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Research report

The morphological changes of pyramidal and spiny stellate cells in the primary visual cortex of chronic morphine treated cats

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ABSTRACT

Morphine exposure may have a negative effect on the receptive field properties of neurons in primary visual cortex of cats. The present experiment used morphological methods in order to investigate whether chronic morphine treatment also affects dendritic characters of these neurons. According to the Sholl analysis and dendritic branch order analysis, we obtained the dendritic length and calculated the spine density on dendrites of the pyramidal neurons in layer III and the spiny stellate neurons in layer IV. The results showed that morphine exposure induced significant decreases in the total dendritic length and spine density on both pyramidal and spiny stellate neurons. The further branch order analysis revealed that spine density was decreased at every (first to fourth) branch order of dendrites of pyramidal and spiny stellate neurons. Decrease in dendritic length of the pyramidal neurons was observed only at the fourth branch order, while the spiny stellate neurons had shorter dendrite at the second and third branch order. These findings may underlie the degradation of receptive field properties of the primary visual cortex neurons following chronic morphine exposure.

1. Introduction

Chronic administration of morphine or heroin, which can manipulate opioid system in the brain, is known to produce alteration of physiological properties and morphological changes in the structure of neurons. The mesolimbic dopaminergic system comprising the circuit of VTA (ventral tegmental area)/NAcc (nucleus accumbens)/PC (prefrontal cortex) is implicated in the dependence of opiate substances. The opiate receptors, crucial for the opiate effects on the brain, are broadly distributed in this system [34,35]. By binding to these receptors, opiate can excite VTA dopamine neurons and VTA-DA projection neurons [25,42], and inhibit neurons in PC and NAcc [16,19]. Numbers of studies have shown the morphologic dendritic changes of this system [49,50]. In opiate-treated rats, it has been found that the decrease of long-term potentiation (LTP) in hippocampus, abundant in κ- and μ-opiate receptors [45], is accompanied by the descent of dentritic spiny density [48]. Significant concentrations of opiate receptors have also been observed in the visual system of rats, cats and macaques [30,64,68]. The fact thus leads to the speculation that visual system may be subject to opiate modulation. The previous studies have documented the existence of functional alterations in the visual system related to opiate abuse, including down-regulated visual sensitivity in humans, decreased visual discriminative function in rats and impaired visual responses properties of primary visual cortex (area 17) neurons in cats and rats [18,52,65]. Recent studies of our laboratory have revealed degradation of receptive field properties of the visual cortical neurons in morphine-treated cats [21,22]. We suggest that these alterations could also accompany morphine-related morphological changes of neurons in visual system.

The distribution of neurons with long and local association pathways in cat neocortex is well established [11]. Most pyramidal neurons in layer III of primary visual cortex have axons projecting out of the striate cortex to furnish the corticocortical projection with other cortical areas or forming local projection within primary visual cortex. And these neurons are modulated by local neurons (inhibitory) in the same layer and projection neurons (excitatory) from other layers [7,11,37]. Spiny stellate neurons in layer IV of area 17 contact with excitatory and inhibitory neurons within-layer, projection neurons from other layers and neurons from LGN. All of these neurons compose feedforward and feedback modules. Therefore, spiny stellate neurons play an important role in forming the informational processing circuit [1,7,11,43].

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Table 1
Description about the gender, weight, injection volume of each cat

Cats (morphine, control group)	Gender (M/F)	Weight (kg)	Dose (mg) (weight[a] 10 mg/kg)	Volume (ml)
Morphine	M	2.8	28	2.8
Morphine	M	2.3	23	2.3
Morphine	M	2.1	21	2.1
Control	M	2.5	25	2.5
Control	M	2.3	23	2.3
Control	M	2.1	21	2.1

[a] The concentration of morphine HCl in our present study was 10 mg/ml.

As mentioned above, many studies have been done to investigate alterations in the opiate-treated animals. However, the morphologic integrity of neurons providing identified local-circuits and corticocortical circuits has not been revealed with respect to morphine-related changes. We wanted to determine whether chronic morphine exposure leads to changes of the dendritic arborization of pyramidal cells in layer III and spiny stellate cells in layer IV of primary visual cortex of the cat.

2. Experimental procedures

2.1. Subjects and drug exposure

The experiments were performed on six healthy adult (2–4 years old) male cats (2–3 kg). Three cats were randomly allocated to the morphine-treated group and three cats to the control group. All of these cats were obtained from the Laboratory Animal Center, University of Science and Technology of China. Protocols of morphine treatment were similar to those used by other researchers [15,21,61]. Morphine HCl (10 mg/kg) was administered by cervical subcutaneous injection twice per day at 9:00 a.m. and 9:00 p.m. for 10 days. Control cats were treated similarly by saline instead of morphine (Table 1). All animal treatments were strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Tissue preparation

Twelve hours after the last injection of morphine or saline, cats were deeply anesthetized with ketamine HCl (80 mg/kg), and then perfused through the heart with 2000 ml of 0.9% physiological saline solution, followed by 1000 ml of fixative solution containing 4% paraformaldehyde dissolved in 0.1 M phosphate buffer (PB, pH 7.2–7.4). Brains were removed from the skull, and then blocks of tissue containing the area 17 (posterior to bregma 22–26 mm, lateral to midline 1–4 mm) [46] were dissected out and processed by Golgi-Cox stain method, according to the procedures described by Gibb and Kolb [17]. The blocks were first stored in the dark for 14 days in Golgi-Cox solution then 5 days in 30% sucrose. Blocks were sectioned at a thickness of 120-μm in the coronal plane with a vibratome (VT1000S, Leica). All sections were collected on 2% gelatin-coated slides and stained with Kodak D-76 for 30 s, washed with water three times, followed by Kodak Film Fix for 20 min, and then washed with water, dehydrated, cleared, and mounted using a resinous medium.

2.3. Neuron-selection criteria and morphological analysis

We analyzed two types of neurons, the pyramidal neuron in layer III and the spiny stellate neuron in layer IV. All of these neurons, which were clearly shown under the microscope, were photographed at a magnification of 400× (BX-60, Olympus Microscope).

