SYNAPSE 33:160–162 (1999)

Short Communication

Morphine Alters the Structure of Neurons in the Nucleus Accumbens and Neocortex of Rats

TERRY E. ROBINSON[1]* **AND BRYAN KOLB**[2]
[1]*Department of Psychology and Neuroscience Program, The University of Michigan, Ann Arbor, Michigan 48109, USA*
[2]*Department of Psychology, University of Lethbridge, Lethbridge, Alberta T1K 3M4, Canada*

KEY WORDS Golgi; dendrites; dendritic spines; pyramidal cells; sensitization; opiates; plasticity; prefrontal cortex; parietal cortex

ABSTRACT Rats were given repeated injections of 10 mg/kg of morphine and were then left undisturbed for 24–25 days before their brains were processed for Golgi-Cox staining. Prior exposure to morphine decreased the complexity of dendritic branching and the number of dendritic spines on medium spiny neurons in the shell of the nucleus accumbens and on pyramidal cells in the prefrontal and parietal cortex. It is suggested that some of the long-term behavioral consequences of repeated exposure to morphine may be due to its ability to reorganize patterns of synaptic connectivity in the forebrain. **Synapse 33:160–162, 1999.**

The ability of opiates to influence the activity of ascending catecholamine neurons is thought to contribute to many of their effects on behavior (Wise and Bozarth, 1987). Furthermore, when given chronically opiates induce neuroadaptations in catecholamine systems, some of which are thought to contribute to the long-term sequelae associated with repeated opiate use and abuse, including tolerance, sensitization, dependence, and addiction (Nestler, 1996). Most studies on opiate-induced neuroadaptations have focused on the ability of these drugs to alter biochemical processes and/or the electrophysiological characteristics of neurons. However, we have recently found that repeated treatment with psychostimulant drugs, such as amphetamine or cocaine, alters not only biochemical processes but also the morphology of cells in brain regions that are innervated by catecholamine neurons (Robinson and Kolb, 1997, 1999). Given the similar effects of psychostimulants and opiates on catecholamine neurotransmission, and the increase in illicit opiate use during the late 1990s (NIDA Monitoring the Future data; www.nida.nih.gov), we asked whether repeated exposure to morphine might also alter the morphology of cells in brain regions innervated by catecholamine neurons.

Female Sprague-Dawley rats approximately 3 months old (225–250 g) at the beginning of the experiment received an i.p. injection of either saline or 10 mg/kg of morphine sulfate (weight of the salt) once each day for 5 consecutive days, followed by 2 drug-free days, and this procedure was repeated for 4 weeks. This injection regimen was used because it has been shown to produce sensitization to morphine's psychomotor activating effects (Jeziorski and White, 1995) and because upon the discontinuation of drug treatment there are no obvious overt signs of withdrawal (K.A. Trujillo and F.J. White, personal communications). After the last injection the animals were left undisturbed for 24–25 days, after which time they were deeply anesthetized with sodium pentobarbital and perfused intracardially with 0.9% saline. The brains were removed and processed for Golgi-Cox staining using procedures described previously (Gibb and Kolb, 1998). Neurons in three brain regions were selected for analysis: medium spiny neurons in the shell of the nucleus accumbens, layer V pyramidal cells in the prefrontal cortex (Cg3), and layer III pyramidal cells in the parietal cortex (Par1; anatomical terminology after Zilles, 1985). Five cells in each hemisphere were drawn using camera lucida at a magnification of 250x by a person blind to treatment conditions. The dendritic surface was quantified both by counting the number of branches at each order from the cell body and by counting the number of ring

*Correspondence to: Dr. Terry E. Robinson, Biopsychology Program, Department of Psychology, The University of Michigan, 525 E. University (East Hall), Ann Arbor, MI 48109, USA. E-mail: ter@umich.edu

