Morphine effects on opioid, cannabinoid, and stress-related receptors in the locus coeruleus

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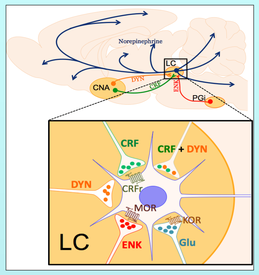
Introduction

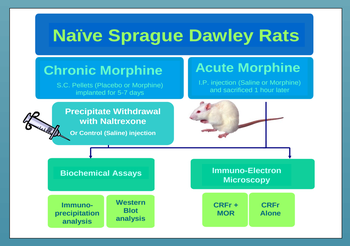


The locus coeruleus (LC) is finely tuned by co-regulation between the endogenous opioids, enkephalin (ENK) and dynorphin (DYN), and CRF.

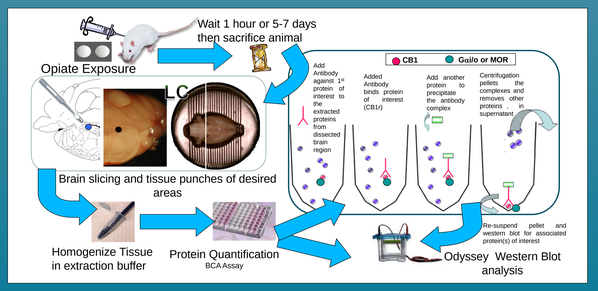
- Endogenous opioids and corticotropin releasing factor (CRF), reciprocally regulate LC neuronal activity[1-5].
- Chronic morphine exposure sensitizes the LC to the stimulatory effects of CRF[6].
- Neurochemical adaptations in the LC occur after exposure to drugs of abuse. For example, ENK levels significantly decrease following chronic morphine treatment[7]. Several G-protein coupled receptors (GPCR) that utilize the adenylate cyclase 2nd messenger system, mediate the integration of signals within the LC.
  - A high concentration of the μ-opioid receptor (MOR) exists in the LC, which associates with an inhibitory Gαi/o resulting in hyperpolarizing effects[8].
  - The cannabinoid receptor, CB1r, associates with Gαi/o after binding the endogenous cannabinoid, anandamide, or synthetic analogs like WIN.
  - The CRF receptor, CRFr1, is associated with a stimulatory Gαs, ultimately increasing LC activity.
  - Co-localization of both MOR with CB1r and MOR with CRFr1 in the LC has been previously demonstrated by the Van Bockstaele laboratory[9-10]. Additionally, co-localization of CRFr1 with CB1r[11], and opposing activity of WIN, and CRF-induced effects have been found[12].
- **Based on these lines of evidence, the LC is uniquely poised to be a site of convergent actions of opioid, cannabinoid, and stress pathways that modulate norepinephrine (NE) output.**
- **The goals of the study were to elucidate the role and regulation of salient GPCRs following opioid exposure in the LC; including cellular distribution, co-localization, association, and potential mechanisms of integration.**
- **Investigating the intersection between these signaling systems after morphine exposure may uncover broader circuit-based interactions that impact noradrenergic signaling and potentially elucidate novel targets for the treatment of opioid addiction.**

Methods

Subjects & Treatments

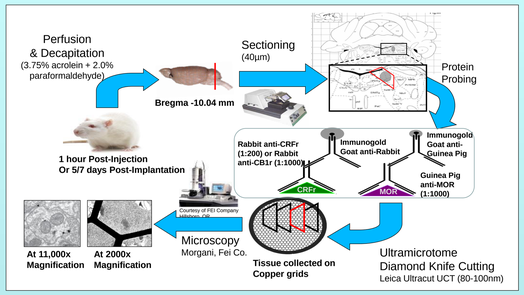


Western Blotting & Co-Immunoprecipitation

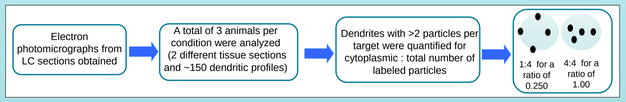


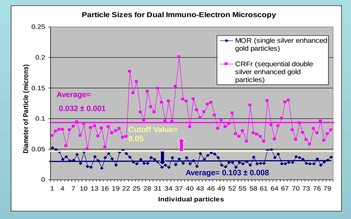
Methods (cont'd)