To obtain all of the dendritic branches, we photographed a series of pictures for each neuron at the same location and different focal planes. And then dendrites of each order were photographed at high power (1000×). However, some spines and dendrites below or above the plane of view may be invisible. Thus, this measurement is apt to underestimate the total of spiny number and the total dendritic length.

In our experiment, each dendritic tree was quantified by the following parameters for each cell: (1) total dendritic length and spine density; (2) dendritic length of each branch order; (3) spine density of each branch order.

The criteria used to select the two types of neurons for photographing and analysis were same as those described in the previous studies: for the pyramidal neurons [26,54,63], (1) location of the cell soma in layer III (Fig. 1), (2) full impregnation of the neurons, (3) medium triangular soma, (4) presence of at least three primary basilar dendritic shafts, each of which branched at least once, (5) no morphological changes attributed to Golgi-Cox stain; for the spiny stellate neuron [32,36], (1) location of the cell soma in layer IV (Fig. 1), (2) presence of a star-like dendritic arborization, (3) spiny dendrites, (4) absence of an apical dendrite, (5) no morphological changes attributed to Golgi-Cox staining.

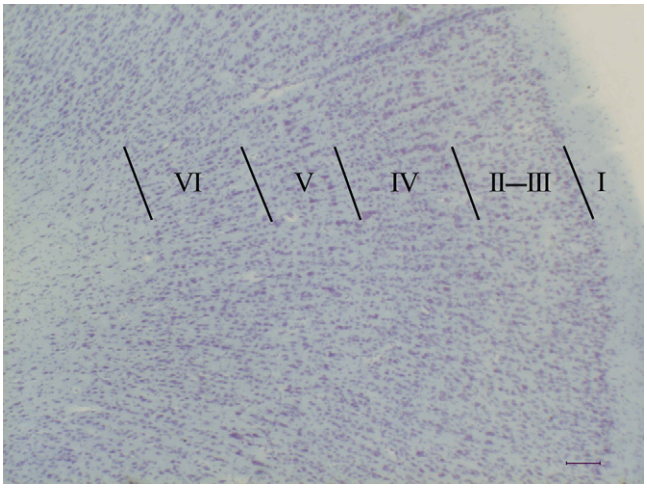

Fig. 1. Coronal section of a nissl-stained primary visual cortex of cat showing the regions of analyses. Layer III and IV were analyzed. Scale bar = 500 μm.

For each neuron, the analysis of dendritic length was done by using a procedure according to the Sholl's method [29,53]. We placed concentric rings (10 μm between rings) over pictures of neurons and counted the number of intersections of the dendrites with the concentric rings. The length of every branch order of dendrites was the number of intersections multiplied by the ring spacing (10 μm), and the total dendritic length was the summation of the length of all dendritic branches. Spine density was defined as the number of spines per unit length and was estimated by photographing a segment of dendrite. We calculated the exact length (10 μm) of the dendritic segment which was chosen from the pictures of dendrites, at the proximal part of all the branch orders (magnification of 1000×). The number of spines along that length was counted and then the spine density was expressed as spines/10 μm.

Data from the neurons' spine densities, dendritic length as well as the Sholl analysis of the number of ring intersections were analyzed by one-way-ANOVA ($P < 0.05$ was considered significant).

3. Results

3.1. Dendritic morphology

We used one out of every 3 s and got about 5 s per animal. From those sections, about 6 neurons per section were chosen and 204 neurons in all were collected for analysis, including 154 pyramidal cells (84 from the control group and 70 from the morphine group) and 50 spiny stellate cells (25 from each group). The Golgi-Cox-impregnated neurons of the visual cortex were identified by their characteristics. The pyramidal neurons have a triangular soma shape, an apical dendritic extension toward the pial surface, and numerous dendritic spines. The spiny stellate neurons have a star-like dendritic arborization with a high density of spines around its soma.

From the pictures of these neurons, dendritic shafts and spines of pyramidal and spiny stellate neurons in layers III/IV of primary visual cortex were entirely filled with Golgi-Cox stain (Fig. 2A, B and E, F, respectively). All of these neurons exhibited extensive den-

