#### FUNCTIONAL NEUROANATOMY OF MORPHINE-INDUCED ABSTINENCE, TOLERANCE, AND SENSITISATION

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

Adam S. Hamlin B.Sc. (Hons)

2006

**Pain Management Research Institute Department of Anaesthesiology** Faculty of Dentistry, Medicine & Pharmacy **University of Sydney** 

#### **Statement of Authorship**

This thesis is submitted to the University of Sydney in fulfilment of the requirement
for the degree of Doctor of Philosophy. All experiments were performed by the
author except where acknowledged. The text presented in this thesis is original unless
otherwise stated.

Signature_	Date

#### Acknowledgements

Thank you to my supervisor Dr Peregrine Osborne for having enough faith in me to move me to Sydney, and for teaching me what it is to be a scientist. I am especially going to miss our long discussion on how the brain works. Thank you to Associate Professor Janet Keast for allowing me into her 3<sup>rd</sup> year neuroscience program all those years ago, even though technically I wasn't eligible (that really set me on the path that I now find myself) and for the generous use of her laboratory and equipment. Thank you to Drs Pascal Carrive and John Leah for their assistance in the development of the immunohistochemical technique. Thank you to Dr. Gavan McNally and Professor Fred Westbrook at the Department of Psychology at the UNSW for the use of their behavioural laboratories and their assistance with the development of the behavioural techniques. Finally, thank you to all the people I met both in the lab and in Sydney for making my PhD a thoroughly enjoyable experience.

The research conducted during my PhD candidature was supported by a National Health and Medical Research Council of Australia Dora Lush postgraduate scholarship and project grants 157158 and 209577 and project grant 10007151 awarded by the Australian Research Council.

#### **Table of Contents**

Statement of Authorship	ii
Acknowledgements	iii
Table of Contents	iv
Abbreviations	xii
Publications	xvi
CHAPTER 1	

### Introduction

1.1 Aims	2
1.2 The function of neural plasticity in opioid-induced behaviour	2
1.3 Anatomical mapping of inducible transcription factor proteins in	
the exploration of the functional neuroanatomy of drugs of abuse	5
1.4 Acute-morphine abstinence	9
1.4.1 Acute-morphine abstinence	9
1.4.2 Anatomy of withdrawal	11
1.5 Psychomotor sensitisation	16
1.5.1 Incentive-sensitisation theory of addiction	16
1.5.2 Morphine-induced psychomotor sensitisation	17
1.5.3 The role of the basal ganglia in psychomotor sensitisation	19
1.5.4 The use of inducible transcription factors in the study of psychomotor	
sensitisation	23
1.6 References	27

#### **CHAPTER 2**

## Induction of c-Fos and zif268 in the central extended amygdala parallels hyperalgesia induced by naloxone following systemic morphine in drug naïve

#### rats

2.1 Summary of results
<b>2.2 Introduction</b>
2.3 Materials and methods
2.3.1 Animals and drug treatments
2.3.2 Tissue processing and immunohistochemistry54
2.3.3 Neuronal counting
2.3.4 Immersion tail-flick testing
2.3.5 Statistical Analysis
<b>2.4 Results</b>
2.4.1 Naloxone induced hyperalgesia in morphine-pre-treated rats59
2.4.2 Induction of c-Fos in divisions of the central amygdala occurred in
parallel with morphine analgesia and abstinence hyperalgesia60
2.4.3 Induction of c-Fos in the central extended amygdala
2.4.4 Induction of zif268 in morphine pre-treated rats injected with naloxone67
2.4.5 Induction of c-Fos-positive neurons in the basolateral amygdala,
midbrain dopamine areas and striatum required prolonged activation of
morphine70
2.4.6 Naloxone administered after morphine does not induce c-Fos in
pathways regulating descending pain inhibition
2.5 Discussion

2.5.1 The role of the extended amygdala in acute morphine absti	nence78
2.5.2 The role of the central amygdala in opioid induced descend	ling
pain inhibition	82
2.5.3 The amygdala contributes to both descending pain inhibition	on
and facilitation	82
2.5.4 Mechanisms of itf protein induction by naloxone after a bri	ief morphine
exposure	88
2.5.5 Kappa-opioid receptor modulation of anti-opioid systems	90
2.5.6 Prolonged morphine exposure is required to induce c-Fos	protein92
2.5.7 Conclusion	93
2.6 References	94
CHAPTER 3  Opioid-sensitisation alters the expression and distribution proteins induced by morphine in the nucleus accumbens and	
regions	
3.1 Summary of results	104
3.2 Introduction	105
3.3 Materials and methods	106
3.3.1 Animals and drug treatments	106
3.3.2 Tissue processing and immunohistochemistry	107
3.3.3 Neuronal counting	109
3.3.4 Behavioural testing	109
3.3.5 Statistical analysis	111

