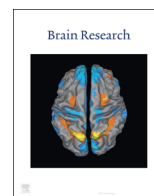




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## Review

# Reflections on: “A general role for adaptations in G-Proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function”



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## ABSTRACT

In 1991 we demonstrated that chronic morphine exposure increased levels of adenylyl cyclase and protein kinase A (PKA) in several regions of the rat central nervous system as inferred from measures of enzyme activity in crude extracts (Terwilliger et al., 1991). These findings led us to hypothesize that a concerted upregulation of the cAMP pathway is a general mechanism of opiate tolerance and dependence. Moreover, in the same study we showed similar induction of adenylyl cyclase and PKA activity in nucleus accumbens (NAc) in response to chronic administration of cocaine, but not of several non-abused psychoactive drugs. Morphine and cocaine also induced equivalent changes in inhibitory G protein subunits in this brain region. We thus extended our hypothesis to suggest that, particularly within brain reward regions such as NAc, cAMP pathway upregulation represents a common mechanism of reward tolerance and dependence shared by several classes of drugs of abuse. Research since that time, by many laboratories, has provided substantial support for these hypotheses. Specifically, opiates in several CNS regions including NAc, and cocaine more selectively in NAc, induce expression of certain adenylyl cyclase isoforms and PKA subunits via the transcription factor, CREB, and these transcriptional adaptations serve a homeostatic function to oppose drug action. In certain brain regions, such as locus coeruleus, these adaptations mediate aspects of physical opiate dependence and withdrawal, whereas in NAc they mediate reward tolerance and dependence that drives increased drug self-administration. This work has had important implications for understanding the molecular basis of addiction.

*Original Article Abstract: “A general role for adaptations in G-proteins and the cyclic AMP system in mediating the chronic actions of morphine and cocaine on neuronal function”.*

Previous studies have shown that chronic morphine increases levels of the G-protein subunits  $G_{i\alpha}$  and  $G_{o\alpha}$ , adenylyl cyclase, cyclic AMP-dependent protein kinase, and certain phosphoproteins in the rat locus coeruleus, but not in several other brain regions studied, and that chronic morphine decreases levels of  $G_{i\alpha}$  and increases levels of adenylyl cyclase in dorsal root ganglion/spinal cord (DRG–SC) co-cultures. These findings led us to survey the effects of chronic morphine on the G-protein/cyclic AMP system in a large number of brain regions to determine how widespread such regulation might be. We found that while most regions showed no regulation in response to chronic morphine, nucleus accumbens (NAc) and amygdala did show increases in adenylyl cyclase and cyclic AMP-dependent protein kinase activity, and thalamus showed an increase in cyclic AMP-dependent protein kinase activity only. An increase in cyclic AMP-dependent protein kinase activity was also observed in DRG–SC co-cultures. Morphine regulation of G-proteins was variable, with decreased levels of  $G_{i\alpha}$  seen in the NAc, increased levels of  $G_{i\alpha}$  and  $G_{o\alpha}$  amygdala, and no change in thalamus or the other brain regions studied. Interestingly, chronic treatment of rats with cocaine, but not with several non-abused drugs, produced similar changes compared to morphine in G-proteins, adenylyl cyclase, and cyclic AMP-dependent protein kinase in the NAc, but not in the other brain regions studied. These results indicate that regulation of the G-protein/cyclic AMP system represents a mechanism by which a number of opiate-sensitive neurons adapt to chronic morphine and thereby develop aspects of opiate tolerance and/or dependence. The findings that chronic morphine and cocaine produce similar adaptations in the NAc, a brain region

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important for the reinforcing actions of many types of abused substances, suggest further that common mechanisms may underlie psychological aspects of drug addiction mediated by this brain region. © 1991. This article is part of a Special Issue entitled *SI:50th Anniversary Issue*.

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### 1. Introduction: drug abuse research circa 1990

This manuscript was published four years into my first term as a new assistant professor in the Department of Psychiatry at Yale University. At that time, virtually nothing was known about how drugs of abuse such as opiates and stimulants change the brain to produce the range of behavioral abnormalities that define a state of addiction. In the opiate field, there was nearly exclusive focus on the opioid receptor itself, which had not yet been cloned. The notion that molecules beyond the receptor might be involved in adaptations to chronic opiate exposure was novel—unheard of really, as was pointed out to me in the reviews of my first NIH grant application in 1987 which was not funded: “There is no evidence to suggest that postreceptor signaling is important for opiate addiction.” The grant was ultimately funded on the second try in 1988. Its goal was to study whether chronic opiate exposure altered proteins downstream of opioid receptors in a range of brain regions implicated in mediating the consequences of chronic opiate exposure including physical dependence, analgesic tolerance, and changes in reward and motivation associated with addiction.

