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Chronic morphine exposure alters the dendritic morphology of pyramidal neurons in visual cortex of rats

Yanfei Li [a,b], Hao Wang [a], Lei Niu [a], Yifeng Zhou [a,*]

[a] *Hefei National Laboratory for Physical Sciences at Microscale and School of Life Science, University of Science and Technology of China, Hefei, Anhui 230027, PR China*
[b] *Department of Cell biology, Anhui University, Hefei, Anhui 230039, PR China*

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Abstract

Repeated treatment of psychotropic drugs produces changes in brain and behavior that far outlast their initial neuropharmacological effects. The nature of persistent drug-induced neural plasticity is of interest because it is thought to contribute to the development of drug dependency and addiction. To determine if chronic morphine treatment alters the morphology of visual cortical neurons, we statistically examined the dendrites of layer III pyramidal neurons in the primary visual cortex of both morphine-treated and saline-control rats. Compared with control rats, the pyramidal cells of morphine-treated animals showed a significant decrease in the total dendritic length (24%) and a significant reduction (27%) in the dendritic spine density of dendritic arborization at the level of the second branch order. Our results suggest that some of the persistent neurobehavioral consequences and cognitive impairment resulting from repeated exposure to morphine may involve a reorganization of synaptic connectivity in visual cortical neurons.

Keywords: Golgi; Dendrite spines; Opiates; Pyramidal cells; Visual cortex; Plasticity

Chronic exposure to opiates often leads to opiate dependency. Previous studies showed that morphine-like drugs decreased visual sensitivity in humans [33], impacted visual discrimination performance in rats [11] and affected cortical potentials evoked from optic chiasm stimulation in cats [44]. Recent work in our lab also showed that chronic morphine exposure influenced the response properties of cortical neurons in cats and impaired short-term synaptic plasticity depression in the geniculo-cortical visual pathway of rats [12]. The brain is rich in opiate receptors, particularly in the visual system of cat [43], macaque [45] and the rat [24] where significant concentrations of opiate receptors are observed, suggesting that the visual system is subject to powerful opiate modulation. Manipulation of the opioid system in the brain is known to produce neuronal changes, including dendritic process modifications [30]. However, the underlying mechanism is still unknown.

Previous studies suggest that normal excitatory and inhibitory synaptic transmission is crucial for the development and maintenance of visual cortical function. Specifically, GABAergic synaptic transmission is significantly influenced by opiates [5,23,41]. Furthermore, reports indicate that repeated usage of addictive drugs leads to multiple adaptive neuronal responses [30,32]. For example, repeated administration of addictive drugs, which produce changes in psychological function, not only alters brain biochemistry but also modifies brain structure, such as modifying patterns of synaptic connectivity [10,19]. So far, it is known that systemic morphine exposure could reduce the dendritic complexity and spine density on pyramidal neurons in the motor cortex, although the effect of larger and longer basal dendritic arbors by chronic morphine in different brain regions has been reported as well [1]. Additionally, it is well known that dendrites and dendritic spines are the loci of most excitatory synapses, which are subject to experience-dependent structural plasticity and are considered as foci of related study [13,18,20,26]. Therefore, determining how drugs alter dendrites and dendritic spines within neural circuits is essential for understanding how drug dependency leads to life-long changes in behavior and psychological function.

* Corresponding author at: School of Life Science, University of Science and Technology of China, Hefei, Anhui 230027, PR China. Tel.: +86 551 3601436; fax: +86 551 3607014.
E-mail address: zhouy@ustc.edu.cn (Y. Zhou).

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This study aimed to determine if there were alterations in the morphology of visual cortical neurons following chronic morphine treatment. Moreover, we attempted to elucidate the mechanisms responsible for the effects of chronic morphine treatment on the response properties of visual cortical neurons. By Golgi-Cox staining, a method, which is sensitive to examination of neuronal morphology changes [10]. We quantitatively measured the dendritic length and spine density of layer III pyramidal neurons in the visual cortex of morphine-treated rats and control rats to see whether chronic morphine administration modifies neuronal dendritic morphology.

