studies have explored the global changes in the expression profile of PSD-associated proteins upon morphine treatment. We have initiated studies to identify proteins in the purified PSD fraction by tandem mass-spectrometry and develop a protein database in the mouse hippocampus. Of the 94 proteins identified, 72 were previously shown to be localized to the PSD, and 22 are novel. In order to characterize changes in PSD-associated protein profile in the mouse hippocampus following chronic morphine treatment, we used isotope-coded-affinity-tag (ICAT) for the quantitative analysis of differences in protein levels by mass spectrometry. These results show that chronic morphine treatment leads to significant increase in the level of 5 proteins and decrease in the level of 6 proteins. These include proteins involved in protein trafficking, cytoskeletal organization and metabolism. These studies show that proteomics can serve as a valuable tool to explore global changes in protein expression during various paradigms of morphine addiction.

T38 MORPHINE-INDUCED CHANGES IN PRESYNAPTIC ACTIVE ZONE PROTEINS IN THE MOUSE HIPPOCAMPUS ***N.S. Abul-Husn (1), J.A. Morón (1), R. Wang (2), L.A. Devi (1). (1)Dept. Pharmacol. & Biol. Chem., (2)Dept. Human Genetics, Mount Sinai School of Medicine, New York, NY USA*** Chronic opiate use produces alterations in synaptic plasticity in various brain regions, including the hippocampus. This can be mediated by changes in the postsynaptic density or in the presynaptic active zone (PAZ), where vesicle trafficking and neurotransmitter release take place. To date, relatively fewer studies have focused on presynaptic mechanisms of opiate-induced plasticity. To address this, we have initiated studies to characterize proteins in the hippocampal PAZ fraction, and have identified 140 proteins by tandem mass spectrometry. A comparison of two-dimensional gels from saline- and morphine-treated samples revealed that 21 PAZ proteins were differentially modulated upon morphine treatment. Among these, three proteins showed statistically significant changes. Sequence analysis revealed one of these to be NSF, a protein involved in synaptic vesicle trafficking. This is consistent with a presynaptic role for morphine in modulating neurotransmitter release. Overall, these studies should provide insight on presynaptic mechanisms of opiate-induced plasticity. Support: DA08863, DA019521 (LAD)

T39 ROLE OF GABA-A RECEPTOR α6 SUBUNIT IN MORPHINE TOLERANCE ***N. Guo (1), E. Kozela (2), P. Popik (2) , L. Yu (1), J.T.A. Meij (1) (1) Dept. Cell Biol., Neurobiol. & Anat., Univ. Cincinnati, Cincinnati, OH, USA, 2 Inst. Pharmacol., Polish Acad. Sci., Kraków, Poland*** Concurrent administration of NMDAR antagonist with morphine prevents opioid tolerance. We used this paradigm in a gene array screen (Affymetrix MG-U74v2). C57BL/6 mice were bi-daily injected with either memantine (non-tolerant control), morphine (tolerant), or memantine preceding morphine (non-tolerant) for 5 d. Pooled PAG RNA samples were used to generate probes. The results showed enhanced signal for GABAA receptor α6 subunit in both non-tolerant groups vs tolerant. This was surprising because, normally, GABAA α6 is exclusive to cerebellar granule cells. To investigate whether it played a role in the response to chronic morphine, mice were injected i.c.v. with GABAA α6 antisense, missense or saline on day 1,3,5 and every 24 h after. From day 6-10, s.c. memantine and/or morphine were also given. On day 11, mice were tested in a cumulative morphine dose–tail flick response assay. I.c.v. antisense, but not missense or saline, prevented the rightward shift in the dose-response curve in morphine-treated mice. Thus, inhibiting GABAA α6 expression blocked morphine tolerance.

T40 CHRONIC MORPHINE UPREGULATES Gα12 AND CYTOSKELETAL PROTEINS IN CHO CELLS EXPRESSING THE CLONED MU OPIOID RECEPTOR ***H. Xu (1), X.Y. Wang (1), D. Zimmerman (1), E.S. Boja (2), J. B. Wang (3), E.J. Bilsky (4), R.B. Rothman (1) (1) CPS, IRP, NIDA, NIH, DHHS, Baltimore, MD USA, (2) LBC, NHLBI, NIH, Bethesda, MD USA, (3) Univ. Maryland, Baltimore, MD, (4) Univ. of New England College of Osteopathic Medicine, Biddeford, ME, USA*** Chronic morphine treatment decreased expression of Gα-i2 (64%) and Gα-i3 (60%), had no effect of Gα-o, and increased Gα-12 (66%) expression in CHO cells expressing the cloned human mu opioid receptor (hMOR-CHO cells). Chronic morphine treatment enhanced thrombin-stimulated RhoA activity and expression of α-actinin, a cytoskeletal anchoring protein, in hMOR-CHO cells. Proteomic analysis of 2D spots prepared from hMOR-CHO cells showed that morphine treatment affected the expression of proteins associated with morphological changes. Up-regulation of Ga12 and α-actinin by chronic morphine was also observed in mouse brain. Viewed collectively, these findings indicate, for the first time, that chronic morphine enhances the Ga12-associated signaling system, which is involved in regulating cellular morphology and growth, supporting other findings that chronic morphine may alter cellular morphology, in addition to cellular function.

T41 DIFFERENTIAL EFFECTS OF KAPPA OPIOID AGONISTS ON G PROTEIN EXPRESSION IN CELLS EXPRESSING THE CLONED HUMAN KAPPA OPIOID RECEPTOR ***R.B. Rothman , X.Y. Wang, T.S. Benaderet, C.M. Dersch, H. Xu (1) CPS, IRP, NIDA, NIH, DHHS, Baltimore, MD USA*** Previous work suggested that different kappa opioid agonists may act via binding to different domains of the kappa receptor. Thus, we sought differences in the effects of 6 kappa agonists ((-)-EKC, DynA1-13, (-)-U50,488, salvinorin A, U69,593and (-)-etorphine) on various end-points: agonist-induced changes in the high-affinity [^{35}S]GTP-γ-S binding sites in CHO cells expressing the cloned human kappa opioid receptors (hKOR-CHO) and chronic treatment-induced regulation of G protein expression and gene expression. The 6 agonists demonstrated quantitative, but not qualitative, differences in their ability to increase the Bmax and lower the Kd of the high affinity [^{35}S]GTP-γ-S binding site. No agent affected expression of Gα-i2 or Gα-o. All drugs except (-)-U50,488 increased expression of Gα-12. All drugs except DynA1-13 decreased expression of Gα-i3. Based on the differential effects of (-)-U50,488 and DynA1-13 on G protein expression, we used the Aligent mouse array to probe for differential transcriptional responses between these two agonists. These data are being analyzed and will be presented at the meeting.

T42 LPS-STIMULATED INTERLEUKIN-6 mRNA IS REDUCED BY THE KAPPA-SELECTIVE LIGAND, U50,488 IN A MOUSE MONOCYTE-LIKE CELL LINE ***A.L. Parkhill, J.M. Bidlack Dept. Pharmacology and Physiology, Univ. Rochester School of Medicine and Dentistry, Rochester, NY, USA*** Kappa opioids modulate various immune responses both *in vivo* and *in vitro*, including the production of proinflammatory cytokines, such as interleukin-6 (IL-6). The goal of this study was to determine whether this modulation was on the level of transcription. P388D1 cells, a mouse monocyte-like cell line, were stimulated with the antigen, lipopolysaccharide (LPS), in the presence or absence of the kappa-selective ligand, U50,488. Treatment with U50,488 significantly reduced LPS-stimulated IL-6 mRNA as measured by RT-PCR. This effect was mediated through the kappa opioid receptor, because nor-BNI, a kappa-selective antagonist, blocked this inhibition. Surprisingly, U50, 488 had no effect on the levels of LPS-stimulated interleukin-1β or tumor necrosis factor-αmRNA. The reduction of antigen-elicited IL-6 mRNA by kappa opioids may contribute to the immunomodulatory effects of these compounds *in vivo*. Support: K05-DA00360, DA04355

T43 PHA -DEPENDENT KAPPA AGONIST - INDUCED IL-7 RECEPTOR mRNA EXPRESSION IN R1.1 THYMOMA CELL LINE ***M. Khimich, J.M. Bidlack Dept. Pharmacology and Physiology, Univ. Rochester School of Medicine and Dentistry, Rochester, NY, USA*** Endogenous stimulation of kappa opioid receptor (KOR) has been shown to provide a positive signal for intrathymic T-cell maturation. However, the kappa-selective agonist U50,488 has been shown to decrease the level of expression of IL-7R on primary murine thymocytes, which, taking into consideration an extremely important role of IL-7R in thymocyte survival, is a direct immunosuppressive effect. To further elucidate the role of KOR in thymocyte development, in current study, we investigated the influence of U50,488 administration on IL-7R α chain transcription in the R1.1 thymoma cell line, which was derived from very immature double negative mouse thymocytes. Using RT-PCR, we showed that U50,488 induced IL-7R α chain mRNA expression in the R1.1 thymoma cell line. The effect was PHA – dependent and was completely blocked by the kappa-selective antagonist nor-BNI. The data obtained suggest that the effect of opioid administration may depend on the stage of T cell differentiation in thymus. Support: K05-DA00360, DA04355

T44 CHANGES IN PHOSPHORYLATION STATE OF CONNEXIN43 AT ASTROCYTIC GAP JUNCTIONS IN THE MOUSE SPINAL CORD INDUCED BY CHRONIC *IN VIVO* TREATMENT WITH MORPHINE ***M. Suzuki, M. Narita, M. Narita, K. Niikura, A. Nakamura, T. Suzuki Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan*** It is widely accepted that prolonged exposure to morphine induces adaptive changes, resulting in its tolerance to analgesia/antinociception. We previously reported that mice tolerant to morphine exhibited the production of reactive astrocytes associated with activating PKC in the mouse spinal cord. Here we show that repeated treatment with morphine induced a significant increase in the immunoreactivity for PKC-dependent phosphorylated form of connexin43 (Cx43), which is present on the astrocytes in the mouse spinal cord. Furthermore, intrathecal pretreatment with the blockers of gap junctional channels, carbenoxolone and Gap27, suppressed the antinociceptive response by morphine, suggesting that gap junctional communication in the mouse spinal cord play a role in the expression of antinociception induced by morphine. These results indicate that repeated treatment with morphine induces the change in astrocytic gap junctional communication associated with the increase in the phosphorylated form of Cx43, which may affect the development of tolerance to morphine-induced antinociception.

T45 REGULATION OF G-PROTEIN COUPLED RECEPTOR ENDOCYTOSIS BY PHOSPHO-LIPASE D2 ***T. Koch, D. Wu, L. Yang, L.O. Brandenburg, V. Höllt Dept. Pharmacology and Toxicology, Otto-von-Guericke Univ. Magdeburg, Magdeburg, Germany*** We have recently shown that the mu-opioid receptor (MOR1) is associated with the phospholipase D2 (PLD2), a phospholipid-specific phosphor-diesterase located in the plasma membrane. We further demonstrated that in HEK293 cells coexpressing MOR1 and PLD2, treatment with DAMGO led to an increase in PLD2 activity and an induction of receptor endocytosis, whereas morphine, which does not induce opioid receptor endocytosis failed to activate PLD2. We report here that activation of PLD2 is mediated also by other GPCRs like delta opioid receptor (DOR) and cannabinoid receptor (CB1). Furthermore, inhibition of PLD2 activity by primary alcohol or overexpression of a dominant negative PLD2 (nPLD2) impaired the agonist-induced endocytosis of MOR1, DOR, and CB1 receptor. This indicates that activation of PLD2 is essential for the induction of agonist-induced GPCR-endocytosis. Moreover we provide evidence that the constitutive endocytosis of a mu-opioid receptor splice variant (MOR1D) is also mediated by PLD2-dependent pathway. These data indicate the important role for PLD2 in the regulation of agonist-dependent and agonist-independent GPCR-endocytosis.

T46 CHRONIC STRESS ALTERS GENE EXPRESSION IN DYNORPHIN KNOCKOUT AND WILDTYPE MICE ***J.D. Lowe, P. Amieux, J. McLaughlin, C. Chavkin Dept Pharmacology, Univ. Washington, Seattle WA USA*** Previous studies showed that repeated forced swim caused dynorphin (Dyn) activation of the kappa opioid receptor that resulted in analgesia, immobility and potentiation of cocaine conditioned place preference. To identify the underlying genetic mechanisms, we compared the stress responses of prodynorphin gene knockout (KO) mice with wildtype (WT) littermates and mice pretreated with the KOR selective antagonist norBNI. WT mice showed a robust increase in plasma corticosterone levels following stress; the responses of matched Dyn KO or norBNI pretreated mice were not significantly different than WT. Using cDNA microarrays corresponding to the NIA 23K library, we found stress-induced changes in gene expression in the ventral striatum that could be confirmed by QRT-PCR, however there were only subtle differences between WT, Dyn KO, and norBNI pretreated mice. Additionally, we found increased FosB expression levels in the ventral striatum of both WT and Dyn KO mice following stress. Although we have not found a strong kappa opioid receptor dependence, we have identified genes that are regulated by swim stress which may in part contribute to the stress-induced potentiation of cocaine place preference.

T47 NEUROPETIDE REGULATION IN MICE CHRONICALLY TREATED BY MORPHINE ***F.M. Décaillot (1), F.Y. Che (2), L. Fricker (2), L.A. Devi (1). (1) Mt. Sinai School of Medicine, New York, USA, (2) Albert Einstein College of Medicine, New York, NY, USA*** Chronic morphine administration is known to affect a number of neuropeptide systems and this could participate in the behavioral effects of opiates. To quantitate global changes in neuropeptides levels, we used a previously described neuropeptide isolation method from the brains of mice lacking Carboxypeptidase E (Cpefat/fat). Carboxypeptidase E is a major enzyme in the biosynthesis of numerous neuroendocrine peptides. We used a differential labeling procedure with stable isotopic tags and mass spectrometry to quantitate the relative changes in a number of hypothalamic and striatal peptides in Cpefat/fat mice chronically treated with morphine. Over 100 peptides were found including a number of fragments of proenkephalin, prothyrotropin-releasing hormone, secretogranin II, chromogranin A and B, protackykinin B, provasopressin, progonadotrophin releasing hormone and pro-SAAS. Overall, most of them were unchanged (within 10-15% of saline control) but a small number of peptides were consistently increased or decreased >40% by morphine administration. Taken together, the results provide interesting insights into possible cross-talk between opioids and other endogenous systems and suggest further experiments to link candidate peptides with long term effects of morphine. Support: DA008863 and DA019521 (LAD), DA019521 (LDF)

T48 DENDRITIC SPINE FORMATION AND LOCALIZATION OF NMDA AND AMPA RECEPTORS IN PRIMARY HIPPOCAMPAL NEURONS ARE CONTROLLED BY SIGMA-1 RECEPTORS ***S.-Y. Tsai, T. Hayashi, T.-P. Su Cellular Pathobiology Unit/DPS/ CNRS/IRP/NIDA /NIH/DHHS, Baltimore, MD, USA*** Sigma-1 receptors (Sig-1R) at the ER are implicated in CNS diseases (depression, demyelination, amnesia, and drug abuse) and are found to compartmentalize ER lipids and promote the lipid raft formation at the plasma membrane. We showed that Sig-1R increase NMDA (NR1) and AMPA (GluR2/3) receptors in lipid raft fractions from rat primary hippocampal neurons (HN). NR1 and GluR2/3 exist at the dendritic spine (DS). We hypothesize therefore that Sig-1R recruit NR1 and GluR2/3 to the DS by providing rafts at the DS. Data supporting the hypothesis are reported. A knockdown of Sig-1R in HN with siRNA against Sig-1R depletes GM1 ganglioside that is a critical component of lipid rafts. The siRNA-treated HN form fewer DS, possess more and longer filopodia, show a stunned growth and development of dendrites, exhibit few dendritic branchings, lose the synapse markers PSD-95 and synapto-physin, and fail to recruit NR1 or GluR2/3 to the DS: they clusters and localizes solely at the dendritic shafts. Thus Sig-1R provide rafts for the neuron, thus facilitating synapse formation by anchoring receptors or ion channels to the postsynaptic region.

T49 MORPHINE REGULATION OF PC1/3 AND PC2: IMPLICATION FOR THE SWITCH FROM DRUG USE TO DRUG ABUSE ***A. Anghel (1), K. Lutfy (1,2), Y. Liu (1), Y. Nie (1) and T.C. Friedman (1) (1)Endocrinol, Charles Drew Univ., Los Angeles, CA, (2)Pharm. Sci., Western Univ., Pomona, CA USA*** We hypothesized that morphine regulates prohormone convertases (PC1/3 and PC2, which control the biosynthesis of biologically active opiates) via phosphorylation of CREB (p-CREB). Rats were treated with morphine for 1 or 7 day(s) and PC1/3, PC2 and p-CREB levels were measured by Western blot and immunohistochemistry. One-day morphine treatment significantly decreased PC1/3, PC2 and p-CREB protein expression in anterior and intermediate pituitary and p-CREB expression in the hypothalamus. Seven-day morphine treatment increased PC1/3, PC2 and CREB-P protein expression in the same areas. Morphine decreased PC1 promoter activity by 30% and PC2 promoter activity by 79% in GH3 cells stably transfected with the mu opiate receptor, an effect absent when constructs with mutated CRE(s) were used. Morphine decreased PC2 mRNA levels (determined by RT-PCR) and PC2 enzymatic activity in a time- and dose-dependent manner. Our results suggest that opiates induce a biphasic effect on PC1/3 and PC2 expression. This action may mediate the switch from drug use to drug abuse. Support: R01DA14659, R24DA017298 (TCF)

T50 MU AND KAPPA OPIOIDS DIFFERENTIALLY MODULATE VENTRAL TEGMENTAL AREA OUTPUTS DEPENDING ON EFFERENT TARGET ***E.B. Margolis (1), H. Lock (1), V. Chefer (2), T. Shippenberg (2), G.O. Hjelmstad (1), H.L. Fields (1) (1) Ernest Gallo Clinic & Research Center, UCSF, Emeryville, CA, USA (2) Integrative Neuroscience Section, DHHS/NIH, NIDA/IRP, Baltimore, MD USA*** Ventral tegmental area (VTA) dopamine (DA) neurons are involved in motivational responses to opioids. Subpopulations of VTA neurons project to the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC). We hypothesized that mu and kappa opioid effects in the VTA differ with projection target. Whole-cell recordings showed that DA neurons that project to the mPFC, but not the NAc, were inhibited by the kappa opioid receptor agonist U69593. Accordingly, intra-VTA U69593 administration decreased DA release in the mPFC, but not the NAc, during microdialysis. Although mu

opioid effects were heterogeneous to both projection targets, microdialysis showed a significant increase in DA release in both regions during mu agonist administration into the VTA. Further, mu agonists inhibited a significant number of non-DA VTA neurons projecting to the mPFC. These results demonstrate that distinct subpopulations of VTA neurons with different CNS projection targets and transmitter content can be independently controlled by the opioids.

T51 NEUROPETIDE REGULATION IN MICE CHRONICALLY TREATED BY MORPHINE ***F.M. Décaillot (1), F.Y. Che (2), L. Fricker (2), L.A. Devi (1) (1) Mt. Sinai School of Medicine, New York, NY, USA, (2) Albert Einstein College of Medicine, New York, NY, USA*** Chronic morphine administration is known to affect a number of neuropeptide systems and this could participate in the behavioral effects of opiates. To quantitate global changes in neuropeptides levels, we used a previously described neuropeptide isolation method from the brains of mice lacking Carboxypeptidase E (Cpefat/fat). Carboxypeptidase E is a major enzyme in the biosynthesis of numerous neuroendocrine peptides. We used a differential labeling procedure with stable isotopic tags and mass spectrometry to quantitate the relative changes in a number of hypothalamic and striatal peptides in Cpefat/fat mice chronically treated with morphine. Over 100 peptides were found including a number of fragments of proenkephalin, prothyrotropin-releasing hormone, secretogranin II, chromogranin A and B, protackykinin B, provasopressin, progonadotrophin releasing hormone and pro-SAAS. Overall, most of them were unchanged (within 10-15% of saline control) but a small number of peptides were consistently increased or decreased >40% by morphine administration. Taken together, the results provide interesting insights into possible cross-talk between opioids and other endogenous systems and suggest further experiments to link candidate peptides with long term effects of morphine. Support: DA008863, DA019521 (LAD), DA019521 (LD)

T52 PROTEOMIC ANALYSIS OF THE EFFECT OF MORPHINE TREATMENT ON THE EXPRESSION PROFILE OF PSD-ASSOCIATED PROTEINS IN MOUSE HIPPOCAMPUS ***J.A. Morón (1), N. Abul-Husn (1), G. Dolios (2), R. Wang (2), L. Devi (1) (1) Dept. Pharmacology and Biological Chemistry, (2) Dept. Human Genetics, Mount Sinai School of Medicine, New York, NY, USA*** The postsynaptic density (PSD) contains proteins involved in cell signaling that are thought to play a role in opiate addiction and dependence. However, not many studies have explored the global changes in the expression profile of PSD-associated proteins upon morphine treatment. We have initiated studies to identify proteins in the purified PSD fraction by tandem mass-spectrometry and develop a protein database in the mouse hippocampus. Of the 94 proteins identified, 72 were previously shown to be localized to the PSD, and 22 are novel. In order to characterize changes in PSD-associated protein profile in the mouse hippocampus following chronic morphine treatment, we used isotope-coded-affinity-tag (ICAT) for the quantitative analysis of differences in protein levels by mass spectrometry. These results show that chronic morphine treatment leads to significant increase in the level of 5 proteins and decrease in the level of 6 proteins. These include proteins involved in protein trafficking, cytoskeletal organization and metabolism. These studies show that proteomics can serve as a valuable tool to explore global changes in protein expression during various paradigms of morphine addiction. Support: DA008863, DA019521 (LAD)

Consequences of receptor activation and physiology

T53 KAPPA-OPIOID RECEPTOR ACTIVATION OF p38 MAP KINASE: ROLES OF RECEPTOR PHOSPHORYLATION AND ARRESTIN ***M.R. Bruchas, J.D. Lowe, A. Francois, C. Chavkin Dept. Pharmacol, Univ. Washington, Seattle, WA, USA*** G-protein receptor kinase phosphorylation of the kappa-opioid receptor (KOR) serine 369 and subsequent arrestin (Arr) binding mediates desensitization. Arr association has also been suggested to enable G-protein coupled receptor activation of mitogen-activated protein kinases (MAPK). Coscia et. al has shown that KOR activates ERK MAPK in C6 glioma cells. In the current study we determined if the KOR activates p38 MAPK and furthermore, to determine if this activation was receptor phosphorylation and Arr-dependent. Primary cultured mouse striatal cells and AtT-20 cells transfected with KOR-GFP treated with U50,488 showed enhanced phospho-p38 staining by westerns and confocal analysis. The maximal effect was evident after 15 min and blocked by norBNI. Astrocytes isolated from KOR-knockout mice did not show U50-stimulated p38 phosphorylation. AtT-20 cells expressing GRK-insensitive KOR(S369A) did not show U50,488 stimulated p38 phosphorylation; however, transfection of the dominant positive Arr restored U50,488-induced p38 phosphorylation in KOR(S369A) expressing AtT20. These results suggest that KOR may activate p38 in primary cultured astrocytes and transfected cells by a GRK/Arr-mediated process.

T54 ACTIVITY-STATE-SENSITIVE ANTIBODIES TO OPIOID RECEPTORS ***A. Gupta, F.M. Décaillot, O. Tkalych, L.A. Devi Dept. Pharmacology and Biological Chemistry, Mount Sinai School of Medicine, New York, NY USA*** The N-terminal region of many G-protein coupled receptors has been known to undergo an activity-dependent conformational change. We explored the idea that antisera directed against this region should be able to discriminate between the receptor activity states. To test this possibility, we generated antibodies to the N-termini of mu and delta opioid receptors, CB1 cannabinoid receptors, and α-2a adrenergic receptors. We find that antisera against all four receptors exhibit receptor-type selectivity and enhanced binding to activated receptors. These antisera are sensitive enough to discriminate between the various activity states of the receptor, suggesting that they could serve as useful tools to screen and characterize receptor type-specific ligands and/or receptor mutants. These antisera also serve as valuable probes to study endogenous receptors since we are able to monitor the time course and extent of receptor activity in the brain following peripheral drug administration. Taken together, these receptor-specific antibodies serve as powerful tools to probe the spatio-temporal dynamics of GPCR activation *in vitro* as well as *in vivo*. Support: DA08863, DA019521 (LAD).

T55 N.P. Murphy NOCICEPTIN RECEPTOR KNOCKOUT MICE DISPLAY ALTERED ETHANOL SENSITIVITY ***K. Sakoori, N.P. Murphy Neural Circuit Mechanisms Res. Group, RIKEN Brain Sci. Inst., Wakoshi, Japan*** Previous studies suggest ligands acting at the nociceptin receptor (NOP) can modulate the abuse liability of ethanol. Consequently, the current study sought evidence of a role for endogenous nociceptin in ethanol sensitivity by comparing female NOP receptor knockout and wild-type mice. NOP receptor knockout mice showed reduced preferences to ethanol over water during free access in a bottle choice paradigm, and tended towards reduced ethanol preference during restricted access. Although tendencies towards reduced absolute ethanol intake were observed, the overall reduced preference was due largely to higher water intake in NOP receptor knockout mice. NOP receptor knockout mice showed stronger locomotor responses to acute ethanol administration, but conditioned place preference and sedative responses were comparable between genotypes. Upon recovery from a sedative dose of ethanol, NOP receptor knockout mice showed lower locomotion than wild-type mice. No differences in ethanol metabolism or taste reactivity were found between genotypes. These results show endogenous nociceptin makes a select contribution to ethanol sensitivity, particularly locomotor responses, in addition to being a determinant of water balance.

T56 MU AND KAPPA OPIOID RECEPTORS ACTIVATE ERK/MAP KINASE VIA DIFFERENT PKC ISOFORMS AND SECOND MESSENGERS IN ASTROCYTES ***C.J. Coscia, P.D. Haas, A.L. Clark, J.W. Hahn, A. Kiss, M.M.Belcheva Dept. Biochem. and Mol.Biol., St. Louis Univ. Sch. Med., St. Louis, MO USA*** The mu agonist, DAMGO induces a transient stimulation of ERK phosphorylation, whereas kappa agonist, U69,593, engenders sustained ERK activation. Here we demonstrate that acute U69,593 and DAMGO stimulate ERK phosphorylation by utilization of different second messengers and protein kinase C (PKC) isoforms upstream of the growth factor pathway in astrocytes. Evidence was gained to implicate calmodulin and PKCepsilon in DAMGO stimulation of ERK. DAMGO activation of PKCepsilon and ERK was insensitive to selective inhibitors of Ca2+ mobilization, but it was blocked upon phospholipase C inhibition. These results suggest a novel mechanism wherein upon DAMGO binding, calmodulin is released from the mu receptor and activates phospholipase C. Phospholipase C then generates diacylglycerides that stimulate PKCepsilon activity. In contrast, U69,593 appears to act via phosphoinositide-3-kinase, PKCzeta and Ca2+ mobilization. Our findings suggest that the differential mechanism of upstream signaling may contribute to the distinct outcomes on ERK modulation induced by chronic mu and kappa opioids. Support: DA05412

T57 INVOLVEMENT OF EPIDERMAL GROWTH FACTOR RECEPTOR TRANS-ACTIVATION IN THE MORPHINE-INDUCED REWARDING EFFECT IN MICE ***T. Takeuchi, M. Narita, Y. Yajima, T. Suzuki Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut., Sci., Tokyo, JAPAN*** Recent biochemical evidences have demonstrated that transactivation of epidermal growth factor receptor (EGFR) in response to activation of mu-opioid receptor involves autocrine/paracrine shedding of a soluble EGFR ligand, which result from proteolytic cleavage of a membrane-anchored precursor by matrix metalloproteases (MMP). Here, we show that the EGFR tyrosine kinase inhibitor AG 82 given intracerebroventricular (i.c.v) caused a significant inhibition of morphine-induced place preference without changing in the hyperlocomotion produced by a single injection of morphine. Futhermore, the morphine-induced rewarding effect was significantly suppressed by the i.c.v. injection of MMP inhibitor GM6001. These findings suggest that the MMP-dependent transactivation of EGFR through mu-opioid receptor is associated with the development of morphine-induced rewarding effect.