By the mid-1980s it was known that opioid receptors signal through inhibitory G proteins comprised of  $G_{\alpha i}/G_{\alpha o}$  and  $G_{\beta \gamma}$  subunits, which inhibit adenylyl cyclase and protein kinase A (PKA), among other actions. Very little was known about the substrates of PKA that would presumably mediate some of the functional consequences of opioid receptor signaling. We approached this question using an unbiased approach, which would be called proteomics today: we prepared extracts from several discrete brain regions of rats, exposed chronically to morphine or sham treatment, under conditions that inhibited protein kinases and protein phosphatases. We then added purified PKA+<sup>32</sup>P-labeled ATP to phosphorylate proteins in the extracts and resolved the phosphorylated proteins by 2D gel electrophoresis and autoradiography. Our initial study focused on locus ceruleus (LC), the major noradrenergic nucleus in brain, which had been implicated at the time in mediating physical opiate dependence and withdrawal (Koob et al., 1992). We identified altered phosphorylation of several known substrates of PKA (Guitart and Nestler, 1989, 1990; Guitart et al., 1990), suggesting that PKA activity itself might be induced by morphine, something we confirmed directly (Nestler and Tallman, 1988). This also prompted us to investigate whether adenylyl cyclase is regulated in LC by chronic morphine and indeed found this to be the case (Duman et al., 1988). Finally, we found that this induction of adenylyl cyclase and PKA was associated with increased levels of  $G_{\alpha i}$  and  $G_{\alpha o}$  in LC in response to chronic morphine administration (Nestler et al., 1989). Our findings with adenylyl cyclase and PKA were reminiscent of studies a decade earlier by Sharma et al. (1975), who showed that repeated

exposure of cultured neuroblastoma × glioma cells to morphine led to increased levels of cAMP upon removal of the morphine. Our hypothesis was that the concerted upregulation of the cAMP pathway, namely, induction of adenylyl cyclase and PKA, in LC represents a homeostatic adaptation to chronic opiate exposure which serves to oppose opiate action (tolerance) and drive physical withdrawal symptoms upon removal of the opiate (dependence), thus representing the first known molecular mechanism of opiate tolerance and dependence established in vivo. It would take us through most of the 1990s and beyond to provide direct causal evidence for this hypothesis; more about that below.

Our findings in LC, and a related report concerning adenylyl cyclase by Stanley Crain and colleagues in dorsal root ganglion (DRG)-spinal cord (SC) co-cultures (Makman et al., 1988), prompted the study highlighted in this issue (Terwilliger et al., 1991), in which we surveyed whether changes in G proteins and the cAMP pathway are seen in other CNS regions in response to chronic morphine. The study relied on very crude methods: measures of G protein subunits by Western blotting and of adenylyl cyclase and PKA catalytic activity in crude extracts of discrete brain regions dissected from morphine- or sham-treated rats. It was not possible to perform Western blots on adenylyl cyclase and PKA subunits at that time due to the lack of suitable antibodies. We found no evidence for regulation of these proteins in most brain regions studied with a few notable exceptions: induction of adenylyl cyclase and PKA activity in nucleus accumbens (NAc) and amygdala, and induction of PKA only in thalamus; we also demonstrated induction of PKA in DRG–SC co-cultures. Changes in G protein subunits were more variable. These findings prompted us to update our hypothesis stated above: that upregulation of the cAMP pathway represents a general mechanism of opiate tolerance and dependence exhibited by many types of central neurons and mediates diverse behavioral consequences of chronic opiate exposure depending on the functioning of the affected neurons and their circuitry. Thus, whereas cAMP pathway upregulation in LC contributes to physical opiate dependence and withdrawal, the same adaptations in NAc—a key brain reward region—might mediate reward tolerance and dependence, and so on. This general hypothesis was supported by related work in the myenteric plexus of the gut (Wang and Gintzler, 1995).

Moreover, because the NAc had at that time come to be seen as a shared neural substrate for the rewarding actions of all drugs of abuse (Di Chiara and Imperato, 1988; Koob and Bloom, 1988), we tested whether cocaine induced similar molecular changes in this brain region. Our findings of equivalent adaptations in G protein subunits, adenylyl cyclase, and PKA in NAc of rats treated chronically with cocaine, but not with several classes of non-abused drugs, led us to hypothesize further that upregulation of the cAMP pathway in this brain region represents a mechanism of reward

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