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Chronic morphine-induced neuronal morphological changes in the ventral tegmental area in rats are reversed by electroacupuncture treatment

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ABSTRACT

The aim of this study was to observe the effect of electroacupuncture (EA) on chronic morphine-induced neuronal morphological changes in the ventral tegmental area (VTA) in rats at electron-microscopic level. Fourteen days of administering escalating doses of morphine induced pathological morphological changes of neurons in the VTA: the rough endoplasmic reticulum swelled, membrane configuration of the nucleus and mitochondria blurred, and structure of myelin sheath changed. Both 2 and 100 Hz EA treatment reversed the morphological alterations induced by chronic morphine administration. The findings provide new evidence that EA may serve as a potential therapy in treating opiate addiction.

Keywords Electroacupuncture (EA), morphine dependence, morphine withdrawal, neuronal morphological changes, ventral tegmental area (VTA), rat.

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INTRODUCTION

The ventral tegmental area (VTA) is involved in the initiation and development of drug addiction. Various biochemical and physiological adaptations in the VTA neurons (both dopaminergic neurons and GABAergic neurons) following chronic exposure to morphine have been reported (Diana *et al*. 1999; Manzoni & Williams 1999; Spiga *et al*. 2003; Steffensen *et al*. 2006; Nugent, Penick & Kauer 2007), and neuroplastic changes within VTA neurons are believed to contribute to drug addiction (Nestler 1997, 2004). However, very little is known about the morphological changes of the VTA neurons associated with chronic morphine treatment at the electron-microscopic level. In the present study, we focused on the ultrastructural characteristics of neurons in the VTA.

Our previous results have shown that electroacupuncture (EA) can reduce craving in addicted individuals (Wu *et al*. 1999; Shi *et al*. 2004) and reverse morphine-induced cell size reduction of VTA dopamine neurons (Chu *et al*. 2007), but the mechanisms still need to be further clarified. So the second aim of this study was to investigate the effect of EA on the ultrastructural characteristics of VTA neurons in chronic morphine-treated rats.

MATERIALS AND METHODS

Subjects

Twelve male Sprague–Dawley rats, weighing 180–200 g at the beginning of the experiment, were obtained from the Institute of Animal Research, Chinese Academy of Science, Beijing. They were housed four per chamber, in a standard 12:12-hour light/dark cycle (light on at 7 AM), with food and water *ad libitum*.

The room temperature was maintained at 22 ± 1°C. The rats were habituated to the environment and handled daily for 5 days before the experiment. The experimental procedures were approved by the Committee on Animal Care and Use of the Peking University.

Morphine and EA administration

Morphine hydrochloride, purchased from the Pharmaceutical Factory of Qinghai, China, was dissolved in

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sterile saline and administered twice daily (at 8 AM and 8 PM) for 14 days as described (Diana *et al.* 1999). Briefly, the initial dose administered was 20 mg/kg, and was increased by 20 mg/kg every other day until the 14th day of treatment, reaching a dose of 140 mg/kg for the last injection. Morphine doses up to 100 mg/kg were administered s.c. in a volume of 1 ml/kg, whereas higher doses were administered i.p. in a volume of 1 ml/0.1 kg. Normal saline (NS) control rats received an equal volume of saline.

Rats chronically treated with morphine for 14 days were randomly assigned to the following groups: (1) morphine group: morphine abstinence for 14 days before sacrifice, without any further treatment; (2) 2 Hz EA group: after chronic morphine administration, rats were gently restrained in specially prepared holders and stimulated with 2 Hz (0.6 ms pulse width) EA twice (30 minutes per session) a day for 3 days, followed by once a day for 7 days, totaling 13 sessions in 10 days; and (3) 100 Hz EA group: rats were treated the same way as that in the 2 Hz EA group, except that 100 Hz (0.2 ms pulse width) was used.

The EA treatment was executed as follows: 12 hours after the last injection of the drug, two stainless steel needles of 0.3 mm diameter were inserted into each hind leg, one in the acupoint ST36 (5 mm lateral to the anterior tubercle of the tibia), and the other in the acupoint SP-6 (at the level of the upper border of the medial malleolus, posterior border of the tibia). Constant current square-wave electric stimulation generated by a programmed pulse generator, HANS LH-800 (Peking University of Astronautics and Aeronautics Aviation, Beijing, China), was given via the two needles for a total of 30 minutes. The intensity of the stimulation was increased stepwise from 0.5 to 1 mA, and then to 1.5 mA, with each step lasting for 10 minutes.

On the 14th day after the last injection of drugs, all groups of rats were sacrificed by decapitation, and the brains were taken for study under a transmission electron microscope.