T58 INDUCTION OF c-FOS AND ZIF268 IN THE CENTRAL EXTENDED AMYGDALA PARALLELS HYPERALGESIA INDUCED BY NALOXONE FOLLOWING SYSTEMIC MORPHINE IN DRUG NAÏVE RATS ***P.B. Osborne (1), A.S. Hamlin (1), G.P. McNally (2) (1) Pain Management Res. Inst., Univ. Sydney, Australia, (2) Dept. Psychology, UNSW, Australia*** High doses of naloxone administered after systemic morphine can induce hyperalgesia. In the present study, 5 mg/kg naloxone administered 30 min after 10 mg/kg morphine, caused hyperalgesia measured by tail-flick in rats, and induced c-Fos and zif268 immunoreactive neurons in dorsal and ventral capsular central amygdala, lateral IPAC and laterodorsal BST. No induction in these regions occurred when morphine pretreated rats were injected with saline or 0.05 mg/kg naloxone, which did not induce hyperalgesia. Other amygdala and striatal regions were activated by administration of either morphine, or naloxone, but no other statistically significant non-additive synergistic increases in c-Fos neurons were identified. Many regions activated by morphine - e.g. basolateral amygdala, dorsal striatum and substantia nigra-required prolonged agonist exposure, as increases in c-Fos-positive neurons were reduced or absent when morphine was antagonised after 30 min. These data implicate the region identified as "nociceptive" amygdala in pain facilitation caused by acute opioid abstinence.

T59 SELECTIVE ACTIVATION OF ACCUMBENS PROJECTION PATHWAYS BY MORPHINE INDUCED CATALEPSY AND STEREOTYPY ***A.S. Hamlin (1), G.P. McNally (2), R.F. Westbrook (2), P.B. Osborne (1) (1) Pain Management Res. Inst., Univ. Sydney, Australia and (2) Dept. Psychology, UNSW, Australia*** Stereotypic behaviour can develop when catalepsy induced by morphine fades with repeated drug administrations. As the nucleus accumbens (ACB) is implicated in these motor behaviours we used dual-label immunohistochemistry to map ACB activation by c-Fos expression. Administering morphine daily to rats over 14 d caused fewer c-Fos neurons to be induced in the ACB shell when morphine was administered 24h later. This reduction was specific to prodynorphin-negative (striatopallidal) neurons. In the caudal accumbens core, prodynorphin-positive (striatonigral) neurons expressing c-Fos increased. We next measured behaviour and c-Fos in the same rats when morphine was administered two weeks after drug pre-treatment. In the accumbens core, we identified correlations between catalepsy and c-Fos induction in matrix (striatopallidal) neurons ($r2 = 0.42$, $P < 0.01$), and stereotypy and c-Fos induction in patch (striatonigral) neurons ($r2 = 0.60$, $P < 0.001$). These results suggest morphine induced stereotypy could be caused by increased activation of striatonigral projections from the ACB core.

T60 DELTA-1-STIMULATION DOWN REGULATES DELTA-2-RESPONSES IN HEART ***S.H. Deo, S. Johnson-Davis, M.A. Barlow, D. Yoshishige, J.L. Caffrey Univ. North Texas Health Science Center, Fort Worth, TX, USA*** Delta-1-receptor stimulation mimics the cardioprotective effect of ischemic preconditioning. Ultra-low dose, MEAP improved vagal transmission (vagotonic) and lowered heart rate by stimulating local delta-1-receptors in the sinoatrial (SA) node. The vagotonic effect was consistent with the known cardioprotective effect of acetylcholine. However, higher doses of MEAP were vagolytic via delta-2-stimulation. A study was designed to the test whether sustained delta-1-stimulation down regulates opposing delta-2-responses. Opioids were delivered into the SA node by microdialysis during vagal stimulation. Deltorphin was added periodically to evaluate delta-2-mediated vagolytic responses. After exposure to the delta-1-agonist, TAN-67, the vagolytic effect of deltorphin fell from 75 % inhibition to 25%. The response was also eroded when repeated deltorphin was administered alone without added TAN-67. In both cases, pretreatment with the delta-1-antagonist, BNTX prevented the erosion. Once down regulated however, the original vagolytic response to deltorphin was not restored by acute BNTX. Delta-1-stimulation in heart appears to down regulate opposing delta-2-responses. Support: AHA TX Affiliate.

T61 KNOCKOUT OF THE MU OPIOID RECEPTOR ENHANCES SURVIVAL OF PROGENITOR CELLS IN THE ADULT HIPPOCAMPUS ***G. Harburg (1), F.S. Hall (2), A. Harrist (3), I. Sora (4), G.R. Uhl (2), A. Eisch (1) (1) Dept. Psychiatry, U.T. Southwestern Med. Ctr., Dallas, TX, (2) Molec Neurobio. Branch, NIDA-IRP, NIH, Baltimore, MD, (3)Univ. Penn. Sch. Med., Philadelphia, PA, (4) Dept. Neurosci., Tohoku Univ. Grad. Sch. Med., Sendai, Japan*** Chronic exposure to the largely mu opioid receptor (MOR) agonist morphine decreases proliferating progenitor cells in the adult hippocampus (Eisch et al, 2000). To further study the impact of MOR on adult neurogenesis, we examined the birth and survival of progenitor cells in adult MOR1 knockout mice (Sora et al, 1997). Mice were injected with BrdU and sacrificed two hours or four weeks later. Two hours post-BrdU, mice had no significant differences in numbers of proliferating cells, regardless of genotype. In contrast, four weeks post-BrdU, heterozygote and homozygote knockout mice had 157% and 154% the number of wild-type BrdU immunoreactive cells. The majority of these cells had neuronal morphologies and colocalized with the neuronal marker NeuN. In

concordance with the increased numbers of cells maturing into neurons, the MOR KO animals also had larger hippocampal granule cell layers. These findings underscore roles for MOR in progenitor cell survival and/or maturation.

T62 ETHYNYLESTRADIOL-INDUCED CHOLESTASIS CAUSES SCRATCHING IN RATS ***S. Inan, A. Cowan Dept. Pharmacology, Temple Univ. School of Medicine Philadelphia, PA USA*** Pruritus is a complication of some liver diseases. Lack of an animal model limits investigation of possible antipruritic agents. We examined first if ethynylestradiol (EE)-induced cholestasis causes scratching in rats and second, as a preliminary study, whether nalfurafine, a kappa agonist, would antagonize such scratching. Male SD rats (175-200 g; n=20) were injected s.c. with either 2 mg/kg 17α ethynylestradiol or vehicle (50% propylene glycol in water) once a day for 14 days. On day 13, the animals were placed in individual observation boxes to acclimate. One min after the regular injection of EE, the number of body scratches was counted for 30 min. On day 14 after acclimation only the EE group was injected s.c. at -20 min with either nalfurafine (0.02 mg/kg) or saline and scratching counted for 30 min after EE. Heart blood was drawn for measurement of serum bile acid levels to confirm cholestasis. The incidence of scratching was greater in the EE group than in the vehicle group (32 + 5.7 and 14 + 3.8, respectively, $p< 0.01$). Serum bile acid levels were higher in the EE group compared to the vehicle group (131 + 14 and 41.9 + 16 micromol/L, respectively, $p< 0.001$). The EE group did not gain weight. Our preliminary data with one low dose of nalfurafine showed a decreased number of scratches compared to saline. We propose that EE-induced cholestasis in rats can provide a model to investigate possible antipruritic effects of kappa agonists in cholestatic pruritus.

15:30 – 18:50 **Symposium IV Prescription and non-prescription drug abuse Marriott Ballroom**
Chair F. Vocci, Cochair M.B. Max

15:30 – 15:55 **S22 F. Vocci OPIATE ABUSE PATTERNS IN THE UNITED STATES: A CHANGING SCENE** ***F. Vocci Division of Pharmacotherapies and Medical Consequences of Drug Abuse, National Institute in Drug Abuse, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA*** A remarkable increase in opiate abuse has been reported in the United States since the early 1990s. Two availability factors have been cited as fueling the increase in abuse: 1) high "potency" heroin with concentrations as high as 72 %; and 2) the increased prescribing and diversion of prescription opiates. The high potency heroin allows use by the intranasal (method of choice by American adolescents) and pulmonary routes of administration. The average age of initiation of heroin use has dropped from age 27 to 19. Consequently, adolescent treatment admissions tripled in the 1990s in the US Treatment Episode Data Set (TEDS). Abuse of prescription opiates has also become increasingly common. The Monitoring the Future study, a survey of 8th, 10th, and 12th graders, has noted a three-fold increase in prescription opiate abuse since 1991. The 2003 National Survey on Drug Use and Health reported that 2.5 million people endorsed non-medical use of prescription opiates in 2002, a five-fold increase since 1990. Treatment admissions for prescription opiates have surpassed heroin admissions in TEDS.

15:55 – 16:20 **S23 J. White BUPRENORPHINE IMPLANTS (PROBUPHINE®) FOR TREATMENT OF OPIOID DEPENDENCE: CLINICAL TRIAL RESULTS** ***J. White (1), J. Bell (2), J. Saunders (3), P. Williamson (1), M. Makowska (3), D. Lissin (4), A. Jacobs (4); (1) Univ of Adelaide, Australia, (2) The Langton Center, Sydney, Australia, (3) Univ of Queensland and Royal Brisbane and Women's Hospital and Prince Charles Hospital Districts, Brisbane, Australia, (4) Titan Pharmaceuticals, S. San Francisco, CA USA*** Probuphine is a novel subcutaneous implant dosage form of buprenorphine (BPN) for the treatment of opioid dependence. The implant is inserted subcutaneously into a site such as the inner upper arm, with the aim of achieving sustained and stable plasma BPN concentrations over 6 months following a single treatment. Each implant is a solid matrix of BPN and ethylene vinyl acetate, and measures 26 mm in length by 2.4 mm in diameter. The capacity to provide opioid-dependent patients with a continuous source of an effective treatment will potentially improve BPN treatment outcome by reducing the need for frequent sublingual (SL) BPN dosing, eliminating daily visits to the clinic/pharmacy for supervised dispensation, decreasing variable blood levels observed after SL BPN administration, and diminishing the risk of abuse and diversion of SL BPN. An open-label, 2-dose-group, 6-month clinical study of Probuphine is presented. Patients maintained on 8mg SL BPN were switched to 2 Probuphine implants, and patients maintained on 16mg SL BPN were switched to 4 Probuphine implants. Plasma levels were related to number of implants: peak levels were 2.00±0.41 ng/ml with 2 implants, and were 3.02±0.67ng/ml with 4 implants. Steady-state levels were maintained for 6 months: 0.38±0.12 ng/ml and 0.74 ±0.24 ng/ml for 2 and 4 implants, respectively. Probuphine controlled withdrawal symptoms,

heroin use and cravings in both groups. Supplemental SL BPN was administered to (or required by) 5 of the 12 patients: average number of days on supplemental SL BPN for both dose groups was 2.8 days. No significant adverse events (AEs) were noted, and no AEs required removal of the implants. Minor, transient procedural AEs occurred in 7 patients (implant site reaction, pain) and resolved with no additional treatment. Other AEs were known side effects of BPN. This study shows that opioid-dependent patients can be successfully switched from maintenance SL BPN to Probuphine implants, providing 6 months of sustained plasma levels of BPN, suppression of heroin use, and control of withdrawal and cravings. Further safety and efficacy studies are underway.

16:20 – 16:40 *Coffee break*

16:40 – 17:05 **S24 M.B. Max RECENT DEVELOPMENTS IN THE DRUG TREATMENT OF CHRONIC PAIN** ***M.B. Max Pain and Neurosensory Mechanisms Branch, National Institute of Dental and Craniofacial Research, National Institutes of Health, Department of Health and Human Services, Bethesda, MD, USA*** Dr. Max will review new data about the use of opioids in chronic pain. Several recent clinical trials suggest that opioids may surpass the leading non-opioids such as tricyclic antidepressants and gabapentin in their relief of most patients with diabetic neuropathy and postherpetic neuralgia, but that patients with major primary afferent loss may be opioid resistant, presumably because of the loss of presynaptic spinal opioid receptors. Pain researchers are beginning to use candidate gene studies to probe the reasons for differences in susceptibility to pain and to benefit and toxicity from opioid analgesics. Data will be reviewed from studies of the development of temporomandibular pain in normals, of chronic pain following lumbar diskectomy for sciatica, and of effectiveness of postoperative opioids.

17:05 – 17:30 **S25 W. Ling HYPERALGESIA: PUTTING ASUNDER WHAT GOD HATH JOINED TOGETHER?** ***W. Ling Integrated Substance Abuse Programs, Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine, University of California at Los Angeles, CA, USA*** Transient and reversible hyperalgesia, a cardinal feature of acute pain, prevents further tissue damage and promotes healing. Persistent hyperalgesia confounds the evaluation of chronic pain and complicates their management especially in patients exposed to opioids chronically, either from an opioid addictive disorder or from treatment of their chronic pain, as it now appears increasingly evident that hyperalgesia results from the persistence of pain as well as from chronic administrations of opioids. The shared mechanism appears to be the chronic activation of the NMDA receptor that underlies central sensitization involved in pain modulation and in apparent opioid analgesic tolerance. For the clinician the dilemma is how to tease apart the hyperalgesia as a result of chronic pain from the hyperalgesia that comes from chronic opioid exposure, and what to do with these patients. Specifically, can this opioid induced hyperalgesia be prevented or overcome without disturbing the ability of opioids to provide pain relief. Can man put asunder what God hath joined together? The answer, increasingly, appears to be “yes”.

17:30 – 17:45 **S26 D.C. Mash NORIBOGAINE: A METABOLITE OF THE NATURALLY OCCURRING SUBSTANCE IBOGAINE MEDIATES THE BENEFICIAL EFFECTS OF THE DRUG ON OPIATE WITHDRAWAL AND DEPENDENCE** ***D.C. Mash (1), J. Pablo (1), L. Duque (1), F.R. Ervin (2) , W.L. Hearn (1), J.D. Kamlet (3) (1) Dept. Neurology and Pharmacology, Univ. Miami Sch Medicine, Miami, FL, (2) Dept. Psychiatry and Human Genetics, McGill Univ., Montreal, Canada, (3) Mt. Sinai Medical Center, Miami, FL*** The indole alkaloid ibogaine naturally occurs in the plant Tabernanthe Iboga, which is native to equatorial Africa. Informal addict self-help groups have provided testimonials that ibogaine blocks opiate withdrawal signs and provides long-term benefits on opiate consumption in the weeks to months after taking ibogaine. Preclinical studies demonstrate that ibogaine dose-dependently decreases withdrawal in morphine-dependent rats. We have obtained a large open label case series in human volunteers. We have examined 272 addicts (male = 202; females = 72), who were dependent on drugs or alcohol. Pharmacokinetic and safety data were obtained to determine the metabolism and clearance of ibogaine and the relationship of behavioral or adverse effects to dose. Pharmacokinetic modeling demonstrates that Ibogaine is a pro-drug that is converted by cytochrome P4502D6 to an active metabolite – noribogaine. Objective physician ratings demonstrate that oral doses of ibogaine promote rapid detoxification from heroin and methadone. We determined if ibogaine would diminish drug craving using a multi-dimensional craving questionnaire for heroin (HCQN-29). Questions were asked about the positive reinforcing effects of the drug or the expectation of the outcome from using a drug of choice or the alleviation of withdrawal states. Subjects undergoing opiate detoxification reported significantly decreased drug craving for opiates on five measures taken from the HCQN-29 scales at 36 hrs post-treatment. Noribogaine has high intrinsic activity and binds in a pseudoirreversible manner to mu-opioid receptors. The

targeted actions of noribogaine at mu receptors may explain ibogaine's ability to reduce withdrawal symptoms in opiate-dependent humans.

17:45 – 18:00 **S27 B.A. Moore TREATMENT OUTCOME OF NONMEDICAL PRESCRIPTION OPIATE USERS IN OFFICE-BASED BUPRENORPHINE TREATMENT: COMPARISON WITH HEROIN AND COMBINED USERS** ***B.A. Moore, D.A. Fiellin, R.S. Schottenfeld Psychiatry and Internal Medicine, Yale University School of Medicine, New Haven, CT, USA*** Our recent trial involving 24-weeks of office-based buprenorphine/naloxone maintenance (N = 200) mirrors national trends indicating increased non-medical prescription (NMP) opiate abuse. During the first year of the trial 6% of patients reported exclusive NMP opiate use compared to 27% in the last year, with oxycodone being the most common reported (67%). We compared those who reported only heroin use (148), heroin and NMP opiates (21), or only NMP opiates (31). Patients who reported only NMP opiate were younger (.02), had fewer years of opiate use (.007), were more likely to be white (.01), less likely to have had a previous drug detoxification (.02), less likely to have ever participated in methadone maintenance (.001), less likely to report injection drug use (<.001), more likely to complete buprenorphine induction (.009), remained in treatment longer (.01), were more likely to complete treatment (.006), had more weeks of continuous opiate abstinence in treatment than heroin only users (.03) and twice as likely to achieve 6-weeks of continuous opiate abstinence during treatment compared to heroin only users (<.001, 67% vs. 32%). Users of both heroin and other opiates were between these groups on all measures. The current findings suggest that while the number of treatment seeking individuals dependent on NMP opiates has recently increased, these individuals respond well to buprenorphine/naloxone maintenance in an office-based primary care setting. Support: DA09803, DA19246

18:00 – 18:15 **S28 J. Prosser NEUROPSYCHOLOGICAL CORRELATES OF PROLONGED ABSTINENCE IN OPIOID ADDICTION** ***J. Prosser (1), L. Cohen (1), E.D. London (2), I. Galynker (1) (1) Dept. of Psychiatry, Beth Israel Medical Center, New York, NY, (2) Neuropsychiatric Institute, University of California at Los Angeles, CA*** This study tests the hypothesis that former opiate addicts who have detoxified from methadone maintenance therapy have less pronounced cognitive impairment than patients continuing long term methadone maintenance therapy. A series of neuropsychological tests were administered to 29 former heroin addicts receiving methadone maintenance treatment, 27 former heroin addicts withdrawn from all opiates (average duration of abstinence = 10.8 months), and 29 healthy controls. Both methadone maintained and abstinent subject groups performed worse than controls on tasks that measured verbal function, visual-spatial analysis and memory, and resistance to distractibility. There were no statistically significant differences in test performance between the methadone group and abstinent group in 3 of 4 cognitive tasks examined. Cognitive impairment was positively correlated with a composite score of personality ($r = .594$, $p < .001$), but did not correlate with drug use history. Our results suggest that former opiate addicts who are drug-free have a similar degree of cognitive impairment as patients receiving methadone maintenance.

18:15 – 18:30 **S29 J. Dorsey OPIOID AND NONOPIOID ANALGESICS: REPORTED FATAL EXPOSURES 1983-2003** ***J. Dorsey L. Earle Adult Addiction Clinic, Anne Arundel County Health Department Annapolis, MD*** The American Association of Poison Control Centers has a database of over 36 million poison exposure cases. To ascertain the fatalities associated with opioid/non-opioid analgesics, we reviewed the annual reports published 1984-2004. Most fatalities occurred in individuals ages 20-49. The analgesics are in 6 groups: acetaminophen only, acetaminophen plus opioid, aspirin only, aspirin plus opioid, opioids, and non-steroidal anti-inflammatory drugs. The latter have the highest exposure, but the lowest associated fatality. In contrast, opioids have the lowest exposure, but the highest fatality. The non-opioids represent over 70% of the exposures, but less than 50% of the fatalities. Opioids/opioid combinations represent less than 30% of the exposures, but over 50% of the fatalities. Combining the groups into three larger groups for exposures shows: non-opioids>opioids>opioid combinations, for fatalities/1000 opioids>opioid combinations>non-opioids. Intentional suicide represents the primary reason for fatal exposures. Intentional misuse or abuse is the second. Since this review did not involve utilization or prescribing data, comparisons between availability and exposure were not possible.

18:30 – 18:45 **S30 S.C. Roerig REDUCED ABUSE LIABILITY OF BIVALENT MU AGONIST-DELTA ANTAGONIST COMPOUNDS IN THE CONDITIONED PLACE PREFERENCE (CPP) ASSAY** ***S.C. Roerig (1), N.R. Lenard (1), D.J. Daniels (2), P.S. Portoghese (2) (1) Dept. of Pharmacol, LSUHSC-S, Shreveport, LA, (2) Dept. of Medicinal Chem., Univ. of Minn., Minneapolis, MN*** Treatment of moderate to severe pain with opioids is limited by their abuse liability. We developed a series of 6 compounds with the mu agonist α-oxymorphamine

connected by a spacer (19-26 angstroms) to the delta antagonist naltrindole (bivalents). All bivalents are effective in the tail flick assay. Antinociceptive tolerance and physical dependence do not develop to those with longer spacers when administered i.c.v. for 3 d. Here, we investigated their effects in the CPP assay. Mice were conditioned with i.v. morphine, α-oxymorphamine with a spacer attached (monovalent), or 3 of the bivalents. Place preference developed to morphine and the monovalent, but not to the bivalents. In another group, place preference to morphine was established then extinguished. Acute administration of morphine or the monovalent, but not the bivalents, reinstated the morphine place preference. Taken together, these results suggest that the bivalents may have lower abuse liability for opioid-naïve patients and reduced potential for inducing relapse in previously addicted patients.

18:45 – 19:00 **S31 S.L. Chen CHRONIC NALOXONE INDUCED REWARDING AND CRAVING EFFECTS IN KN-S196A MICE** ***S.L. Chen (1), P.L. Tao (1), P.P. Yang (1), P.Y. Law (2), H.H. Loh (2), (1) Department of Pharmacology, National Defense Medical Center, Taipei, Taiwan, R.O.C. (2) Department of Pharmacology, University of Minnesota, Minneapolis, USA*** Previously we have introduced the S196A mutation into the mouse mu-opioid receptor by a knock-in strategy and found that opioid antagonists, such as naloxone and naltrexone, elicited antinociceptive effects similar to that of partial agonists. However, chronic treatment of the homozygous mice (KN-S196A) with naltrexone did not produce the expected tolerance, whereas less physical dependence was observed than with chronic morphine treatment. In the present study, we further investigated whether naloxone would induce the rewarding/craving effects in the KN-S196A mice by using conditioned place preference (CPP) test. We found that chronic morphine treatment (5 mg/kg, s.c., 6 days) induced the rewarding and craving effects in both KN-S196A and wild type mice. However chronic naloxone treatment (15 mg/kg) induced rewarding/craving effects in the KN-S196A mice but aversive effects in the wild type mice. These data in conjunction with previous reports suggest that local expression of S196A mutant in spinal cord and systemic administration of naloxone could be the strategy of choice in controlling chronic back pain.

Wednesday, July 13

7:00 – 8:30 **Continental breakfast** **Marriott Ballroom Foyer**

8:30 – 9:30 **P4 Plenary Lecture Richard Huganir Marriott Ballroom**
Regulation of glutamate receptors and brain functions

Richard L. Huganir, Department of Neuroscience, Howard Hughes Medical Institute, Johns Hopkins University School of Medicine, Baltimore MD USA Neurotransmitter receptors mediate signal transduction at the postsynaptic membrane of synaptic connections between neurons in both the central and peripheral nervous systems. We have been studying the molecular mechanisms in the regulation of neurotransmitter receptor function and synaptic function. Recently we have focused on glutamate receptors, the major excitatory receptors in the brain. Glutamate receptors can be divided into two major classes: AMPA and NMDA receptors. AMPA receptors mediate rapid excitatory synaptic transmission while NMDA receptors play important roles in neuronal plasticity and development. Studies in our laboratory have found that both AMPA and NMDA receptors are multiply phosphorylated by a variety of protein kinases. Phosphorylation regulates several functional properties of these receptors including conductance and membrane targeting. For example, phosphorylation of the GluR1 subunit of AMPA receptors by multiple kinases including PKA, PKC and CaM kinase II regulates the ion channel function and membrane trafficking of the receptor. The phosphorylation of glutamate receptors is regulated by neuromodulators such as dopamine, norepinephrine and serotonin as well as by drugs of abuse such as cocaine and may be important for the modulation of excitatory synaptic transmission by these agents. In addition, we have demonstrated that the phosphorylation of AMPA receptors is regulated during cellular models of learning and memory such as long-term potentiation (LTP) and long-term depression (LTD). Moreover, phosphorylation of GluR1 is required for the expression of these forms of plasticity and for the retention of spatial memory. We have also recently shown that phosphorylation of another AMPA receptor subunit, GluR2, is required for LTD in cerebellar Purkinje cells. These studies demonstrate the importance of glutamate receptor phosphorylation in the regulation of synaptic transmission and plasticity underlying information processing in the brain. These modes of regulation may be critical for the initial neuronal responses to drugs of abuse and the resulting synaptic plasticity that may be important for drug dependence and addiction.

9:30 – 12:30 **Symposium V Opioid receptor regulation Marriott Ballroom**
Chair J.B. Wang,, Cochair C. Evans

9:30 – 9:55 **S32 J..B. Wang M** ***Dept. of Pharmaceutical Sci., School of Pharmacy, Univ. Maryland, Baltimore, USA*** Mu opioid receptor (MOR) phosphorylation plays a significant role in modulating receptor function. Results from various studies of cell lines expressing cloned opioid receptors suggest that the status of MOR phosphorylation could be influenced by ligand specific receptor conformation and multiple protein kinases and specific signaling molecules. Attempts have been made to identify specific phosphorylation sites and their involvement in the cellular regulation of the receptor function. This presentation describes the current level of knowledge regarding MOR phosphorylation with an emphasis on the mechanisms that regulate the receptor phosphorylation process. In our laboratory, a protein kinase C interactive protein (PKCI) was identified to undergo specific interactions with MOR. The PKCI protein contains 126 amino acids and is a ubiquitous member of the histidine triad (HIT) protein family. We provide novel evidence that the PKCI modulates function of the MOR, antagonizes receptor-mediated inhibition of adenylyl cyclase and attenuates PKC-induced MOR phosphorylation. Mice lacking PKCI show enhanced analgesic responses to acute morphine, but more rapid analgesic tolerance to repeated morphine.