Received 14 January 1999; Accepted 15 January 1999

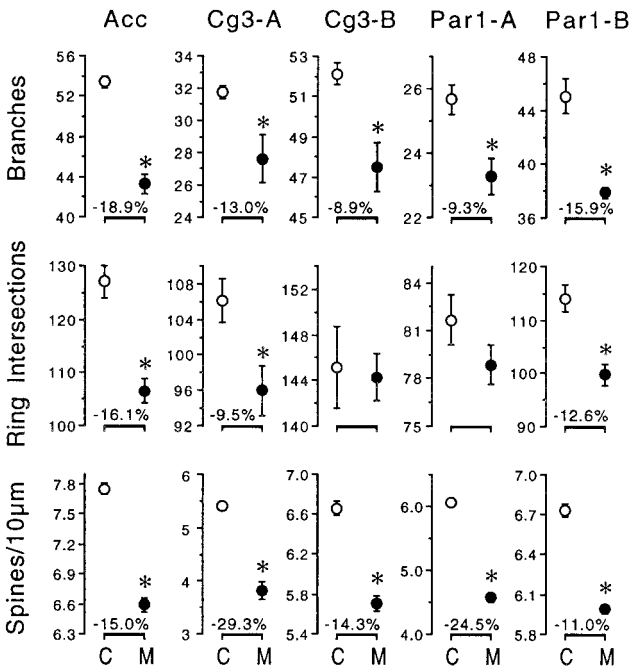

Fig. 1. Morphine-induced changes in dendritic morphology. The three rows proceeding from top to bottom show the mean ± SEM number of branches, number of ring intersections (rings), and number of spines/10 µm, in the shell of the nucleus accumbens (Acc), the prefrontal cortex (Cg3-A, apical dendrites; Cg3-B, basilar dendrites) and the parietal cortex (Par1-A and Par1-B, apical and basilar dendrites, respectively). Where error bars are not evident, they are smaller than the diameter of the symbol. Open circles (C) indicate the control group (n = 10) and closed circles (M) the morphine-treated group (n = 10). The control group is the same as reported in Robinson and Kolb (1999), and all animals were treated and the brains processed at the same time. Asterisks indicate statistically significant group differences (t-tests; t-values range from 7.5–423 and *P*-values from < 0.01–0.0001), and the numbers above the horizontal axis indicate the average percent decrease from control.

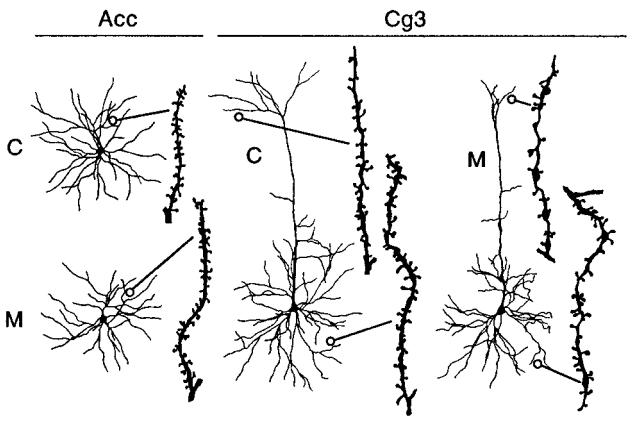

Fig. 2. Camera lucida drawings of representative nucleus accumbens medium spiny neurons (Acc) from a control (top left, C) and morphine-treated rat (bottom left, M), and prefrontal cortex Layer V pyramidal cells (Cg3) from a control (middle) and morphine treated rat (right). Sample dendritic segments used to count spine density are shown at high power to the right of each cell (the actual segments represented are indicated by the pointers).

intersections using an overlay of concentric rings (see Kolb et al., 1998, for details). For cortical cells, the apical and basilar dendrites were quantified separately. In addition, the density of dendritic spines was estimated by drawing at least 10 µm long segments from the terminal tips at high power (1,000x) and counting the number of spines. Statistical analyses were conducted after averaging across cells within hemispheres (i.e., hemisphere was the unit of analysis).

In animals treated with morphine there was a marked *decrease* in the complexity of dendritic branching and the density of dendritic spines on neurons in all three brain regions examined (Figs. 1, 2). There was a 9–19% decrease in the number of dendritic branches ($P < 0.01$) and in three of the five regions a comparable decrease in dendritic length as estimated by the number of ring intersections (Fig. 1). There was an especially marked decrease in the density of dendritic spines in all regions ($P < 0.001$), ranging from an 11% loss of spines from Par1 basilar dendrites to a 29% loss of spines from Cg3 apical dendrites (Fig.1).

If given early during development it is known that morphine can decrease the complexity of dendrites on cells in the cerebral cortex and hippocampus (Ricalde and Hammer, 1990), and because blockade of opioid receptors can have the opposite effect it is thought that endogenous opioid systems are important for normal neuronal development (Hauser et al., 1987, 1989). However, to our knowledge this is the first report that repeated exposure to a relatively low dose of morphine in adult rats can produce a persistent decrease in the dendritic branching and spine density in the cerebral cortex and nucleus accumbens. Many studies have established that changes in dendritic structure seen in Golgi material, similar to those reported here, are indicative of alterations in synaptic organization (Greenough et al., 1990; Kolb et al., 1998). Our results suggest, therefore, that in adult rats moderate doses of morphine, not sufficient to produce overt physical dependence, can produce widespread alterations in patterns of synaptic connectivity in the forebrain, including brain regions implicated in mediating morphine's rewarding effects.