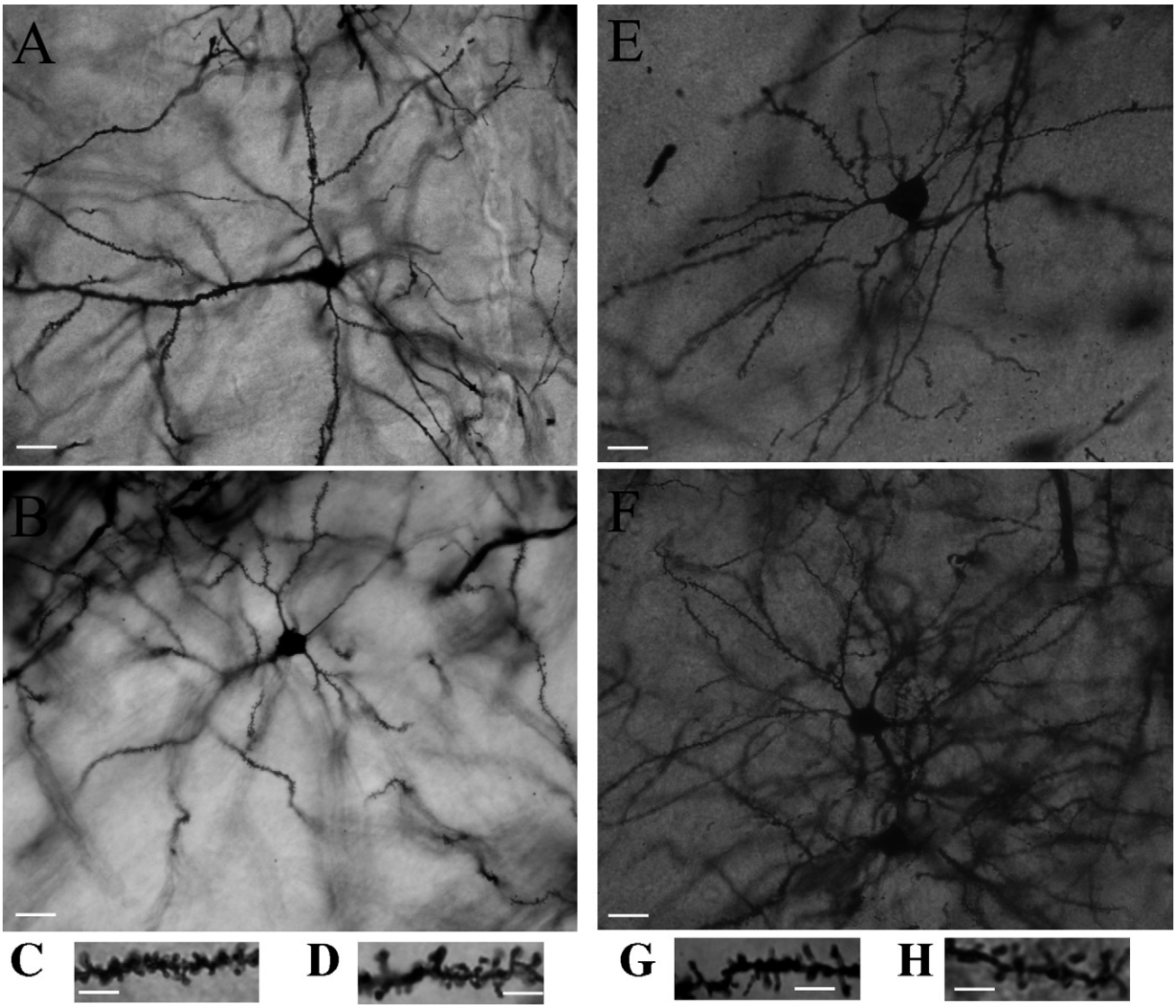

Fig. 2. Photomicrographs showing Golgi-Cox impregnated dendritic arborization and dendritic spines of pyramidal neurons in layer III (A and B) and spiny stellate neurons in layer IV (E and F) of primary visual cortex in cats. A, E show the result of morphine-exposed cats and B, F show the result of saline-control cats. The spiny segments of the dendrites are also indicated in this picture, C came from A, D from B, G from E, H from F. Scale bar = 10 μm (A, B, E and F). Scale bar = 4 μm (C, D, G and H).

dritic branching with a large number of spines (Fig. 2C, D and G, H, respectively).

3.2. Sholl analysis of dendritic arbors

The complexity of dendritic trees of pyramidal and spiny stellate cells was assessed using Sholl analysis (Fig. 3A and B). Significant morphine-related decreases in the numbers of intersections between the dendrites and the Sholl circles occurred only between 50–100 μm from the neuronal somata in pyramidal cells and 50–120 μm in spiny stellate cells (Fig. 3A; $P < 0.05$, $P < 0.001$, Fig. 3B; $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively, *t*-test).

3.3. Quantitative analyses of dendritic length and spine density

According to Sholl analysis, the average total dendritic lengths of pyramidal cells and spiny stellate cells in the morphine-treatment cats were 483.86 ± 14.57 and 488.00 ± 36.35 μm, respectively (means ± S.E.M.), which was significantly different from that in the saline-control group (563.53 ± 14.83 μm for pyramidal neuron and 680.80 ± 44.63 μm for spiny stellate neuron). The total dendritic lengths of pyramidal cells in layer III and spiny stellate cells in layer IV of the visual cortex in the morphine group were obviously shorter than that of the control group (Fig. 4A; $P < 0.001$ and $P < 0.01$, respectively, *t*-test).

Similarly, an obvious morphine-related decrease occurred in dendritic spine densities of pyramidal and spiny stellate cells. Results revealed the decreased spine density of pyramidal cells (42.3% decrease, $P < 0.001$, *t*-test) and spiny stellate cells (39.2% decrease, $P < 0.001$, *t*-test) in the morphine-treated group (Fig. 4B).

3.4. Branch order analysis

The average dendritic branch length per branch order of pyramidal cells and spiny stellate cells was shown in Fig. 5. Decreases in dendritic length of the pyramidal neurons were prominent at the fourth branch order, while the spiny stellate neurons had shorter dendrite at the second and third branch order (Fig. 5A and B).

The spine changes were further analyzed by measuring the spine density per 10 μm per branch order (Fig. 6). On both kinds of neu-

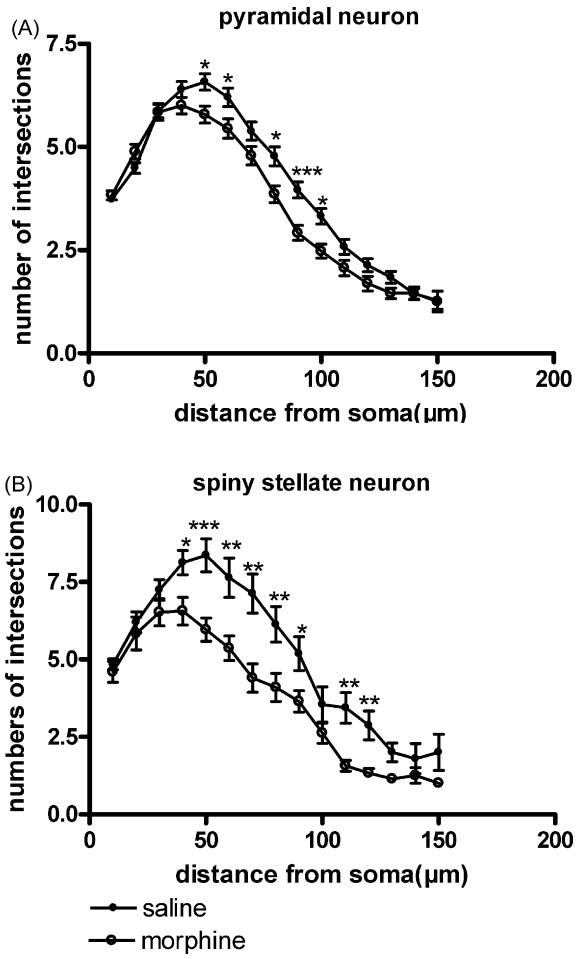

Fig. 3. Sholl analysis of dendrites of pyramidal neurons (A) and spiny stellate neurons (B). Hollow circles indicate the morphine group (A, $n=70$; B, $n=25$) and solid circles correspond to the saline cats (A, $n=84$; B, $n=25$). Asterisks indicate statistically significant differences across the dendritic tree ($^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$). Values represent means ± S.E.M. The abscissa and ordinate represent distance from the soma, and the numbers of intersections.