9:55 – 10:15 *Coffee break*

10:15 – 10:40 **S33 G. Hendersen ROLE OF PROTEIN KINASE C IN MU-OPIOID RECEPTOR DESENSITIZATION AND MORPHINE TOLERANCE *IN VITRO* C.P. Bailey, E. Kelly, G. Henderson *Department of Pharmacology, University of Bristol, Bristol, UK*** In mature rat locus coeruleus neurones morphine produces less mu-opioid receptor (MOR) desensitization than full agonists such as DAMGO (Bailey et al. (2003) J Neurosci 23:10515-10520). We have shown that MOR desensitization can be enhanced by protein kinase C (PKC) activation either through Gq-coupled M3 muscarinic receptor activation or directly by the phorbol ester, PMA (Bailey et al. (2004) Mol Pharmacol 66:1592-1598). The enhancement of desensitization required agonist occupancy of the receptor and was homologous in that cross-tolerance to α2 adrenoceptors on the same neurones was not observed. We have now gone on to study the role of PKC in cellular tolerance to morphine in mature rat brainstem slices incubated for 6 – 9 hours in morphine *in vitro*. To measure the level of tolerance we compared the change in the maximum responses to morphine (a partial agonist) and DAMGO (a full μM, a concentration agonist) in morphine-treated and untreated slices. At 1 likely to be achieved in the brain by an analgesic dose, morphine alone induced no tolerance over this time period. Whereas, when slices were incubated with 1 μM morphine and either oxotremorine-M (10 μM), a muscarinic agonist, or PMA (1 μM) a moderate level of tolerance was observed that could be prevented by the inhibitor of conventional PKC isoforms, Go6976 (1 μM). In contrast, incubating slices with 30 μM morphine alone induced a high level microM). of tolerance that was also preventable by Go6976 (1 μM). These data demonstrate a crucial role for conventional isoforms of PKC in the development and maintenance of cellular tolerance to morphine *in vitro*. This correlates well with the observation that in the intact animal morphine tolerance can be reversed by PKC inhibitors and by antagonists of various receptors that directly or indirectly couple to PKC.

10:40 – 11:05 **S34 L.M. Bohn β-ARRESTIN-2 AND μ OPIOID RECEPTOR REGULATION IN MICE *L.M. Bohn The Ohio State University, College of Medicine, Depts of Pharmacology & Psychiatry, Columbus, OH, USA*** Our work has focused on how mu opioid receptor (MOR) regulation can lead to alterations in various physiological responses induced by opiates such as morphine. By employing a combination of physiological, behavioral and biochemical studies in the genetically modified mice, we have sought to identify the contribution of β-arrestin mediated regulation of the MOR to opiate-mediated biological effects in vivo. We have found that by genetically ablating β -arrestin-2 (Barr2) in mice, morphine-induced physiologies are profoundly altered. Barr2 knockout mice display: enhanced and prolonged analgesia; dramatically reduced antinociceptive tolerance; enhanced dopamine release; more drug reinforcement; and no apparent difference in developing physical dependence as compared to morphine effects in the wild-type controls. We have now evaluated how morphine-induced side effects, such as respiratory suppression and constipation are effected in these animals. Taken together our findings suggest that the path of receptor regulation may vary with regard to the receptor environment and that this may manifest in vastly different physiological responses induced by opiate agonists. Support: DA14600, DA18860.

11:05 – 11:30 **S35 V. Höllt MU OPIOID RECEPTOR INTERNALIZATION AND DESENSITIZATION *V. Höllt, G. Grecksch, T. Koch, M. Pfeiffer, S. Schulz, R. Stumm, D. Wu Dept. Pharmacol. & Toxicol., Otto von***

Guericke University, Magdeburg, Germany It is well known that opioids differ in their ability to induce mu receptor (MOP-r) endocytosis. For instance morphine or buprenorphine are much less able to cause MOP-r internalization than methadone, fentanyl, or opioid peptides. We recently demonstrated in MOP-r expressing HEK293 cells that the endocytotic potencies of wide variety of opioids are negatively correlated with their ability to cause receptor desensitization/tolerance indicating that endocytosis counteracts tolerance by inducing fast receptor reactivation by receptor recycling. In addition, daily application of morphine and buprenorphine to rats resulted in a progressive development of tolerance against the analgesic effect, whereas the administration of equieffective doses of etonitazene, a drug which causes rapid receptor recycling failed to cause tolerance. MOP-r endocytosis (e.g. by the opioid peptide DAMGO) is preceded by phosphorylation of several serine/theonine residues, particularly of Ser-375 at the COOH-tail. Mutation of Ser-375 to alanine or the overexpression of RKIP (Raf kinase inhibitor protein) an inhibitor of GRK-2 (G-protein-coupled receptor kinase 2) inhibited the DAMGO-induced receptor internalization. During the course of DAMGO-induced receptor recycling a rapid dephosphorylation occurs. In contrast, morphine which does not induce endocytosis causes a less intense, but prolonged phosphorylation at Ser-375. We showed recently that DAMGO and other opioids which induce receptor endocytosis, activate phospholipase D2 (PLD2) in contrast to morphine. In addition, inhibition of PLD2 activation by primary alcohols or by overexpression of a negative dominant PLD2 resulted in the inability of DAMGO to induce receptor endocytosis indicating an important role of PLD2 in opioid-induced internalization. Moreover, the observation that opioids differ in their ability to activate PLD2 indicates that additional signal transduction pathways can be induced by internalizing opioids as compared to non-internalizing opioids. The lipoproteins M6a and synaptophysin were found by yeast two hybrid screens to interact with MOP-r. These proteins strongly affect agonist-induced MOP-r internalization when co-expressed in HEK293 cells.In summary, activation of PLD2 results in agonist-induced endocytosis and recycling of MOP-r which, in turn, causes rapid receptor reactivation by dephosphorylation counteracting the development of tolerance.

11:30 – 11:45 **S36 L.-Y. Liu-Chen COMPARTMENTALIZATION OF THE KAPPA OPIATE RECEPTOR IN LIPID RAFTS ATTENUATES AGONIST-INDUCED ACTIVATION** ***W. Xu, S.-I. Yoon, P. Huang, Y.L. Wang, P. Chong, L.-Y. Liu-Chen Dept Pharmacol, Temple Univ Med Sch, Philadelphia, PA USA*** We determined if opioid receptors in the rat brain and human kappa opioid receptor (hKOR) expressed in CHO cells were localized in lipid rafts and if changes in cholesterol contents affected hKOR signaling. Lipid rafts were prepared from rat brain membranes and CHO cells using a detergent-free method and fractionation through a sucrose gradient. The majority of endogenous opioid receptors, FLAG-hKOR and Giα1-3 were present in fractions of low sucrose density, coincided with high levels of caveolin-1, flotilin-1 and cholesterol, markers of rafts/caveolae. Pretreatment with 2% methyl β-cyclodextrin reduced cholesterol content, disrupted lipid rafts, shifted caveolin-1 and FLAG-hKOR and Giα1-3 to higher density fractions, increased the affinity of U50,488H for the hKOR and enhanced U50,488H-induced [^{35}S] [^{35}S] GTPγS binding and p42/44 MAP kinase phosphorylation. Cholesterol replenishment returned FLAG-hKOR, caveolin-1 and Giα1-3 to lipid rafts and reverted hKOR signaling to the control. These findings indicate that endogenous opioid receptors and FLAG-hKOR are located in lipid rafts, which may restrict the coupling of hKOR to G proteins.

11:45 – 12:00 **S37 E. Navratilova PEPTIDE AND NON-PEPTIDE AGONISTS USE DIFFERENT MECHANISMS TO REGULATE THE HUMAN DELTA-OPIOID RECEPTOR** ***E. Navratilova, E.V. Varga, D. Stropova, S. Waite, W.R. Roeske, H.I. Yamamura The University of Arizona, Tucson, Arizona, USA*** Prolonged agonist activation of the human delta-opioid receptor (hDOR) leads to receptor desensitization by regulatory mechanisms that include receptor phosphorylation, internalization and down-regulation. We hypothesized that peptide (deltorphin II, DPDPE) and non-peptide (SNC80) agonists regulate the receptor differently using different receptor domains. We examined regulation of the hDOR by peptide and non-peptide agonists in Chinese hamster ovary cells transfected with wild type and several mutant hDOR cDNAs. We found that the C-terminus was obligatory for hDOR regulation by peptide agonists, while SNC80 was able to regulate the hDOR by utilizing other intracellular domains. Furthermore, S363 within the C-terminus played an important role in receptor desensitization and down-regulation by peptide agonists. In contrast, mutation of S363 did not significantly alter hDOR desensitization and down-regulation by SNC80. To conclude, we demonstrate that in addition to well-documented differential regulation of opioid receptors by morphine, differences also exist between peptide and non-peptide agonist-mediated desensitization of the hDOR. Support: NIH

12:00 – 12:15 **S38 D.E. Selley ACUTE ADAPTATION OF MU OPIOID RECEPTORS TO MORPHINE OCCUPANCY** ***D.E. Selley, A. Sparta, K.L. Scoggins, M.P. Cassidy, W.L. Dewey, L.J. Sim-Selley Dept.***

Pharmacology & Toxicology, Virginia Commonwealth University, Richmond, VA The literature is conflicting as to whether morphine acutely desensitizes or internalizes mu opioid receptors (MOR). Therefore, we examined the concentration- and time-dependence of morphine-induced receptor desensitization and internalization in MOR-expressing CHO cells. Results showed that relatively high concentrations of morphine (≥0.3 μM) and long pretreatment times (≥6 hr) of MOR-CHO cells were required to attenuate morphine-stimulated [^{35}S]GTPγS binding in membranes prepared from these cells. In contrast, inhibition of cAMP accumulation in intact MOR-CHO cells by morphine was significantly desensitized after 2 hr of morphine exposure. Morphine also stimulated MOR internalization in CHO cells as indicated by both immunohisto-chemical visualization and [^{3}H]CTAP binding to MOR in intact cells. However, the potency, maximal effect and rate of morphine-stimulated MOR internalization was less than that of the full agonist DAMGO. These results indicate that morphine can acutely desensitize and internalize the MOR in CHO cells, and highlights the importance of measuring MOR desensitization in intact cells. Support: DA10770, DA10647

12:15 – 12:30 **S39 J.R. Traynor ENDOGENOUS RGS PROTEINS DIFERENTIALLY MODULATE FULL AND PARTIAL MU OPIOID AGONISTS AT ADENYLYL CYCLASE** ***M.J. Clark, J.R. Traynor Dept. of Pharmacol., University of Michigan, Ann Arbor, MI, USA*** We have previously shown that endogenous regulators of G protein signaling (RGS) proteins reduce mu opioid mediated inhibition of adenylyl cyclase through Go, although the effect with morphine is more pronounced than with DAMGO. This study tests the hypothesis that partial agonists are more susceptible to the action of RGS proteins than full agonists using both Go and Gi2 proteins. Stable clones of C6 cells expressing the mu-opioid receptor and different levels of pertussis toxin-insensitive and RGS-sensitive or RGS-insensitive α subunit of Go or Gi2 were compared for inhibition of adenylyl cyclase by the full mu opioid agonist DAMGO and the partial agonists morphine, buprenorphine and nalbuphine. Compared to DAMGO the relative efficacy of partial agonists was increased significantly in the RGS-insensitive Go and Gi2 clones over RGS-sensitive Go and Gi2 clones with similar expression levels. The potency for inhibition of adenylyl cyclase was increased slightly for both full and partial agonists in the RGS-insensitive Go and Gi2 clones compared with clones expressing RGS-sensitive G proteins. Thus partial agonists are more sensitive to the action of RGS proteins. Support: DA04087

12:30 – 14:00 **Lunch** **Marriott**

12:30 – 14:00 **Executive committee meeting** **Marriott First Floor Meet Rm**

14:00 - 15:30 **NIDA Update & Grant Writing Workshop for Young Investigators**
L. Miner, R. Liu (*NIDA*) with E Unterwald, Mark Green and others

This Workshop is designed to prepare early career researchers to write and submit successful NIH/NIDA-funded proposals. This session will provide an overview of NIDA's priority research areas, current funding mechanisms and the peer-review process. It will present strategies to effectively navigate the NIH grant-writing system.

Afternoon and evening otherwise free

Thursday, July 14

7:00 – 8:30 **Continental breakfast** **Marriott Ballroom Foyer**

8:30 – 9:30 **P5 Plenary Lecture** **A.G. Phillips** **Marriott Ballroom**
Memory and addiction: Double duty for corticolimbic circuits

A.G. Phillips, Department of Psychiatry and Institute of Mental Health, Univ. of British Columbia, Vancouver, Canada There is a growing awareness that memory functions play a critical role in the development and maintenance of addictive behaviors. This lecture will review recent data implicating transcortical glutamategic pathways and subcortical dopamine projections to the medial prefrontal cortex and ventral stiatum in working memory function. Evidence for cortical control of dopaminergic activity in the ventral striatum will then be described as a prelude to a discussion of cortico-limbic pathways involved in relapse to drug-seeking behavior. Specific experiments will demonstrate important roles for glutamatergic projections from both the ventral subiculum and the basolateral nucleus of the amygdala to the ventral striatum and the role of different members of the glutamate and dopamine receptor

families. Psychostimulant-induced behavioral sensitization also has been shown to be dependent of aspects of the same cortico-limbic circuitry, giving rise to the hypothesis that drug-induced craving may also involve experience-dependent changes in synaptic plasticity within glutamatergic projections to the ventral striatum. We have strong evidence for a role of long-term depression within the ventral striatum as a critical factor in the expression of behavioral sensitization. The implications of these data for the role of memory in addictive behavior will be discussed.

9:30 – 12:30 **Symposium VI New opioid compounds Marriott Ballroom**
Chair W. Schmidt, Cochair I. Carroll

9:30 - 9:55 **S40 W. Schmidt EXCITING TIMES FOR NOVEL OPIOIDS, 2005** ***W. K. Schmidt, Renovis, Inc., South San Francisco, CA, 94080, USA*** Advances in opioid therapeutics have generally come slowly and have been dominated more by advances in dosage formulation technology than in novel therapeutics. Before our understanding of the molecular nature of opioid receptors and signal transduction, most of the advances in opioid therapeutics were dominated by full or partial agonist compounds working on spinal and supraspinal mu opioid receptors where therapeutic differences were heralded by milligram potency, synthetic pedigree, or pharmacokinetics. Naltrexone and nalmefene advanced non-selective opioid reversal and addiction therapeutics a generation ago; newer delivery systems may improve efficacy and compliance. Advances in both analgesia and opioid antagonism may come from an appreciation of the role of peripheral opioid receptors in analgesia and gastrointestinal function. Peripherally-acting opioid antagonists (alvimopan, methylnaltrexone) are on the horizon for treating opioid bowel dysfunction and postoperative ileus. Clinical studies have demonstrated analgesic efficacy with locally or topically-administered opioids acting as peripheral opioid agonists; studies with peripherally-acting mu and kappa opioid agonists have shown promise although peripheral vs. central selectivity remains a challenge. Compounds which combine activity at mu and delta opioid receptors or at opioid and non-opioid receptors promise the possibility of newer centrally-acting opioid analgesics which may be devoid of respiratory depression, euphoria, dysphoria, addiction liability, and other CNS actions of current opioid analgesics. Other therapeutic opportunities may become increasingly important as we think beyond pain/analgesia/addiction to anxiety, depression, schizophrenia, neuroendocrine function, immune modulation, GI function, and other ancillary areas where modulation of opioid receptors may be used for therapeutic benefit.

9:55 – 10:15 *Coffee break*

10:15 – 10:40 **S41 I. Carroll SELECTIVE KAPPA OPIOID RECEPTOR ANTAGONIST JDTIC BLOCKS STRESS-INDUCED REINSTATEMENT OF COCAINE REINFORCED RESPONDING AND HAS ANTIDEPRESSANT-LIKE EFFECTS IN RATS** ***I. Carroll (1), P. Beardsley (2), J. Howard (3) (1) Org. & Med. Chem., Research Triangle Inst., Research Triangle Park, NC, (2) Dept. Pharmacol. & Toxicol., School of Med., Virginia Commonwealth Univ., Richmond, VA, (3) Howard Assoc., LLC, Research Triangle Park, NC USA*** During the last few years, we have conducted research directed toward development of selective kappa opioid receptor antagonists. The major overall goal was to discover and develop pharmacotherapies to treat cocaine relapse. These studies lead to discovery of the highly potent and selective kappa opioid receptor antagonist JDTic. In the [^{35}S] GTP-γ functional assay, JDTic showed no agonist activity at levels of 10 μM, possessed a Ke of 0.01 nM for the kappa receptor, and is 341- and 7930-fold selective for the kappa receptor relative to mu and delta receptors. JDTic reversed antinociception of kappa agonists in mice and squirrel monkeys and antagonized kappa agonist-induced diuresis in rats. It showed activity using subcutaneous, intramuscular, and oral routes of administration. In this study, we determine whether JDTic could reduce the ability of a stressor (intermittent footshock) to reinstate responding previously reinforced with cocaine infusion and to have antidepressant-like effects in the forced swim test (FST) in rats. JDTic prevented stress-induced relapse in a rat cocaine self-administration paradigm and significantly decreased immobility and increased swimming time in the FST in rats, a test that suggests antidepressant activity. Stress and depression are two states during cocaine abstinence that are known to precipitate relapse. The fact that JDTic can attenuate both stress and depression makes it a candidate for development as a pharmacotherapy for cocaine relapse. Its merit as a potential pharmacotherapy is further supported by its highly favorable toxicity profile in several *in vitro* tests, its low toxicity in mice and rats, and its opioid receptor selectivity in a 61 assay NovaScreen.

10:40 – 11:05 **S42 B.L. Roth NOVEL KOR LIGANDS REVEAL MODE OF BINDING OF SALVINORIN A** ***B.L. Roth (1), F. Yan (1), T. Vortherms (1), J. Stuart (2), J. Zjawiony (2) R. Westkaemper (3) (1) Dept. Biochemistry, Case Western Reserve University Medical School, Cleveland, OH, (2) Dept. Pharmacology, School of***

Pharmacy, University of Mississippi, University, MS; (3) Medicinal Chemistry, Medical College of Virginia, Richmond, VA USA Salvinorin A is a naturally-occurring hallucinogenic diterpenoid from the plant Salvia divinorum that selectively and potently activates κ-opioid receptors (KORs). Salvinorin A is unique in that it is the only known lipid-like molecule which selectively activates a peptidergic G-protein coupled receptor (GPCR); salvinorin A is also the only known non-nitrogenous opioid receptor agonist. In this manuscript we identify key residues in KORs responsible for the high binding affinity and agonist efficacy of salvinorin A. Surprisingly, we discovered that salvinorin A was stabilized in the binding pocket by a interactions with tyrosine residues in helix 7 (Tyr313 and Tyr320) and in helix 2 (Tyr119). Intriguingly, activation of KORs by salvinorin A required interactions with the helix 7 tyrosines Tyr312, Tyr313, and Tyr320 and with Tyr139 in helix 3. By contrast, the prototypical nitrogenous KOR agonist U69593 and the endogenous peptidergic agonist dynorphin A (1-13) showed differential requirements for these three residues for binding and activation. We also employed a novel approach whereby we examined the effects of cysteine-substitution mutagenesis on the binding of salvinorin A and an analogue with a free sulfhydryl group—salvinorinyl-2-thiol. These data imply that structurally diverse agonists utilize different residues within a binding surface for binding and activation of peptide receptors. We discovered that residues predicted to be in close proximity—especially Tyr 313--to the free thiol of salvinorinyl-2-thiol when mutated to Cys showed enhanced affinity for 2-thiosalvinorin B. Taken together, these findings imply that the diterpenoid salvinorin A utilizes unique residues within a commonly shared binding pocket to selectively activate KORs (Supported by NIDA and NIMH).

11:05 -11:20 **S43 H. Umeuchi NALFURAFINE HYDROCHLORIDE (TRK-820): A POSSIBLE NEW ANTIPURITIC AGENT** ***H. Umeuchi (1) , Y. Togashi (2), K. Nakao (2), K. Takeshita (1) , K. Kawamura (2) , T. Kurokawa (2) , M. Itoh (2) , T. Endoh (2) , H. Miyakawa (3) , J. Kamei (4), H. Nagase (5), K. Okano (2) (1) Pharmaceutical Clinical Research Dept., Toray Industries Inc., Chiba, Japan, (2) Pharmaceutical Research Laboratories, Toray Industries Inc., Kanagawa, Japan, (3) Fourth Department of Internal Medicine, Teikyo Univ. Sch. of Med., Kanagawa, Japan, (4) Department of Pathophysiology and Therapeutics, Hoshi Univ., Sch. Pharm. Pharm. Sci., Tokyo, Japan, (5) Department of Medical Chemistry, Sch. Pharma. Sci., Kitasato Univ., Tokyo, Japan*** Pruritus is defined as an unpleasant cutaneous sensation, which provokes the desire to scratch. Antihistamine-resistant pruritus is more common in clinical settings and is observed in patients with many diseases such as atopic dermatitis, cholestasis and chronic renal failure. Recently, the involvement of mu-opioid system on itch pathway has been suggested, i.e. elevation of endogenous mu-opioid tone in itchy patient and central originated itching with epidural and intrathecal morphine. According to the recent studies, kappa-opioid system produces many opposite effect to mu-opioid system, indicating the possibility that the activation of kappa-opioid system exert antipruritic effect. Nalfurafine hydrochloride (TRK-820) has a unique chemical structure and an agonistic activity to kappa-opioid receptor. 1) Nalfurafine reduced the pruritogen-induced scratching behavior, intracisternal morphine in addition to intradermal histamine and substance P in mice or monkeys. 2) Nalfurafine was also effective against spontaneous scratching behavior in NC/Nga mice and MRL/lpr mice, atopic dermatitis and autoimmune diseases, respectively. 3) The antipruritic effect of nalfurafine was abolished by nor-BNI, a kappa-opioid receptor antagonist, pretreated intracerebroventricularly. Those results suggest that the mechanism of systemic administration of the kappa-opioid receptor agonist on antipruritic effect may be the blockage of itch pathway by antagonizing the activation of central mu-opioid receptor. In the clinical trials, moreover, nalfurafine showed an antipruritic effect against severe uremic pruritus. Nalfurafine is likely to reduce itch sensation itself and has potential therapeutic values against antihistamine-resistant pruritus in a new concept.

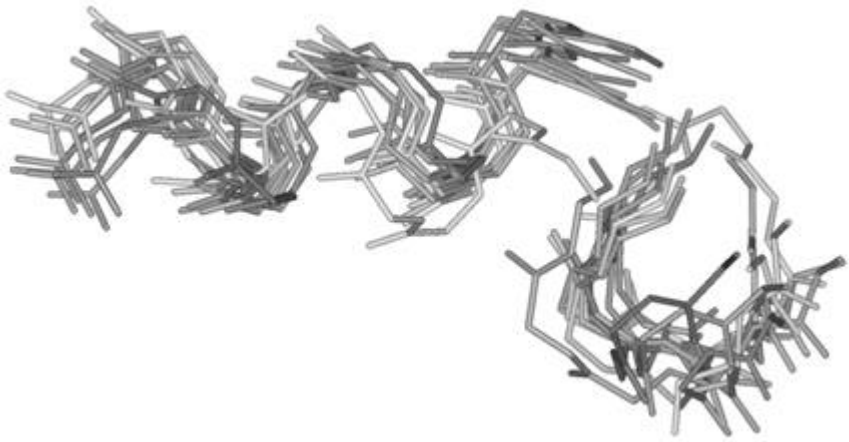
11:20 – 11:35 **S44 H. Schmidhammer NOVEL, HIGHLY POTENT OPIOID AGONISTS AND ANTAGONISTS IN THE MORPHINAN SERIES** ***H. Schmidhammer Dept. of Pharmaceutical Chemistry, University of Innsbruck, Innsbruck, Austria*** The cornerstone in the development of opioid agonistic 14-alkoxymorphinans was the synthesis of the highly potent analgesic 14-O-methyloxymorphone in 1984. This mu opioid receptor agonist shows high antinociceptive potency but also the usual side effects associated with morphine type compounds. Further development led to the very potent analgesic 14-methoxymetopon (HS 198) which exhibited considerably less pronounced side effects than morphine although interacting predominantly with mu opioid receptors. Introduction of arylalkyl substituents such as a phenylpropoxy group in position 14 gave rise to a series of extremely potent analgesics with binding affinities in the subnanomolar range to all three opioid receptor types. Very recently, ionizable 6-amino acid derivatives of 14-O-methyloxymorphone which are not capable of crossing the blood-brain barrier were developed. These compounds show very high antinociceptive potency in acute and inflammatory pain models by interacting with mu opioid receptors located in the periphery and thus do not exhibit the usual CNS related

side effects of centrally acting opioid analgesics. Replacing the methyl substituent at the morphinan nitrogen in this class of 14-alkoxy substituted morphinans by cyclopropylmethyl or allyl resulted in highly potent opioid antagonists and partial agonists.

11:35 -11:50 **S45 C. Mitch DISCOVERY OF NEW OPIOID RECEPTOR ANTAGONISTS** ***C. Mitch, Discovery Chemistry Research and Technologies, Eli Lilly and Company, Indianapolis, IN, USA*** Many nonpeptide opioid antagonists are structurally derived from the morphinan scaffold, as exemplified by naloxone, naltrexone and nalmefene. The phenylpiperidine structural series has also been a rich source of opioid antagonist compounds, as exemplified by LY255582, alvimopan and JDTic. Our laboratories have recently prepared phenylpiperidine analogs with carboxamide substitution on the aryl ring and results will be presented showing them to have potent opioid antagonist activity along with good oral bioavailability in rat. Additionally, a new structural scaffold with good opioid antagonist activity that is structurally unrelated to either the morphinan or phenylpiperidine platforms will be discussed.

11:50 – 12:05 **S46 E.E. Codd THE NOVEL DELTA OPIOID RWJ-394674 IS BIOTRANSFORMED TO THE POTENT MU OPIOID, RWJ-413216** ***E.E. Codd, J.R. Carson, R.W. Colburn, S.L. Dax, D. Desai-Krieger, R.P. Martinez, L. McKown, L.A. Neilson, P.M. Pitis, P. Stahle, D.J. Stone, A. Streeter, W.N. Wu, S.P. Zhang J&J PRD, Spring House, PA USA*** Although the mu opioid receptor is the primary target of marketed opioid analgesics, several studies suggest the advantageous effect of combinations of mu and delta opioids. The novel compound RWJ-394674 bound with high affinity to the delta opioid receptor, and somewhat weaker affinity to the mu opioid receptor. GTPγS binding assays demonstrated its delta agonist and mu antagonist functionality. Surprisingly given its delta opioid profile, mouse hot plate (48°C) evaluation of RWJ-394674 revealed potent oral antinociception, accompanied by a moderate Straub tail. Probe antagonist studies demonstrated attenuation of the antinociception by delta and mu subtype selective antagonists. *In vitro* studies demonstrated that RWJ-394674 was metabolized to RWJ-413216, a potent mu opioid agonist. Pharmacokinetic studies in the rat revealed that oral administration of RWJ-394674 rapidly gave rise to RWJ-413216, which itself demonstrated potent oral antinociceptive effect. RWJ-394674 is a delta opioid agonist that appears to augment its antinociceptive effect through biotransformation to a novel mu opioid selective agonist.