Observation of neuron morphology in the VTA using transmission electronic microscope

The rats were decapitated, and the VTA was removed from the appropriate sections under a dissecting microscope. The tissues were then placed in a fixative solution of 2% sodium cacodylatebuffered glutaraldehyde, pH 7.4, for 6 hours. After being rinsed in a buffered solution of saccharose, the tissue samples were postfixed for 2 hours in 1% osmium tetraoxide, dehydrated and flat embedded in epoxy resins. The semi-thin sections were obtained from the tissue blocks in a Leica ultramicrotome (Leica Corporate, Solms, Germany) equipped with glass knives. The sections were stained with toluidine blue and then coverslipped. From the surface of these trimmed blocks, ultrathin sections ranging from 90 to 100 nm were obtained with a diamond knife and mounted in single-slot grids, which had previously been covered with formvar film. The sections were double stained with aqueous solutions of uranium acetate and lead citrate, and observed and photographed in a JEM-100CXII electron microscope (JEOL, Tokyo, Japan).

RESULTS

Obvious ultrastructural alterations occurred in the VTA of the rats receiving chronic morphine. Multiple 2 or 100 Hz EA treatments improved the pathological changes.

Rough endoplasmic reticulum (RER)

As shown in Fig. 1, compared with NS, chronic morphine treatment resulted in a fragmentation and degranulation, as well as a vacuolar change, of the RER in the VTA neurons. In addition, the orderliness of the RER and polyribosome was lost. Both the 2 and 100 Hz EA treatments reversed the pathological changes of the RER induced by chronic morphine administration.

Mitochondria

Elongated mitochondria with lamellar cristae and continuous mitochondrial membranes were seen in the NS group (Fig. 2). Damages to the mitochondrial membranes and cristae were observed in the morphine group. The mitochondria were rounded in the morphine group, with flaked content and membrane disorganization. In the 2 and 100 Hz EA groups, the pathological changes of mitochondria induced by chronic morphine treatment were improved.

Nucleus

In the NS group, the nucleus had a round shape and regular contours with an easily seen double membrane (Fig. 3). The nucleus chromatin was homogeneously distributed in the NS group. On the contrary, in the morphine-treated rats, apparent indentations were present in the nuclei. However, most of the nucleus in the 2 and 100 Hz EA groups was normal.

Myelin sheath

Empty cavity and lamellar separation were seen within the myelin sheath in the morphine group (Fig. 4). Both 2 and 100 Hz EA treatment enabled the myelin sheath to recover to a tightly arranged state.

DISCUSSION

The main findings of the present study were that chronic morphine treatment induced pathological alterations on

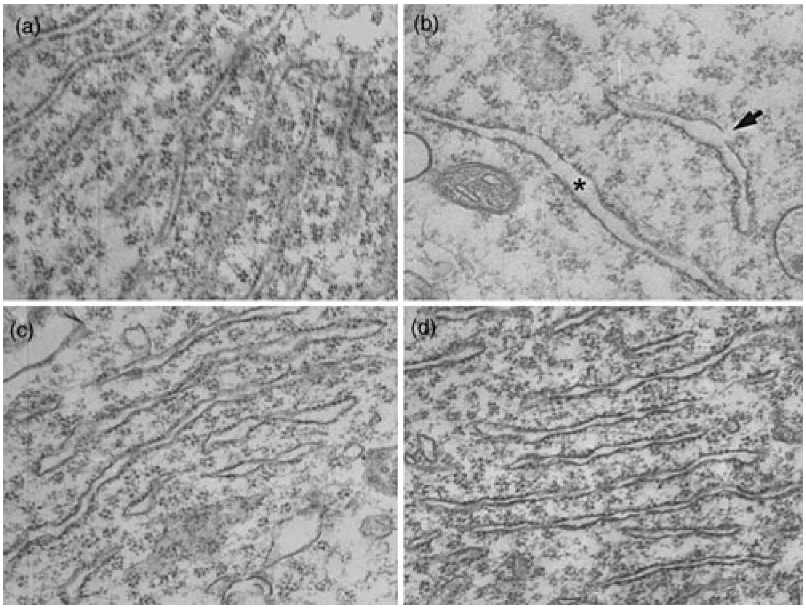

Figure 1 RER (29 000×) taken from the: (a) NS group; (b) morphine group; (c) 2 Hz EA group; and (d) 100 Hz EA group. Chronic morphine induced disorganized (arrowhead) and swollen RER cisternae (asterisk). Both the 2 and 100 Hz EA treatments improved the changes. EA = electroacupuncture; NS = normal saline; RER = rough endoplasmic reticulum