Twenty male Sprague–Dawley (200–230 g) rats were obtained from the Laboratory Animal Center, An'hui Medical University (Hefei, China). Rats were housed in groups and maintained with food and water ad libitum on a 12 h light/dark cycle. Ten rats were administered with morphine (10 mg/kg) (SC injection) twice per day at 12 h intervals for 10 days as described previously [28,39]. Control rats were treated similarly, except that normal saline (NS) was used instead. All animal treatments were strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Three hours after the last injection, the rats ($n = 10$ per group) were deeply anesthetized with urethane and perfused intracardially with 0.9% saline solution. The brains were removed and prepared for processing by Golgi-Cox staining, according to procedures described by Gibb and Kolb [9,29]. Next they were sectioned at 120-μm thickness in the coronal plane at the level of visual cortex (ranging from AP 1.0–5.0, Lateral 6.0–9.6 mm) [27] using a Rotary Type Microtome (American Optical).

The Golgi-impregnated pyramidal neurons of the visual cortex were readily identified by their characteristic triangular soma shape, apical dendritic extension toward the pial surface, and numerous dendritic spines. As with that described previously [37,42]. Only neurons with the following criteria were used for quantitative analysis: (1) location of the cell soma in layer III of visual cortex (Fig. 1); (2) full impregnation of the neurons; (3) presence of at least three primary basilar dendritic shafts, each of which branched at least once; (4) no morphological changes attributed to Golgi-Cox staining.

At least three neurons from each visual cortex region of each hemisphere per animal were photographed at a magnification of 400× (BX-60, Olympus Microscope) by a person blind to experimental groups [19]. One hundred and two neurons were calculated in all. For each neuron, the dendritic tree, including all branches, was quantified by Sholl analysis as follows [36]. One dendrite was examined per neuron. A transparent grid with concentric rings, equivalent to 10-μm spacing, was placed over the dendrite picture, and the number of ring intersections was used to estimate the total dendritic length (TDL) [19,37]. The spine density, which was defined as the number of spines per unit length [19], was estimated by photographing a length of dendrite (at least 10 μm long), then the exact length of a randomly chosen dendritic segment was calculated, and the number of spines along that length was counted and expressed as spines/10 μm.

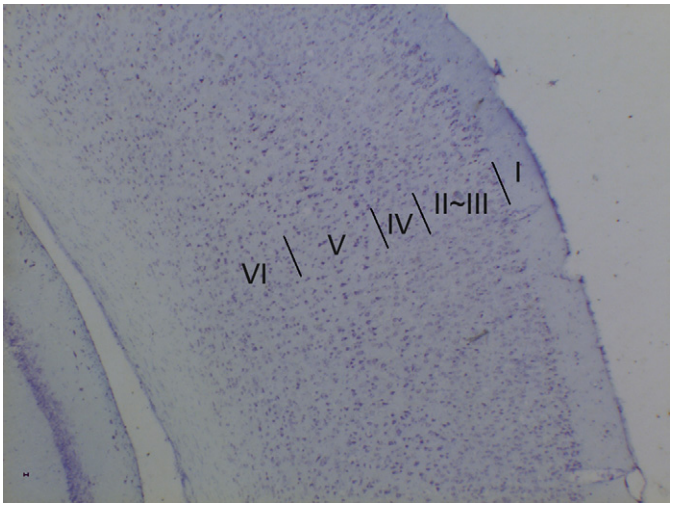

Fig. 1. Coronal section of a Nissl-stained visual cortex of rat showing the regions of analyses. Layer III of visual cortex was analyzed. Scale bar = 60 μm.

Spines were counted at the proximal part of the second order branch of pyramidal neurons. In some cases, the entire branch was 40-μm long or more. The spine number was counted along the entire length of the branch, and the spine density was then calculated and expressed as spines/10-μm. No attempt was made to correct for the fact that spines directly below and above the plane of view could not be seen, so this measure necessarily underestimates the total dendritic length.

Data from the neurons' spine densities, dendritic lengths as well as the Sholl analysis of the number of ring intersections were analyzed by averaging across cells per hemisphere (i.e., hemisphere was the unit of analysis) for each animal, and group differences were assessed using *t-test* (P value of <0.05 was considered significant).