12:05 – 12:20 **S47 R. Polt BIOUSIAN GLYCOPEPTIDES [ENDORPHIN ANALOGUES] PENETRATE THE BBB** ***R. Polt (1), R.D. Egleton (2), E.J. Bilsky (3), L. Yeomans-Maldonado (1), M. Dhanasekaran (1), C.M. Keyari (1), I. Alves (1), F. Porreca (2), V.J. Hruby (1), P. Davis (2), H.I. Yamamura (2) (1) Carl S. Marvel Labs, Department of Chemistry, Univ. of Arizona, Tucson, AZ (2) Dept. of Pharmacology, AZ Health Sciences Center, Tucson, AZ (3) Dept. of Pharmacology, Univ. of New England, Biddeford, ME USA***



Glycosylated endorphin analogues designed to penetrate the blood-brain barrier (BBB) have been studied by circular dichroism (CD) and by 2D-NMR in the presence of water; TFE/water; SDS micelles; and in the presence of both neutral and anionic bicelles. In water, the glycopeptides show nascent helix behavior & random coil conformations. In all cases, the glycopeptides were largely helical in the presence of membrane bilayer models (micelles or bicelles). Plasmon waveguide resonance (PWR) studies showed hen egg phosphatidyl choline (PC) bilayers produce amphipathic

helices lying parallel to the membrane surface, with dissociation constants (KDs) in the low nanomolar to micromolar concentration range. Two low energy states are suggested for the glycosylated endorphin analogues, a flexible aqueous state and a restricted membrane bound state. Biousian behavior is crucial for penetration of the BBB *via* transcytosis. Support: N00014-02-1-0471 (ONR)

12:30 – 15:30 **Lunch and Poster Session III** **Historic Inns**

Structure-Activity Relationships

Th1 A STUDY WITH CHIMERIC PEPTIDES OF Met-ENKEPHALIN and FMRFa: EFFECT OF HALOGENATION AND C-TERMINAL MODIFICATION ***K. Hanif, K. Gupta, S. Gupta, S. Manikandan, S. Pasha Institute of Genomics and Integratuve Biology, Delhi, India*** A chimeric peptide of Met-enkephalin and FMRFa, YFa (YGGFMKKKFMRFa) was synthesized to understand role of MERF. In previous study, YFa produced dose dependent, naloxone reversible antinociception and delayed development of tolerance to morphine. To improve bioavailability and better central nervous system entry of chimeric peptide, a halogenated analogue of YFa, [D-Ala^2, pCl^4] YAGFMKKKFMRFa, was synhesized. Octanol/saline distribution studies confirmed that due to chlorination, [D-Ala^2, pCl^4] YFa showed more lipophilicity and significanltly enhanced analgesia upon i.p. administration. YGGFMKKKFMRa, another analogue of YFa designed to test the importance of C-terminal RFamide on antinociception of YFa, produced significantly lower analgesia upon i.p. administration. So chlorination seems to have enhanced lipophilicity of [D-Ala^2, pCl^4] YFa to cross BBB more effectively and thus produced more analgesia. Lowered analgesia by [Des-Phe^{12}]YFa indicated that chimeric peptide, besides opioids receptors, is possibly interacting with non opioid binding sites (e.g. anti-opioid receptors) also. Biophysical studies showed YFa has a propensity to adopt α-helix which was confirmed by preliminary data of molecular modeling.

Th2 GLYCOSYLATION OF ENKEPHALINS PROMOTES PENETRATION OF THE BBB ***L. Yeomans-Maldonado (1), C.M. Keyari (1), R.D. Egleton (2), E.J. Bilsky (3) R. Polt (1) (1) Carl S. Marvel Labs, Dept. Chemistry, Univ. of Arizona, Tucson, AZ (2) Dept. Pharmacology, AZ Health Sciences Center, Tucson, AZ (3) Dept. Pharmacology, Univ. of New England, Biddeford, ME USA*** Opioid peptides do not typically cross the blood-brain barrier (BBB). Their therapeutic use has been severely limited due to pharmacokinetic issues such as serum stability as well as their limited BBB permeability. Previous work with delta-opioid glycopeptide agonists has shown that these compounds have extended serum lifetimes, and cross the BBB to produce potent analgesia in mice. Both mixed mu/delta selective enkephalin glycopeptides (e.g. Tyr-dThr-Gly-Phe-Leu-[Glycosyl]Ser-CONH2) and highly mu-selective glyocopeptides (e.g. Tyr-dAla-Gly-[N-Me]Phe-[Glycosyl]Ser-CONH2) show enhanced penetration of the BBB. CD and NMR studies indicate that these peptides are unstructured (random coil) in H2O, but associate with micelles where they adopt preferred turn structures that may be relevant to both BBB transport as well as binding to opiate receptors. Support: ONR (N00014-02-1-0471).

Th3 β-ENDORPHIN BIOTRANSFORMATION IN THE RAT STRIATUM: EVIDENCE FOR THE EXTRACELLULAR ACTIVITY OF INSULIN-DEGRADING ENZYME ***B. Reed, B.T. Chait, M. J. Kreek Rockefeller Univ., New York, NYUSA*** Numerous studies have investigated behavioral effects of β-endorphin (BEND), but the potential for biotransformation of BEND in the extracellular space has, to our knowledge, not been addressed. Using methodology we developed for dynorphin A(1-17) extracellular processing in the rat brain, utilizing microinfusion/microdialysis and MALDI mass spectrometry, we investigated BEND biotransformation in the striatum of Fischer rats. We infused 1.0 nmol BEND and observed rapid cleavage resulting in BEND(1-18) and various N-terminal truncation fragments. In *in vitro* studies using isolated striatum, we observed BEND (1-18) and (1-17) as well as the complement peptides (19-31) and (18-31), in addition to (2-18), (2-17), and (20-31). Addition of an aminopeptidase inhibitor prevents observation of BEND (20-31), (2-17), and (2-18), with no effect on the initial cleavage fragments. The pattern of cleavage sites including Leu17-Phe18 and Phe18-Lys19 is consistent with published *in vitro* studies of BEND cleavage by purified insulin degrading enzyme. Support: DA00049, DA05130 (MJK), NCRR Grant RR00862 (BTC)

Th4 FROM A SPECIFIC ANTAGONIST TO POTENT AGONISTS – BIOLOGICAL AND PHARMACOLOGICAL ACTIVITIES OF NOVEL DERIVATIVES OF CYPRODIME ***M. Spetea (1), F. Schüllner (1), R.C. Moisa (2), I.P. Berzetei-Gurske (2), M.D. Aceto (3), L.S. Harris (3), A. Coop (4), H. Schmidhammer (1) (1) Dept. of Pharmaceut. Chem., Univ. of Innsbruck, Austria, (2) SRI International, Biosci.***

Div., Menlo Park, USA, (3) Dept. Pharmacol. Toxicol., Virginia Commonwealth Univ., Richmond, USA, (4) Dept. Pharmaceut. Sci., Univ. Maryland, Sch. Pharmacy, Baltimore, MD, USA Cyprodime was reported as the first non-peptidic, competitive pure and specific mu opioid receptor antagonist. We have recently reported on the major impact of a phenylpropoxy group in position 14 in morphinan-6-ones to interact with opioid receptors. 14-Phenylpropoxy derivatives of cyprodime were developed and characterized by biological and pharmacological methods. A significant increase in affinity at mu receptors and greater mu selectivity were observed compared to cyprodime. In the [^{35}S] GTPγS functional assay, all tested compounds were partial agonists at mu and delta receptors, while they showed antagonism or some partial agonism at kappa receptors. Pre-incubation of rat brain membranes with the new derivatives resulted in wash-resistant inhibition of mu receptors, while cyprodime acted as a reversible ligand. When tested in vivo in mice, these compounds were considerably more potent than morphine in different analgesic tests. In conclusion, introduction of a 14-phenylpropoxy substituent leads to a profound alteration in the pharmacological profile of cyprodime.

Th5 THE ANTINOCICEPTION INDUCED BY TAPS: INVOLVEMENT IN THE ENDOGENOUS KAPPA-OPIOID PEPTIDE ***R. Urushiyama (1), K. Ito (1), H. Watanabe (1), A. Yonezawa (1), H. Mizoguchi (1), C. Watanabe (1), T. Fujimura (2), K. Murayama (2), T. Sakurada (3), S. Sakurada (1) (1) Dept. of Physiol. and Anat., Tohoku Pharmaceut. Univ., Sendai, Japan, (2) Div. of Biochem. Anal. Ctr. Lab. of Med. Sci., Juntendo Univ. School of Med., Tokyo, Japan, (3) Dept. of Biochem., Daiichi Coll. of Pharmaceut. Sci., Fukuoka, Japan*** Intrathecal (i.t.) injection of H-Tyr-D-Arg-Phe-Sar-OH (TAPS), an N-terminal tetrapeptide analogue of dermorphin, produced an antinociceptive effect in the mouse. The effect of TAPS was completely abolished by i.t. pretreatment with either a selective mu-opioid receptor antagonist β-funaltrexamine or a mu1-opioid receptor antagonist naloxonazine, indicating that the antinociception induced by TAPS is mainly mediated through the situmulation of mu1-opioid receptor. However, i.t. pretreatment with nor-binaltorphimine, a kappa-opioid receptor antagonist, or an antiserum against dynorpihin B(1-13) also significantly attenuated the TAPS-induced antinociceptive effect. These results indicate that the antinociception induced by i.t.-administered TAPS is mediated by the stimulation of mu1-opioid receptor, which may promote the release of dynorphin B(1-13) in the mouse spinal cord.

Th6 ANTINOCICEPTIVE PROPERTY OF A NOVEL DERMORPHIN TETRAPEPTIDE ANALOG AMIDINO-TAPA ***K. Moriyama, K. Ohwada, H. Mizoguchi, C. Watanabe, A. Yonezawa, S. Sakurada Dept. of Physiol. and Anat., Tohoku Pharmaceut. Univ., Sendai, Japan*** Antinociceptive property of a novel dermorphin tetrapeptide analog amidino-TAPA was characterized in the mouse tail-flick test. Intrathecal (i.t.) administration of amidino-TAPA showed a potent and long-lasting antinociception. Amidino-TAPA-induced antinociception was significantly attenuated by i.t. pretreatment with mu-opioid receptor antagonist β-funaltrexamine, mu1-opioid receptor antagonist naloxonazine, kappa-opioid receptor antagonist nor-binaltorphimine and delta-opioid receptor antagonist naltrindole. Moreover i.t. pretreatments with antisera against endogenous kappa-opioid peptides dynorphin A, dynorphin B and α-neo-endorphin, and endogenous delta-opioid peptide [Leu5]-enkephalin attenuated the antinociception induced by amidino-TAPA. The present results suggest that amidino-TAPA given spinally stimulates novel mu1-opioid receptor different from heretofore known mu1-opioid receptor, and subsequently increases the release of endogenous kappa opioid peptides dynorphin A, dynorphin B, α-neo-endorphin, and endogenous delta opioid peptide [Leu5]-enkephalin to produce antinociception.

Th7 FUNCTIONAL SIGNIFICANCE OF A METHYL GROUP: MODULATING KAPPA OPIOID ANTAGONIST ACTIVITY THROUGH CONFORMATIONAL RIGIDITY ***S. Runyon, H. Navarro, F.I. Carroll RTI, Research Triangle Park, NC USA*** The discovery of trans-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines by Zimmerman et al. introduced a structurally unique class of opioid antagonists. Prior to this development, opioids typically required N-allyl or N-cyclopropylmethyl substituents to obtain functional antagonism. trans-3,4-Dimethyl-4-(3-hydroxyphenyl)piperidines were unique since the functional properties of this structural class appeared to be modulated by the configuration and presence of the 3-position methyl group as opposed to the N-substituent. We have synthesized and evaluated a series of 6-(3-hydroxyphenyl)-3-azabicyclo[3.1.0]hexanes as conformationally rigid trans-3,4-dimethyl-4-(3-hydroxyphenyl)piperidines. N-[trans-3'-(2-Methylphenyl)-2'-propenyl]-6-methyl-6-(3-hydroxyphenyl)-3-azabicyclo[3.1.0]- hexane possessed a Ke value of 2.9 nM as a kappa antagonist in [^{35}S] GTPγ S *in vitro* functional assays and showed no agonist activity at 10 uM. A more detailed understanding of this structural class may lead to potent and selective kappa antagonists for the treatment of opioid addiction. Support: DA09045

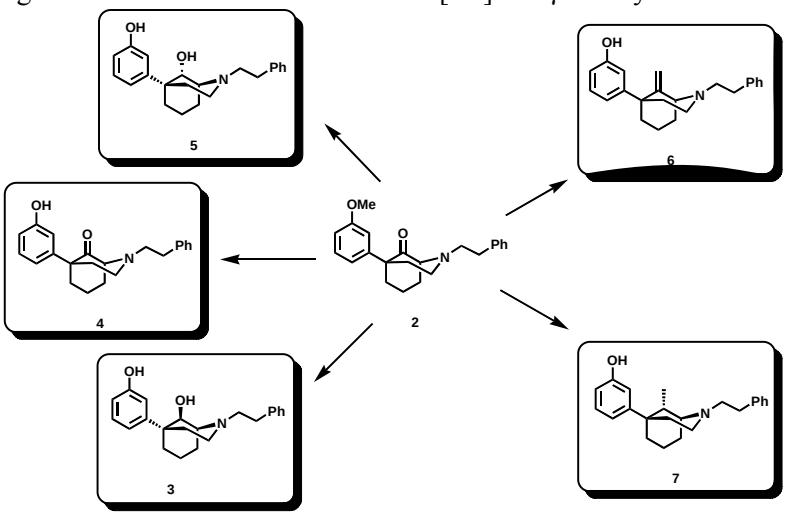
Th8 DIFFERENTIAL ANTINOCICEPTIVE EFFECTS OF [DMT1]ENDOMORPHIN-1 and [DMT1] ENDOMORPHIN-2 IN MICE ***Y. Jinsmaa (1), Y. Fujita (2), K. Shiotani (2), A. Miyazaki (3), T. Li (2), Y. Tsuda (2,3,4), Y. Okada (2,3,4), A. Ambo (5), Y. Sasaki (5), E. Marczak (1), S.D. Bryant (1), L.H. Lazarus (1) (1) Med. Chem. Group, Lab of Pharmacol. Chem., Natl. Inst of Environ. Health Sci, Res. Triangle Park, NC, USA, (2) Grad. Sch. Food Med. Sci, (3) Faculty Pharm. Sci, Dept. Med. Chem. and (4) High Tech. Res. Center, Kobe Gakuin Univ., Kobe, Japan, (5) Dept. Biochem., Tohoku Pharm. Univ., Sendai, Japan*** Replacement of the Tyr by Dmt (2',6'-dimethyl-L-tyrosine) in endomorphin-1 and -2 changes the opioid receptor subtypes involved in antinociception of the compounds. Using hot-plate (supraspinal) and tail-flick (spinal) assays in the combination with opioid antagonists (NAL, NTI, β-FNA, NAZ), we analyzed the antinociception of [Dmt1]endomorphin-1 and [Dmt1] endomorphin-2 in mice. The endomorphin analogues were more potent than their natural endogenous peptides. Furthermore, our data indicated that spinal effect of compounds primarily was mediated by mu2- and delta-receptors; however, while a supraspinal mechanism of [Dmt1]endomorphin-2 involved both mu1/mu2-, only mu2-subtype was responsible for the effect of [Dmt1]endomorphin-1. Therefore, dimethylation of Tyr enhanced mu- and delta-receptor activity of the compounds.

Th9 SYNTHESIS OF N-PHENETHYL PARA-E- AND PARA-F-OXIDE-BRIDGED PHENYL- MORPHANS ***J. Zezula (1), L.B. Singer (1), A.K. Przybyl (1), J. Deschamps (2), D. Parrish (2), A.E. Jacobson (1), K.C. Rice (1) Lab. Medicinal Chemistry, NIDDK, NIH, DHHS, Bethesda, MD, (2) Lab. for the Structure of Matter, Naval Research Lab., Washington DC USA*** With the synthesis of these oxide-bridged phenylmorphans and others formerly prepared we have a series of isomeric compounds essential for our study of the three-dimensional pattern needed for optimum binding to opioid receptors as agonists or antagonists and, as well, to explore the effect of the N-substituent on opioid activity. This work describes the synthesis of racemic and enantiopure N-phenethyl derivatives of para-e- and para-f-phenylmorphans from commercially available 2-fluorophenyl acetonitrile. Utilizing our reported synthetic sequence, key intermediates were prepared. The oxide-bridge was closed by base-induced cyclization of alcohols onto the fluorinated aromatic ring as recently reported for the e-isomer. Demethylation was achieved in two steps, and direct alkylation gave the corresponding N-phenethyl derivatives. The nitro substituent was converted into hydroxyl in three steps. Resolutions of selected intermediates succeeded via formation and recrystallization of diastereomeric salts with chiral acids.

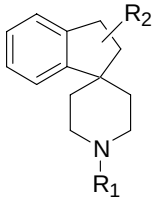
Th10 A PORTRAIT OF POTENT MU- AND DELTA-OPIOID RECEPTOR LIGANDS ***L.H. Lazarus (1), S.D. Bryant (1), Y. Jinsmaa (1), E. Marczak (1), Y. Okada (2), Y Tsuda (2), Y. Fujita (2), T Li (2), K. Shiotani (2), A. Miyazaki (2), A. Ambo (3), Y. Sasaki (3), G. Balboni (4), S. Salvadori (5) (1)Med. Chem., LPC, NIEHS, RTP, NC, (2) Fac. Pharm. Sci., Kobe Gakuin Univ., Kobe, Japan, (3)Tokoku Pharm. Univ., Sendai, Japan, (4) Dept. Toxicol., Univ. Cagliary, Cagliary, Italy, (5) Dept. Pharm. Sci., Univ. Ferrara, Ferrara, Italy*** Tail-to-tail condensation of Dmt (2',6'-dimethyl-L-trosine) or Dmt-Tic (1,2,3,4-tetrahydroisoquinoline-3-carboxylate), with diaminoalkane or 3,6-aminoalkyl-pyrazinone gave opioids with high affinities and bioactivities. While Dmt-NH-X only exhibited weak mu properties, bis-[Dmt-NH]-alkyl had Ki(mu) <0.1 nM, weak Ki(delta) and mu antinociception. Alkyl-pyrazinone linker maintained high mu affinity, increased mu selectivity and transit through the BBB. Symmetric or asymmetric dimeric Dmt-Tic had high Ki(delta) 0.06-1.5 nM, Ki(mu) 0.4-6 nM, potent delta antagonism (pA2, 10.3-11.2) and weak mu agonism (>3 uM). Affinity and bioactivity of dimeric ligands depend on length and chemical composition of linker, which augments activity. Small size and computational model of delta receptor exclude binding to multiple receptors. Data suggest multiple recognition sites in a receptor.

Th11 SYNTHERSIS AND EVALUATION OF NOVEL ENANTIOMERIC N-Phenylethyl-5-phenylmorphans ***A.-C. Hiebel (1), G. De Martino (1), R.B. Rothman (2), C.M. Dersch (2), J. Deschamps (3), A.E. Jacobson (1), K.C. Rice (1) (1) Lab. Med. Chem., NIDDK, NIH, Bethesda, MD USA (2) Clin. Psychopharm. Sec., NIDA, NIH, Baltimore, MD USA (3) Lab. Struct. Matter, Naval Res. Lab., Washington, DC USA*** New enantiomeric *N*-phenylethyl-5-phenylmorphans were synthesized and submitted for testing for their specific binding to the mu, delta and kappa opioid receptors. Over the years, the opioid system has been intensively investigated. Simplification of the morphine skeleton has yielded a variety of ligands such as the 5-phenylmorphan class of analgesics. This class of compounds was first examined by May in the 50's and has yielded a broad range of agonists, antagonists and inverse agonists of the opioid receptors. In continuation with the extensive work that has been ongoing in our laboratory on the 5-phenylmorphans, compounds (+)- and (-)-3-7 were synthesized from common intermediates (+)- and (-)-2 (Scheme 1). The optical resolution of the *N*-Me precursor of 2 was accomplished by selective salt crystallization. The (+)- and (-

)-isomers of 3-7 have been submitted for evaluation of their opioid receptor binding affinity profile. The higher affinity ligands were further examined in the [^{35}S]GTPγS assay.



Th12 SPIROCYCLIC INDANES AS LIGANDS FOR THE NOP (ORL-1) RECEPTOR ***R.R. Goehring, X. Zhou, J.-C. Huang, L.J. Barnett, Q. Sun, S.F. Victory, K.J. Valenzano, W.S. Miller, S. Shan, D.J. Kyle Discovery Research, Purdue Pharma, L.P., Cranbury, NJ, USA*** The NOP (formerly ORL-1) receptor is the most recently identified opioid receptor. Despite the homology with other opioid receptors, the NOP receptor and its native ligand, Nociceptin/Orphanin FQ (N/OFQ), are clearly distinct. Classical opioids bind poorly to the NOP receptor. In addition, N/OFQ has little affinity for the mu, kappa and delta opioid receptors. As part of a program to identify novel ligands for the NOP receptor, high throughput screening identified the spirocyclic indane/piperidine ring system as a useful scaffold. Synthetic exploration has lead to a series of potent and selective NOP antagonists which were shown to bind competitively with N/OFQ to a common binding site at the NOP receptor. The SAR in this series, atypical for NOP ligands, will be presented.



Th13 THE NEW DELTA OPIOID ANTAGONIST, TYR-TIC–(2S,3R)βMEPHE-PHE-OH REVEALS DISTINCT DELTA SITES IN RAT AND MOUSE BRAIN ***M. Szucs (1), G.Toth (1), I. Kertesz (1), L. Bakota (2), K. Gulya (2), J. Pintar (3), E. Birkas (1) (1) Biological Research Center, Szeged, (2) Dept. Cell Biol., SZTE, Szeged, Hungary, (3) UMDNJ, Piscataway, NJ, USA*** Tyr-Tic-(2S,3R)βMePhe-Phe-OH and [3H]Tyr-Tic-(2S,3R)βMePhe-Phe-OH (specific activity 53.7 Ci/mmol) were synthesized. A single site binding with a KD=0.28 +/- 0.001 nM and Bmax=155 +/- 6.6 fmol x mg protein –1 was detected in rat brain membranes. Na+ increased the binding affinity showing the antagonist character of the new ligand. There were fewer binding sites with higher affinity in wt mouse brain. No specific labeling was detected with receptor autoradiography in DOR-KO mouse brain. Competition binding assays revealed that the new ligand is highly delta specific. Interestingly, unlabeled Tyr-Tic-(2S,3R)βMePhe-Phe-OH displaced more binding than the the prototypic delta ligands Ile5,6-deltorphin II and naltrindole in mice but not in rats. Naltrindole and Tyr-Tic-(2S,3R)βMePhe-Phe-OH also differed in their ability to block the stimulating effect of the delta agonist DTLET in the [^{35}S] GTPγS functional assay in mouse brain membranes. These results support the existence of delta opioid receptors with distinct ligand binding profile. Support: OTKA TS 049817 research fund

Th14 FURTHER STUDY OF THE STRUCTURE-ACTIVITY RELATIONSHIPS OF NEOCLERODANE DITERPENES AT OPIOID RECEPTORS ***T. Prisinzano (1), W. Harding (1), K. Tidgewell (1), M. Schmidt (1), C.M. Dersch (2), R.B. Rothman (2) (1) Division of Medicinal and Natural Products Chemistry, University of Iowa, Iowa City, IA, (2) Clinical Psychopharmacology Section, IRP, NIDA, NIH, DHHS, Baltimore, MD USA*** The

neoclerodane diterpene, salvinorin A, was recently reported to be a potent and selective kappa opioid receptor agonist *in vitro* and *in vivo*. Salvinorin A is a hallucinogen isolated from the Mexican mint plant Salvia divinorum. Currently, S. divinorum and salvinorin A are gaining popularity as unscheduled hallucinogens available for purchase over the internet. Interestingly, salvinorin A bears no structural similarity to classical hallucinogens, dissociatives, or opioid receptor ligands. Currently, there is little information available as to why this compound is selective for kappa opioid receptors. One approach is to systematically alter the structure of salvinorin A and examine the effects on opioid receptor affinity and activity. Here, we report the identification of the first neoclerodane diterpene that is a κ opioid receptor agonist *in vitro* and *in vivo*. This represents the identification of a novel structural class of κ opioid receptor agonists.

Th15 XENOPUS LAEVIS PRODYNORPHIN SEQUENCE REVEALS NOVEL FAMILY MEMBERS OF THE OPIOID PEPTIDES ***S. Benyhe (1), P. Pattee (2), S.R. Nagalla (2), E-A. Ilie (1), G. Toth (1), A. Borsodi (1) (1) Inst. Biochem., Biol. Res. Ctr., Hungarian Acad. Sci., Szeged, Hungary (2) Center for Biomarker Discovery, Oregon Health and Sci. Univ., Portland, Oregon USA*** To identify potential new members of the opioid peptides we used the opioid 'message' motif (Tyr Gly-Gly-Phe) as an anchor sequence to clone partial cDNA's from the African clawed frog, Xenopus laevis, using RT-PCR. Two distinct isoforms (Xendorphin A and B) of an opioid prohormone were isolated from a Xenopus brain cDNA library that could generate multiple novel opioid ligands, same or different from known members of this family. Both precursor isoforms are present in a wide variety of tissues including the brain. Two potential hexadecapeptides, Xen-dorphin-1A and 1B, exhibited naloxone – reversible opioid agonist activity in frog (Rana esculenta) and rat brain membranes using a [^{35}S] GTPγS assay. *In vitro* radioligand binding assays demonstrated high kappa receptor affinities of Xen-dorphin-1B and -A. Xendorphin-1 peptides are unique in that these contain a novel 'Xenopus' enkephalin sequence, Ile5-enkephalin. Beside Xendorphin-1, the propeptide cDNAs encode also for a big peptide with potential opioid activity (Xendorphin-3 with a length of 43 amino acids), α-neo-endorphin and Xenopus dynorphin A. Cloning and characterization of the Xen-dorphin peptides provides new evidence for the potential presence of other members in the opioid superfamily. Support: NKTH-RET-DNT-2004 grant from the Fund Management Directorate of the Ministry of Education, Budapest, Hungary

Th16 MORPHINE-6-GLUCURONIDE INDUCES CONDITIONED PLACE PREFERENCE WHEREAS MORPHINE-3-GLUCURONIDE INDUCES CONDITION PLACE AVERSION IN MICE ***V. Olsen, M. Handal, Å. Ripel, F. Boix, J. Mørland Norwegian Institute of Public Health, Division of Forensic Toxicology and Drug Abuse, Oslo, Norway*** In humans, morphine is conjugated to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). M6G has been shown to produce analgesia, whereas M3G did not possess analgesic activity. The effects of the morphine-glucuronides in reward and induction of permanent neurobiological changes related to development of drug dependence have not been well studied. Handal et al have showed that M6G induced locomotor activity that lasted longer than morphine, whereas M3G did not increase locomotor activity. Conditioned place preference (CPP) is considered to be a more specific test for drug reward than locomotor activity. In the present study CPP was registered after s.c. injections of different doses of morphine, M3G and M6G in male C57BL/6J-Bom mice, using a 2 compartment counterbalanced paradigm. Morphine and M6G induced CPP, whereas M3G caused condition place aversion (CPA). The magnitude of the CPP and CPA was dose dependent. Thus, conditions influencing morphine metabolism and the ratio between M3G and M6G might affect the rewarding properties of morphine and its precursor heroin.