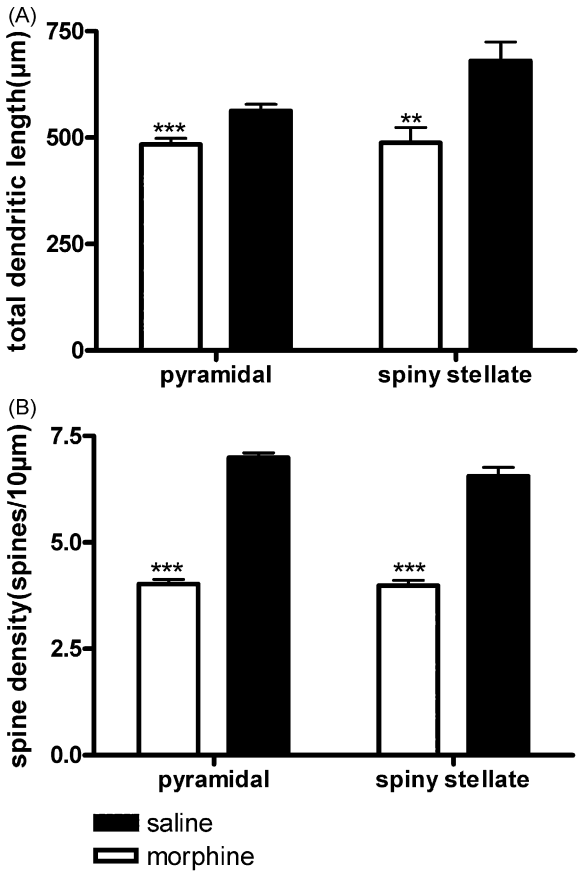

Fig. 4. Histograms showing total dendritic length and the density of dendritic spines (spines/10 μm) in visual cortex layer III pyramidal neurons and layer IV spiny stellate neurons of cats treated with saline or morphine. The bars represent the values of mean plus S.E.M. Dendritic length was estimated using the Sholl analysis. The dendritic length was decreased in morphine-exposed animals (Fig. 3A; pyramidal neuron, $^{***}P<0.001$; spiny stellate neurons, $^{**}P<0.01$). Spine densities were decreased in the morphine-exposed group compared with the control group (Fig. 3B; pyramidal neurons, $^{***}P<0.001$; spiny stellate neurons, $^{***}P<0.001$). The different effects of morphine on pyramidal neurons and spiny stellate neurons is statistically significant (Fig. 3A; the total length, $^{**}P<0.01$; Fig. 3B; the spiny density, $^{**}P<0.01$).

rons, spine density decreased significantly at the first three branch orders. Such decrease at the fourth branch order was only observed at the pyramidal neurons, but not at the spiny stellate neurons (Fig. 6A and B; $P<0.001$, *t*-test).

4. Discussion

The experiments presented here show that chronic morphine exposure induces the morphological alteration of the pyramidal and spiny stellate neurons in area 17 of cats. The method of morphine administration used in our present study is known to produce significant tolerance and dependence to the drugs in rats [13,61]. Another study has revealed that chronic morphine administration in adult cats (2.0 mg/kg, 15 days) once a day can elicit tolerance to the behavioral effects of the drug [20]. De Andres and Caballero [8] have found that this tolerance developed to the effects of morphine upon both non-rapid eye movement (NREM) and rapid eye movement (REM) sleep. Chronic morphine exposure in cats using this dosage also can induce their behavior changes, such as salivation, licking, swallowing, and urination [9]. In our previous electrophysiological experiments in morphine-treated cats, this dosage clearly resulted in the alteration of receptive field properties in primary visual cortex. Additionally, all morphine-treated cats showed some behavioral changes compared with control cats (salivation, diarrhea and abnormal movement) [21]. Therefore, we selected the 10 mg/kg dosage for chronic morphine administration on cats.

We photographed a series of pictures for each neuron to quantify the morphology of dendritic surface. Although this method rendered most dendritic tree clearly visible in these pictures, some practical problems do exist at the present study. For example, sectioning tissue at 120 μm could not include the whole dendrite tree. Additionally, some branches may be absent in pictures of each neuron due to the limited number of focal planes. Also, difficulties in estimating tissue shrinkage under Golgi-Cox staining procedure may induce some degree of spatial distortion and then lead to underestimation of the dendritic length of these neurons. Furthermore, spines lying on the backside of the dendrites that were pointing directly up at the viewer cannot be seen reliably with standard light microscopy [14,60]. Thus, in our study, the spine density measured with the light microscope may be inevitably underestimated. A better solution is the three-dimensional reconstruction of neurons at high magnification using confocal microscopy. Yet this method cannot be expected to eliminate such underestimation [51,60] and the process is extremely time-consuming when analyzing large neocortical neurons [51]. Fortunately, these methodological limitations equally affected all neurons in our samples and, therefore, had no direct effects on