Dendritic branching and dendritic spine densities of the visual cortex were measured by Golgi-Cox staining in rats subjected to either chronic morphine exposure or saline-control. The Golgi-Cox impregnation procedure clearly filled the dendritic shafts and spines of pyramidal neurons in the visual cortical layers III (Fig. 2). The observed effects of morphine treatment on the morphology of pyramidal cells in visual cortex were consistent with data reported in other brain region [30]. Fig. 3 shows that chronic morphine exposure significantly decreased spine density of both apical and basilar dendrites of pyramidal cells (27% decrease, $P < 0.01$) in the visual cortex (5.65 ± 0.34spines/10 μm of morphine-treated rats compared with that of saline-control 7.76 ± 0.45spines/10 μm). Additionally, compared with saline-treated rats, we found that many spines in morphine-treated rats appeared to be appreciably shortened, as indicated by stubby stems (see Fig. 2 B and D). These results indicate that chronic morphine treatment induces morphological changes in visual cortical neurons.

As measured by Sholl analysis, the total dendritic length of the pyramidal cells in visual cortex was significantly lower in morphine-treated rats (568.30 ± 35.63 μm) than in saline-treated controls (750.58 ± 78.15 μm) ($P < 0.05$) (Fig. 4). The analysis of intersection per radius of shell shows that

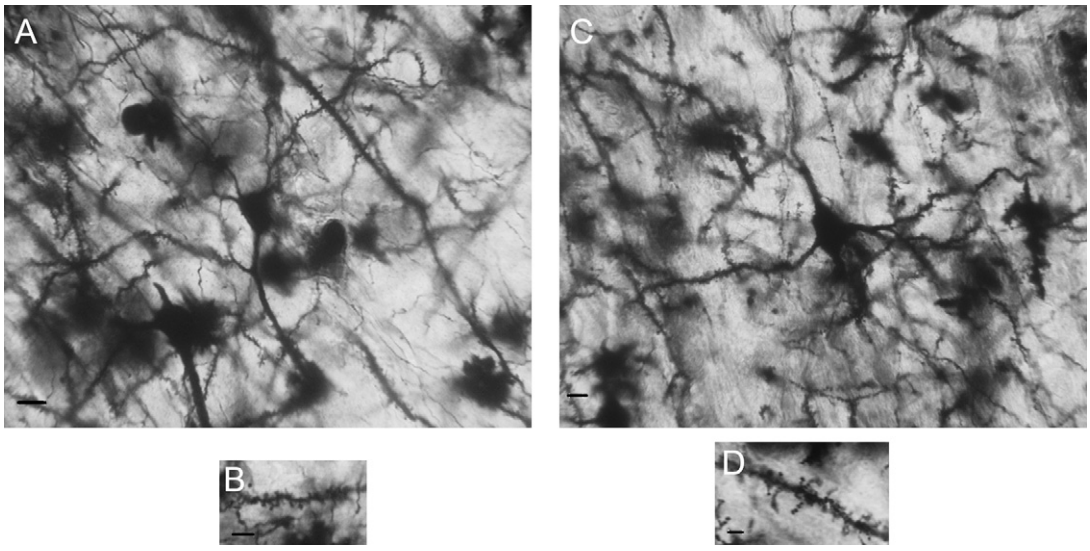

Fig. 2. Photomicrographs illustrating Golgi-Cox impregnated dendritic arborization and dendritic spines of pyramidal neurons in visual cortex layer III. Morphine-exposed rats (A and B) and saline-control rats (C and D). The dendrites of morphine-dependent rats become thinner (A) while dendritic spines become fewer and shorter (B). The morphology of dendrites and dendritic spines observed in saline-control rats are depicted in figure C and D respectively. Scale bar = 30 μm (A and C). Scale bar = 15 μm (B and D).

morphine-treated rats had fewer intersections per shell or less dendritic arborization than saline-treated rats (Fig. 4).