The mechanism by which morphine alters dendritic structure is not known, but it may be related to its ability to alter neurofilament proteins or other cytoskeletal proteins (Beitner-Johnson et al., 1992; Boronat et al., 1998). Indeed, it was recently reported that chronic morphine administration via a subcutaneous implant produces a 25% decrease in the size of dopamine cell bodies in the ventral tegmental area (Sklair-Tavron et al., 1996). In this latter study, nondopaminergic cells in the ventral tegmental area were unaffected, suggesting the effects of chronic morphine treatment were highly localized. The present results suggest, however, that morphine treatment may in fact promote a widespread reorganization of synaptic connectivity in the forebrain

(although, of course, there may also be differences in the neuroadaptive processes engendered by continuous vs. intermittent morphine treatment; Robinson and Becker, 1986).

It is especially interesting that the effects of morphine on dendritic structure were in the opposite direction of those seen in comparable studies with psychostimulant drugs, and appeared to be more widespread (Robinson and Kolb, 1997, 1999). For example, repeated amphetamine treatment *increased* the length of dendrites on medium spiny neurons in the nucleus accumbens and the length of apical (but not basilar) dendrites of pyramidal cells in prefrontal cortex, but had no effect on the length of either apical or basilar dendrites of pyramidal cells in the parietal cortex (Robinson and Kolb, 1997). How the changes in dendritic structure produced by either psychostimulants or morphine alter information processing in these neural circuits, and thus potentially contribute to the many long-term functional consequences of repeated exposure to drugs of abuse, including tolerance, sensitization, dependence, and addiction, is not known (Nestler, 1996; Sklair-Tavron et al., 1996). Nevertheless, it is becoming increasingly clear that in exploring the mechanisms by which potentially addictive drugs produce persistent effects on brain function and behavior it will be necessary to look beyond brain biochemistry, to anatomical circuitry (Robinson and Kolb, 1997, 1999; Sklair-Tavron et al., 1996), as is the case for other forms of experience-dependent plasticity (Greenough and Bailey, 1988; Harris and Kater, 1994; Kolb et al., 1998).

REFERENCES

Beitner-Johnson D, Guitart X, Nestler EJ. 1992. Neurofilament proteins and the mesolimbic dopamine system: common regulation by chronic morphine and chronic cocaine in the rat ventral tegmental area. J Neurosci 12:2165–2176.

Boronat MA, Olmos G, Garcia-Sevilla JA. 1998. Attenuation of tolerance to opioid-induced antinociception and protection against morphine-induced decrease of neurofilament proteins by idazoxan and other I-2-imidazoline ligands. Br J Pharmacol 125:175–185.

Gibb R, Kolb B. 1998. A method for vibratome sectioning of Golgi-Cox stained whole rat brain. J Neurosci Methods 79:1–4.

Greenough WT, Bailey CH. 1988. The anatomy of a memory: convergence of results across a diversity of tests. Trends Neurosci 11:142–147.

Greenough WT, Withers GS, Wallace CS. 1990. Morphological changes in the nervous system arising from behavioral experience: what is the evidence that they are involved in learning and memory? In: Squire LR, Lindenlaub E, editors. The biology of memory, Symposia Medica Hoechst. New York: F.K. Schattauder Verlag. p 159–185.

Harris KM, Kater SB. 1994. Dendritic spines: cellular specializations imparting both stability and flexibility to synaptic function. Annu Rev Neurosci 17:341–371.

Hauser KF, McLaughlin PJ, Zagon IS. 1987. Endogenous opioids regulate dendritic growth and spine formation in developing rat brain. Brain Res 416:157–161.

Hauser KF, McLaughlin PJ, Zagon IS. 1989. Endogenous opioid systems and the regulation of dendritic growth and spine formation. J Comp Neurol 281:13–22.

Jeziorski M, White FJ. 1995. Dopamine receptor antagonists prevent expression, but not development, of morphine sensitization. Eur J Pharmacol 275:235–244.

Kolb B, Forgie M, Gibb R, Gorny G, Rowntree S. 1998. Age, experience and the changing brain. Neurosci Biobehav Rev 22:143–159.

Nestler EJ. 1996. Under siege: the brain on opiates. Neuron 16:897–900.

Ricalde AA, Hammer RP Jr. 1990. Perinatal opiate treatment delays growth of cortical dendrites. Neurosci Lett 115:137–143.

Robinson TE, Becker JB. 1986. Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Res Rev 11:157–198.

Robinson TE, Kolb B. 1997. Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. J Neurosci 17:8491–8497.

Robinson TE, Kolb B. 1999. Repeated exposure to either amphetamine or cocaine alters the morphology of neurons in the nucleus accumbens and prefrontal cortex. Eur J Neurosci 11:1598–1604.

Sklair-Tavron L, Shi W-X, Lane SB, Harris HW, Bunney BS, Nestler EJ. 1996. Chronic morphine induces visible changes in the morphology of mesolimbic dopamine neurons. Proc Natl Acad Sci USA 93:11202–11207.

Wise RA, Bozarth MA. 1987. A psychomotor stimulant theory of addiction. Psychol Rev 94:469–492.

Zilles K. 1985. The cortex of the rat. Berlin: Springer-Verlag.