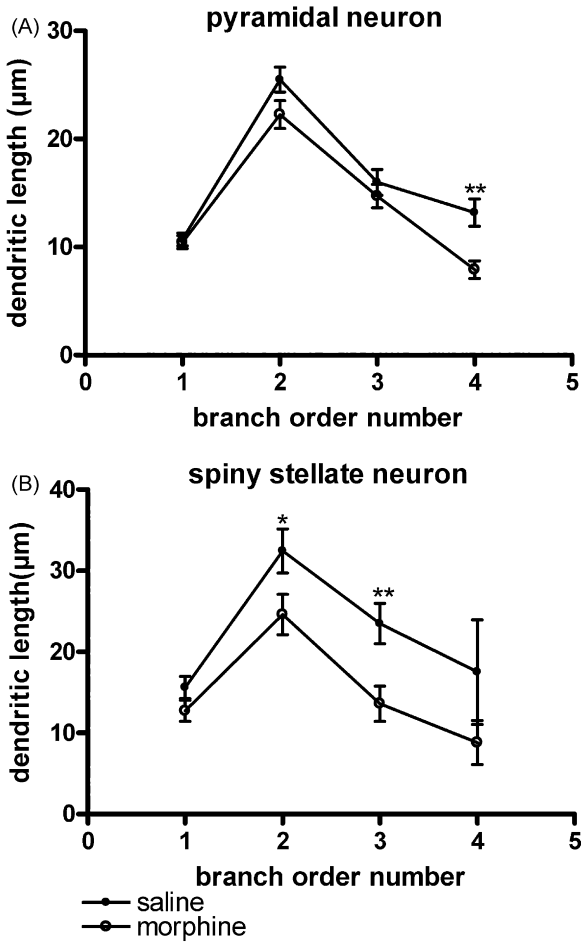

Fig. 5. Branch order analyses of average dendritic length at each branch order for the pyramidal (A) and spiny stellate neuron (B) in morphine and saline cats. Statistically significant differences are indicated by asterisks ($^{*}P < 0.05$, $^{**}P < 0.01$). Values represent means ± S.E.M. Morphine-related dendritic length decreases in the forth branch order of the pyramidal neuron and the second and third branch order of the spiny stellate neuron.

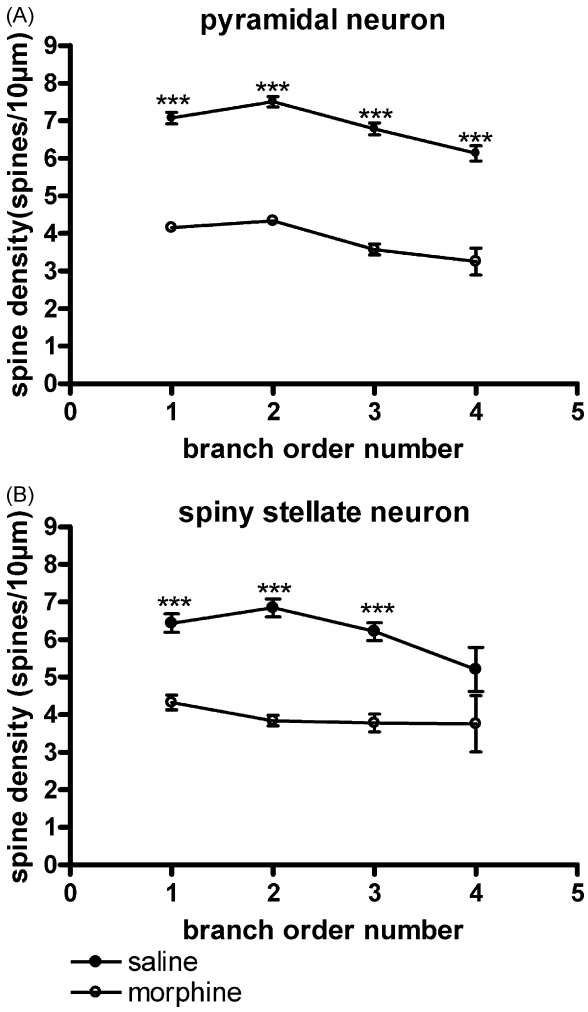

Fig. 6. Branch order analyses for spine densities at each branch order for the pyramidal (A) and spiny stellate neurons (B) in morphine and saline cats. Statistically significant differences are indicated by asterisks ($^{***}P < 0.001$). Values represent means ± S.E.M.

the observed differences in dendritic length and spine density of neurons between morphine-treated and normal cats.

In the present study, we observed that the dendritic length and spine density decreased greatly in layer III/IV of area 17. On the layer III, morphine-related decreases in dendritic length occurred only at the forth branch order for pyramidal neurons. On the layer IV, however, those changes were mainly at the second and third order for spiny stellate neurons. Sholl analyses revealed that morphine-related dendritic regressive changes were restricted to a very limited portion of the dendrites in both kinds of neurons. Those specific alterations of dendritic length on certain branch order may reflect that morphine selectively affected these portions of the dendritic tree. Previous studies have shown that dendrites mainly receive excitatory projections and that different domains of dendrites receive distinct synaptic inputs, for instance, proximal dendrites receive excitatory inputs from local sources (collaterals in the same area or from an adjacent area) [11,41,57]. Also, some spines on certain locations of the dendrites have a greatly influence on the integration of excitatory synaptic inputs. For example, spines which locate on the distal dendrite can compensate for their distance from the soma by scaling their conductance in order to normalize their somatic influence [66]. Additionally, voltage-gated channels also distribute on specific locations of dendrites or typical neurons. Many of these channels can affect the integration of synaptic potentials. Hyperpolarization-activated cation (HCN) channels are expressed in a somatodendritic gradient along the apical dendrites of layer V pyramidal neurons. Deactivation of HCN channels reduces EPSP (excitatory postsynaptic potentials) duration and results in a slight hyperpolarization following the IPSP (inhibitory postsynaptic potentials), hence limits the expected distance-dependence of EPSP temporal summation in the soma [33,67]. Furthermore, distinct populations of GABAergic interneurons have important effects on the information processing. Some GABAergic interneurons target specific dendritic domains on pyramidal neurons [10,56]. Therefore, we suggest that preferential alteration of certain branch orders of dendrites on our study may be due to the changes of information processing and may result in alterations of receptive field properties in area 17 of morphine-treated cats [21,22]. Further studies of the affects of morphine upon area 17 will help clarify the relationship between changes of function and structure consequent to chronic morphine exposure on cats.

