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# GABA INCREASES STIMULUS SELECTIVITY OF NEURONS IN PRIMARY VISUAL CORTICES OF CATS CHRONICALLY TREATED WITH MORPHINE

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**Abstract—Chronic exposure to opiates leads to maladaptive changes in various functions of the mammalian brain, including properties of neuronal response in the visual pathway. In the present study, we used multibarreled microelectrodes to study the effects of electrophoretic application of GABA or the $GABA_A$ receptor antagonist bicuculline on the properties of individual V1 neurons in cats which were chronically treated with morphine (MTCs) or saline (STCs). The results showed that the application of either GABA or bicuculline significantly altered spontaneous activity as well as orientation selectivity and signal-to-noise ratios of visually evoked responses in both MTCs and STCs. While administration of bicuculline exerted a much stronger effect on neuronal responses of V1 neurons of the STCs, administration of GABA resulted in improved visual function mainly in MTCs. Most importantly, GABA-treated cells in area V1 of the MTCs displayed similar responses to those in STCs. These results are consistent with the idea that: (1) there is a decrease in GABA-mediated inhibition in area V1 of cats exposed chronically to morphine, and (2) this decrease contributes strongly to the apparent degradation of neuronal function observed in animals exposed chronically to morphine.** **© 2013 IBRO. Published by Elsevier Ltd. All rights reserved.**

**Key words: primary visual cortex, orientation selectivity, GABA, bicuculline.**

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*Abbreviations:* BMI, bicuculline methiodide; CRT, cathode ray tube; EEG, electroencephalograph; FFT, fundamental Fourier component; LGN, lateral geniculate nucleus; LTP, long-term potentiation; MTC, morphine-treated cats; OB, orientation bias; PSTH, Post-stimulus time histogram; SEM, standard error of the mean; STC, saline-treated cats; STN, signal-to-noise ratio.

## INTRODUCTION

Opiate abuse is an important social and medical problem throughout the world. It has been suggested that exposure to opiates may not only result in physical and psychological dependence but may also lead to many other behavioral changes, such as reduced visual sensitivity (Rothenberg et al., 1979) in humans, disordered behavior responses in kittens (Burgess and Villablanca, 2007), abnormal visual discrimination performance in rats (Grilly et al., 1980), and disrupted visual sensitivity in pigeons (Nielsen and Appel, 1983). Widespread maladaptive changes in neuronal structure and response properties (Rothenberg et al., 1979; Di Chiara and North, 1992; Robinson and Kolb, 1997, 1999; Pu et al., 2002; Wang et al., 2006; Li et al., 2010) induced by opiate exposure are thought to be the substrate of these disrupted behaviors.

Previous studies in our laboratory have revealed that, when compared with normal or saline-treated cats, cats treated chronically with morphine exhibit significantly different neuronal response properties, such as higher spontaneous activities, higher visually evoked responses and lower signal-to-noise ratios in both the lateral geniculate nucleus (LGN) (He et al., 2005a) and V1 (He et al., 2005b). A degraded signal-to-noise ratio leads to decreased ability of the neural system to discriminate signal from background, which therefore may have some relationship with the disrupted visual functions, such as the reduced visual sensitivity, described above. Additionally, decreased response modulation, extended time course of response and visual response latencies were also found in the visual pathway of cats given chronic morphine treatment (He et al., 2005c; Long et al., 2008). Most notably, orientation and direction selectivity, which play important roles in the perception of form (Hubel, 1988) and motion (Albright and Stoner, 1995),were also found to be impaired in the LGN (He et al., 2005a) and V1 (He et al., 2005b). All these findings suggest that chronic morphine exposure has a substantial influence on neurons in the visual pathway. Although a number of studies have proved that opiate receptors are expressed extensively in the visual system (Wise and Herkenham, 1982; Lewis et al., 1983; Walker et al., 1988), and that chronic opiate exposure results in morphological changes in neurons in the visual cortex (Li et al., 2007b;

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Hu et al., 2008), the precise mechanism by which chronic opiate exposure affects the functions of neurons in the visual system remains an open question.