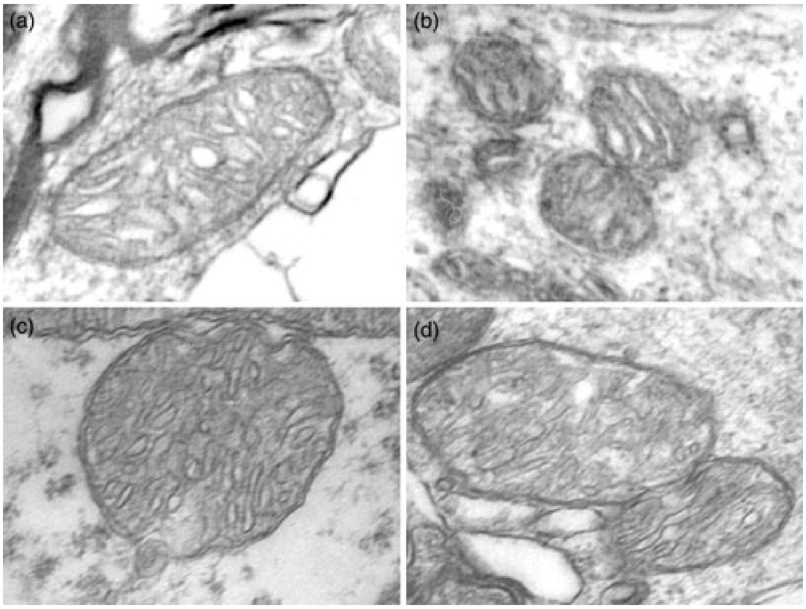

Figure 2 Mitochondria (29 000×) taken from the: (a) NS group; (b) morphine group; (c) 2 Hz EA group; and (d) 100 Hz EA group. The membrane and cristae of the mitochondria were disorganized in the morphine group. Both the 2 and 100 Hz EA treatments improved the changes. EA = electroacupuncture; NS = normal saline

the VTA ultrastructure, and that these alterations could be reversed by EA. After chronic morphine treatment, the striking feature observed in the cell body was the fragmentation and degranulation of the RER. Some of the RER distended and showed vacuolar changes. Disaggregation of the free polyribosome was also seen. Because the integrity of the RER and polyribosome is closely related to protein biosynthesis (Csala, Banhegyi & Benedetti 2006), the changes previously mentioned might reflect a reduction or stop of protein synthesis. The mitochondria act as the energy factories of the cells by converting organic materials into energy in the form of adenosine triphosphate (ATP) via the process of oxidative phosphorylation (dam-Vizi & Chinopoulos 2006). Impaired mitochondria may lead to energy scarcity. The nucleus contains all the information that the cell needs to do specific jobs, such as grow and divide, with the information stored in DNA molecules. The myelin sheath facilitates the transmission of nerve impulses. The disorganization of the RER, mitochondria, nucleus and myelin sheath may lead to the abnormal synthesis of structural and functional protein and the disordered communication between cells, thus underlining the neuronal dysfunction of the VTA in morphine-treated rats.

It has been reported that morphine treatment lead to ultrastructure changes of the neurons in the hypothalamus, caudate nucleus, locus coeruleus and hippocampus (Garcia-Estrada *et al.* 1988; Kolusheva 1988; Miao *et al.*

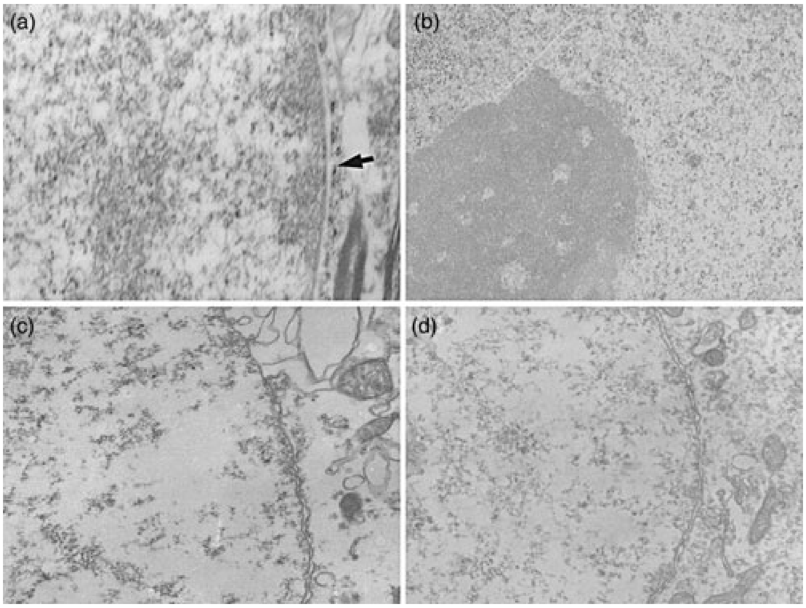

Figure 3 Nucleus (14 000×) taken from the: (a) NS group; (b) morphine group; (c) 2 Hz EA group; and (d) 100 Hz EA group. Regular contours with double membrane (arrow) were seen in the NS group. Both the 2 and 100 Hz EA treatments reversed the apoptosis-like changes of the nucleus induced by the chronic morphine treatment. EA = electroacupuncture; NS = normal saline