Regressive dendritic changes in cortical pyramidal cells after morphine treatment have been reported in the primary somatosensory cortex and hippocampus of rats [47], the NAcc and neocortex of rats [49], and the visual cortex of rats [31]. Our study extends these findings by showing the dendritic morphological changes of spine stellate neurons in layer IV of area 17 in cats. And the changes

of this kind of neuron are also in line with that of pyramidal neurons in previous findings [31,47,49]. The alteration of spine stellate neurons in layer IV of area 17, which have important effects on visual information processing [1,7,11,43], may directly result in the change of function of the higher visual cortex and LGN.

We further analyzed the spine density on every dendritic branch order of these layer III pyramidal neurons and layer IV spiny stellate neurons. Spine density decreased uniformly among the different branch orders in the two types of neurons. In morphine-treated cats, the spine density was decreased by 42.3% in pyramidal cells and 39.2% in spiny stellate cells. This apparent reduction of dendritic density produced by morphine in cats is consistent with previous findings on adult rats [48–50].

It is widely known that dendrites with affluent spines, which are rich in receptors and receive synaptic inputs, are a striking feature of excitatory neurons. The spines vary considerably in their size and shape, and are highly plastic. However, their functional significance is not clearly understood. They might increase the dendritic surface area in order to optimize the packing of a large number of synapses onto a given length of dendrite [28,58]. Additionally, the plasticity of the spines may be available to contribute to learning and memories [5,27,38]. Tsay and Yuste [62] have found that spines play an important role in regulating the electrical properties of the neuron. Therefore, the alteration of spine density observed in our study may reflect the loss of some specific spines and reduce the dendritic surface area. These spiny alterations of dendrites might be due to the changes of information processing in the visual system. In the future, we will use other new methods to check the change of spine size and shape. That will be useful for explaining the changes of receptive field properties of the visual system.

The decreases of dendritic length and spine density may cause the alteration of pyramidal and spiny stellate neurons' effects on the within-cortex and corticocortical connections. These neurons thus may presumably interact with other neurons differently than before. Therefore, we speculate that these alterations of spiny and length of dendrites may result in functional degradations of the visual system in morphine-treated cats [21,22] through changes of the information processing circuit.

How morphine cause changes in the dendritic arborization of the pyramidal and spiny stellate neurons of the visual cortex is not clear. It is possible that those morphine-induced changes described here are associated with the fact that repeated exposure to morphine can compromise neuronal integrity in many cortical regions. For example, repeated treatment with morphine alters growth factor [39], neurofilament proteins and other cytoskeletal proteins that regulate neuronal and synaptic structure [4,24] in cerebral cortex, decreases axonal transport in VTA-NAcc system [2], and decreases the size of dopamine cell bodies in VTA [55]. All of these may contribute to alterations of dendritic arborization. The dendritic changes can also be explained at gene level. For example, one report has showed the increase of the expression of pro-apoptotic Fas receptor and the decrease of the expression of anti-apoptotic Bcl-2 oncoprotein in the cerebral cortex of rats [3]. These results may be related to a morphine-induced inhibition of neurogenesis in the dentate granule cell layer [12] and the decrease in frontal lobe volume as seen in opioid addicts [44].

In conclusion, our present study showed the fact that brain changes following opiate substances treatment were accompanied by significantly morphological changes of neurons, such as the spine density and the length of dendrite. Although a lot of work has been done, the mechanism of opiate dependence is still unclear [6,23,40,59]. It is worth finding the distribution of receptors and the release of neurotransmitters which may directly explain the degradation of functional degradation of the opiate-treatment animal. Additionally, the anterograde and retrograde labeling combined with electron microscopy may supply much information about excitatory and inhibitory projections. In one word, future work is needed to further explore the relationship between the morphological changes in dendrites and the changes of electrophysiological properties in visual cortex.

Conflict of interest

We declare that what we have done in this research has no conflict of interests with others published in the public journals.

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References

[1] B. Ahmed, J.C. Anderson, K.A.C. Martin, J.C. Nelson, Map of the synapses onto layer 4 basket cells of the primary visual cortex of the cat, Journal of Comparative Neurology 380 (1997) 230–242.
[2] D. Beitner-Johnson, E.J. Nestler, Chronic morphine impairs axoplasmic transport in the rat mesolimbic dopamine system, Neuroreport 5 (1993) 57–60.
[3] M.A. Boronat, M.J. Garcia-Fuster, J.A. Garcia-Sevilla, Chronic morphine induces up-regulation of the pro-apoptotic Fas receptor and down-regulation of the anti-apoptotic Bcl-2 oncoprotein in rat brain, British Journal of Pharmacology 134 (2001) 1263–1270.
[4] M.A. Boronat, G. Olmos, J.A. Garcia-Sevilla, Attenuation of tolerance to opioid-induced antinociception and protection against morphine-induced decrease of neurofilament proteins by idazoxan and other I2-imidazoline ligands, British Journal of Pharmacology 125 (1998) 175–185.
[5] J. Bourne, K.M. Harris, Do thin spines learn to be mushroom spines that remember? Current Opinion in Neurobiology 17 (2007) 381–386.
[6] D.E. Bredesen, P. Mehlen, S. Rabizadeh, Apoptosis and dependence receptors: a molecular basis for cellular addiction, Physiological Reviews 84 (2004) 411–430.
[7] E.M. Callaway, Local circuits in primary visual cortex of the macaque monkey, Annual Review of Neuroscience 21 (1998) 47–74.
[8] I. De Andres, A. Caballero, Chronic morphine administration in cats: effects on sleep and EEG, Pharmacology, Biochemistry, and Behavior 32 (1989) 519–526.
[9] I. De Andrés, M. Garzón, J.R. Villablanca, The brain stem but not forebrain independently supports morphine tolerance and withdrawal effects in cats, Behavioural Brain Research 148 (2004) 133–144.
[10] J. DeFelipe, Types of neurons, synaptic connections and chemical characteristics of cells immunoreactive for calbindin-D28K, parvalbumin and calretinin in the neocortex, Journal of Chemical Neuroanatomy 14 (1997) 1–19.
[11] R.J. Douglas, K.A. Martin, Neuronal circuits of the neocortex, Annual Review of Neuroscience 27 (2004) 419–451.
[12] A.J. Eisch, M. Barrot, C.A. Schad, D.W. Self, E.J. Nestler, Opiates inhibit neurogenesis in the adult rat hippocampus, Proceedings of the National Academy of Sciences of the United States of America 97 (2000) 7579–7584.
[13] G.H. Fan, L.Z. Wang, H.C. Qiu, L. Ma, G. Pei, Inhibition of calcium/calmodulin-dependent protein kinase II in rat hippocampus attenuates morphine tolerance and dependence, Molecular Pharmacology 56 (1999) 39–45.
[14] M.L. Feldman, C. Dowd, Loss of dendritic spines in aging cerebral cortex, Anatomy and Embryology 148 (1975) 279–301.
[15] E.D. French, S.A. Vasquez, R. George, Behavioral changes produced in the cat by acute and chronic morphine injection and naloxone precipitated withdrawal, European Journal of Pharmacology 57 (1979) 387–397.
[16] J.L. Giacchino, S.J. Henriksen, Systemic morphine and local opioid effects on neuronal activity in the medial prefrontal cortex, Neuroscience 70 (1996) 941–949.
[17] R. Gibb, B. Kolb, A method for vibratome sectioning of Golgi-Cox stained whole rat brain, Journal of Neuroscience Methods 79 (1998) 1–4.
[18] D.M. Grilly, R.F. Genovese, M.J. Nowak, Effects of morphine, d-amphetamine, and pentobarbital on shock and light discrimination performance in rats, Psychopharmacology (Berl.) 70 (1980) 213–217.
[19] R.L. Hakan, C. Eyl, S.J. Henriksen, Neuropharmacology of the nucleus-accumbens—systemic morphine effects on single-unit responses evoked by ventral pallidum stimulation, Neuroscience 63 (1994) 85–93.
[20] C.M. Harris, J.R. Villablanca, J.W. Burgess, I. De Andres, Reassessing morphine effects in cats. III. Responses of intact, caudate nuclei-lesioned and hemi-