It has been shown that chronic opiate exposure significantly affects various neurotransmission systems, including the glutamatergic (Martin et al., 1999a,b; Pu et al., 2002; Zeng et al., 2006) and GABAergic systems (Vaughan et al., 1997; Cruz et al., 2004; Laviolette et al., 2004; Li et al., 2007a; Madhavan et al., 2010). It is known that GABA plays an important role in the maintenance of neuronal performance in the visual pathway. Decreased GABA-mediated inhibition often leads to a decline in function of neurons involved in signal-to-noise ratio, orientation and direction selectivity (Eysel et al., 1990, 1998; Sato et al., 1996; Crook et al., 1997; Liu et al., 2007). On the basis of these findings, we hypothesized that chronic morphine exposure results in a decrease in GABA-mediated inhibition in area V1, which contributes to the apparent degradation of neuronal functions described above. As shown in a previous study (Leventhal et al., 2003), a simple way to test this hypothesis is to explore the effects of administration of GABA and bicuculline, a type of $GABA_A$ receptor antagonist, on neuronal function. If the proposed hypothesis is true, GABA administration would result in greater improvement in neuronal function in chronic morphine-treated cats (MTCs) while the application of bicuculline would exert a much stronger effect on neuronal responses in saline-treated cats (STCs). We therefore used multi-barreled microelectrodes to study the effects of electrophoretic application of GABA and bicuculline on the response properties of individual V1 cells in MTCs and STCs. Our findings provided strong support for the hypothesis.

## EXPERIMENTAL PROCEDURES

### Animals and morphine exposure

The experiments were performed on seven healthy adult male cats (2–3 kg), three of which comprised the morphine-treated group; the remaining four cats were used as a control saline-treated group. The protocol for morphine treatment was identical to that described previously (Pu et al., 2002; He et al., 2005a,b,c). Morphine HCl (10 mg/kg) was administered by cervical subcutaneous injection twice per day at 9:00 AM and 9:00 PM for 10 days before the electrophysiological experiments. Saline-treated cats were treated similarly, except that the saline (0.9%) was used. During the recording procedure, morphine or saline was injected in the same way.

All cats were examined using an ophthalmoscope before the experiment to ascertain that they had no optical defects or obvious retinal problems that would impair their visual function.

This study was performed in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the University of Science and Technology of China.

### Preparation for recording

On the 11th day, the animals were prepared for extracellular single-cell recording. The methods of preparation and single-cell recording have been described previously (Shou et al., 1996; Hua et al., 2006; Li et al., 2008). Cats were anaesthetized prior to surgery with ketamine HCl (20 mg/kg, intramuscular injection); following this, intravenous and tracheal cannulae were inserted. After surgery, each animal was placed in a stereotaxic apparatus. A long-acting anesthetic (1% lidocaine HCl) was applied to all wound margins and pressure points. The nictitating membranes were retracted using neosynephrine (0.5%). The pupils were dilated maximally with atropine, and contact lenses were used to protect the eyes from desiccation. A mixture of urethane (20 mg/kg/h) and gallamine triethiodide (10 mg/kg/h) was infused intravenously to maintain anesthesia and paralysis. The heart rate (about 180–220 pulses/min) and electroencephalograph (EEG) were monitored to assess the level of anesthesia. End-expiratory $CO_2$ was maintained at approximately 4%; body temperature was maintained at 38 °C. A craniotomy (8 mm diameter) was performed 4 mm posterior to the ear bars in the midline, and the dura was removed. We also used published visuotopic maps of areas 17 (Tusa et al., 1978) to identify the areal location of cells. After introduction of the electrode assembly, the opening was covered with a 4% solution of agar in saline. Action potentials of isolated cortical cells were recorded extracellularly using microelectrodes with impedances of 2–5 MΩ (filled with 4 M NaCl).