Immuno-Electron Microscopy



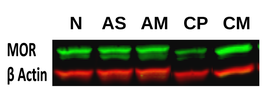
Quantification of Immuno-Electron Microscopy



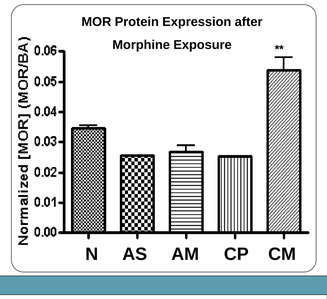


Results

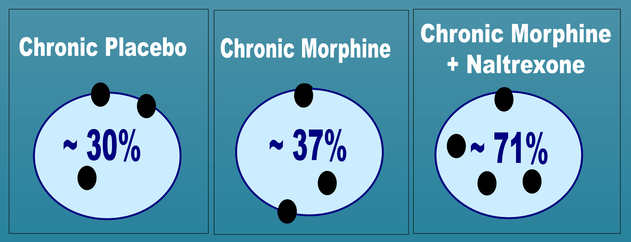
Morphine Effects on MOR



N	Naïve
AS	Acute Saline
AM	Acute Morphine
CP	Chronic Placebo
CM	Chronic Morphine



Western blot analysis after acute (1 hour post-intraperitoneal, i.p injection) and chronic morphine (7 day pellet subcutaneous, s.c. implantation) for MOR and relevant controls. (n= 3 animals/condition) As shown in E, there was a significant increase in protein expression after chronic exposure. (**p< 0.01 by ANOVA, Tukey's post-test)



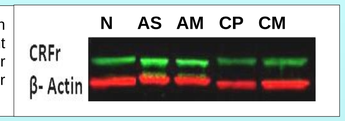
Electron microscopy showed subcellular localization of MOR in LC dendrites following placebo pellet implantation, 5 day treatment with morphine pellets (2x 75g, NIDA), and 5 day morphine + subcutaneous naltrexone(100mg/kg) injection. (n= 3 animals/condition) in Scavone et. al. 2009.

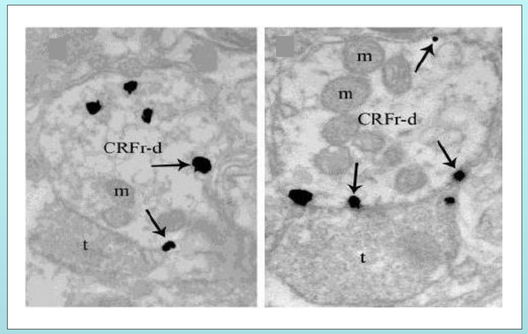
A slight internalization of MOR after chronic exposure (30% to 37%) and marked internalization with naltrexone induced withdrawal (71%) was observed.

Results (cont'd)

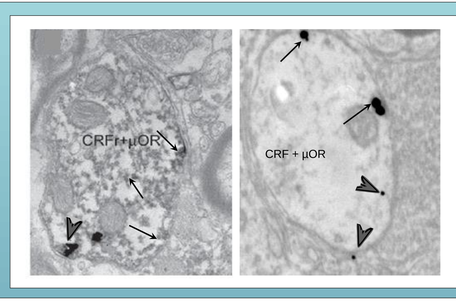
Morphine Effects on CRFr

Western blot analysis of CRFr protein levels demonstrated no significant change in CRFr expression after morphine suggesting a trafficking or receptor associated change.



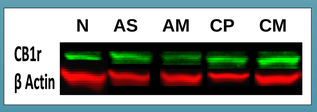


Electron micrographs from placebo pellet (Left) and morphine 7 day pellet (Right) singly labeled for CRFr. A trend exists where more CRFr is on the plasma membrane after morphine exposure compared to naïve or placebo dendritic profiles. m= mitochondria, t= axon terminal

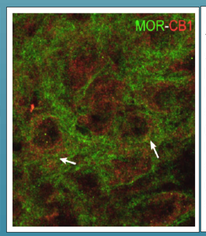


Electron photomicrographs showing co-localization of CRFr + MOR within LC dendritic profiles from naïve animals. CRFr is labeled with peroxidase enhanced antibodies and MOR is labeled with gold antibodies and silver enhancement (Left, from Reyes et. al. 2007). Dual immunogold labeling of receptors (Right) with sequentially silver enhanced gold antibodies against CRFr yielding large electron dense particles (> 0.5 microns) and single enhanced small electron dense particles labeled for MOR (< 0. 5 microns).