3.4 Results	112
3.4.1 Morphine pre-treatment sensitised behaviours induced by a subsequent	<b>L</b> .
morphine challenge	112
3.4.2 Sensitisation altered the pattern of c-Fos and Fos-related proteins expr	essed
by neurons in the ventral striatum after a morphine challenge	113
3.4.3 Relative persistence of Fos proteins induced by morphine in the striatur	m
and accumbens	120
3.4.4 Morphine induced c-Fos in dopamine neurons in the interfascicular	
nucleus but not the ventral tegmental area or substantia nigra	120
3.4.5 Chronic morphine pre-treatment induced c-Fos in the capsular division	ı
of the central amygdala	125
3.4.6 Morphine pre-treatment does not alter induction of Fos by morphine in	
cortex, thalamus and basolateral amygdala	127
3.5 Discussion	127
3.5.1 Opioid-sensitisation alters the expression of Fos related proteins	
induced by morphine in accumbens neurons	129
3.5.2 Induction of c-Fos in the regions that provide dopamine and glutamate	
projections to the accumbens was not altered by morphine sensitisation	132
3.5.3 Chronic morphine increases c-Fos in the capsular division of the	
amygdala	133
3.5.4 Conclusion	134
3.6 References	135

#### **CHAPTER 4**

## Morphine increases c-Fos in patch striatonigral projection neurons in the nucleus accumbens core in drug-sensitised rats

4.1 Summary of results
<b>4.2 Introduction</b>
4.3 Materials and methods
4.3.1 Animals and drug treatments
4.3.2 Tissue processing and immunohistochemistry
4.3.3 Neuronal counting
4.3.4 Statistical analysis
<b>4.4 Results</b>
4.4.1 A history of intermittent morphine does not alter the distribution of Fos
induced by morphine in the nucleus accumbens shell
4.4.2 A history of intermittent morphine prior to the morphine challenge injection
increased c-Fos in patch striatonigral neurons in the caudal accumbens core152
<b>4.5 Discussion</b>
4.5.1 Functional anatomical organization of the nucleus accumbens156
4.5.2 A morphine injection in drug-sensitised rats increases c-Fos expression
in the accumbens core
4.5.3 Distribution of Fos induced by morphine in the accumbens shell
4.5.4 Conclusion
<b>4.6 References</b>

#### **CHAPTER 5**

# Network-level changes in the distribution of c-Fos in the accumbens core predicts tolerance to catalepsy and sensitisation of stereotyped behaviours in morphine-sensitised rats

5.1 Summary of results	171
5.2 Introduction	173
5.3 Materials and methods	179
5.3.1 Animals and drug treatments	179
5.3.2 Behavioural testing	180
5.3.3 Tissue processing and immunohistochemistry	181
5.3.4 Neuronal counting	183
5.3.5 Statistical analysis	183
5.4 Results	184
5.4.1 A history of intermittent morphine produced tolerance to catalepsy and	
sensitisation of stereotyped behaviours	184
5.4.2 A history of intermittent morphine increased c-Fos in the patch	
compartments of the nucleus accumbens core	185
5.4.3 A history of intermittent morphine increased c-Fos in the	
ventrolateral striatum	194
5.4.4 Network level changes in the distribution of c-Fos in the nucleus	
accumbens predicted tolerance and behavioural sensitisation	195
5.4.5 c-Fos induction in the ventrolateral striatum predicted the level of	
stereotyped behaviours induced by acute morphine challenge	200
5.5 Discussion.	200

5.5.1 Tolerance to catalepsy and sensitisation of stereotyped behaviours are	2
persistent changes	202
5.5.2 Large or small test environments had no effect on morphine-induced	
Behaviours	204
5.5.3 Network-level changes in the distribution of c-Fos protein in the accus	mbens
core predicts morphine-induced catalepsy and behavioural sensitisation	206
5.5.4 A history of intermittent morphine did not alter the distribution of c-Fe	os in the
accumbens shell	212
5.5.5 Stereotypy induced by morphine does not lead to a patch-predominant	t
pattern of Fos expression in the striatum	213
5.5.6 Stereotypy induced by morphine leads to an increase in Fos expressio	n
in the ventrolateral striatum	214
5.5.7 Morphine increases c-Fos expression in the ventromedial striatum in	drug-
naïve rats exposed to the large testing environment	216
5.5.8 Conclusion	217
5.6 References	218
CHAPTER 6	
General Discussion	
6.1 Summary of results	228
6.2 Acute morphine-abstinence	230
6.3 Morphine-induced tolerance and psychomotor sensitisation	241
6.4 Future directions	246