### Visual stimulation

The visual stimulation was identical to that used previously (Li et al., 2008). Computer-controlled visual stimuli were generated on a gamma-corrected Sony G220 CRT monitor (1024 × 768, 100 Hz), placed 57 cm from the eyes of the cats. The mean luminance of the display was 45.2 cd/m$^2$, and the environmental luminance on the cornea was approximately 0.1 lux. The program that generated the stimulus was written in MATLAB (Mathworks, Natick, MA, USA), using the extensions provided by the high-level Psychophysics Toolbox (Brainard, 1997) and low-level Video Toolbox (Pelli, 1997). We used drifting sinusoidal gratings as stimuli (2 Hz), at five cycles per trial (2.5 s). The Michelson contrast of the stimuli was 99%. Each stimulus orientation was presented in two trials for a total of 10 cycles/orientation. Blanks (4 s) of the same mean luminance as the grating stimuli were interleaved with stimulus trials to determine the spontaneous firing rate and to prevent response adaptation. We selected an optimal stimulus size and spatial frequency for each cell during stimulation of the dominant eye. Subsequently, a set of sinusoidal gratings with optimal stimulus parameters, moving in 24 different directions (0–345° scale with an increment of 15°) was used to compile the orientation tuning curves. The direction of movement of each stimulus was orthogonal to its orientation.

### Drug application

The methods of application of GABA and bicuculline were the same as used generally before (Leventhal et al., 2003; Li et al., 2008) GABA and bicuculline were delivered through multibarrel electrode arrays containing four pipette channels. Two of the barrels were used to hold GABA and bicuculline, one was filled with NaCl (4 M) to record the action potentials of the cells and the final barrel was filled with vehicle solution (pH 4.5) to balance the current. The following drug solutions were prepared for each experiment: GABA (0.5 M, pH 3.5) and bicuculline methiodide (BMI; 5 mM, pH3.5). All solutions for use in the microiontophoresis were prepared immediately before the experiments, filtered, and kept at 4 °C for subsequent use. Administration of one drug at a time was accomplished by passing +20 to +50 nA through the barrel. Mean and standard error of the mean (SEM) of ejecting currents of GABA and BMI in MTCs were 47.70 ± 0.83 nA (mean ± SEM) and 47.03 ± 0.96 nA, and in STCs were 48.24 ± 0.69 nA and

47.76 ± 0.81 nA. Retaining currents of −20 nA were applied simultaneously through the remaining barrels in order to prevent leakage of the other drugs.

### Data collection and analysis

After the neuronal signal had been amplified with a microelectrode amplifier (DAGAN, Minneapolis, Minnesota, USA), action potentials were fed into a window discriminator with an audio monitor. Action potentials of single neurons, identified by their unique shape and amplitude, were monitored both visually (on an oscilloscope) and acoustically (over the audio monitor). When a single unit was isolated, the cell's receptive field was carefully mapped by consecutively presenting a series of computer-generated light spots on the CRT. The original voltage traces were digitized using a data acquisition board (National Instruments, USA) controlled by IGOR software (WaveMetrics, Portland, Oregon, USA). The original data were saved for analysis offline. We also confirmed single units offline with spike size matching. Only well-isolated cells satisfying strict criteria for single-unit recording were included for further analyses.

The ratio of the fundamental Fourier component (FFT1) to the mean component (FFT0) of the response to a neuron's optimal drifting sinusoidal grating provides a measurement of the relative response modulation. When the ratio is above 1, the neuron is classified as a simple cell. If the ratio is below 1, it is classified as a complex cell (Skottun et al., 1991; Bardy et al., 2006).

Post-stimulus time histograms (PSTHs) of the neuronal responses were obtained for further analysis. The responses of a cell to the sinusoidal gratings were defined as the amplitude of FFT 1 (in simple cells) or FFT0 (in complex cells) of the PSTH integrated over a time equal to the stimulus modulation period. A straightforward and objective method was used to classify cells as simple or complex (Skottun et al., 1991). For simple cells, the FFT1 value of each stimulus orientation was used to draw the orientation tuning curve. While for complex cells, the FFT0 value was used.

The signal-to-noise ratio (STN) of a cell was defined as the ratio of the visually evoked response (FFT1 and FFT0 value in the optimal direction for simple and complex cells, respectively) to the spontaneous activity of this cell. To avoid data skewing or overestimation, all spontaneous activities below 1 spike per second were set equal to 1 spike per second for signal-to-noise analysis.