spherectomized animals following chronic administration and precipitated withdrawal, Pharmacology, Biochemistry, and Behavior 21 (1984) 929–936.
[21] L. He, X. Li, T. Hua, P. Bao, R. Ma, Y. Zhou, Chronic morphine exposure affects the visual response properties of V1 neurons in cat, Brain Research 1060 (2005) 81–88.
[22] L.H. He, X.R. Li, T.M. Hua, P.L. Bao, Y.F. Zhou, Degradation of response modulation of visual cortical cells in cats with chronic exposure to morphine, Neuroscience Letters 384 (2005) 168–171.
[23] S.E. Hyman, R.C. Malenka, Addiction and the brain: the neurobiology of compulsion and its persistence, Nature Reviews 2 (2001) 695–703.
[24] P.E. Jaquet, M. Ferrer-Alcon, P. Ventayol, J. Guimon, J.A. Garcia-Sevilla, Acute and chronic effects of morphine and naloxone on the phosphorylation of neurofilament-H proteins in the rat brain, Neuroscience Letters 304 (2001) 37–40.
[25] S.W. Johnson, R.A. North, Opioids excite dopamine neurons by hyperpolarization of local interneurons, Journal of Neuroscience 12 (1992) 483–488.
[26] E.G. Jones, Varieties and distribution of non-pyramidal cells in the somatic sensory cortex of the squirrel monkey, The Journal of Comparative Neurology 160 (1975) 205–267.
[27] H. Kasai, M. Matsuzaki, J. Noguchi, N. Yasumatsu, H. Nakahara, Structure-stability-function relationships of dendritic spines, Trends in Neurosciences 26 (2003) 360–368.
[28] C. Koch, A. Zador, The function of dendritic spines: devices subserving biochemical rather than electrical compartmentalization, Journal of Neuroscience 13 (1993) 413–422.
[29] B. Kolb, M. Forgie, R. Gibb, G. Gorny, S. Rowntree, Age experience and the changing brain, Neuroscience and Biobehavioral Reviews 22 (1998) 143–159.
[30] M.E. Lewis, A. Pert, C.B. Pert, M. Herkenham, Opiate receptor localization in rat cerebral cortex, The Journal of Comparative Neurology 216 (1983) 339–358.
[31] Y. Li, H. Wang, L. Niu, Y. Zhou, Chronic morphine exposure alters the dendritic morphology of pyramidal neurons in visual cortex of rats, Neuroscience Letters 418 (2007) 227–231.
[32] J.S. Lund, Spiny stellate neurons, in: E.G. Jones, A. Peters (Eds.), The Cerebral Cortex, vol. 1, Plenum Press, New York, 1984, pp. 255–308 (Chap. 7).
[33] J.C. Magee, Dendritic lh normalizes temporal summation in hippocampal CA1 neurons, Nature Neuroscience 2 (1999) 508–514.
[34] A. Mansour, C.A. Fox, S. Burke, F. Meng, R.C. Thompson, H. Akil, S.J. Watson, Mu, delta, and kappa opioid receptor mRNA expression in the rat CNS: an in situ hybridization study, The Journal of Comparative Neurology 350 (1994) 412–438.
[35] A. Mansour, C.A. Fox, R.C. Thompson, H. Akil, S.J. Watson, Mu-Opioid receptor mRNA expression in the rat CNS: comparison to mu-receptor binding, Brain Research 643 (1994) 245–265.
[36] H. Markram, M. Toledo-Rodriguez, Y. Wang, A. Gupta, G. Silberberg, C. Wu, Interneurons of the neocortical inhibitory system, Nature Reviews 5 (2004) 793–807.
[37] K.A. Martin, D. Whitteridge, Form, function and intracortical projections of spiny neurones in the striate visual cortex of the cat, Journal of Physiology 353 (1984) 463–504.
[38] M. Matsuzaki, N. Honkura, G.C. Ellis-Davies, H. Kasai, Structural basis of long-term potentiation in single dendritic spines, Nature 429 (2004) 761–766.
[39] C.J. Messer, A.J. Eisch, W.A. Carlezon Jr., K. Whisler, L. Shen, D.H. Wolf, H. Westphal, F. Collins, D.S. Russell, E.J. Nestler, Role for GDNF in biochemical and behavioral adaptations to drugs of abuse, Neuron 26 (2000) 247–257.
[40] E.J. Nestler, G.K. Aghajanian, Molecular and cellular basis of addiction, Science (New York, NY) 278 (1997) 58–63.
[41] R. Nieuwenhuys, The neocortex. An overview of its evolutionary development, structural organization and synaptology, Anatomy and Embryology 190 (1994) 307–337.
[42] M.C. Nowycky, J.R. Walters, R.H. Roth, Dopaminergic neurons: effect of acute and chronic morphine administration on single cell activity and transmitter metabolism, Journal of Neural Transmission 42 (1978) 99–116.
[43] A. Peters, A. Fairén, Smooth and sparsely-spined stellate cells in the visual cortex of the rat: a study using a combined Golgi-electron microscopic technique, The Journal of Comparative Neurology 181 (1) (1978) 129–171.