6.5 Conclusion	248
( ( Defense	240
6.6 References	249

#### **Abbreviations**

3V third ventricle
ac anterior commissure
AC anterior cingulate
AcbC nucleus accumbens core
AcbSh nucleus accumbens shell
ANOVA analysis of variance
AStr amygdalostriatal transition zone
BG basal ganglia
BLA basolateral amygdala
BST bed nucleus of the stria terminalis
BSTld lateral-dorsal bed nucleus of the stria terminalis
BSTv ventral bed nucleus of the stria terminalis
CALB calbindin
cAMP cyclic-AMP
cc corpus callosum
CeA central nucleus of the amygdala
CeC capsular central amygdala
CeL lateral central amygdala
CeM medial central amygdala
CGRP calcitonin gene-related peptide
CM centromedial thalamus
CRF corticotropin releasing factor
CRH corticotropin releasing hormone

ct commissural stria terminalis

CTX cortex

D1 dopamine type 1 receptor

D2 dopamine type 2 receptor

dl dorsolateral striatum

dm dorsomedial striatum

DOR delta-opioid receptor

dPAG dorsal periaqueductal gray

ENK enkephalin

FRA Fos-related-antigen

GABA Gamma-Aminobutyric Acid

GP globus pallidus

HPA-axis hypothalamic pituitary adrenal-axis

IC intercalated cell groups

IF interfascicular nucleus

IL infralimbic cortex

IL-1β interleukin 1β

i.p. intraperitoneal

IP interpeduncular nucleus

IPAC interstitial nucleus of the posterior limb of the anterior commisure

IPACl lateral interstitial nucleus of the posterior limb of the anterior commisure

IPACm medial interstitial nucleus of the posterior limb of the anterior commisure

IR immunoreactivity

IPAG lateral periaqueductal gray

KOR kappa-opioid receptor

LA lateral amygdala

LPB lateral parabrachial nucleus LC locus coeruleus LH lateral hypothalamus LV lateral ventricle M/- morphine/no injection control M/M morphine/morphine M/N5 morphine/naloxone (5mg/kg) M/N0.05 morphine/naloxone (0.05mg/kg) MOR mu-opioid receptor M/S morphine/saline ot optic tract PAG periaqueductal gray PB phosphate buffer PBT-X phosphate buffer containing 2% normal horse serum/0.2% Triton-X-100 PB parabrachial nucleus PBS phosphate buffer saline pDyn prodynorphin PFC prefrontal cortex PPN pedunculopontine nucleus PrL prelimbic cortex PV paraventricular thalamus PVN paraventricular nucleus of the hypothalamus NeuN anti-neuronal nuclei NHS normal horse serum

NMDA *N*-Methyl-D-aspartate

NTS nucleus of the solitary tract

RVM rostral ventromedial medulla

S/- saline/no injection control

s.c. subcutaneous

SEM standard error of the mean

SI substantia innominata

SId dorsal substantia innominata

S/N5 saline/naloxone (5mg/kg)

S/N0.05 saline/naloxone (0.05mg/kg)

SN substantia nigra

SNc substantia nigra pars compacta

SNr substantia nigra pars reticulata

SpC spinal cord

S/S saline/saline

Str striatum

TH tyrosine hydroxylase

THAL thalamus

vl ventrolateral striatum

VLM ventrolateral medulla

vlPAG ventrolateral periaqueductal gray

vm ventromedial striatum

VP ventral pallidum

VTA ventral tegmental area.

#### **Publications**

#### Peer reviewed manuscripts:

Hamlin, A.S., McNally, G.P., and Osborne, P.B. Activation of the lateral central extended amygdala parallels abstinence hyperalgesia induced by naloxone in drug naïve rats after a brief exposure to morphine. *submitted* 

Buller, K., Hamlin, A.S., and Osborne, P.B. Dissection of peripheral and central endogenous opioid modulation of systemic interleukin-1b responses using c-fos expression in the rat brain. Neuropharmacology, 49, 230-242.

Hamlin, A.S., Buller, K., Day, T.A., and Osborne, P.B. (2004) Effect of naloxone precipitated morphine withdrawal on c-fos expression in rat CRH neurons in the paraventricular hypothalamus and extended amygdala. Neuroscience Letters, 362, (1), 39-43.

Hamlin, A.S., Buller, K., Day, T.A., and Osborne, P. (2001) Peripheral withdrawal recruits distinct central nuclei in morphine-dependent rats. Neuropharmacology, 41, 574 – 581.