We used orientation bias to measure the strength of orientation selectivity (Worgotter and Eysel, 1987; Thompson et al., 1994; Leventhal et al., 2003; He et al., 2005b; Li et al., 2008). Orientation bias (OB) is a global measurement that is influenced by all of the data points on the tuning curve, and is calculated as follows:

$$OB = \left| \frac{\sum_k R_k e^{i2\theta_k}}{\sum_k R_k} \right|$$

where $R_k$ is the mean spike rate in response to a grating drifting with angle $\theta_k$ (in radians). The OB averages the responses for the two directions of motion at each orientation. This value is quite robust to noise in the data and provides a bounded range from 0 to 1, with 0 indicating complete insensitivity to orientation and 1 indicating a response to only one orientation.

The changes in OB induced by administration of GABA and bicuculline were defined by $\Delta OB_1$ and $\Delta OB_2$:

$$\Delta OB_1 = OB_{GABA} - OB_{control}$$

$$\Delta OB_2 = OB_{control} - OB_{BMI}$$

Here, $OB_{control}$, $OB_{GABA}$, and $OB_{BMI}$ represent the OB before drug application, after GABA administration and after bicuculline application, respectively.

Data are presented as means ± standard error of the mean unless otherwise indicated.

## RESULTS

We studied 86 cells in the primary visual cortex of three morphine-treated cats (abbreviated as MTCs) and 109 cells in four saline-treated cats (abbreviated as STCs). The recording conditions were required to keep stable for at least 2 h, which would be sufficient to accomplish a complete set of tests with GABA and BMI iontophoresis; the following analysis includes only those neurons that survived throughout the whole series of tests (74 MTCs and 85 STCs); and the cells (12 MTCs and 24 STCs) which did not finish all tests are not included. As found in previous research (Nelson, 1991; Li et al., 2008), no significant differences were observed between simple cells and complex cells in the effects of GABA and bicuculline. Therefore our data were collapsed across cell types.

### Effects of GABA and bicuculline on orientation selectivity

The effects of GABA and bicuculline on the responses of one typical cell in MTC and one in STC are illustrated in Fig. 1. Before drug administration, the cell in MTC responded almost equally well to all orientations and therefore exhibited weak orientation selectivity (Fig. 1A). The administration of GABA increased the orientation selectivity of this neuron greatly (Fig. 1B), while the application of the GABA antagonist, bicuculline, reversed it (Fig. 1C). Ten minutes after the cessation of drug administration, the neuron reverted to the pre-application state (Fig. 1D). In contrast, the typical cell in STC exhibited moderate orientation selectivity before drug application (Fig. 1E). It was less affected by the administration of GABA (Fig. 1F) but was more strongly affected by bicuculline (Fig. 1G) when compared with the typical cell in MTC. Again, 10 min after the discontinuation of drug administration, the drug-induced changes disappeared (Fig. 1H).

A comparison of the changes in OB of neurons in MTCs and STCs during the administration of GABA and bicuculline is shown in Fig. 2. It is clear that the orientation selectivity improved after GABA administration, while decreased after bicuculline application. Finally, most of the drug-induced changes disappeared 10 min after the drug administration. A two-way within-subject ANOVA (Group × Drug) was conducted to reveal the drug-induced changes. It was found (Fig. 2) that the main effect of Drug ($F_{3,155} = 52.893$, $p < 0.001$) and the two-way interaction ($F_{3,155} = 6.047$, $p = 0.001$) were significant, which suggests the existence of complicated drug-induced changes in orientation selectivity. A series of post hoc analyses were then conducted on the basis of these findings. The results indicated that the neurons in MTCs were significantly ($F_{1,\ 157} = 5.637$, $p = 0.019$)