Th17 NALFURAFINE CAUSES SPECIES-SPECIFIC DIURESIS THROUGH KAPPA OPIOID RECEPTORS ***S. Inan (1), D.Y.W. Lee (2), L.Y. Liu-Chen (1), Z. Ma (2), B. Cohen (2), A. Cowan (1) (1) Dept. Pharmacology, Temple Univ. School of Medicine, Philadelphia, PA, (2) McLean Hospital, Harvard Medical School, Belmont, MA, USA*** Activation of kappa opioid receptors causes water diuresis in mice and rats. We examined possible diuretic effects of two new kappa opioid agonists, nalfurafine and salvinorin A, in these species. U50,488 served as the standard kappa agonist. Mice (male SW, 25-30 g) and rats (male SD, 200-250 g) received ad lib water and food before s.c. U50,488 (0.03-10 mg/kg in mice and 0.10-10 mg/kg in rats), nalfurafine (0.0015-0.05 mg/kg in mice and 0.005-0.04 mg/kg in rats), salvinorin A (1-40 mg/kg in mice and 1-10 mg/kg in rats) or vehicle. The animals (n= 6-10) were placed individually in metabolism cages with no food and water for 5 h. Neither nalfurafine nor salvinorin A increased urine flow in mice. An inverted U-shaped dose response curve was obtained with U50,488 with maximum diuresis at 0.1 mg/kg. Nalfurafine and U50,488 caused diuresis in rats in a dose-dependent manner. Salvinorin A had no marked effect. Preinjection (-30 min) with GNTI (0.1 mg/kg, s.c.), a kappa opioid antagonist, blocked the diuretic effect of

U50,488 (0.1 mg/kg) in mice (p= 0.0008) and nalfurafine (0.02 mg/kg) in rats (p= 0.0004). Our results show that the diuretic effect of nalfurafine is species-specific and is mediated by kappa opioid receptors. The lack of diuretic effect with salvinorin A may be due to its rapid metabolism.

Th18 A STUDY WITH CHIMERIC PEPTIDES OF Met-ENKEPHALIN AND FMRF: EFFECT OF HALOGENATION AND C-TERMINAL MODIFICATION ***K. Hanif, K. Gupta, S. Gupta, S. Manikandan, S. Pasha Institute of Genomics and Integratuve Biology, Delhi, India*** A chimeric peptide of Met-enkephalin and FMRFa, YFa (YGGFMKKKFMRFa) was synthesized to understand role of MERF. In previous study, YFa produced dose dependent, naloxone reversible antinociception and delayed development of tolerance to morphine. To improve bioavailability and better central nervous system entry of chimeric peptide, a halogenated analogue of YFa, [D-Ala2, pCl4] YAGFMKKKFMRFa, was synhesized. Octanol/saline distribution studies confirmed that due to chlorination, [D-Ala2, pCl4] YFa showed more lipophilicity and significanltly enhanced analgesia upon i.p. administration. YGGFMKKKFMRa, another analogue of YFa designed to test the importance of C-terminal RFamide on antinociception of YFa, produced significantly lower analgesia upon i.p. administration. So chlorination seems to have enhanced lipophilicity of [D-Ala2, pCl4] YFa to cross BBB more effectively and thus produced more analgesia. Lowered analgesia by [Des-Phe12]YFa indicated that chimeric peptide, besides opioids receptors, is possibly interacting with non opioid binding sites (e.g. anti-opioid receptors) also. Biophysical studies showed YFa has a propensity to adopt α-helix which was confirmed by preliminary data of molecular modeling.

Th19 LY255582 INHIBITS BASAL, MORPHINE- AND FEEDING-INDUCED DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS SHELL (NAs) ***M.A. Statnick, A.E. Sahr, B.J. Eastwood, C.H. Mitch Lilly Research Laboratories, Indianapolis, IN USA*** Substantial evidence supports the central opioid system in regulation of goal orientated behaviors. Opioids may mediate these behaviors via modulation of the mesolimbic dopamine (DA) system. In the present study we sought to compare LY255582 and naltrexone on a) morphine-induced, b) basal and c) feeding-induced DA release in the NAs. Male rats implanted with microdialysis probes in the NAs received morphine (10.0 mg/kg, s.c.) in combination with naltrexone or LY255582. Basal DA was assessed in rats receiving either naltrexone or LY255582 alone. Feeding-induced DA release was monitored 1 hour following treatment with LY255582 or naltrexone, respectively. Samples were analyzed with HPLC-ED. Naltrexone and LY255582 reversed morphine-induced increases in NAs extracellular DA levels with similar potencies. Interestingly, naltrexone did not alter basal DA release, while LY255582 significantly decreased basal DA release. Moreover, we found that LY255582, but not naltrexone, inhibited consumption of palatable food and the associated rise in NAs DA levels. These data support that LY255582 exhibits intrinsic efficacy that differs from the morphinan antagonist naltrexone.

Th20 THE DEVELOPMENT OF TOLERANCE TO FENTANYL DERIVATIVES IS RELATED TO THEIR POTENCY AND ANALGESIC DURATION ***T. Wen, J.E. Pintar Dept. of Neurosci, UMDNJ, USA*** Since fentanyl was introduced in the late 50s, multiple fentanyl derivatives have been synthesized. Among them, sufentanyl and alfentanyl are characterized by their exceptionally high potency (5-10 times higher than fentanyl) and short analgesic duration (5-15min), respectively. Although these compounds have been used clinically, their tolerance characteristics and mechanisms of action have not been studied systematically in rodents. In our studies, we established the dose-response curves of morphine, fentanyl, sufentanyl and alfentanyl in C57BL/6J WT, DOR-1KO and MOR-1KO mice. Our data first demonstrate that morphine and all fentanyl derivatives tested produce their analgesic effect through the MOR-1, while DOR-1 does not play a role in acute analgesia. The analgesic timecourse curves for each drug were also determined. At the dose of ED80, the tolerance curves for each drug were tested for 10 days. In comparison with morphine, sufentanyl showed a significant delay in the development of tolerance, while an intermediate delay was seen for fentanyl and alfentanyl. Thus, the onset of tolerance to fentanyl derivatives is negatively correlated to the potency of the drug and positively correlated to its analgesic duration.

Th21 DEVELOPMENT OF OPIOID GLYCOPEPTIDES AS TREATMENTS FOR ACUTE AND CHRONIC PAIN ***J. Lowery (1), R. Paolino (1), L. Yeomans (2), J. Bidlack (3), R. Polt (2), E. Bilsky (1) (1) Univ. New England, Biddeford, ME; (2) Univ.Arizona, Tucson, AZ; (3) Univ. Rochester Medical Center, Rochester, NY USA*** Enkephalin stability and CNS bioavailability can be increased by addition of a serine glycoside. We compare the delta-selective glycopeptides with that of a highly mu-selective glycopeptide, LYM-147 (Tyr-DAla-Gly-[N-Me]Phe-[Lactosyl]Ser-CONH2) in mouse models of nociception and in assays that pick up mu-mediated side-effects. LYM-147 exhibited extraordinary potency following i.c.v.administration in the 55°C tail-flick assay; A50 value (95% CI) of 2.2 pmol (1.7-

3.0 pmol). The compound was also very potent following i.v. and s.c. injection; A50 values of 1.15 (0.82-1.64) and 2.60 (1.89-3.56) µmol/kg, respectively. We next compared side-effect profiles of LYM-147 with our delta/mu agonists. LYM-147 produced greater stereotypic circling and increased locomotor activity compared to equieffective antinociceptive doses of the delta/mu compounds. LYM-147 also produced greater levels of physical dependence as indexed by naloxone-precipitated withdrawal. We are currently assessing effects on GI transit and respiratory depression.

Th22 *IN VITRO* AND *IN VIVO* CHARACTERIZATION OF OPIOID INVERSE AGONISTS AND NEUTRAL ANTAGONISTS ***E. Bilsky (1), D. Wang (2), W. Sadée (2), (1) Univ. New England, Biddeford, ME; (2) Ohio State Univ., Columbus, OH USA*** Our group and others have previously provided evidenced of increased basal activity at opioid receptors in tissues exposed to morphine. *In vitro* and *in vivo* evidence suggests that naloxone and naltrexone act as inverse agonists in the opioid dependent state, whereas several close analogs of these compounds exert a more neutral antagonist profile. The current studies provide additional data correlating the degree of opioid exposure to changes in basal signaling in various brain regions. We also provide updated pharmacological data on several of the naltrexone analogs and their *in vitro* and *in vivo* profiles of activity at the cloned opioid receptors and respective heterodimers. The results indicate that the level of basal signaling following morphine exposure is tissue/region dependent. 6ß-Naltrexol appears to be a neutral antagonist at MOR and DOR, and an inverse agonist at KOR in the opioid dependent state. In contrast, 6ß-naltrexamide appears to be a neutral antagonist at all three cloned opioid receptors. The *in vivo* significance of these findings is currently being investigated.

Th23 A NOVEL MU OPIOID AGONIST BASED ON SALVINORIN A ***K.J. Tidgewell (1), R.A. Moyer (3), L.M. Bohn (3), W.W. Harding (1), C. Dersch (2), R.B. Rothman (2), T.E. Prisinzano (1) (1) College of Pharmacy, Univ. Iowa, (2) IRP, NIDA, NIH, DHHS, Baltimore, MD, (3) Depts. Pharmacology & Psychiatry, Ohio State Univ USA.*** Mu opioid receptor ligands are used to treat pain and heroin dependence. The major active component of the hallucinogenic sage Salvia divinorum, salvinorin A, is a potent and selective kappa receptor agonist. Salvinorin A analogs were synthesized and herkinorin was found to have significant affinity and activity at mu opioid receptors using the [125I]-IOXY and [^{35}S]GTP-γ-S assays, respectively. Salvinorin A and herkinorin also show significant antinociceptive effect in both the first and second phases of formalin induced pain in rats. Interestingly, 24-hour pre-treatment with the kappa selective antagonist nor-BNI enhanced the anti-nociceptive effects of herkinorin. Herkinorin is a novel mu opioid ligand with analgesic activity and may act through a unique mechanism of action.

Th24 PHARMACOKINETICS AND BEHAVIORAL EFFECTS OF SALVINORIN A, A NATURALLY OCCURRING KAPPA-OPIOID HALLUCINOGEN, IN NON HUMAN PRIMATES ***E.R. Butelman (1), M.D. Schmidt (2), M.S. Schmidt (2), W.W. Harding (2), K. Tidgewell (2), D.J. Murry (2), M.J. Kreek (1), T.E. Prisinzano (2) (1) Rockefeller Univ., New York NY, USA, (2) Univ. Iowa, Iowa City IA, USA*** Salvinorin A, the main active compound from the widely available hallucinogenic plant, Salvia divinorum, is a high efficacy kappa-opioid agonist *in vitro*. The *in vivo* effects of salvinorin A have only been studied under a limited set of conditions, and its *in vivo* pharmacokinetics have not been reported in any species. The pharmacokinetics of bolus i.v. salvinorin A (0.032 mg/kg) were studied in 4 rhesus monkeys (2 male, 2 female), using a recently developed LC-MS technique. Salvinorin A had a relatively short elimination half-life (approximately 57 min); salvinorin B (a known inactive metabolite) was not robustly detected in these studies. Salvinorin A (0.032-0.1 mg/kg; i.v.) caused dose-dependent, sedative-like, unresponsiveness to environmental stimuli (n=3 males). These behavioral effects of salvinorin A were prevented by naltrexone (0.32 mg/kg, s.c.) and reversed by nalmefene (0.1 mg/kg, i.v.). Support: DA11113, DA17369 (ERB); DA05130, DA00049 (MJK), U. of Iowa Biol. Sci. Funding Program.

Th25 CHARACTERIZATION OF A PUTATIVE NOVEL ENDOGENOUS PEPTIDE LIGAND FOR THE MU OPIOID RECEPTOR ***B. Anton (1), M. Matus (1), H. Gompfh (2), J.C. Calva (1), A. Salazar (1), R. Arreola (1), L. Parra-Gamez (3) R. Acevedo (1), P. De los Heros (4), B. Peng (5), S.L. Cruz (6), G. Gamba (4), C. Allen (2), J. Pintar (5), P. Leff (1). (1) Instituto Nacional Psiquiatria-RF Mexico, (2) CROET Oregon Hlth. & Sci. Univ. Portland, OR, USA, (3) Fac. Med., Dpto. Anatomia, UNAM, (4) IIB & INCMNSZ, Mexico, (5) UMDNJ-RBJ Med. Sch., Piscataway, NJ, USA, (6) Dpto. Farmacol. Cinvestav, Mexico*** Several molecular approaches have been used for searching for endomorphin-1 (E-1) and endomorphin-2 (E-2) precursor(s) protein(s) and its cloning has remained as an elusive molecular achievement. In the present study, antigen affinity-purified antibodies for E-1-2 were used for immunoscreening brain cDNA expression libraries transfected into E. coli. A positive transformant expressing

endomorphin-like immunoreactivity (E-1-2-LI) was detected on blotted nitrocellulose filters. After repeated subcloning steps, a single cDNA was cloned and characterized at structural level. This cDNA encodes an mRNA containing a single ORF coding for a deduced 91 aa polypeptide. Furthermore, this protein displays structural features of a prepropeptide protein precursor and encodes a peptide sequence of 8 aa flanked by endopeptidase processing consensus motifs. Moreover, this peptide contains a non alfa-amidated endomorphin-like peptide sequence at its C-terminal domain. Competitive radioligand binding assays showed a high affinity binding profile of this peptide for mu opiod receptors (i.e., Ki= 2-10 nM). In contrast to E-1-2, this peptide behaved as a full agonist for this opioid receptor subtype in dose-response assays stimulating the [^{35}S] GTP-γ-S binding. Furthermore, *in vitro* agonist-induced mu opioid receptor internalization assays on CHO cells showed the agonist role of this peptide on this opioid receptor subtype. This peptide was capable to induce in a dose-response manner a significant decrease in the cell firing of hypothalamic neurons, and effect blocked by CTOP. *In vivo*, this peptide showed significant antinociceptive effect after ICV injection and potent facilitatory actions on sexual behavior after local injection in medial preoptic region in rodent. Finally, the mRNA encoding the protein precursor from this peptide showed a wide distribution throughout the CNS and lymphoid tissue from spleen in rodent, which suggests a putative role of this novel peptide system into a wide range of functions in brain and immune system. Support: INP-2040 & Fundacion Gonzalo Del Rio Arronte

Th26 PLASMON WAVEGUIDE RESONANCE (PWR) SPECTROSCOPY, A NOVEL AND SENSITIVE TOOL TO EXAMINE LIGAND BINDING TO THE HUMAN CANNABINOID RECEPTOR ***T. Georgieva (1), E. Varga (1), M. Eaton (1), Z. Salamon (2), V.J. Hruby (2), W.R. Roeske (1), G. Tollin (2), H.I. Yamamura (1); Depts Medical Pharmacology (1), Biochemistry (2), Univ. Arizona, Tucson, AZ, USA*** PWR can provide information about conformational changes in lipid-embedded proteins by measuring resonant electron oscillations (plasmons) generated by a polarized laser light in a thin dielectric layer-coated silver film on the surface of a prism (resonator). Resonance is detected at an incident angle determined by the thickness and refractive index of the proteolipid deposited on the resonator surface. We have employed this novel method to study ligand binding to the human cannabinoid receptor (hCB1). A recombinant epitope-tagged hCB1 protein was solubilized and partially purified using affinity chromatography. The purified receptor was incorporated into a lipid bilayer on the surface of the PWR resonator. PWR spectroscopy demonstrated that cannabinoid agonists exhibit high affinity (Kd=0.2 nM and 5nM, for CP 55,940 and WIN 55,212-2, respectively) to the purified lipid-embedded hCB1. Further investigations are in progress to study the effect of structurally different cannabinoids on the conformation of the hCB1. Support: NIH.

Memory

Th27 DELTA OPIOID RECEPTORS MODULATE MEMORY PROCESSES: EVIDENCE FROM BEHAVIORAL ANALYSIS OF KNOCK-OUT MICE ***G. Scherrer (1), S. Grappi (2), F. Hartmann (1), A. Matifas (1), C. Gambarana (2) and B.L. Kieffer (1) (1) IGBMC, CNRS/INSERM/ULP, UMR7104, Illkirch, France (2) Dept. Neuroscience, Pharmacology Unit, Univ. Siena, Siena, Italy*** Our first behavioral analysis of delta knockout mice has revealed deficiencies in emotional responses. We now have extended our observation of the mutant mice. We found that delta knockout mice exhibit both spontaneous hyperactivity and habituation deficit, suggesting a participation of delta receptors in the regulation of exploratory activity. Because this phenotype can result from defects in learning and memory processes, we challenged mutant mice in several memory paradigms. In a two-phase Y-maze exploration test mutant mice display impaired preference for the novel arm. In vanilla sugar appetitive behavior, mutant mice show significantly increased wrong choices when searching for vanilla pellets. In Pavlovian fear conditioning mutant mice display significant decreased freezing response to the context. In accordance with the receptor expression in hippocampus and amygdala, those results suggest that enkephalins modulate memory processes through activation of delta receptors.

Th28 EFFECTS OF KAPPA-OPIOID RECEPTOR AGONIST ON THE DEFICITS OF LEARNING AND MEMORY IN MICE ***T. Mamiya, J. Inagaki, T. Asanuma, M. Ukai Dept. Chem. Pharmacol., Fac. Pharm., Meijo Univ., Nagoya, Japan*** It is well known that opioid system regulates the learning and memory. In our previous study, both the activations of mu- and delta-opioid receptors induce the impairment of the ability, whereas very low doses of mu-opioid receptor agonist attenuate the scopolamine-induced dysfunction. It has been reported that kappa-opioid receptor agonists (dynorphin and U-50,488H) improve the deficits induced by chemicals, ischemia and carbon monoxide. Taken together, because it is possible that kappa-opioid receptor agonists have anti-amnesic effects, we evaluated the U-50,488H on the Aβ25-35 protein-induced learning and memory deficits in novel recognition task in mice. We used male ICR strain mice (6 weeks old, Nihon SLC Co. Ltd., Shizuoka) in this study. Aβ25-35 (3 nmol/ 3

μL/ mouse, i.c.v.) (Bachem AG) was administered after the 4 day-incubation for aggregation. Seven days later, the novel recognition test, which is a nonaversive task that relies on a mouse's natural exploratory behavior, was done. When mice were exposed to two new objects in the training session, control (vehicle-treated) mice preferred the novel object. The administration of Aβ25-35 showed the exploration for each object at the same extent, suggesting that Aβ25-35 impaired the memory retention in this task. U-50,488H reversed the memory retention deficits by Aβ25-35 to the control level, indicating that U-50,488H may have an anti-amnesic effect. Western blotting analysis revealed that Aβ25-35 reduced the phosphorylation of CaMKIIα subunit in the brain. U-50,488H also attenuated the decrease in this phosphorylation. These results suggest that U-50,488H may improve the memory deficits via NMDA receptor/CaMKII cascade.

Th29 A UNIQUE PATTERN OF BEHAVIORAL EFFECTS OF BIG DYNORPHIN, A PRODYNORPHIN DERIVED PEPTIDE IS MEDIATED THROUGH NMDA RECEPTORS ***A. Kuzmin (1,2), N. Madjid (1), S.O. Ogren (1), L. Terenius (2), G. Bakalkin (2. Depts (1) Neurosci. and (2) Clinical Neurosci., Karolinska Institutet, Stockholm, Sweden*** We previously reported that big dynorphin (Big Dyn) consisting of dynorphin A (Dyn A) and dynorphin B (Dyn B) produces nociceptive behavior mediated through the NMDA receptors when administered i.t. in low fmol amounts (Tan-No et. al., 2002). Here we studied effects of Big Dyn administered i.c.v. on behaviour in mice using the passive avoidance, open field and elevated plus maze tests. Big Dyn, Dyn A and Dyn B enhanced aversive learning. Big Dyn also increased locomotor and exploratory activity and produced anxiolytic – like action in the open field test, and increased time spent in the open branches of the elevated plus maze apparatus with no changes in general locomotion. Dyn A and Dyn B acted through kappa-opioid receptors whereas effects of Big Dyn were mediated through NMDA receptors. These data suggest that Big Dyn has a unique pattern of memory enhancing, locomotor and anxiolytic-like effects different from those of the prodynorphin-derived ligands for kappa-opioid receptors.

Th30 PERSISTENT DISRUPTION OF AN ESTABLISHED MORPHINE CONDITIONED PLACE PREFERENCE FOLLOWING RECONDITIONING ***M.H. Milekic, S.D. Brown, C. Castellini, C.M. Alberini Dept. Neuroscience, Mt. Sinai School of Medicine, New York, NY USA*** In human addicts, establishment of compulsive addiction responses such as craving and drug-seeking behavior is frequently evoked by exposure to reminder cues. This response has been hypothesized to rely on a memory-like consolidation processes. We tested whether inhibiting consolidation-like processes erases addiction behavior. We found that, like conventional memories, both a new and an established morphine conditioned place preference (mCPP) are persistently disrupted if protein synthesis is inhibited either during conditioning or following reactivation, respectively. Importantly, disruption of an established mCPP requires that reactivation evokes a concomitant re-experience of both the conditioned context and the internal state induced by the drug. The established CPP can be abolished by selectively inhibiting protein synthesis in the hippocampus (HC), basolateral amygdala (BLA) or nucleus accumbens (NAc), but not in the ventral tegmental area (VTA). The loss of mCPP appears to be permanent as it does not re-instate after further conditioning. Support: R21 DA 017672, Hirschl Foundation (CMA)

Regulation of Opiate Systems

Th31 MOR EXPRESSION IS INDUCED IN DENTATE GYRUS GRANULE CELLS AFTER FOCAL CEREBRAL ISCHEMIA AND STIMULATION OF ENTORHINAL AFFERENTS ***R. Stumm, H. Rüthrich, V. Höllt. Inst. Pharmacol./Toxicol., Otto-von-Guericke-Univ. Magdeburg, Germany*** Focal ischemia in the cerebral cortex affects the inducibility of long-term potentiation (LTP) in the hippocampus. This impairment of hippocampal function may result from excessive activation of cortico-hippocampal afferents and subsequent perturbation of hippocampal LTP-relevant transmitter systems including opioids. Here, we tested if focal ischemia and electrical afferent stimulation influence expression of the MOR in the rat hippocampus. After focal ischemia, the entire ipsilateral cortical hemisphere and hippocampus experienced sustained excitation as indicated by a long-lasting increase in the expression of ARG mRNA, a marker for neuronal activity. Expression of MOR mRNA was dramatically increased in granule cells, which contain very low MOR mRNA levels under normal conditions, but not in GABAergic neurons, which express the MOR constitutively. In contrast to the dentate gyrus, MOR expression was unaltered in the hippocampus and in non-infarcted cortical areas. Repetitive high frequency stimulation of cortico-hippocampal afferents induced strong MOR mRNA expression throughout the granular layer. Weak tetanization sufficient to induce LTP and ARG expression did not influence MOR expression. This provides evidence for upregulation of MORs in granule cells experiencing sustained excitation by cortical afferents. In activated, MOR-expressing granule cells, inhibitory opioids may counterregulate glutamatergic excitation by cortical inputs.

Th32 COMPARISON OF MU-OPIOID RECEPTOR DESENSITIZATION IN RAT LOCUS COERULEUS NEURONS AND IN HEK293 CELLS ***E.A. Johnson, E. Kelly, G. Henderson, C.P. Bailey Dept. Pharmacology, Univ. Bristol, UK*** In the present study we have directly compared MOR desensitization in HEK293 cells with rat locus coeruleus (LC) neurons. HEK293 cells were stably transfected with MOR1 receptors and transiently transfected with the G-protein activated inwardly rectifying K+ (GIRK) channel. Horizontal rat brain slices containing the LC were prepared. GIRK currents were measured by whole-cell patch clamp recordings, and used as real-time measures of MOR activation. To compare directly desensitization elicited by different MOR agonists (morphine and DAMGO), receptor-saturating concentrations (10-30 □M) of each were applied for 7-10min. In HEK293 cells, morphine and DAMGO both caused high degrees of receptor desensitization (morphine = 84±1%; DAMGO = 73±1%), whereas in rat LC neurons, DAMGO and Met-Enk caused significantly more desensitization than morphine (morphine = 10±2%; DAMGO = 64±2%). In rat LC neurons but not in HEK293 cells, activation of PKC increased the extent of agonist-induced desensitization whereas in HEK293 cells PKC inhibitors reversed a component of desensitization These findings demonstrate profound differences in agonist-induced MOR desensitization between HEK293 cells and rat LC neurons.

Th33 MU-OPIOID RECEPTOR (MOR) DESENSITIZATION AND TRAFFICKING BY MORPHINE AND 6-MONOACETYL-MORPHINE ***C.P. Bailey, E. Braksator, S.J. Mundell, E. Johnson, E. Kelly, G. Henderson Dept. Pharmacology, Univ. Bristol, UK*** 6-Monoacetyl-morphine (6-MAM) is the major breakdown product of heroin found in aqueous solutions and plasma. In this study we have compared MOR activation, desensitization and trafficking elicited by morphine and 6-MAM to that elicited by DAMGO. MOR activation and desensitization were studied by whole-cell patch-clamp recording from rat locus coeruleus neurones. The maximum responses evoked by receptor saturating concentrations of 6-MAM and morphine were similar but smaller than that produced by the full agonist DAMGO indicating that 6-MAM and morphine are both partial agonists. The responses evoked by 6-MAM and morphine both desensitized at similar rates and extents, and significantly less than desensitization caused by DAMGO. In HEK293 cells stably transfected with MOR1 receptor-saturating concentrations of morphine and 6-MAM caused only small amounts of MOR1 internalisation whereas DAMGO induced significantly greater MOR1 internalisation. Arrestin-2 translocation was only seen following MOR1 activation by DAMGO, not by morphine or 6-MAM. These data show no functional differences in the effects of 6-MAM and morphine both of which act as partial agonists at the MOR.

Th34 FEEDBACK REGULATION OF OPIOID RECEPTOR ENDOCYTOSIS BY ADENYLYL CYCLASES ***H. Ammer, A.I. Giesen, R. Schulz Institute of Pharmacology, Toxicology and Pharmacy, University of Munich, Germany*** High-efficacy agonists induce tolerance by opioid receptor (OR) desensitization, internalization and down-regulation. In contrast, morphine circumvents these adaptations producing tolerance by compensatory changes at the post-receptor level. One of these reflects sensitization of adenylyl cyclase (AC), which also accounts for the development of dependence. Here we report that in [delta]OR transfected COS-7 cells chronic morphine treatment significantly increases agonist-induced receptor endocytosis in the presence of co-transfected AC isoforms that mediate the induction of AC supersensitivity. Studies with various AC isoforms (types II, V, VI) and constructs thereof (AC V-C1) revealed that regulation of [delta]OR endocytosis requires functional interaction of inhibitory G[α]-subunits with their associated AC isoforms, which in turn promotes up-regulation of GRK2 levels during chronic morphine treatment and a more pronounced agonist-induced redistribution of GRK2 and [β]arrestin1 towards the cell membrane. These results indicate that persistent inhibitory AC signaling produces feedback regulation of the intracellular machinery involved in agonist-stimulated OR desensitization.