#### **Published Abstracts:**

Hamlin, A.S., McNally, G.P., Westbrook, R.F., and Osborne, P.B. (2005) Selective activation of accumbens projection pathways by morphine induced catalepsy and stereotypy. *International Narcotics Research Conference*.

Hamlin, A.S., McNally, G.P., and Osborne, P.B. (2005) Induction of c-Fos and zif268 in the central extended amygdala parallels hyperalgesia induced by naloxone following systemic morphine in drug-naïve rats. *International Narcotics Research Conference*.

Hamlin, A.S., McNally, G.P., and Osborne, P.B. (2004) Naloxone administered to non-dependent, morphine-treated rats induces c-Fos throughout the central extended amygdala, and inhibits c-Fos induction in striatal circuits. *Society for Neuroscience*.

Hamlin, A.S., McNally, G.P., and Osborne, P.B. (2004) Fos induction by naloxone-precipitated withdrawal from morphine is the same in the central amygdala of drugnaïve and dependent rats. *Addictions* 2004.

Hamlin, A.S., McNally, G.P., Westbrook, R.F., and Osborne, P.B. (2004) Network level changes in Fos-related-proteins in the rat nucleus accumbens predicts the expression of morphine-induced behavioural sensitisation. *Addictions* 2004.

Hamlin, A.S., McNally, G.P., Westbrook, R.F. and Osborne, P.B. (2003) Morphine pre-treatment alters Fos expression in the rat striatum, produces tolerance to catalepsy and sensitises stereotypy. *International Narcotics Research Conference*.

Hamlin, A.S., McNally, G.P., Westbrook, R.F. and Osborne, P.B. (2003) Induction of Fos-immunoreactive nuclei in the nucleus accumbens by acute morphine is altered by chronic morphine exposure. *Proceedings of the Australian Neuroscience Society*.

Osborne, P.B., Hamlin, A.S., McNally, G.P., and Westbrook, R.F. (2002) Differential induction by chronic morphine of c-Fos and pan-Fos immunoreactive neurons in reward circuits. *Society for Neuroscience*.

Schmid, K.L., Hamlin, A., Hess, R., and Field, D.J. (2001) The effect of noise targets with differing spatial frequency amplitude spectra on emmetropization in chicks.

Investigative Ophthalmology and Visual Science (ARVO suppl.) 42, (4), p. S58.

Schmid, K.L., Hamlin, A.S., and Wildsoet, C.F. (2001) Myopic defocus limits for emmetropization under controlled viewing conditions in chick. *Proceedings of the Australian Neuroscience Society*. 12, p. 204.

Hamlin, A.S., Buller, K.M., Day, T., and Osborne, P.B. (2000) Patterns of fos induction produced by opioid withdrawal in brain "stress" and "reward" pathways. *Proceedings of the Australian Neuroscience Society.* 11, p. 35.

Hamlin, A.S., Buller, K.M., Day, T., and Osborne, P.B. (2000) Fos induction in brain by opioid withdrawal precipitated outside the central nervous system. *Proceedings of the Australian Neuroscience Society.* 11, p.248.

#### **Invited talks**:

Hamlin, A.S. (2005). The role of the central amygdala in acute morphine abstinence induced hyperalgesia. *Kolling Institute seminar series*.

Hamlin, A.S. (2004). Network level changes in Fos related proteins in the rat nucleus accumbens predicts morphine induced stereotyped behaviours. *Sun, Surf, & Science Colloquium, Kioloa*.

Hamlin, A.S., McNally, G.P., Westbrook, R.F., & Osborne, P.B. (2003) Repeated morphine administration in rats alters Fos expression in the ventromedial striatum and nucleus accumbens, produces tolerance to catalepsy and sensitises stereotypy. *XI Annual Royal North Shore Scientific Meeting*.

Hamlin, A.S., McNally, G.P., Westbrook, R.F., & Osborne, P.B. (2003) Differential expression of Fos in the rat striatum by acute morphine challenge in chronically pretreated animals. *Sun, Surf, & Science Colloquium Kioloa*.

Hamlin, A.S., McNally, G.P., Westbrook, R.F., & Osborne, P.B. (2003) Differential expression of Fos and c-Fos immunoreactive nuclei in the rat forebrain by acute morphine challenge in chronically pre-treated animals. *Gene Regulation in Brain, Australian Neuroscience Society Satellite Meeting, Heron Island.* 

Hamlin, A.S. (2002). Functional Neuroanatomy of opioids in the rat forebrain. *Sun, Surf, & Science Colloquium, Kioloa*.

Hamlin, A.S. (2000) "When Stingrays Attack" Centre for Marine Science, Opening of the Stradbroke Island Research Station.