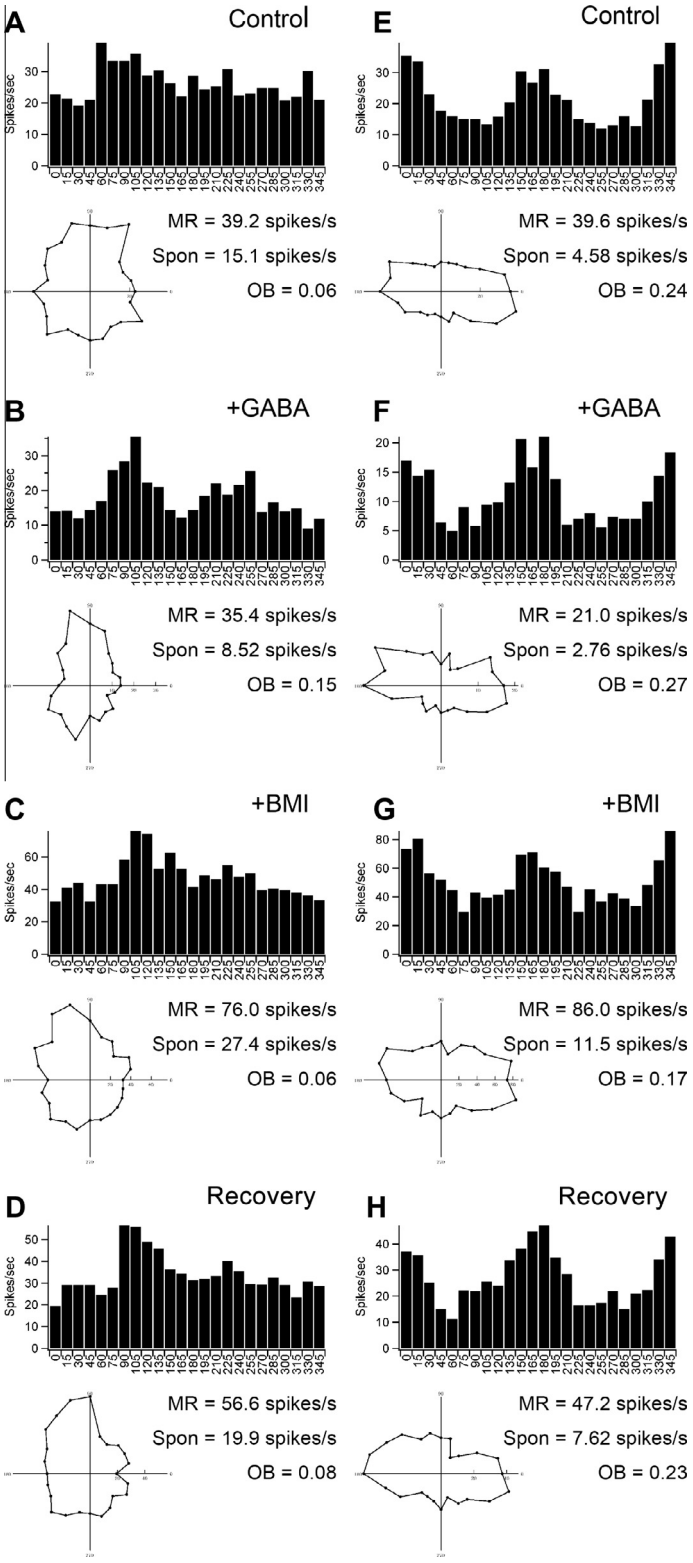


**Fig. 1.** Response properties for one typical cell in MTC and one typical cell in STC. The maximum (peak) responses (MR), spontaneous activities (Spon) and orientation biases (OB) are shown for each condition. A typical cortical cell in MTC with lack of orientation selectivity is shown in (A). After GABA application (B), this cell exhibited moderate orientation selectivity. The peak of response and the spontaneous activity of this cell decreased. GABA application was then discontinued, and bicuculline application began (C). Bicuculline reversed the effects of GABA. Ten minutes after the discontinuation of drug administration, the drug-induced changes disappeared (D). A tuning curve of a typical cell in STC is shown in (E). This cell exhibits moderate orientation selectivity, which was little affected by the administration of GABA (F). On the other hand, application of bicuculline resulted in a more than 100% increase in both peak response and spontaneous activity, and a large reduction in orientation selectivity (G). Ten minutes after the cessation of drug administration, this cell also reverted to the pre-application state (H).