Th35 DOWNREGULATION OF KOP-R IN BRAINS OF RATS WITHDRAWN FOR 14 DAYS FROM AN ESCALATING DOSE "BINGE" COCAINE ADMINISTRATION PARADIGM ***A. Bailey, R. Gianotti, A. Ho, M.J. Kreek Lab. Biology of Addictive Diseases, Rockefeller Univ., New York, NY, USA*** There is evidence showing that the opioid systems play an important role in cocaine addiction and withdrawal. To determine whether cocaine and/or chronic withdrawal from cocaine alters the kappa- opioid system, we performed quantitative autoradiographic mapping of kappa opioid receptors (KOP-r) in the brains of rats treated with escalating dose "binge" cocaine and of rats withdrawn from cocaine for 14 days. Rats were injected with saline or cocaine three times daily at 1 h intervals for 14 days. Similarly treated rats were withdrawn from cocaine for 14 days. A significant increase in KOP-r binding was detected in brains of cocaine treated rats. In sharp contrast, there was a significant decrease of KOP-r binding in the

septum and basolateral amygdala of rats withdrawn from cocaine, compared to rats at the end of 14 days cocaine administration. These results reconfirm that KOP-r undergo upregulation in response to chronic "binge" cocaine. The observed decrease in KOP-r binding which was shown in two brain regions of cocaine withdrawn animals might contribute to the decrease in dysphoria a long time after the discontinuation of the drug.

Th36 MECHANISMS OF MU OPIOID RECEPTOR TRANSCRIPTIONAL REGULATION BY INTERLEUKIN-4 ***J. Kraus, C. Börner, S. Kolbitz, V. Höllt Dept. Pharmacology, Magdeburg University, Germany*** Expression of mu opioid receptors (MOR) in immune cells is strictly regulated and normally repressed. However, the cytokine interleukin-4 (IL-4) strongly induces de novo transcription of the gene via the transcription factor STAT6, which binds at nt – 997 to the MOR promoter, as previously reported. Here we show, that in the Jurkat T cell model IL-4, and IL-4-inducing agents like cannabinoids (see abstract Borner et al), upregulate the MOR gene not only directly via STAT6, but also via additional mechanisms involving the transcription factor GATA3. In this scenario, STAT6 first transactivates expression of GATA3, as demonstrated by knocking out STAT6 by decoy oligonucleotides. Then, GATA3 binds at nts –963 and –954 to the MOR gene promoter and additionally transactivates the MOR gene, as demonstrated in transfection experiments and with decoy oligonucleotides directed against GATA3. Suggesting a physiological function of MOR also in T helper cell biology, the IL-4-inducible transcription of MOR is completely repressed by interferon-γ (200 ng/ml), which is characteristical for T helper cell type 2 genes.

Th37 DIFFERENTIAL EXPRESSION OF THE KAPPA OPIOID RECEPTOR ON MONOCYTES/ MACROPHAGES ***C.M. Tipton, J.M. Bidlack Dept. Pharmacology and Physiology, Univ. Rochester, Rochester, NY, USA*** Previous studies have demonstrated functionality of the kappa opioid receptor (KOR) on monocytes/macrophages in modulating cytokine/chemokine production, phagocytosis, and chemotaxis. In the current study, the human monocyte cell line, U-937, was used to study changes in KOR gene expression in response to activation by lipopolysaccharide (LPS) and differentiation by phorbol myristate acetate (PMA). Expression of KOR mRNA was analyzed via semi-quantitative RT-PCR and quantitative real-time PCR. Results showed that KOR mRNA increased 3-fold with LPS stimulation and 10-fold when LPS is added with interferon-γ. This increase of KOR mRNA did not occur until approximately 12 hr post-stimulation, suggesting an indirect mechanism of upregulation possibly through the release of inflammatory cytokines/ chemokines. Past studies indicate that KOR activation leads to a suppression of inflammatory cytokine production. Therefore, regulation of KOR expression by inflammatory cytokine release could serve as a negative feedback mechanism for inflammatory processes. Support: K05-DA00360, DA04355

Th38 THE EXPRESSION OF PRODYNORPHIN GENE IS TRANSCRIPTIONALLY INHIBITED BY LIPOPOLYSACCHARIDE TREATMENT IN U-937 MONOCYTE/MACROPHAGE CELLS ***B. Sun, J.M. Bidlack Dept. Pharmacology and Physiology, Univ. Rochester, School of Medicine, Rochester, NY, USA*** As the endogenous kappa opioid peptides in the central nervous system, dynorphins are also present in the immune system and possess immunomodulating properties. In this study, we investigated the expression of prodynorphin (PDYN) gene, which encodes the precursor of dynorphins, in human monocyte/macrophage U-937 cells. Initially, we observed a detectable level of PDYN mRNA measured with a standard RT-PCR method in U-937 cells, but not in Jurkat T cells and Raji B cells. Further RT-PCR analyses using primers covering each exon of the PDYN gene showed that U-937 cells expressed the adult brain-type PDYN mRNA. Most interestingly, activation of U-937 cells with lipopolysaccharide (LPS) led to LPS concentration- and treatment duration-dependent decreases in PDYN mRNA levels. Furthermore, LPS treatment decreased the PDYN gene promoter activity. Taken together, our results suggested the U-937 cells expressed adult brain-type PDYN mRNA which was down-regulated by activation of the cells with LPS due to an inhibition of PDYN gene transcription. Support: K05-DA00360, DA04355

Th39 ACTIVATION OF MOUSE MICROGLIAL CELL LINE BY IFN-γ AND LPS LEADS TO DOWN-REGULATION OF DOR mRNA ***S. Sumagin, J.M. Bidlack Dept. Pharmocology and Physiology Univ. Rochester School of Medicine and Dentistry, Rochester, NY, USA*** Microglia play a vital in a number of neuro-inflammatory diseases. Because there are, as of yet, no well characterized human microglial cell lines, and primary microglial cultures are difficult to obtain, the mouse microglial cell line BV-2 has been used. Our hypothesis was that opioid receptors were present on the mouse microglial cell line BV-2, and that activation of these cells with LPS and IFN-g would lead to altered levels of opioid receptors. We used RT-PCR to detect mRNA levels for the three opioid receptors mu, delta, and kappa. Delta opioid receptor (DOR) mRNA was expressed in BV-2 cells in untreated cells and under all treatment conditions when cells were treated for 3, 6, 12, and 24 hr, but that treatment with LPS, IFN-g,

or a combination of both, decreased the expression of DOR mRNA. Neither mu nor kappa opioid receptor mRNA was detected, regardless of treatment type, or time (3, 6, 12, 24 hr). The BV-2 mouse microglial cell line can be used as a model to study the bidirectional effects of DOR interactions with microglia. Support: K05-DA00360, DA04355, T32 DA07232

Th40 MODULATION OF BASAL MU OPIOID RECEPTOR (MOR) ACTIVITY IN MORPHINE-DEPENDENT MICE ***D. Wang (1), E.J. Bilsky (2), W. Sadée (1) (1) Dept. Pharmacol., Ohio State Univ., Columbus, Ohio, (2) Dept. of Pharmacol., Univ. New England, Biddeford, ME, USA*** MOR displays basal (constitutive, spontaneous) signaling activity, as shown for other GPCRs. Thus, inverse agonists could cause adverse effects at least in part by suppressing basal MOR activity, while neutral antagonists block the receptor without altering basal signaling. Measurements of basal MOR signaling in brain tissues, in naïve and morphine-dependent mice demonstrate that agonist pretreatment causes moderate MOR desensitization but paradoxically enhances basal MOR signaling. Moreover, naloxone, a neutral antagonist in untreated tissues, turns into an inverse agonist after morphine pretreatment. In contrast, 6β-naltrexol remains neutral under all conditions. Naloxone's potency in eliciting withdrawal increases dramatically in the opioid-dependent state, implying that basal MOR activity plays a key role in maintaining dependence. We have measured the effects of agonists, neutral antagonists, and inverse agonists on MOR signaling in brain sections of mice made increasingly dependent on morphine. While agonist stimulation, measured with [^{35}S]GTPγS binding, decreased less than 50%, inverse activities increased, matching the magnitude of the agonist effects in highly dependent mice. These results support a physiological role of basal MOR signaling in dependence. Support: DA04166

Th41 MORPHINE-INDUCED GASTROINTESTINAL TRANSIT IN β-ARRESTIN2 KNOCK OUT MICE ***K.M. Raehal, L.M. Bohn Depts. Pharmacology & Psychiatry, Ohio State Univ. College of Medicine, Columbus, OH*** Morphine is a potent analgesic but also produces adverse side effects including constipation which limits its clinical utility. Morphine produces its effects primarily through activation of the mu opioid receptor, a G protein-coupled receptor (GPCR). We have previously shown that mice lacking the GPCR regulator ββ-arrestin2 (Barr2), display enhanced and prolonged morphine analgesia. In this study we ask whether morphine's effects on gastrointestinal transit are also enhanced in these animals. Using both *in vivo* and ex vivo methods, we demonstrate that the Barr2 knockout mice do not display more gastrointestinal inhibition but rather, they have less constipation following morphine compared to wild-type mice. To gain a better understanding of the mechanisms underlying differences in GI motility between the two genotypes, we are investigating central versus peripheral effects of opiates on this system. Support: DA14600, DA18860.

Th42 CANNABINOIDS INDUCE MU OPIOID RECEPTOR TRANSCRIPTS IN JURKAT T CELLS ***C. Börner, J. Kraus, V. Höllt Dept. of Pharmacology and Toxicology, Univ. Magdeburg, Germany*** Behavioural studies provided evidence for interactions between opioid- and cannabinoid systems on multiple levels. However, little is known about molecular events characterizing such interactions. Here, we report that activation of the cannabinoid receptor type 2 (CB2) in Jurkat T cells leads to an upregulation of mu opioid receptor mRNA. Incubation of the cells for 48 h with delta-9-tetrahydrocannabinol, or the CB2-specific agonist JWH015, but not with the CB1-specific agonist R(+) methanandamide induces mu opioid receptor transcripts. This effect is mediated via interleukin-4 (IL-4), since the mu opioid receptor mRNA induction is blocked with an IL-4 receptor antagonist. Consequently, transcriptional induction of IL-4 by cannabinoids is observed. In addition, we demonstrate that STAT5 is the main transcription factor involved in the cannabinoid-mediated upregulation of IL-4. We previously reported that induction of mu opioid receptors by IL-4 is also found in neuronal cells. Therefore, mu opioid receptor upregulation by CB2-specific agonists and IL-4 may contribute to analgesic effects of peripheral, CB2-specific cannabinoids.

Th43 THE DELTA OPIOID RECEPTOR (DOR) IN NG108-15 CELLS AND EXPRESSED IN CHO CELLS LOCALIZES IN LIPID RAFTS ***P. Huang, W. Xu, S.-I. Yoon, C. Chen, P.L.-G. Chong, L.-Y. Liu-Chen Depts. Pharmacol. and Biochem., Ctr. Subs. Abuse Res., Temple Univ. Med. Sch., Philadelphia, PA USA*** We examined whether the DOR was localized in lipid rafts and, if so, whether cholesterol (CHL) depletion by methyl-β-cyclodextrin (MCD) affected DOR properties. NG108-15 or CHO cells expressing an FLAG-mouse DOR were homogenized in a detergent-free buffer containing 0.5 M Na2CO3 and fractionated through a discontinuous (5%/35%/45%) sucrose gradient. [^{3}H]diprenorphine binding and immunoblotting showed that the majority of DOR (>90%) was in fractions of low-sucrose density enriched in CHL, flotillin-1 and caveolin-1, markers of lipid rafts. Pretreatment of NG108-15 cells

and CHO cells with 2% MCD for 1 h reduced CHL by ~50%, disrupted lipid rafts and shifted DOR to fractions of higher sucrose density. In NG108-15, MCD treatment attenuated DPDPE-induced [^{35}S]GTPγS binding and [3H]diprenorphine binding by ~ 60% and 40%, whereas in CHO it potentiated DPDPE-induced [^{35}S]GTPγS binding by ~200% without changing [^{3}H] diprenorphine binding. Whether the discrepancy is due to the lack of caveolin-1 in NG108-15 and whether agonists induce DOR translocation out of lipid rafts in both cell lines are under investigations. Support: NIDA, PA-DOH

Th44 THE MU-OPIOID RECEPTOR IN HEK 293 CELLS IS MODULATED BY CO-EXPRESSED METABOTROPIC GLUTAMATE RECEPTOR 5 ***H. Schröder, T. Koch, S. Schulz, V. Höllt, Dept. Pharmacology and Toxicology, Otto-von-Guericke Univ. Magdeburg, Magdeburg, Germany*** Since opioid analgesia and tolerance are modulated by metabotropic glutamate receptors (mGluR) we studied the effect of coexpression of mGluR5 on the MOP-r function. It was found that coexpression of MOP-r and mGluR5 is accompanied by an increased affinity and density of binding sites for [^{3}H]-MPEP, a non-competitive, allosteric mGluR5 antagonist, whereas the [^{3}H]-DAMGO binding sites remained unchanged. However, the DAMGO-induced GTPγS binding and cAMP-inhibition of MOP-r is decreased when mGluR5 is coexpressed but could be restored in the presence of the antagonist MPEP. Other tested competitive antagonists or agonists had no effect. Coimmunoprecipitation experiments indicate the existence of MOP-r/mGluR5 heterodimers. It is hypothesized that heterodimerization decreases MOP-r coupling efficiency and that MPEP affects heterodimerization and normalizes MOP-r coupling. This idea is supported by the finding that the DAMGO-induced desensitization of MOP-r is blocked when the coexpressed mGluR5 is inhibited by MPEP. The data are discussed in the light of the possible colocalization of MOP-r and mGluR5 in the spinal cord and CNS.

Th45 POTENTIATION OF DELTA OPIOID RECEPTOR BINDING BY ACTIVATION OF 5HT3 RECEPTORS ***D. Paul, L. Minor Dept. Pharmacology, LSU Health Sciences Center, New Orleans, LA USA*** Delta opioid receptor (DOR) agonists and 5HT3 receptor agonists produce synergistic analgesia when injected intrathecally. DORs are GPCRs that are sensitive to divalent cations, whereas 5HT3 receptors are ligand-gated cation channels. Both are found on the terminals of the sensory primary neurons within the spinal dorsal horn. The synergy between these two receptor types may be due to a potentiation of DOR affinity produced by the increase in intracellular divalent cations produced when 5HT3 receptors are stimulated. Thus, we assessed the change in affinity and association rate of the DOR ligand [^{3}H]naltrindole produced by 3μM 2-methyl-5-HT (2M5HT), a 5HT3 receptor agonist, in intact NG108-15 cells. The affinity of DORs for [^{3}H]naltrindole increased 4-fold in the presence of 2M5HT in intact cells, but not in membrane preparations. This shift was blocked by the 5HT3 receptor antagonist tropisetron. The association rate shifted 3-fold in the presence of 2M5HT. These result are evidence that ligand gated ion channels can modulate the function of GPCRs by altering the intracellular ion concentration, and thereby changing the affinity of the G-protein coupled receptor for its ligands.

Th46 NALTREXONE UP-REGULATES DELTA OPIOID RECEPTOR BINDING WITHOUT INCREASING MATURE RECEPTOR PROTEIN ***K.M. Wannemacher (1), P.N. Yadav (2), M. Doligosa (1), R.D. Howells (1,2) (1) Dept. Biochem. Mol. Biol., UMDNJ-Grad. Sch. Biomed Sci. (2) UMDNJ-NJ Med. Sch., Newark, NJ, USA*** The effect of naltrexone on the expression of the FLAG-tagged delta opioid receptor (DOR) expressed in transfected HEK293 cells was studied. [^{3}H]Diprenorphine exhibited a Kd of 1.6 nM to the tagged receptor. Naltrexone inhibited [^{3}H]diprenorphine binding with a Ki of 2.0 nM. Following a 24 h treatment of cells with 1 uM naltrexone, the Bmax was increased nearly 2-fold as assessed by 3[H]diprenorphine binding, with no apparent change in Kd. In contrast, western blotting showed no corresponding increase of the major 62 kDa DOR immunoreactive species. However, naltrexone decreased the level of a minor immunoreactive species migrating with an apparent MW of 42 kDa. This species probably represents the fully translated unglycosylated receptor, based on its molecular weight as well as its inability to bind to wheat germ agglutinin-agarose. In addition, naloxone treatment also up-regulated [^{3}H]diprenorphine binding without an increase in immunoreactivity. Further studies will explore the mechanism of antagonist-mediated up-regulation of delta opioid receptor binding sites. Support: DA09113

Th47 THE HISTONE DEACETYLASE INHIBITOR, TRICHOSTATIN A, STIMULATES EXPRESSION OF THE DELTA OPIOID RECEPTOR IN HEK 293 CELLS ***P.N. Yadav (1), K.M. Wannemacher (2), M. Balan (2), R.D. Howells (1,2) (1) Dept. Biochem. & Mol. Biol., UMDNJ-New Jersey Medical School, (2) UMDNJ-Graduate School of Biomedical Sciences, Newark, NJ, USA*** The modulation of chromatin structure by histone deacetylase (HDAC) and subsequent repression of gene expression of various cell cycle regulatory genes has been reported as a

major factor in the development of various diseases. In this study, the effect of a selective inhibitor of HDAC, trichostatin A, on expression of the FLAG-tagged delta opioid receptor (DOR) in an HEK 293 stable cell line was investigated. We have determined that trichostatin A stimulates the expression of DOR by 2-fold, in a time and dose-dependent fashion, as measured by western blotting using a monoclonal anti-FLAG antibody and [^{3}H]diprenorphine binding assays. Similar results were obtained when DOR-expressing cells were treated with sodium butyrate, another HDAC inhibitor. Further studies will address the molecular mechanism underlying the up-regulation of DOR expression by HDAC inhibitors. Support: NIDA DA09113

Th48 DOES FILAMIN REGULATE OPIOID RECEPTORS VIA THE ACTIN CYTO-SKELETON? ***I. Onoprishvili (1), M.L. Andria (1) E.J. Simon (1,2) Depts. (1) Psychiatry, (2) Pharmacology, NYU School of Medicine NY, NY USA*** Our previous studies showed that filamin A associates with the C-tail of the mu opioid receptor (muOR). In melanoma cells(M2), lacking filamin, muOR down-regulation, desensitization and internalization were greatly reduced. To investigate further the role of filamin and whether filamin is acting via the actin cytoskeleton, we prepared two filamin deletions. One has a deletion of the actin binding domain (ABD) and the other (ABD/DD) has deletions of both the actin binding and the dimerization domains. cDNAs of full length and mutant filamins were stably co-transfected with human muOR cDNA into M2 cells. The cells were exposed to DAMGO for 24 hrs and radioligand binding was performed using [^{3}H]diprenorphine. The results of these experiments indicate that the ABD mutant filamin was able to restore muOR down-regulation from 12% in M2 cells to 46%, compared to M2 cells transfected with full length filamin, where muOR down-regulation was 55%. In M2 cells expressing the ABD/DD double deletion, muOR down-regulation was unaffected. The data show that filamin lacking its actin binding site is able to restore most of muOR down-regulation, suggesting the surprising result that at least one of the observed effects of filamin on OR does not seem to require actin binding. Experiments to investigate effects of filamin mutants on muOR desensitization and internalization are in progress. Support: DA00017 (EJS), DA- 07254 Fellowship (IO)

Th49 ROLE OF β-ARRESTIN 1 IN HUMAN DELTA OPIOID RECEPTOR REGULATION ***B. Aguila, L. Coulbault, E. Rippoll, N. Marie, A. Hasbi, P. Jauzac, S. Allouche UPRES EA 3919 Biologie moléculaire et cellulaire de la signalisation, Univ. Caen, France*** Opioid receptors belong to G-protein coupled receptor family and undergo desensitization upon prolonged agonist treatment. The role of opioid receptors desensitization and involvement of arrestins were demonstrated in development of tolerance by using β-arrestin2 KO mice (Bohn et al., 2000). In our laboratory, we study the molecular mechanisms of human delta opioid receptor desensitization in the neuroblastoma cell line SK-N-BE. In the present study, we explored the regulation of opioid receptors by β-arrestin-1 on the inhibition of adenylyl cyclase and the MAPkinases pathway. Different strategies were used: overexpression of this protein or a dominant negative mutant (318-419). Functional and immuno-cytochemical experiments were conducted to determine whether this protein is involved in desensitization, internalization, recycling and resensitization. We found that β-arrestin-1 participates in opioid receptor desensitization upon different agonist's exposure but interestingly this protein modulates rather recycling than internalization as generally reported.

Th50 SERINE 363 PHOSPHORYLATION OF THE DELTA OPIOID RECEPTOR MEASURED BY A PHOSPHOSPECIFIC ANTIBODY ***M. Pfeiffer, S. Schulz, R. Stumm, V. Höllt Dept. Pharmacology, Univ. Magdeburg, Germany*** We examined agonist-dependent phosphorylation of Ser363 in stably transfected HEK293 cells using an antibody that selectively recognizes the Ser363-phosphorylated form of mouse DOR. When DOR-expressing cells were treated with agonists and subjected to Western blot analysis Ser363-phosphorylation of receptors was detectable within 2min and increased steadily throughout 30min incubation period. No phosphorylation was detected in untreated cells or in cells expressing the receptor without the primary phoshorylation site Ser363. Using confocal microscopy we observed a rapid phosphorylation of Ser363 followed by internalization of the receptor after exposure to DPDPE or etorphine. Ser363-phosphorylated receptors were first detectable at the plasma membrane within 2min. After 30min the majority of DORs were confined to perinuclear clusters of vesicles. Exposure to partial agonist buprenorphine induced Ser363-phosphorylation but no internalization of DOR. After removal of the agonist DPDPE we observed Ser363-phosphorylated receptors over a long period (up to 120min). In contrast, after removal of etorphine DOR was rapidly dephosphorylated within 30min and recycled to the plasma membrane.

Th51 DIFFERENTIAL REGULATION OF HUMAN DELTA OPIOID RECEPTOR BY AGONISTS ***B. Aguila, N. Marie, A. Hasbi, P. Jauzac, S. Allouche UPRES EA 3919, Biologie moléculaire et cellulaire de la signalisation, Univ. Caen, France*** In the present study, we explore the regulation of human delta-opioid receptors endogenously

expressed in the neuroblastoma cell line SK-N-BE by different opioid agonists in term of desensitization and trafficking. We found that short term treatment induced a rapid desensitization to a different extent in the presence of various agonists except for morphine which promoted a modest reduction of the inhibition of adenylyl cyclase by 20% after 1h. However, delta-selective agonists induced a more profound desensitization than enkephalins or etorphine. In immunocytochemical experiments, we observed that these agonists induced delta receptors internalization except for morphine and ARM-390. After non-selective delta ligands exposure, the opioid receptors were able to recycle and resensitize to a greater extent than treatment upon delta-selective agonists that partially target the opioid receptors to degradation. In conclusion, we found a complex relationship between receptor trafficking; desensitization.

Th52 LACK OF EVIDENCE FOR AGONIST-SPECIFIC CONFORMATIONS OF THE MU-OPIOID RECEPTOR USING PERTUSIS TOXIN INSENSITIVE G PROTEINS ***M.J. Clark, C.A. Furman, T.D. Gilson, J.R. Traynor Dept. Pharmacology, Univ. Michigan, Ann Arbor, MI, USA*** Agonist-specific conformational states of the mu-opioid receptor with coupling preferences to different Gi/o proteins could provide for agonist-directed trafficking of intracellular signaling. To examine this we have transfected pertussis toxin-insensitive mutants of the α subunits of Gi/o proteins into C6cells expressing a mu-opioid receptor (C6mu) and compared the degree of stimulation of [^{35}S] GTPγ S binding by a series of mu-opioid agonists in membranes from pertussis toxin treated cells. The rank order of efficacy was retained across all the pertussis toxin insensitive Gi/o subtypes: etorphine ≥ DAMGO ≥ fentanyl = endomorphin-2 = endomorphin-1 ≥ morphine ≥ meperidine > buprenorphine ≥ nalbuphine. The potency for each agonist was highest in cells expressing Gi3 and lowest with Gi1 and Gi2. The rank order of potency was constant across the various G proteins: etorphine >> endomorphin-1 = DAMGO= fentanyl = morphine= endomorphin-2 >>meperidine. Therefore, agonist-directed trafficking is not likely to result from a differential ability of mu-opioids to activate Gi/o proteins. Support: DA04087

Th53 UP-REGULATION OF THE MU OPIOID RECEPTOR BY LIPOPOLYSACCHARIDE IN TPA-DIFFERENTIATED HL-60 CELLS ***J. Beltran, A. Pallur, S.L. Chang Dept. Biol., Seton Hall Univ., S. Orange, NJ, USA*** We previously reported that, *in vivo*, an intraperitoneal (i.p.) injection with the bacterial endotoxin, lipopolysaccharide (LPS; 32mg/kg), leads to up-regulation of mu opioid receptor (MOR) mRNA expression in the mesentery and in peritoneal macrophage cells. *In vitro*, differentiation of HL-60 promyelocytic cells to monocytes/macrophages by treatment with 16 nM 12-O-tetradecanoylphobol 13 acetate (TPA) for 4 d leads to a 2.5 fold increase in MOR mRNA expression and enhanced morphine inhibition of forskolin-induced intracellular cAMP accumulation. We, thus, hypothesized that LPS treatment leads to an increase in the MOR in TPA-differentiated HL-60 cells. In this study, LPS treatment up-regulated MOR mRNA levels in TPA-differentiated HL-60 cells in a concentration-dependent manner. The up-regulation of the MOR mRNA by LPS was associated with an increase in morphine inhibition of forskolin-induced intracellular cAMP accumulation, which was naloxone reversible. In addition, the LPS-induced up-regulation of the MOR occurred concurrently with altered secretion of several cytokines, including TNF-α, IL-1 β, IL-6, and IL-8, by the differentiated cells. Support: DA007058, DA016149 (SLC)

Th54 ROLE OF THE T394 MUTANT IN AGONIST-INDUCED MU OPIOID RECEPTOR INTERNAL-IZATION ***E. Barbier, J.B. Wang Dept. Pharmaceutical Sci., School of Pharmacy, Univ. Maryland, Baltimore, MD, USA*** Site-directed mutagenesis studies in our laboratory and other laboratories showed that threonine 394 (T394) of MOR is a crucial residue for initiation of the MOR phosphorylation. It was further shown that replacement of threonine with alanine could diminish DAMGO-induced desensitization in CHO cells. In order to study the modality of the receptor desensitization, we examined the agonist-induced receptor internalization on the T394A mutant using confocal microscopy. Acute etorphine (500nM) treatment triggered a strong endocytosis of the non mutated MOR. In comparison, T394A mutated MOR displayed a very little internalization. Since T394A is a phosphorylation-deficient mutant receptor as previously demonstrated, the result suggests that MOR phosphorylation is required for MOR internalization. Effects of acute and chronic DAMGO (1μM) on receptor internalization are still in progress. This study will further define the role of MOR phosphorylation in modulating the receptor mediated signal transduction pathway and lead to a better understanding on the mechanism of tolerance and dependence development. MOR-phosphorylation, internalization

Th55 ENKEPHALIN RECULATES INCREASES IN CONSTITUTIVELY-ACTIVE MU RECEPTORS DURING OPIATE WITHDRAWAL ***J. Shoblock, N. Maidment NPI, UCLA, Los Angeles, CA USA*** We previously showed that 20h morphine (M) pretreatment, given to increase constitutive mu receptors (mu*), enhances the

conditioned place aversion (CPA) produced by naloxone (NAL), an inverse agonist, but not 6β-NAL, a neutral antagonist. To further test this, the ability of NAL to produce CPA in the absence of agonist was examined using enkephalin-/- mice (ENK) given 20h M pretreatment. NAL did not produce CPA. Since mu* produced by M has been shown to be short lived *in vitro*, we hypothesized that enkephalin release during M withdrawal was responsible for the increase in mu* previously observed in WT. Therefore, the ability of NAL to produce jumping was measured in WT and ENK mice at different time points after M. Jumping peaked 2h after M treatment. NAL's ability to produce jumping continued to diminish at 4.5h in the ENK mice but increased in WT mice. Next, the ability of NAL to produce CPA in the ENK mice 2h after M treatment, during peak levels of mu*, was determined. NAL, but not 6β-NAL, produced CPA. These data are in agreement with the hypothesis that NAL acts as an inverse agonist to produce physical and psychological withdrawal, and that compensatory increases in enkephalin during M withdrawal produce increases in mu*.