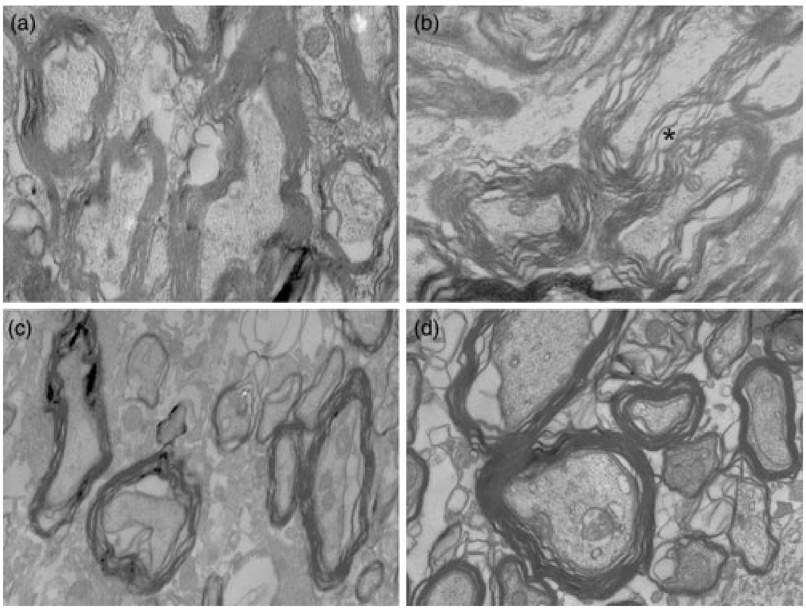

Figure 4 Myelin sheath (14 000×) taken from the: (a) NS group; (b) morphine group; (c) 2 Hz EA group; and (d) 100 Hz EA group. Empty cavity and lamellar separation (asterisk) occurred within the myelin sheath in the morphine group. Both the 2 and 100 Hz EA treatments reversed the changes induced by the chronic morphine treatment. EA = electroacupuncture; NS = normal saline

1997). As far as we know, this is the first time that the changes of the VTA neurons are observed at an electron-microscope level in morphine-treated rats. However, because of the limitation of the experimental method (transmission electronic microscope), we could not distinguish the dopaminergic neurons from the GABAergic neurons in the VTA in the present study. It was found that chronic morphine administration induced cell size reduction of VTA dopaminergic, but not GABAergic, neurons in rats (Sklair-Tavron *et al*. 1996; Russo *et al*. 2007), and we also observed pathological changes of dopaminergic neurons in the VTA using co-immunofluorescence under a light microscope (Chu *et al*. 2007). Thus, we may confer that the ultrastructure changes observed in the present study occurred in dopaminergic neurons.

In the present study, the morphine-induced morphological changes of VTA neurons could be reversed by the 2 or 100 Hz EA treatments. The parameters (intensity and frequency) of EA were used as described (Wu *et al*. 1999; Shi *et al*. 2003; Cui *et al*. 2004; Chen *et al*. 2005), and they are proved to be efficacious by our previous and present study. EA, as a kind of physiotherapy, is being used widely in drug addiction (Whitehead 1978; Ulett, Han & Han 1998; Montazeri, Farahnakian & Saghaei 2002; D'Alberto 2004). Unlike many pharmacotherapies, which usually have aversive side effects, there is little, if any, adverse effect with acupuncture therapy (Lu *et al*. 2004). Brain opioid receptors are reported to be involved in mediating the EA-induced inhibition of morphine-conditioned place preference (Shi *et al*. 2003), which is an animal model to examine the reinforcing properties of drugs of abuse (Tzschentke 2007). Here, we explained the mechanisms of EA at the morphological level. In conclusion, we found that chronic morphine administration induced morphological changes of VTA neurons at the electron-microscope level, which could be reversed by the 2 and 100 Hz EA treatments. The findings suggest new evidence that EA may serve as a potential therapy in treating opiate addiction.

Acknowledgements

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