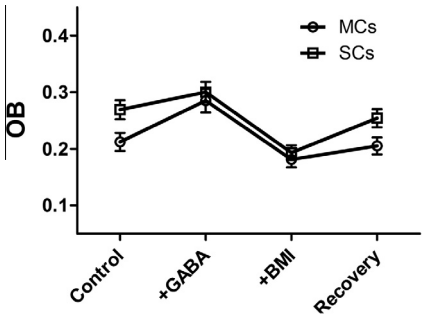


**Fig. 2.** The OB of V1 cortical cells in each condition. Error bars indicate SEM.

less orientation-selective than those in STCs before drug application. For both groups, the administration of GABA induced improvements in orientation selectivity ($F_{1,\ 157} = 30.548$, $p < 0.001$) while BMI decreased it ($F_{1,\ 157} = 64.330$, $p < 0.001$). Neurons in MTCs after GABA application exhibited similar performance levels ($F_{1,157} = 0.366$, $p = 0.546$) to the control responses in STCs, and neurons in STCs after the administration of BMI exhibited similar performance levels ($F_{1,157} = 0.839$, $p = 0.361$) to the control responses in MTCs. Additionally, since compared with those in STCs, neurons in MTCs were more strongly affected by GABA ($F_{1,157} = 4.836$, $p = 0.029$) but were less affected by BMI ($F_{1,157} = 11.300$, $p = 0.001$). Neurons in two groups exhibited similar performance levels after GABA ($F_{1,157} = 0.308$, $p = 0.580$) and BMI ($F_{1,157} = 0.385$, $p = 0.536$) application. In each group, 10 min after the cessation of drug administration, the neuronal responses had reverted to the pre-application state ($F_{1,157} = 3.292$, $p = 0.072$).

## Relationship between GABA-mediated inhibition and orientation selectivity

A previous study (Li et al., 2008) showed that the regulation of the orientation selectivity of a V1 neuron induced by GABAergic inhibition is related to the neuron's orientation selectivity before drug application. Generally speaking, the change in orientation selectivity induced by GABA is greatest in weakly orientation-selective cells, smaller in moderately orientation-selective cells, and minimal in strongly orientation-selective cells, while the change induced by bicuculline exhibits the opposite effect. We therefore investigated this issue in MTCs.

Correlation and linear regression analyses were conducted to examine the orientation selectivity before and after drug application in both MTCs and STCs. The results (Fig. 3A, C) showed that the effects of GABA on OB decreased slightly with the increase of the original OB in both groups. On the other hand, the effects of bicuculline increased substantially with an increase in the original OB (Fig. 3B, D). The decrease was not statistically significant (linear fit, $F_{1,72} = 1.115$, $p = 0.295$ and $F_{1,83} = 0.292$, $p = 0.591$ for MTCs and STCs, respectively) while the increase was significant (linear fit, $F_{1,72} = 43.090$, $p < 0.001$ and