As a kind of brain disorder, drug addiction is considered to be a neuronal adaptation that alters the function of the neuronal circuit, including changes in neuronal plasticity and synaptic transmitter release [25,31,47]. Numerous studies have examined the effects of morphine exposure on specific brain regions and cell types of various species and a variety of results have been reported. For example, intermittent injections of morphine produce a persistent decrease in dendritic branching and spine density of medium spiny neurons in the nucleus accumbens shell and pyramidal cells in sensory cortex [30]. Furthermore, morphine alters neurofilament and other cytoskeletal proteins in the cerebral cortex and ventral tegmental area both in animals [3,17] and human opioid addicts [6,8]. Additionally, chronic morphine exposure reduces axonal transport in the nucleus accumbens shell [2] and decreases the size of dopamine cell bodies in the ventral tegmental area [38]. In this study, we provide direct evidence that chronic morphine administration produces a significant decrease in both total dendrite length and dendritic spine density of neurons in visual cortex. Our results suggest that in adult rats chronic morphine exposure can produce alterations in patterns of synaptic connectivity in the visual cortex.

The vast majority of excitatory synaptic inputs to neocortical pyramidal cells terminate on dendritic spines, which can thus serve as markers, visible by light microscopy, for the locations of these synapses. The basal and proximal oblique dendrites jointly sampled a roughly spherical volume of cortex centered

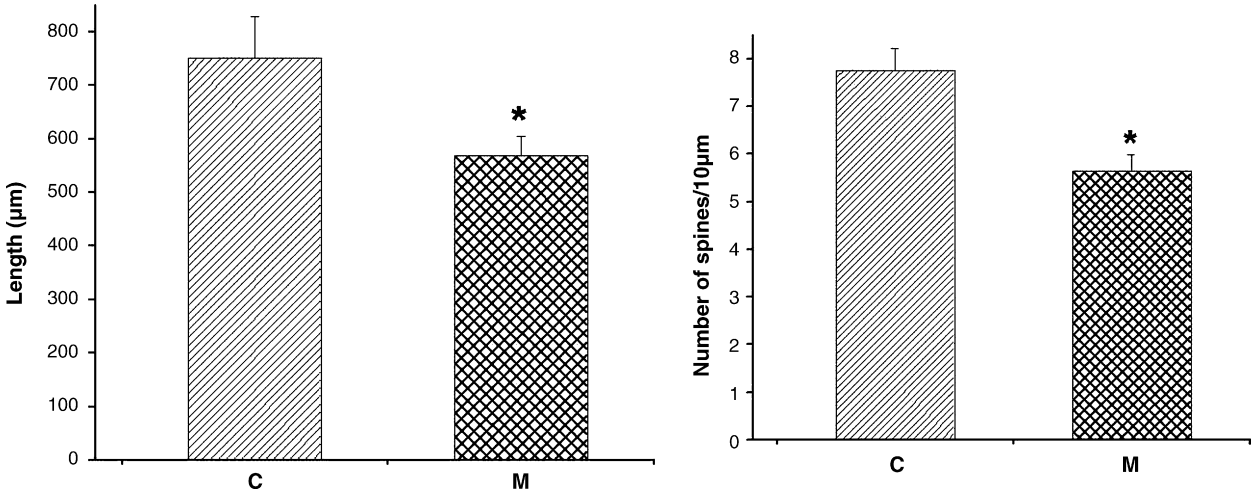

Fig. 3. Quantitative analysis of the density of dendritic spines (spines/10-μm) (left) and total dendritic length (right) in visual cortex layer III pyramidal neurons of rats treated with saline (C) or morphine (M). The bars represent the values of mean plus S.E.M. Spine densities were decreased in the morphine-exposed group compared with the control group ($^{*}P < 0.01$). Dendritic length was estimated using a Sholl analysis. The dendritic length was decreased in morphine-exposed animals ($^{*}P < 0.05$).