[44] L.M. Pezawas, G. Fischer, K. Diamant, C. Schneider, S.D. Schindler, M. Thurnher, W. Ploechl, H. Eder, S. Kasper, Cerebral CT findings in male opioid-dependent patients: stereological, planimetric and linear measurements, Psychiatry Research 83 (1998) 139–147.
[45] L. Pu, P. Bao, N.J. Xu, L. Ma, G. Pei, Hippocampal long-term potentiation is reduced by chronic opiate treatment and can be restored by re-exposure to opiates, Journal of Neuroscience 22 (2002) 1914–1921.
[46] F. Reinoso-Suarez, A.E. Kornmüller, Topographischer Hirnatlas der Katze für experimental-physiologische Untersuchungen, 1961.
[47] A.A. Ricalde, R.P. Hammer Jr., Perinatal opiate treatment delays growth of cortical dendrites, Neuroscience Letters 115 (1990) 137–143.
[48] T.E. Robinson, G. Gorny, V.R. Savage, B. Kolb, Widespread but regionally specific effects of experimenter- versus self-administered morphine on dendritic spines in the nucleus accumbens, hippocampus, and neocortex of adult rats, Synapse (New York, NY) 46 (2002) 271–279.
[49] T.E. Robinson, B. Kolb, Morphine alters the structure of neurons in the nucleus accumbens and neocortex of rats, Synapse (New York, NY) 33 (1999) 160–162.
[50] T.E. Robinson, B. Kolb, Structural plasticity associated with exposure to drugs of abuse, Neuropharmacology 47 (Suppl. 1) (2004) 33–46.
[51] A. Rodriguez, D. Ehlenberger, K. Kelliher, M. Einstein, S. Henderson, J. Morrison, P. Hof, S. Wearne, Automated reconstruction of 3D neuronal morphology from scanning microscopy images, Methods 30 (2003) 94–105.
[52] S. Rothenberg, E.A. Peck, S. Schottenfeld, G.E. Betley, J.L. Altman, Methadone depression of visual signal detection performance, Pharmacology, Biochemistry, and Behavior 11 (1979) 521–527.
[53] D.A. Sholl, Dendritic organization in the neurons of the visual and motor cortices of the cat, Journal of Anatomy 87 (1953) 387–406.
[54] A.B. Silva-Gomez, D. Rojas, I. Juarez, G. Flores, Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats, Brain Research 983 (2003) 128–136.
[55] L. Sklair-Tavron, W.X. Shi, S.B. Lane, H.W. Harris, B.S. Bunney, E.J. Nestler, Chronic morphine induces visible changes in the morphology of mesolimbic dopamine neurons, Proceedings of the National Academy of Sciences of the United States of America 93 (1996) 11202–11207.
[56] P. Somogyi, G. Tamas, R. Lujan, E.H. Buhl, Salient features of synaptic organisation in the cerebral cortex, Brain Research 26 (1998) 113–135.
[57] N. Spruston, Pyramidal neurons: dendritic structure and synaptic integration, Nature Reviews 9 (2008) 206–221.
[58] A. Stepanyants, P.R. Hof, D.B. Chklovskii, Geometry and structural plasticity of synaptic connectivity, Neuron 34 (2002) 275–288.
[59] G. Torres, J.M. Horowitz, Drugs of abuse and brain gene expression, Psychosomatic Medicine 61 (1999) 630–650.
[60] M. Trommald, V. Jensen, P. Andersen, Analysis of dendritic spines in rat CA1 pyramidal cells intracellularly filled with a fluorescent dye, The Journal of Comparative Neurology 353 (1995) 260–274.
[61] K.A. Trujillo, H. Akil, Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801, Science (New York, NY) 251 (1991) 85–87.
[62] D. Tsay, R. Yuste, On the electrical function of dendritic spines, Trends in Neurosciences 27 (2004) 77–83.
[63] E. Vega, J. Gomez-Villalobos Mde, G. Flores, Alteration in dendritic morphology of pyramidal neurons from the prefrontal cortex of rats with renovascular hypertension, Brain Research 1021 (2004) 112–118.
[64] J.M. Walker, W.D. Bowen, L.A. Thompson, J. Franscella, S. Lehmkuhle, H.C. Hughes, Distribution of opiate receptors within visual structures of the cat brain, Experimental Brain Research 73 (1988) 523–532.
[65] D.M. Wilkison, M.J. Hosko, Selective augmentation of visual pathways by morphine in alpha-chloralose-anesthetized cats, Experimental Neurology 77 (1982) 519–533.
[66] S.R. Williams, G.J. Stuart, Role of dendritic synapse location in the control of action potential output, Trends in Neurosciences 26 (2003) 147–154.
[67] S.R. Williams, G.J. Stuart, Voltage- and site-dependent control of the somatic impact of dendritic IPSPs, Journal of Neuroscience 23 (2003) 7358–7367.
[68] S.P. Wise, M. Herkenham, Opiate receptor distribution in the cerebral cortex of the Rhesus monkey, Science (New York, NY) 218 (1982) 387–389.