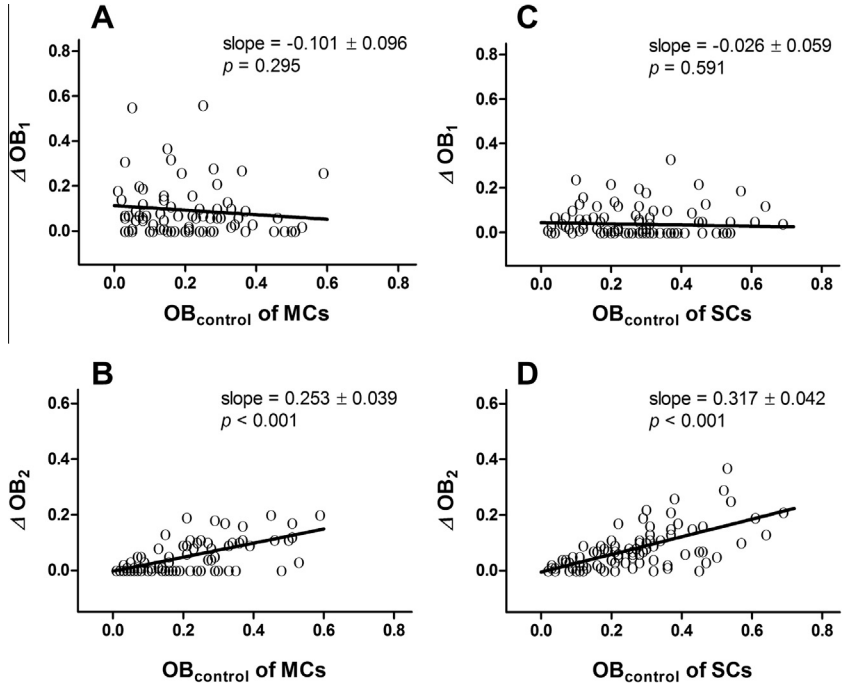


**Fig. 3.** Relationship between the original OB and the OB changes. The effects of GABA on OB decreased slightly with an increase in original OB (A, C), while the effects of bicuculline increased substantially with an increase in original OB in both groups (B, D). Slopes of the linear fitting functions are represented as mean ± SEM.

$F_{1,83} = 57.390$, $p < 0.001$ for MTCs and STCs, respectively). Additionally, the slopes of the linear fit functions were comparable (0.253 ± 0.039 vs. 0.317 ± 0.042 and −0.101 ± 0.096 vs. −0.026 ± 0.059, $p = 0.266$ and 0.242, respectively) between MTCs and STCs. These results supported the previous findings partially (Li et al., 2008), and they suggest that the patterns of regulation of orientation selectivity induced by GABAergic inhibition were similar between the two groups.

### Effect of GABA and bicuculline on spontaneous activity, visually evoked response and signal-to-noise ratio

The effects of GABA and bicuculline on spontaneous activity, visually evoked response and signal-to-noise ratio were also analyzed in MTCs. These results are shown in Table 1 and Fig. 4. In line with the previous findings (He et al., 2005b) in our laboratory, V1 cells in MTCs exhibited higher spontaneous activity, higher visually evoked responses and lower signal-to-noise ratios (Mann–Whitney test, all $p < 0.05$) than those in STCs. Additionally, GABA changed spontaneous activity, visually evoked responses and signal-to-noise ratios in MTCs significantly (Wilcoxon signed ranks test, all $p < 0.05$), which resulted in levels of visual response properties that were closer to those in STCs before GABA application. The application of bicuculline caused the opposite changes (Wilcoxon signed ranks test, all $p < 0.05$) and resulted in larger differences from STCs.

Furthermore, we compared the effects of the drugs on these visual response properties in MTCs and STCs (Table 2). As expected, GABA induced a significantly greater percentage change in the visually evoked response (−40.4% ± 3.2% vs. −54.9% ± 3.0%, Mann–Whitney test, $p < 0.001$) and signal-to-noise ratio (155.8% ± 38.7% vs. 65.4% ± 19.2%, Mann–Whitney test, $p = 0.003$) while BMI had the opposite effect on all three response properties (spontaneous activity, 90.5% ± 16.9% vs. 200.7% ± 28.4%; visually evoked response, 39.5% ± 7.2% vs. 83.1% ± 10.3%; signal-to-noise ratio, −1.4% ± 6.8% vs. −9.8% ± 6.9%; Mann–Whitney test, $p = 0.001$, 0.003 and 0.097, respectively). However, the percentage change in the spontaneous activity seemed to be similar (−45.5% ± 11.4% vs. −51.8% ± 40.1%, Mann–Whitney test, $p = 0.578$) in MTCs and STCs.