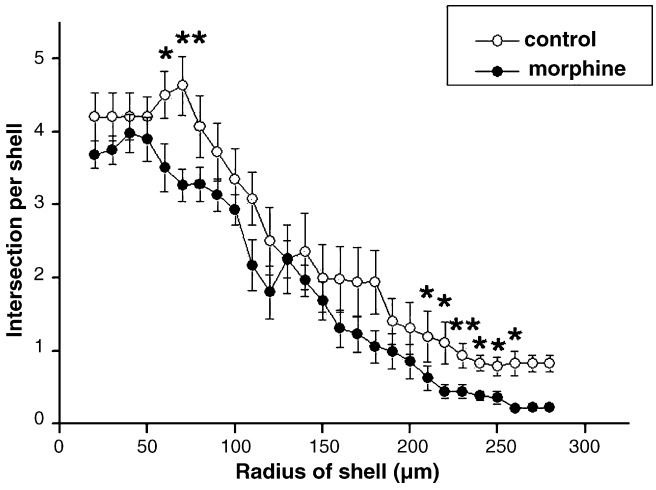

Fig. 4. Sholl analysis of the intersections per shell of visual cortex layer III pyramidal neurons. Closed circles indicate the mean ± S.E.M. of morphine-exposed animals and the open circles correspond to the mean ± S.E.M. of control rats. Where S.E.M. bars are not evident they are smaller than the diameter of the symbol. The morphine treated group showed a decrease in the dendritic length compared with the saline-control group ($^{*}P < 0.05$, $^{**}P < 0.01$).

about the soma, and together they accounted for the substantial majority of the cell's total dendritic shaft membrane area [21]. The distribution of spines varies with respect to cortical layers and dendritic locations. For example, most cells had most of their spines in the layer containing the soma. In visual cortex area, layer II and layer III cells showed a progressive reduction in the proportion of their spines in layers I and II with increasing depth of their soma in the cortex. It seems that the majority of spines were located within a path length of 150 μm from the soma [22]. So we chose layer III pyramidal cells for study and calculated spine densities at the proximal part of the second order branch of pyramidal neurons, and found significant reduction in the spine densities and total length of the dendritic arbors of pyramidal cells in the visual cortex.

Though our analysis of the Golgi-stained material can not tell us exactly which kinds of synapses were modified by morphine or how they were rearranged, the alterations in the spine density presumably reflect a reorganization of the synapses formed on these cells [10,13,16,19,35,46]. The majority of synaptic inputs to neurons are located on either dendrites or dendritic spines, and the amount of synaptic input that a cell receives varies with the amount of dendritic surface available [13]. The afferent axons pass perpendicularly through the dendritic arbors with vesicle-filled varicosities along their length. Apart from its central role of connectivity, dendritic spines also serve other important functions, such as compartmentalization of Ca^{2+} to protect from excitotoxicity, conferring specificity to changes in synaptic efficacy [4]. Indeed, dendritic spines are thought to represent the focus of control over synaptic transmission, thus plastic changes can alter synaptic signaling [35]. For example, an increase in the number of dendritic branches and the number of dendritic spines probably represents an increase in the number of synaptic inputs onto that portion of the dendritic tree, as spines rarely observed left vacant. Furthermore, spines can function to modify synaptic efficacy by modulating the electrotonic characteristics of the synapse [15,26,34,35,40], such as induction and endurance of LTP, by altering fast synaptic neurotransmission [18], or the local chemical environment [35].

Previous studies have reported altered response properties of visual cortical cells and the impaired short-term synaptic plasticity within the geniculo-cortical visual pathway in morphine treated animals [12]. Our findings describing the morphological changes in visual cortical cells of morphine-treated rats could partly explain the underlying mechanism. Dendritic spines serve as excitatory or inhibitory synaptic input sites, and the density of spines on the dendrites is related to the degree of connectivity between these neurons and the axons that pass through their dendritic arbors [7]. Therefore, the decrease in both the dendritic length and the number of dendritic spines we observed may affect the electrophysiological and biochemical properties of visual cortical pyramidal neurons. This interpretation is consistent with the findings that response properties of visual cortical neurons are altered in morphine treated animals [12,14].

In summary, the data presented here suggests that chronic morphine exposure can lead to significant dendritic changes in visual cortical pyramidal neurons. Morphine-derived morphological changes, coupled with a decrease of GABAergic inhibition might contribute to the functional degradation of cells within the visual cortex. The challenge for the future will be in matching such patterns of anatomical connectivity to similar brain changes in human drug users and associated – possibly drug-specific – changes in behavior, psychological and cognitive functions. As the effect of chronic morphine exposure is complex, unraveling the mechanism mediating the degradation of function within the visual system of animals suffering from chronic opiate exposure, poses a major challenge for future research.

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