Th56 DELTA OPIOID RECEPTOR FUNCTION IN MIDBRAIN NEURONS AFTER CHRONIC MORPHINE ***S.P. Hack, E.E. Bagley, B.C.H. Chieng, M.J. Christie Pain Management Research Institute, Univ. Sydney, Australia*** Delta-opioid receptor (DOPr) activation fails to produce inhibition in periaqueductal gray (PAG) nerve terminals, despite neural expression of high densities of the receptor (Vaughan et al., 2003, Brit. J. Pharmacol. 139, 362). Histochemical studies have demonstrated that DOPr is located in an intracellular pool and can be translocated to the surface membrane by a variety of stimuli, including chronic morphine. PAG neurons in slices from untreated mice exhibited mu-opioid receptor but not DOPr mediated inhibition of GABAergic synaptic currents. After chronic morphine treatment, DOPr stimulation inhibited synaptic GABA release onto most neurons. Similar results were observed in rats treated with morphine. Shorter exposure to morphine (up to 4 h *in vitro* or 18 h *in vivo*) did not induce DOPr responses. DOPr responses could not be induced in slices from untreated animals by directly increasing synaptic activity. Induction of DOPr signalling required mu-opioid receptor expression because no DOPr receptor responses were observed in mu-receptor KOs. These results suggest that induction of DOPr mediated actions in PAG by chronic morphine requires prolonged mu-opioid receptor stimulation.

Th57 NANDROLONE DECANOATE AFFECTS THE ENKEPHALIN SYSTEM IN THE FEMALE RAT BRAIN ***K. Magnusson (1) M. Hallberg (1) A.-S. Lindqvist (2) C. Fahlke (2) F. Nyberg (1) (1) Dept. Pharm. Biosci., Div. Biol. Res. on Drug Dependence, Uppsala Univ., Uppsala, Sweden, (2) Dept. Psychol., Gôteborg Univ., Gôteborg, Sweden*** Anabolic androgenic steroids (AAS) have been shown to affect the brain reward system. As a result, AAS may not only cause a dependency but could also serve as a gateway to the use of other drugs. We have previously reported that chronic treatment with the AAS nandrolone decanoate alters the levels of Met-enkephalin-Arg6-Phe7 (MEAP) in various regions of the male rat brain, e.g. regulating drug dependence and aggression. In the present study we have examined the effect of nandrolone decanoate on regional brain tissue levels of MEAP in the female rat brain. Female Wistar rats received daily injections (s.c.) of nandrolone decanoate (15 mg/kg) for two weeks. At the end of the experiment, the animals were sacrificed by decapitation, the brains were dissected and the MEAP-ir was measured using radioimmunoassay. The results are in congruence with previous findings and thus, female rats seems to be affected similarly to male rats regarding the MEAP levels in different brain regions implicated e.g. in drug dependence and aggression.

Th58 MORPHINE PROMOTES PHOSPHORYLATION OF THE DELTA OPIOID RECEPTOR AT SERINE 363 ***D. Stropova, E. Navratilova, M.C. Eaton, E.V. Varga, T.W. Vanderah, W.R. Roeske, H.I. Yamamura Univ. Arizona, Tucson, AZ, USA*** After opioid agonist treatment the delta-opioid receptor undergoes phosphorylation, internalization and down-regulation. It is well documented that the relative propensity of morphine to induce opioid receptor regulation is disproportionally lower then morphine's efficacy for signaling. Although in previous studies morphine-mediated total phosphorylation of the delta-opioid receptor was difficult to demonstrate, in this study, we show that morphine phosphorylates S363 in the C-terminus of the human delta-opioid receptor. We compared phosphorylation of S363 by morphine and deltorphin II and found that morphine-mediated maximal phosphorylation reached 63 +/- 12% of the maximal phosphorylation by deltorphin II. The kinetics of phosphorylation and dephosphorylation was different between the two agonists. We propose that phosphorylation of S363 may play a role in the delta-opioid receptor desensitization by morphine. Support: NIH

Th59 MATURATION OF HUMAN κ OPIOID RECEPTORS (hKOR) EXPRESSED IN CHO CELLS

J.-G. Li, C. Chen, L.-Y. Liu-Chen Dept. Pharmacology, Temple Univ. Sch. Med., Philadelphia, PA, USA In this study, we examined glycosylation of the FLAG-hKOR expressed in CHO cells. By immunoblotting with anti-FLAG antibody, FLAG-hKOR was resolved as a broad and diffuse 55 kDa band and a less diffuse 45 kDa band, indicating that the receptor is glycosylated. Endo H reduced the 45 kDa band to about 39 kDa, but did not change the 55 kDa species. Thus, the 45 kDa protein is located in the ER or Golgi and contains high mannose or hybrid types of N-linked oligosaccharides. PNGase F, which removes all types of N-linked oligosaccharides, changed the 55 kDa to two species of 43 kDa and 39 kDa, suggesting that the 43-kDa band is O-glycosylated. Treatment of cells with tunicamycin, which blocks N-linked glycosylation, resulted in two species of 43 kDa and 39 kDa. The 43 kDa species generated by tunicamycin treatment was not digested by Endo H or PNGase F, but reduced to lower MW bands by neuraminidase and O-glycosidase, indicating that the 43 kDa species contains O-linked oligosaccharides. These results indicate that the hKOR is synthesized as 39-kDa polypeptide and modified by N-glycosylation in the ER followed by trimming and further N-glycosylation and O-glycosylation in the Golgi. Support: NIDA

Th60 CHRONIC ORPHANIN FQ/NOCICEPTIN (OFQ/N) INDUCES MU RECEPTOR INTERNALIZATION ***V.I. Ramirez , D.M. Sherry, K.M. Standifer Univ. Houston, Houston, TX USA*** Morphine is the most widely administered drug in a clinical pain setting. Its use is limited by the development of analgesic tolerance. The opioid receptor-like 1 receptor (ORL-1) and OFQ/N have been implicated in the cellular mechanism of morphine tolerance. In mice, morphine-induced antinociception was reversed by OFQ/N. In morphine-tolerant rats, i.c.v. injections of an antibody against OFQ/N reduced morphine tolerance by 50%. Our lab has shown that chronic treatment of BE(2)-C cells with OFQ/N produced heterologous desensitization of the mu receptor. The aim of this study was to determine if the pronounced mu receptor desensitization following chronic OFQ/N treatment could be attributed to mu receptor internalization. BE(2)-C cells stably expressing rat HA-tagged mu receptor were treated with OFQ/N (1μM) for 24 hr, and mu receptor localization within the cell was visualized by immunofluorescence labeling and microscopy. OFQ/N induced internalization of the mu opioid receptor was blocked with PKC inhibition, similar to desensitization. After chronic OFQ/N exposure, very few mu receptors were colocalized within lysosomal compartments. Therefore, while OFQ/N heterologously induces mu opioid receptor internalization, it does not induce downregulation. Support: DA17380-R01/S

Th61 RGS4 BINDS DIRECTLY TO THE C-TERMINAL TAILS OF THE MU-AND DELTA-OPIOID RECEPTORS TO MODULATE G PROTEIN SIGNALING ***L. Leontiadis (1), H.E. Hamm (2) Z. Georgoussi (1) (1) Lab. Cell. Signal. and Mol. Pharmacol., Inst. Biol., EKEFE "Demokritos", Athens, Greece, (2) Dept. Pharmacol.,Vanderbilt Univ. Sch. Medicine, Nashville, TN, USA*** Opioid receptor signaling mechanisms have demonstrated that the third intracellular loop and the C-terminal tails, are critical in mediating the signal. RGS proteins serve as GTPase activating proteins and effector antagonists and act upon members of G proteins to modulate signaling events. Recent observations reveal that this class of proteins can directly interact with GPCRs and serve as scaffolds regulating their function, indicating that RGS proteins play fundamental roles in physiology and disease. In order to map opioid receptor subdomains important for protein interaction and begin to identify components of a putative signal transduction complex mediated by the intracellular domains of the opioid receptors, we focused on the COOH termini of the mu- and delta-opioid receptors and generated GST fusion peptides from these domains to be used as affinity matrices for screening novel interacting partners in pull down experiments. In this respect we were able to demonstrate for the first time that RGS4 a) binds directly and selectively with both mu- and delta-opioid receptors b) forms a stable heterotrimeric complex with the mu-opioid receptor and active Gα and c) modulates DAMGO-mediated adenylyl-cyclase inhibition in HEK293 cells by acting as effector antagonist.

Interactions,othersubstances

Th62 CANNABINOID–OPIOID RECEPTOR INTERACTIONS IN NEURITE OUTGROWTH IN NEURO 2A CELLS ***I. Gomes (1), C. Rios (2), R. Iyengar (1), L.A. Devi (1) (1)Dept. Pharmacology and Biological Chemistry, Mount Sinai School of Medicine, New York, NY, (2) Weill College of Medicine, Cornell Univ., New York, NY USA*** Several studies have described functional interactions between opioid and cannabinoid receptors although the underlying mechanism(s) are not well explored. We investigated the involvement of direct receptor-receptor interactions by examining the proximity of μ opioid and CB1 cannabinoid receptors in live cells using a proximity-based energy transfer assay. We observe a substantial increase in the energy transfer signal indicating that the receptors are in close proximity. We find that, both in heterologous cells and native tissue, the CB1 receptor-mediated GTPγS binding is attenuated by a μ receptor agonist and vice-versa. We examined the physiological

significance of this interaction in Neuro 2A cells where CB1 receptors induce neurite outgrowth by activating the Rap1-Src-STAT3 pathway. We find that co-administration of DAMGO and HU-210 leads to significant attenuation of neurite outgrowth suggesting that interactions between these receptors have profound implications in neuronal differentiation during development. Support: DA08863, DA019521 (LAD)

Th63 NEURONAL PATTERS OF EXPRESSION OF CB2 CANNABINOID RECEPTOR IMMUNO-REACTIVITY IN THE CENTRAL NERVOUS SYSTEM ***J.-P. Gong (1), E. Onaivi (2), G.R. Uhl (1) Molec Neurobio Branch, NIDA-IRP, NIH/DHSS, Baltimore, MD, (2) Dept. Biol., William Paterson Univ., Wayne, NJ USA*** Two cannabinoid receptors have been characterized and cloned from mammalian tissues. A "central" CB1 receptor is largely expressed in brain and a "peripheral" CB2 receptor largely expressed in the immune system. Recent reports have documented CB2 receptor expression in microglia in brain. We now describe expression of brain CB2 receptor-like immunoreactivity neuronal patterns that support broader CNS roles for this receptor. Two anti-CB2 affinity-purified polyclonal antibodies were raised in rabbits immunized with peptide conjugates that corresponded to amino acids 1-33 and 20-33 of the CB2 receptor. Each displays similar staining patterns in spleen sections, brain sections and Western analyses of brain proteins. These antisera reveal abundant CB2 immunoreactivity (iCB2) in apparent neuronal processes, and apparent glial processes, in a number of brain areas. Cerebellar Purkinje cells and hippocampal pyramidal cells reveal substantial immunoreactivity. iCB2 is also observed in olfactory tubercle, islands of Calleja, cerebral cortex, striatum, thalamic nuclei, hippocampus, amygdale, substantia nigra, periaqueductal gray, paratrochlear nucleus, paralemniscal nucleus, red nucleus, pontine nuclei, inferior colliculus and the parvicellular portion of the medial vestibular nucleus. Cerebellar and hippocampal iCB2 is virtually absent in sections stained with primary serum that was preadsorbed with the immunizing peptide. Primary hippocampal cultures revealed dense CB2 immunostaining in neurons that also stain for neuron specific enolase. The multifocal expression of CB2 immunoreactivity in neuronal and glial patterns in a number of brain regions strongly suggests reevaluation of the possible roles that CB2 receptors may play in the brain. Support: NIDA-IRP

Th64 DIFFERENTIAL LONG-TERM NEUROADAPTATIONS OF GLUTAMATE RECEPTORS IN THE BASOLATERAl AND CENTRAL AMYGDALA AFTER WITHDRAWAL FROM COCAINE SELF-ADMINITRATION IN RATS ***L. Lu , J. Dempsey, Y. Shaham, B. Hope Behav. Nsci. Br, NIDA-IRP, NIH/DHHS, Baltimore, MD USA*** Humans and laboratory animals remain highly vulnerable to relapse to cocaine seeking after prolonged periods of withdrawal from the drug. It has been hypothesized that this persistent cocaine relapse vulnerability involves drug-induced alterations in glutamatergic synapses within the mesolimbic dopamine reward system. Previous studies have shown that cocaine self-administration induces long-lasting neuroadaptations in glutamate neurons of the ventral tegmental area and nucleus accumbens. Here, we determined the effect of cocaine self-administration and subsequent withdrawal on glutamate receptor expression in the amygdala, a component of the mesolimbic dopamine system that is involved in cocaine seeking and craving induced by drug-associated cues. Rats were trained for 10 d to self-administer intravenous cocaine (6-h/d) or saline (a control condition) and were sacrificed after 1 or 30 withdrawal days. Basolateral and central amygdala tissues were assayed for protein expression of the AMPA receptor subunits (GluR1, GluR2) and the NMDA receptor subunits (NR1, NR2A and NR2B). In the basolateral amygdala, GluR1, but not GluR2, levels were increased on days 1 and 30, NR2A levels were increased on day 1, and NR2B levels were decreased on day 30 of withdrawal from cocaine. In the central amygdala, GluR2, but not GluR1, levels were increased on days 1 and 30, NR1 levels were increased on day 30, and NR2A or NR2B levels were not altered after withdrawal from cocaine. These results indicate that cocaine self-administration and subsequent withdrawal induces long-lasting and differential neuroadaptations in basolateral and central amygdala glutamate receptors.

Th65 EFFECT OF AC5 KNOCKOUT IN COCAINE-INDUCED INCREASES IN STRIATAL DOPAMINE LEVELS IN MICE ***Y. Zhang (1), S.D. Schlussman (1), A. Ho (1), Y. Ishikawa (2), M.J. Kreek (1) (1) Lab. Biology of Addictive Diseases, Rockefeller Univ., New York, NY, USA, Depts. Physiology and Medicine, Yokohama City Univ. School of Medicine, Yokohama, Japan*** The reinforcing effects of cocaine are associated with increases in dopamine levels in the striatum. Dopamine binds to D1 and D2-like receptors, resulting in either activation or inhibition of adenylyl cyclase, affecting the cAMP signaling cascade in the direct and indirect striatal output neurons. Both the direct and the indirect pathways provide feedback to the dopaminergic neurons. Type 5 adenylyl cyclase (AC5) is the dominant isoform expressed in the striatum. To examine the role of AC5 in regulating cocaine-induced dopamine release, we measured extracellular dopamine levels in the caudate putamen of AC5 -/- and wild type mice by

in vivo microdialysis. Cocaine was administered to both AC5 -/- and wild type controls (20 mg/kg i.p. x 3 at hourly intervals,). Dialysates were collected every 20 min and dopamine content was determined by HPLC with electrochemical detection. In the basal condition, there were no significant differences in dopamine levels in the dialysate from the caudate putamen between AC-/- and wild type mice. "Binge" cocaine administration significantly increased dopamine levels in both the AC-/- and wild type mice compared with saline controls. However, the percent increase over basal levels of dopamine induced by "binge" cocaine was significantly lower in AC-/- mice compared to wild type controls, $p < 0.001$. In a separate study, we have found that AC-/- mice show reduced locomotor activation after 20mg/kg cocaine, which may be related to the finding here that deletion of AC5 attenuates "binge" cocaine-induced increases in dopamine levels in the caudate putamen. Support: NIH-NIDA P60 DA05130, KO5 DA00049 (MJK)

Th66 PSYCHOSTIMULANT SUPERSENSITIVITY IN THE PROTEIN KINASE-C INTERACTING PROTEIN (PKCI) KNOCKOUT MICE ***E. Barbier, O. Egbulefu, S. Gerani-Diznab, J.B. Wang Dept. of Pharmaceutical Sci., School of Pharmacy, Univ. Maryland, Baltimore, MD, USA*** PKCI is a regulatory protein which inhibiting function has been studied in opioid system. In particular, we previously showed that it inhibits mu opioid receptor (MOR) PKC-dependent phosphorylation and modulates MOR function. However, very little is known about its role in regulating other CNS functions. In the present study, we used the homozygous deleted PKCI -/- mice to assess the involvement of PKCI in psycho stimulants effects. We measured three parameters (total distance traveled, stereotypy and ambulatory counts) of locomotion activity in an open field test for 150 minutes. Acute amphetamine treatment (5 mg/kg i.p.) induced an increase in locomotion activity in both wild-type and the PKCI -/- mice with a much significant effect on the knockout. At a dose of 2.5mg/kg, amphetamine did not have any observable effect in the wild type mice but triggered a 2 times increase in the knockouts' scores. Our results show that PKCI is involved in maintaining basal locomotion activity and in sensitivity towards psychostimulants like amphetamine. Testing for other psychostimulants is currently underway. The study will help us to define the role of PKCI in the action of psychostimulants.

Th67 DOPAMINE RECEPTOR INTERACTIONS WITH ARRESTING IN NEOSTRIATAL NEURONS ***T.A. Macey (1), C. Chavkin (1), K.A. Neve (2) (1) Dept. Pharmacology, UW, Seattle, WA, USA (2) Dept. BEHN, OHSU, Portland, OR, USA*** Dopamine D1 (D1R) and D2 (D2R) receptors have been implicated in drug sensitization. The purpose of this work was to investigate the endogenous D1R and D2R interaction with endogenous arrestins in neostriatal neurons. Agonist treatment increased the colocalization of the D2R with arrestin2, increased translocation of arrestin2 to the membrane, and selectively enhanced the coprecipitation of the D2R and arrestin2. Internalization of the endogenous D2R (40%) was maximal at 20 min and D2R internalization was attenuated with siRNA-induced depletion of arrestins in NS20Y cells. In contrast, there was agonist-induced colocalization of the endogenous D1R and arrestin3, increased translocation of arrestin3 to the membrane, and a selective coprecipitation of the D1R and arrestin3. Agonist treatment of neurons induced D1R internalization (35-45%) that was maximal within 2-5 min, a time course similar to that of the increased colocalization of the D1R with arrestin3. These data indicate that the D1R is preferentially regulated by arrestin3 in neostriatal neurons, whereas the D2R receptor interacts with arrestin2. Similar techniques will be used to investigate mu-opioid receptor desensitization.

Th68 MICROARRAY STUDY OF COCAINE EFFECTS IN HUMAN PRIMARY MICROGLIAL CELLS ***V. Yuferov (1), D. Nielsen (1), S. Hu (2), P. Peterson (2), M.J. Kreek (1) (1) Rockefeller Univ., New York, NY, (2) Univ. Minn. Med. Sch., Minneapolis, MN USA*** Microglia regulates migration and proliferation, and support of brain cells. The genomic responses of microglia to cocaine are largely unknown. This study examined alterations in gene expression in primary human fetal microglial cells after cocaine treatment (10-6 and 10-8 M) for 1, 8 and 24 h or INF-γ (200 U/ml) for 8 h using Affymetrix Human Genome U133A arrays in duplicate. The data was analyzed using GeneSpring 7. Genes with 1.4-fold changes and significant at the $p<0.05$ level (t-test) were considered regulated by cocaine or INF. Cocaine treatment (10-8 M) for 1 h down-regulated 173 genes, including those related to transcriptional and translational processes, and up-regulated only 6 genes, including insulin growth factor 2 receptor. However at 24 h, 156 genes were up-regulated by 10-8 M cocaine, including c-fos, CREBL2, GABA-A receptor-associated protein, and the glutamate transporter, EAAT1. After INF stimulation, there were 463 up- and 779 down-regulated genes involved in immune activation. Hence, cocaine-stimulated microglia may affect neuronal activity in brain. Support: NIDA DA-P60-05130, DA00049, DA12848 (MJK), DA09924 (PP)

Th69 OPIOID OR DOPAMINE D1 RECEPTOR BLOCKADE ENHANCES HYPOTHALAMIC VASOPRESSIN GENE EXPRESSION AFTER ACUTE BINGE COCAINE ***Y. Zhou, V. Yuferov, J. Adomako-Mensah, A. Ho, M.J. Kreek Rockefeller Univ., New York, NY USA*** Recently we found (a) increased arginine vasopressin (AVP) mRNA levels in amygdala in acute withdrawal from chronic steady-dose "binge" cocaine and it is mediated by opioid receptors; and (b) persistent increases in AVP mRNA levels in hypothalamus during chronic withdrawal from chronic escalating-dose "binge" cocaine. Here we determined whether (a) acute (1-d) or chronic (14-d) steady-dose "binge" cocaine (45mg/kg/d) alters hypothalamic AVP mRNA levels; and (b) pretreatment with antagonists for mu opioid receptor (MOP-r, naltrexone), dopamine D1 or D2 receptor (D1R, SCH23390 or D2R, sulpiride) alters AVP mRNA response in acute cocaine. Hypothalamic AVP mRNA levels were unaltered after acute cocaine alone. However, after pretreatment with either naltrexone (1mg/kg) or SCH23390 (2mg/kg), but not sulpiride, acute cocaine resulted in increased hypothalamic AVP mRNA levels. Similar to our recent finding, chronic cocaine did not alter AVP, MOP-r or POMC mRNA levels. Acute or chronic cocaine effects on D2R mRNA levels in hypothalamus or thalamus are under investigation. Our results show that MOP-r and D1R mediate inhibitory effects on acute cocaine-induced hypothalamic AVP gene expression. Support: DA-P60-05130 (MJK)

Th70 COCAINE-INDUCED BEHAVIORAL SENSITIZATION IN MICE LACKING THE ORL-1 RECEPTOR ***R. Baliram, N. Gajawada, P. Kotha, K. Lutfy Dept. Pharm. Sci., Western Univ. Health Sci., Pomona, CA USA*** We have recently shown that orphanin FQ/nociceptin (OFQ/N), the endogenous ligand of the opioid receptor-like (ORL-1) receptor, blocks cocaine-induced behavioral sensitization, raising the possibility that the endogenous OFQ/N/ORL-1 receptor system could be involved in cocaine sensitization. Thus, in the present study, we determined whether the development and/or expression of behavioral sensitization will be altered in ORL-1 receptor knockout (KO) mice. Wild type (WT) and KO mice were habituated to testing chambers for 1 h, injected with cocaine (15 mg/kg, i.p.) and motor activity was recorded for 1 h. The same treatment was given once daily for 3 days. Mice were then tested on day 8 and again on day 30, as described above. Our preliminary results show that both WT and KO mice displayed locomotor sensitization on day 8, the magnitude of which appeared to decrease in WT, but not in KO, mice on day 30. Taken together, our results suggest that the endogenous OFQ/N/ORL-1 receptor system may be involved in long-term neuronal plasticity that develops after repeated cocaine administration. Support: DA 16682 (KL)

Th71 COCAINE-INDUCED BEHAVIORAL SENSITIZATION IN OPIOID PEPTIDE KNOCKOUT MICE ***J. Borse, R. Baliram, N. Gajawada, K. Lutfy Dept. Pharm. Sci., Western Univ. Health Sci., Pomona, CA USA*** The phenomenon of behavioral sensitization is thought to play an important role in the development and maintenance of drug dependency. The endogenous opioid system has been implicated in the addictive properties of cocaine. Thus, we determined whether the development and/or expression of cocaine-induced behavioral sensitization would be altered in mice lacking β-endorphin or enkephalin. POMC or PENK knockout (KO) and wild type (WT) mice were habituated to testing chambers for 1 h, injected with saline or cocaine (30 mg/kg, i.p.) and motor activity was recorded for 1 h. The same treatment was given once daily for 3 days and mice were then tested on day 8. The motor stimulatory action of cocaine was reduced in POMC KO mice, suggesting that β-endorphin may be important for acute motor stimulatory action of cocaine. However, senstization was not different between WT and KO mice. Studies are in progress to determine whether the behavioral changes observed in POMC KO mice will correlate with changes in dopamine and c-fos expression in terminal fields of dopaminergic neurons. Support: DA 16682 (KL), MIDARP DA 017298 (TCF/KL)

Th 72 DEMONSTRATION OF LIGAND-SPECIFIC DELTA OPIOID RECEPTOR CONFORMATIONS BY PLASMON WAVEGUIDE RESONANCE SPECTROSCOPY. ***Eva Varga (1), Isabel Alves (2), Zdzislaw Salamon (3), Teodora Georgieva (1), Victor Hruby (2), William Roeske (1), Gordon Tollin (3), Henry Yamamura (1); Departments of Medical Pharmacology (1), Chemistry (2); Biochemistry (3), University of Arizona, Tucson, USA.*** Plasmon-waveguide resonance (PWR) spectroscopy was used to study ligand-specific conformational changes in the human DOR (hDOR). PWR spectroscopy detects resonant electronic oscillations (plasmons) generated by polarized laser light in a silica-coated silver film on the surface of a prism (resonator). The recombinant epitope-tagged hDOR was purified by affinity chromatography and inserted into a lipid bilayer on the surface of the prism. Interaction of ligands with the lipid-bound hDOR alters the optical characteristics of the resonator. PWR experiments showed that structurally different opioids cause qualitatively and quantitatively different changes in resonance characteristics, indicating ligand-specific hDOR conformations. The affinity of the hDOR to individual G protein types, and its efficacy to promote nucleotide exchange also depended on the structure of the bound ligand. Future development of

opioids that selectively activate G protein types should yield analgesics with fewer side effects. Support: NIH.