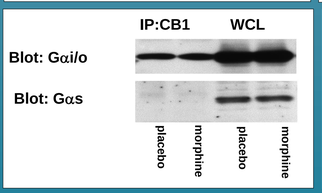
Morphine Effects on CB1r



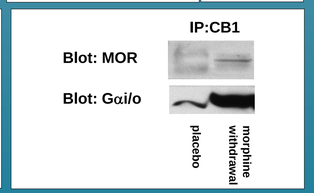
Western blot analysis after acute (AM) and chronic (7 day; CM) morphine exposure showed no significant change in CB1r protein expression compared with acute saline (AS), chronic placebo (CP) or naïve (N) control animals.



Immuno-fluorescence showed co-localization of MOR (green) and CB1r (red) in the LC of naïve animals. (Scavone et. al. 2010).



Immunoprecipitataion studies from LC tissues found after 5 day morphine exposure. Gαi/o association with CB1r is unchanged and does not cause a shift to association with Gαs.

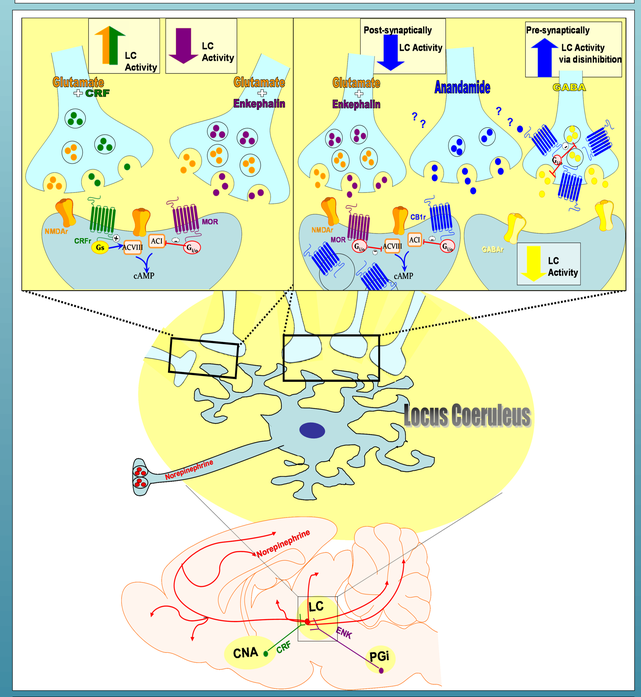


Upon naltrexone-induced withdrawal from morphine, there is an increased association of CB1r with both G α i/o and MOR.

Functional Implications

Utilizing the findings from this study and our knowledge of LC circuitry, the schematic below represents our working hypotheses:

- There is convergence of opioid and CRF neurochemical signals at excitatory synapses on LC dendrites that contain MOR and CRFr.
  - Opposing regulation of the cAMP system can modulate glutamatergic-mediated LC activation.
  - This system is perturbed after chronic opioid exposure with increased sensitization to CRF.
    - Tolerance and the decreased inhibitory capability of opioids may disrupt the reciprocal balance of CRFr and MOR downstream effects, leaving the LC in a more excitable baseline state.
    - Alternatively, association with or altered signaling via MOR could impact CRFr trafficking, whereby a higher concentration of receptors are available to bind CRF, thus potentially increasing LC excitability.
- Cannabinoid receptors further adjust LC activity
  - CB1r is co-localized with MOR post-synaptically on dendrites.
    - Compensatory inhibition via Gαi/o may aid in stabilizing LC activity at equilibrium in the event of chronic opioid-induced MOR tolerance.
    - However, the observed increase in CB1r association with MOR after opioid withdrawal may impact receptor trafficking and the capacity for cannabinoid related inhibition.
  - CB1r has also been identified on pre-synaptic terminals that form inhibitory synapses onto LC neurons.
    - Activation of these receptors may disinhibit GABAergic signaling and result in overall increased LC activation.
- Precise and rapid tuning of LC activation impacts NE release throughout the brain contributing to arousal, attentiveness, emotional and cognitive processing of stressful stimuli, and reinforcement of reward pathways. Understanding the complex integration of signals may enable targeted therapeutic interventions for the treatment of opioid abuse.



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