15:30 – 16:15 **Business Meeting** **Marriott Ballroom**

16:15 – 18:15 **Symposium VII Hot topics** **Marriott Ballroom**

Chair G.R. Uhl, Cochair J.M. Bidlack

16:15 – 16:30 **S48 P. Leff CLONING AND FUNCTIONAL CHARACTERIZATION OF A NOVEL OPIOID PEPTIDE SYSTEM FOR THE MU OPIOID RECEPTOR** ***P. Leff (1), M. Matus (1), J.C. Calva (1), A. Salazar (1), R. Acevedo (1), P. De los Heros (3), H. Gompfh (2), C. Allen (2), B. Peng (4), A. Alagon (5), G. Gamba (3), J. Pintar (4), B. Anton (1) (1) National Institute of Psychiatry, DF, Mexico, (2) CROET Oregon Hlth. & Sci. Univ. Portland, OR, USA, (3) IIB & INCMNSZ, DF, Mexico, (4) UMDNJ-RBJ Med. Sch., Piscataway, NJ, USA, (5) UNAM Biotech. Inst., Morelos, Mexico*** An antibody-based screening approach combined with DNA cloning procedures were used to identify and characterize cDNA(s) coding for protein material containing EM1-2-LI from a whole mouse brain cDNA expression library transfected into cellular expression system of E.coli. For cDNA library screening, antigen affinity-purified antisera against EM-1-2 coupled to [^{125}I]-labeled-IgG were used to detect immunopositive transformants on immunoblotted nitrocellulose filters. Our data showed the identification of a novel cDNA encoding a complete mRNA with an ORF containing a deduced protein named Mexneurine (Mx), displaying structural features of a prepropeptide precursor that encodes three distinct peptide sequences referred to as Mx-1, Mx-2 and Mx-3. *In situ* hybridization studies showed that Mx mRNA is widely distributed throughout the mouse CNS and lymphoid tissue from spleen. Radioligand-binding assays on mouse brain purified membranes showed a high affinity binding (i.e. Ki= 8-10 nM) of the Mx-1 peptide for the mu opiod receptor. Mx-1 and Mx-3 showed a dose response stimulation of [^{35}S] [^{35}S] GTP-γ-S binding on mouse brain purified membrane preparations. Mx-1 induced a significant decrease in the cell firing of hypothalamic neurons whereas Mx-3 induced an increase in pyramidal cell firing at the CA1 region from mouse hippocampus. Our preliminary data support opposite actions of both Mx-1 and Mx-3 peptides on neuronal excitability mediated through distinct GPCRs, suggesting the role of the Mx-1 peptide as a novel endogenous ligand agonist for the mu opioid receptor, whereas Mx-3 could modulate neuronal excitability via distinct novel GPCR(s). Support: INP-2040 & Fundacion Gonzalo Del Rio Arronte.

16:30 – 16:45 **S49 Z. Wang LACK OF OPIOID TOLERANCE AND DEPENDENCE IN S286ACaMKII MUTANT MICE** ***L. Tang, Z.J. Wang Departments of Biopharmaceutical Sciences, Physiology and Biophysics, and Cancer Center, University of Illinois, Chicago, IL, USA*** Calcium/calmodulin-dependent protein kinase II (CaMKII) is a S/T protein kinase that is activated by calcium-activated calmodulin. Studies employing relatively selective chemical inhibitors and antisense oligonucleotides have led to the hypothesis that CaMKII can affect opioid tolerance and dependence either directly or via the learning/memory pathway. In the current study, we tested this hypothesis in mice that express a nonfunctional form of CaMKII (S286ACaMKII). Mutant mice showed normal expression of CaMKII that was not capable of autophosphorylation and activation. Mutant and wild-type littermates had similar basal nociceptive responses to heat in the tail-flick test (48, 52, or 55 C). Wild-type mice developed antinociceptive tolerance to morphine (10mg/kg, twice daily, s.c.); however, S286ACaMKII mutant mice failed to show tolerance to morphine. In the tolerant/dependent mice, naloxone was not able to precipitate significant withdrawal symptoms (jumping, weight loss). These results suggest that activation of CaMKII is required for the development of opioid tolerance and dependence.

16:45 – 17:00 **S50 M.J. Christie OPIOID WITHDRAWAL BUT NOT TOLERANCE IN SINGLE MIDBRAIN NEURONS FROM β-ARRESTIN-2 KNOCKOUT MICE** ***M. Connor, E.E. Bagley, S.P. Hack, B.C.H. Chieng, M.J. Christie. Pain Management Research Institute, University of Sydney, Australia*** After chronic morphine treatment, isolated neurons of the periaqueductal grey (PAG) exhibit opioid receptor tolerance (Connor et al., Br. J. Pharmacol. in press) and withdrawal hyper-excitation of GABAergic cell bodies (Bagley et al., 2005, Neuron, 45, 433), and nerve terminals (Hack et al., 2003, Neuropharmacol., 45, 575). The concentration-response curve for DAMGO inhibition of voltage-gated calcium channel currents from untreated BARR-KOs did not differ from wild-types. After chronic morphine the inhibition produced by DAMGO was reduced in wild-types but was less affected in BARR-KOs. In slices from treated animals, opioid withdrawal induced an opioid-sensitive cation current that was mediated by the GABA transporter-1 (GAT 1) in both wild-type and BARR-KO animals. After chronic morphine, the rate of mIPSCs, in the adenosine A1 receptor antagonist DPCPX, was enhanced during naloxone-precipitated withdrawal *in vitro*, compared to vehicle controls in both wild-type and BARR-KO animals. These findings are consistent with behavioural

studies showing development of opioid dependence but blunted tolerance in BARR-KOs (Bohn et al., 200, Nature 408, 720).

17:00 – 17:15 *Coffee Break*

17:15 – 17:30 **S51 J.E. Pintar INHIBITION OF FOOD INTAKE BY THE OPIOID ANTAGONIST LY255582 IS LOST IN TRIPLE OPIOID RECEPTOR KNOCKOUT MICE** ***M.A. Ansonoff (1), B.J. Eastwood (2), C.H. Mitch (2), M.A. Statnick (2), J.E. Pintar (1) (1) Dept. of Neuroscience and Cell Biology, UMDNJ-RWJMS, Piscataway, NJ, (2) Lilly Research Laboratories, Indianapolis, IN USA*** Treatment with opioid agonists generally enhances food uptake, while treatment with opioid antagonists inhibits food intake. Because many opioid ligands bind at more than one opioid receptor, it has been difficult to determine which receptors stimulate or inhibit food intake. This study examined the ability of LY255582 to inhibit consumption of sweetened condensed milk in pre-trained wild type, MOR-1, DOR-1, KOR-1 and MOR-1/DOR-1/KOR-1 knockout (KO) mice. In wild type mice, LY255582 treatment reduced milk intake at all doses, while in MOR-1/DOR-1/KOR-1 KO mice LY255582 treatment had no effect on milk consumption. In both MOR-1 and DOR-1 KO mice the efficacy of LY255582 was reduced. In KOR-1 KO mice LY255582 treatment inhibited milk intake but, unlike in wild type mice, LY255582 acted dose dependently. We conclude that all three opioid receptors may have a role in the efficacy of LY255582 to inhibit milk consumption. Future studies with additional combinatorial opioid receptor KO mice will clarify how each opioid receptor contributes to LY255582 inhibition of milk intake.

17:30 – 17:45 **S52 M. Narita INVOLVEMENT OF THE NEURONAL MIGRATING REGULATOR, REELIN, IN THE DEVELOPMENT OF TOLERANCE TO MORPHINE-INDUCED ANTI-NOCICEPTION** ***M. Narita (1), J. Khotib (1,2), M. Narita (1), M. Suzuki (1), K. Niikura (1), Y. Yajima (1), S. Syamsiah (2), T. Suzuki (1) (1) Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan (2) Dept. Biomedic. Sci., Facult. Pharm., Airlangga Univ., Surabaya, Indonesia*** Recent advances in cellular and molecular biology have defined components of the Reelin-related signaling pathway that controls neuronal migration and cell positioning in the developing brain. Here, we show that the development of tolerance to morphine-induced antinociception was completely blocked by pre-treatment with both a competitive inhibitor of Reelin, apolipoprotein E2 recombinant, and monoclonal antibody to Reelin. Moreover, pretreatment with the disabled-1 protein inhibitor MG132, dynein inhibitor brefeldin A and cyclin-dependent kinase 5 inhibitor roscovitine, which are all regulated by Reelin, suppressed the morphine-induced antinociceptive tolerance. These findings indicate that Reelin-related signaling pathway is involved in the development of tolerance to morphine-induced antinociception.

17:45 – 18:00 **S53 J Mathews ATTENUATION OF MORPHINE TOLERANCE THROUGH A NOVEL G β/γ MECHANISM** ***J.L. Mathews, T.M. Bonacci, A.V. Smrcka, J.M. Bidlack Dept. Pharmacology and Physiology, Univ. Rochester School of Medicine and Dentistry, Rochester, NY USA*** Opioid analgesics, such as morphine, are the standard therapeutic agents for the treatment of moderate to severe pain. While these agents have demonstrated efficacy, their clinical use is limited by side-effects including analgesic tolerance. One mechanism for altering opioid receptor signaling, and potentially attenuating tolerance, is through the G β/γ subunit. We screened a small molecule library (NCI diversity set) for selective inhibitors of G β/γ subunit signaling and found several compounds that bind to G β/γ subunits and selectively inhibit β/γ dependent signaling *in vitro*. One compound of interest, compound 119, was characterized *in vivo*. When administered i.c.v. in mice, compound 119 caused a leftward shift in the dose-response curves of morphine and DAMGO, 11-fold and 5-fold, respectively. A modest shift was observed with U50,488, 2-fold, and no effect was seen with DPDPE. Of particular interest, compound 119 was able to attenuate acute, antinociceptive tolerance in mice treated concomitantly with both compound 119 and morphine. These preliminary studies suggest that small organic molecules can be designed to specifically regulate G β/γ signaling and that this may have important clinical relevance in the treatment of opioid tolerance. Support: K05-DA00360, DA14251, T32DA07232, GM60286 (AVS)

20:00 – 23:00 **Banquet** **Marriott Ballroom**

L. Fricker, entertainment organizer

Friday, July 15

7:00 – 8:30 **Continental breakfast** **Marriott Ballroom Foyer**

8:30 – 9:30 **P6 Plenary Lecture M. Caron Marriott Ballroom**
Animal models of psychostimulant actions: implications of new GPCR signaling paradigms

Marc G. Caron Department of Cell Biology, Center for Models of Human Disease, Institute for Genome Sciences and Policy, Duke University Medical Center Durham, NC 27710, USA The neurotransmitter dopamine (DA) controls many important physiological functions such as control of locomotion, cognition and affect as well as the mechanisms involved in reward. Dysregulation of the dopaminergic system is thought to underlie several pathological conditions. In the brain the diffusion of DA and the duration of its actions are exquisitely controlled by the ability of presynaptic terminals to re-uptake DA through the DA transporter (DAT). Mice lacking the DAT gene exhibit persistently elevated levels of extracellular DA that mimic the pharmacological treatment of animals with psychostimulants. In the absence of DAT, important neurochemical changes in the presynaptic terminals reveal the essential role of transporter function to the maintenance of neurotransmitter homeostasis. The cellular actions of DA, which are carried out through a series (D1-D5) of 7 transmembrane domain G protein-coupled receptors (GPCR), to produce major behavioral effects are thought to be mediated mostly through the canonical G protein-dependent cAMP signaling pathway. We have used a pharmaco-genetic approach in wild type and animal models to interrogate the possibility that signal transduction mechanisms other than cAMP-dependent pathways might also play a physiological role. We provide evidence that DA receptors control brain-mediated behaviors through a novel signaling pathway that utilizes β-arrestin 2, a known component of the GPCR desensitization machinery. This pathway uses the ability of β-arrestin-2 to act as a cAMP-independent signaling intermediate that scaffolds a complex of protein phosphatase 2A/Akt/PKB and glycogen synthase kinase-3 (GSK-3). In the mouse striatum, increased DA neurotransmission arising either from administration of amphetamine to wild type mice or from the lack of the DA transporter (DAT-KO) in genetically modified mice results in inactivation of Akt and concomitant activation of GSK-3□ and GSK-3□. These biochemical changes are not affected by pharmacological activation of the cAMP pathway but are effectively reverse either by inhibition of DA synthesis or D2-like receptor blockade. Interestingly, D2-class receptors appear to mediate these effects through the formation of signaling complexes containing β-arrestin 2, PP2A and Akt. β-arrestin 2 deficiency in mice results in reduction of dopamine-dependent behaviors and a loss of β-arrestin 2/Akt complexes. Importantly, canonical cAMP-mediated dopamine receptor signaling is not inhibited in the absence of β-arrestin 2 and modulation of cAMP levels does not affect Akt phosphorylation in the striatum. These results provide direct *in vivo* evidence for a novel mechanism of dopamine receptor signaling mediated through a β-arrestin 2/ kinase/phosphatase scaffold. Furthermore, these results establish, *in vivo*, the physiological relevance of the recently appreciated signaling functions of β-arrestins and provide potential new pharmacological targets for dopamine-related psychiatric disorders and drug of abuse.

9:30 – 12:30 **Symposium VIII Psychostimulants, opioids and monoamine interactions Marriott Ballroom**
Chair T Shippenberg, CochairsI Sora, C Chavkin

9:30 – 9:55 **S54 T.S. Shippenberg REGULATION OF MONOAMINE TRANSPORTER FUNCTION AND CELL SURFACE EXPRESSION BY K-OPIOID SYSTEMS: IMPLICATIONS FOR ADDICTION TREATMENT** ***T.S. Shippenberg (1), B. Kivell (1), E. Bolan (1), L. Devi (2), V. Chefer (1) (1) Integrative Neuroscience Section, DHHS NIH/NIDA IRP, Baltimore, MD, (2) Mt Sinai Medical School of Medicine, New York, NY USA*** The repeated administration of kappa opioid receptor (KOPr) agonists prevents alterations in behavior and mesoaccumbal dopamine neurotransmission that occur in response to repeated cocaine use. In contrast, pharmacological inactivation or gene ablation of KOPr results in enhanced responsiveness to cocaine. Evidence that the cocaine-antagonist-like effects of KOPr agonists results from interactions of the KOPr with the dopamine transporter will be presented. Data obtained in native tissue and heterologous cell expression systems will be reviewed which show that acute KOPr activation increases the rate of substrate (dopamine; ASP+) uptake by the dopamine transporter and that this effect is associated with an increase in the velocity of uptake. KOPr activation also results in increased dopamine cell surface expression and decreased transporter internalization. Biochemical and imaging studies indicating a physical basis for the interaction of the KOPr and dopamine transporter will also be presented.

9:55 – 10:15 *Coffee break*

10:15 – 10:40 **S55 I. Sora EXCLUSIVE EXPRESSION OF μ-OPIOID RECEPTORS IN NORADRENERGIC NEURONS REVERSES THE DECREMENTS IN STRESS RESPONSES NOTED IN μ -OPIOID RECEPTOR KNOCKOUT MICE** ***I. Sora (1,2,5), S. Ide (2,3), M. Minami (3), G.R. Uhl (5), K. Ikeda (2) (1) Dept. Psychobiol., Tohoku Univ. Grad. Sch. Med., Sendai, Japan (2) Div. Psychobiol., Tokyo Inst. Psychiatry, Tokyo, Japan (3) Dept. Pharmacol., Hokkaido Univ. Grad. Sch. Pharm. Sci., Sapporo, Japan (4) Lab. Neuropharmacol., Hiroshima International Univ., Hiroshima, Japan (5) Molec. Neurobiol. Branch, NIDA-IRP, NIH/ DHHS, Baltimore, MD USA*** While abundant evidence defines important roles of μ -opioid receptors in reward and nociceptive processes, many μ -opioid receptor roles in stress responses remain to be elucidated. Stressful stimuli enhance functions of central noradrenergic systems that densely express μ receptors, as well as altering hypothalamo-pituitary (HPA) axis components that also express these receptors. Enhanced noradrenergic functions could help subserve important adaptions and improve coping with stress. In the stress induced by tail suspension and forced swims, μ -opioid receptor knockout mice have greater escape mobility and lower corticosterone responses than their wild-type littermates. Both the increased escape mobility and the attenuated stress-induced corticosterone responses are reversed in doubly-genetically-altered mice that display both 1) global μ opiate receptor knockout and 2) focal restoration of μ opiate receptor expression in noradrenergic neurons. This noradrenergic restoration is mediated by a transgene construct in which μ opiate receptor expression is driven by dopamine β-hydroxylase gene promoter sequences. Interestingly, however, selective restoration of μ-opioid receptors in noradrenergic neurons did not change spontaneous locomotion, morphine-induced analgesia, tolerance to morphine or signs of physical dependence on morphine. Expression of μ -opioid receptors in noradrenergic neurons thus appears to play selectively-important roles in animals'sensitivities to and/or responses to stress.

10:40 – 11:05 **S56 M. Morari NOCICEPTIN/ORPHANIN FQ RECEPTOR ANTAGONISTS AS A NOVEL APPROACH FOR THERAPY OF PARKINSON'S DISEASE** ***M. Marti (1), F. Mela (1), M. Fantin (1), C. Fischetti (1), M. Morari (1) (1) Dept. of Exp. and Clinical Medicine, Sect. of Pharmacol., and Neurosci. Center, Ferrara Univ., Ferrara, Italy*** Nociceptin/orphanin FQ (N/OFQ) and its receptor (the NOP receptor) are expressed in the substantia nigra (SN), a brain area containing dopamine neurons that degenerate in Parkinson's disease. We previously reported that reverse dialysis of N/OFQ in the SN reticulata elevated nigral glutamate extracellular levels (Marti et al., Neuroscience 112, 153-160, 2002). Moreover, N/OFQ inhibited the firing of dopaminergic neurons of SN compacta *in vitro* and reduced nigrostriatal dopaminergic transmission *in vivo*, an effect associated with motor impairment and loss of muscle tone (Marti et al., J. Neurosci. 24, 6659-6666, 2004). In that study, a role for endogenous N/OFQ in regulation of nigrostriatal dopaminergic transmission and motor behavior was also disclosed since NOP receptor antagonists elevated nigrostriatal dopaminergic transmission and motor performance, whereas NOP receptor knockout mice outperformed wild-type mice on the rotarod. Recent data also suggest that endogenous N/OFQ is involved in mechanisms underling parkinsonism, since intranigral injection of a NOP receptor antagonist attenuated parkinsonian-like hypokinesia in rats treated with haloperidol (Marti et al., J. Neurochem. 91, 1501-1504, 2004). To investigate more in-depth the role of N/OFQ in Parkinson's disease, NOP receptor peptide (UFP-101) and nonpeptide (J-113397) antagonists were administered in rats made hypokinetic by haloperidol or by 6-hydroxydopamine (6-OHDA) lesion (hemiparkinsonian). UFP-101 (injected into the SN reticulata) and J-113397 (given systemically) attenuated hypokinesia and improved motor function in both models of parkinsonism. In hemiparkinsonian rats, both antagonists improved motor performance on the rotarod with greater potency and efficacy compared to naïve rats, and also attenuated motor asymmetry. UFP-101 and J-113397 reduced glutamate release more potently in SN reticulata of the lesioned rats. Moreover, J-113397 normalized nigral glutamate levels previously elevated by haloperidol. These results will be discussed in relation to studies showing elevation of N/OFQ expression after 6-OHDA treatment, and a partial neuroprotective effect of N/OFQ gene deletion against the DA neuron toxin, MPTP. NOP receptor antagonists may represent a novel therapy for Parkinson's disease.

11:05 – 11:20 **S57 J. McLaughlin PRIOR KAPPA OPIOID RECEPTOR (KOR) ACTIVATION MEDIATES THE STRESS-INDUCED POTENTIATION OF THE COCAINE CONDITIONED PLACE PREFERENCE (CPP) RESPONSE** ***J. McLaughlin (1,2), J. Pintar (3), C. Chavkin (2) (1) Northeastern Univ., Boston MA, (2)Univ. of Washington, Seattle WA and (3)UMDNJ Robert Wood Johnson Med. Sch., Piscataway NJ*** We investigated the mechanism by which forced swim stress (FSS) modulates the potentiation of cocaine CPP. C57Bl/6 mice demonstrated FSS-induced increased analgesia, immobility and a 2-fold potentiation of cocaine CPP, whereas mice lacking KOR did not. Modulation of cocaine CPP was dependent on the time course of KOR activation, as demonstrated by pretreatment with the KOR agonist U50,488 (5 mg/kg i.p.) prior to cocaine conditioning. A 15-min

U50,488 pretreatment reduced cocaine CPP 85%, whereas a 60-min pretreatment doubled cocaine CPP in a nor-BNI sensitive manner. Analgesia induced by U50,488 returned to baseline within 60 min and remained desensitized to a second dose of agonist up to 6 hr later in wildtype mice, but not mice lacking the G-protein receptor kinase 3 (GRK3). Notably, GRK3 (-/-) mice failed to demonstrate FSS-induced potentiation of cocaine CPP. These results suggest the activation and subsequent desensitization of KOR produced the potentiation of cocaine CPP. Support: DA16656, DA16415 (JPM), DA16898, DA15916 (CC)

11:20 – 11:35 **S58 E.M. Unterwald DELTA OPIOID RECEPTOR DESENSITIZATION DURING COCAINE WITHDRAWAL IS ASSOCIATED WITH INCREASED ANXIETY-LIKE BEHAVIORS** ***E.M. Unterwald, S.A. Perrine Dept of Pharmacology & Center for Substance Abuse Research, Temple Univ Sch Med, Philadelphia, PA*** Evidence suggests that delta opioid receptors may play a positive role in modulating anxiety. In humans, heightened anxiety frequently accompanies withdrawal from chronic cocaine and this anxiety is often refractory to classical anxiolytics. We investigated the effects of withdrawal from chronic cocaine on the function of delta receptors and on anxiety. Adult male SD rats received saline or cocaine in a binge-pattern for 14 days. One day later, measures of anxiety on the elevated plus maze were increased in animals undergoing withdrawal from cocaine as compared to saline-injected controls. This anxiety was reversed by the selective delta receptor agonist SNC-80 given one hour prior to testing. The ability of delta receptors to inhibit adenylyl cyclase activity was significantly attenuated in the nucleus accumbens and caudate putamen of rats undergoing cocaine withdrawal. These findings demonstrate that delta opioid receptor signaling is reduced during withdrawal from chronic cocaine and suggest that increases in anxiety-like behavior may be a functional consequence of delta receptor desensitization. Support: DA18326, T32 DA07237, P30 DA13439

11:35 – 11:50 **S59 C. Du OPTICAL BRAIN MONITORING OF COCAINE-INDUCED CEREBRO-VASCULAR AND INTRACELLULAR CALCIUM EFFECTS IN THE LIVING RAT** ***C. Du (1), P.K. Thanos (2), M. Yu (1), S. Rivera (1), H. Benveniste (1) (1) Med. Dept., Brookhaven National Laboratory, (2) Lab. of Neuroimaging, NIAAA,*** NIH Objective is to optically assess the direct effects of cocaine on tissue blood flow, cerebral oxygenation and intracellular calcium in the cocaine-naïve living rat to understand the cerebrovascular effects and cellular mechanisms caused by cocaine Method: Six anesthetized rats were injected intravenously with 1 mg/kg cocaine. Cerebral blood volume, oxygenation and intracellular calcium were simultaneously detected from the cortical surface by optical diffusion and fluorescence spectroscopy. The physiological parameters of ECG, respiration rate, arterial pressure, pCO2 and body temperature were monitored. Results: Cocaine induced 4.1 % and 3.1% decreases in cerebral blood volume and tissue oxygenation respectively in 3-4 min after the cocaine administration when compared to baseline values. In parallel we observed a slight decrease in the mean arterial pressure. Interestingly, intracellular calcium transients were stable for 8.5-min after the cocaine administration before increasing to a maximum of 26.4 +/- 6.0% at 30 min. These suggest that cocaine causes cerebral vasospasm that may lead to borderline brain ischemia or potential stroke.

11:50 – 12:10 **S60 F.S. Hall ROLES FOR DOPAMINE (DAT), SEROTONIN (SERT) AND VESICULAR MONOAMINE 2 (VMAT2) TRANSPORTERS IN D-AMPHETAMINE-CONDITIONED PLACE PREFERENCE** ***F.S. Hall (1), H. Ishiguro (1), C. Mills (1), I. Sora (2), D.L. Murphy (2), K.P. Lesch (1), G.R. Uhl (1) (1) Mol. Neurobiol. Br. NIDA/NIH/DHHS, (2) Dept. Psychobiol.Tohoku Univ., Sendai, Japan, (3) Lab.Clin. Sci. NIMH/NIH/DHHS,Bethesda, MD USA (4) Dept. Psychiat., Univ. Wuerzburg, Germany*** VMAT2 heterozygous knockout (KO) mice display reduced d-amphetamine-conditioned place preference (CPP). It is unknown whether VMAT2 expressed by dopamine, serotonin and/or norepinephrine neurons is involved in this effect. However, since mice with deletions of both DAT and SERT display no cocaine CPP, it seems likely that DAT and/or SERT gene products would be involved in amphetamine reward. We now report assessment of amphetamine CPP (2 mg/kg, 2 conditioning sessions) in the six viable genotypes produced by combining VMAT2 with DAT or SERT knockouts. Amphetamine CPP was virtually eliminated in VMAT2 +/- mice, reduced in DAT -/- mice and enhanced in SERT -/- mice whether tested as single or multiple knockouts. These data support substantial roles for dopamine systems in the reduced amphetamine CPP noted in VMAT2 +/- mice. Support: NIDA-IRP.

12:10 – 12:25 **S61 J.M. Brown DELETION OF THE ppNOCICEPTIN GENE ATTENUATES MPTP -, BUT NOT METHAMPHETAMINE-, INDUCED DOPAMINE DAMAGE** ***J.M. Brown, S. Gouty, V. Iyer, J. Rosenberger, B.M. Cox Dept. of Pharmacology, USUHS, Bethesda MD USA*** Antagonists of the opioid-like peptide

nociceptin/orphanin FQ (N/OFQ) reduce impairment of motor function in an animal model of Parkinson's disease (PD). However, a role for N/OFQ in the associated DA damage has not been investigated. We report that deletion of ppN/OFQ attenuates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-, but not methamphetamine (METH)-induced DA damage. METH or MPTP treated N/OFQ knockout (-/-) or wild-type (+/+) mice were killed by decapitation and brains processed for immunohistochemistry using DA markers tyrosine hydroxylase (TH) and the vesicular monoamine transporter (VMAT). MPTP treatment decreased the number of TH-positive cells in substantia nigra (SN) and TH/VMAT immunoreactivity in caudate-putamen (CPu) of mice+/+. However, mice-/- were resistant to MPTP showing little to no loss of TH/VMAT immunoreactivity in CPu and reduced loss of DA cells in SN. METH also decreased TH/VMAT immunoreactivity in CPu of mice+/+, however, mice-/- showed no protection against the DA deficits induced by METH. It is concluded that removal of endogenous N/OFQ is partially protective against selective DA toxins. Support: DA03102

12:30 **Adjourn**

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