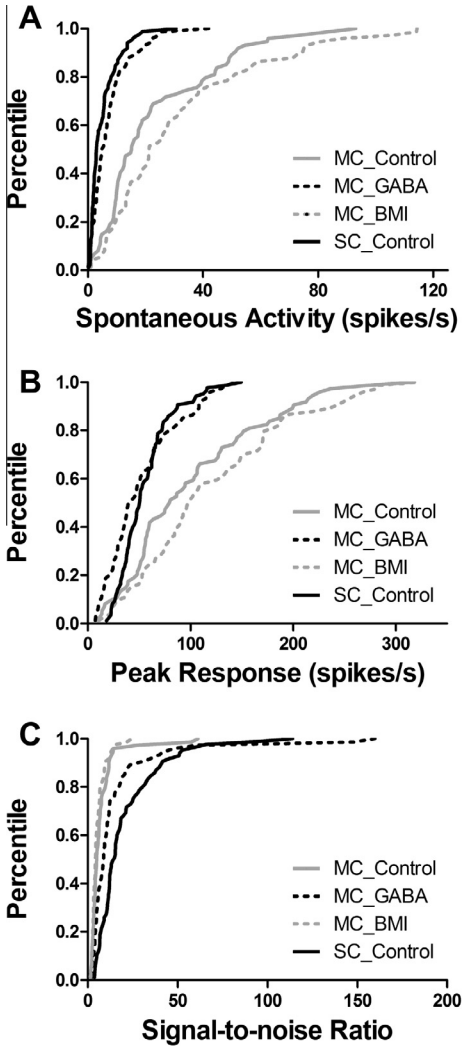


**Fig. 4.** Cumulative distribution of other response properties of V1 cortical cells in each condition. Cortical cells in MTCs exhibited significantly different spontaneous activities (A), visually evoked (peak) responses (B) and lower signal-to-noise ratios (C) than those in STCs. The application of GABA reduced the differences in spontaneous activity, peak response and signal-to-noise ratio between MTCs and STCs. In contrast, bicuculline administration increased these differences.

**Table 1.** Visual response properties of V1 cells before and after drug administration in morphine-treated and saline-treated cats

| | Morphine-treated cats | | | Saline-treated cats |
|---|---|---|---|---|
| | Before | + GABA | + BMI | Before |
| Spontaneous (spikes/s) | 22.95 ± 2.40 | 7.34 ± 0.85[a,**] | 31.38 ± 3.16[a,**] | 5.12 ± 0.56[b,**] |
| Peak response (spikes/s) | 100.40 ± 7.99 | 51.96 ± 4.08[a,**] | 120.40 ± 8.69[a,**] | 55.43 ± 2.89[b,**] |
| Signal-to-noise ratio | 7.49 ± 1.12 | 15.53 ± 2.99[a,**] | 5.60 ± 0.49[a,*] | 20.55 ± 2.06[b,**] |

Data are represented as mean ± SEM. Given that most of the response properties do not exhibit normal distributions (Kolmogorov–Smirnov test, most $p < 0.05$), non-parametric tests are used here.

[a] Wilcoxon signed ranks test.

[b] Mann–Whitney test.

[*] $p < 0.05$ Compared with the visual response properties of MTCs before drug application.

[**] $p < 0.01$ compared with the visual response properties of MTCs before drug application.

**Table 2.** Comparisons of drug effects on spontaneous activity, visually evoked response and signal-to-noise ratio between MTCs and STCs

| | | Before (%) | +GABA (%) | +BMI (%) |
|---|---|---|---|---|
| Spontaneous activity | MTCs | 100 | −(45.5 ± 11.4) | +(90.5 ± 16.9) |
| | STCs | 100 | −(51.8 ± 40.1) | +(200.7 ± 28.4)** |
| Peak response | MTCs | 100 | −(40.4 ± 3.2) | +(39.5 ± 7.2) |
| | STCs | 100 | −(54.9 ± 3.0)** | +(83.1 ± 10.3)** |
| Signal-to-noise ratio | MTCs | 100 | +(155.8 ± 38.7) | −(1.4 ± 6.8) |
| | STCs | 100 | +(65.4 ± 19.2)** | −(9.8 ± 6.9)# |

Data are represented as mean ± SEM. Given that most of these changes do not exhibit normal distributions (Kolmogorov–Smirnov test, most $p < 0.05$), the Mann–Whitney test is used here.

# $0.05 < p < 0.1$ Compared with the percentage change in MTCs.

** $p < 0.01$ Compared with the percentage change in MTCs.

## DISCUSSION

In this study, we investigated the response properties of V1 neurons in MTCs and their relations with GABAergic inhibition by employing microiontophoretic application of GABA and bicuculline. In accordance with a previous study (He et al., 2005b), V1 neurons in MTCs exhibited higher spontaneous activity, higher peak response, lower signal-to-noise ratio and lower orientation selectivity than those in STCs. While administration of bicuculline exerted a much stronger effect on the neuronal responses of V1 neurons in the STCs, administration of GABA resulted in improved visual function mainly in MTCs. Neurons in MTCs, after drug application, therefore exhibited similar responses to those in STCs. We also found analogous patterns in the regulation of orientation selectivity induced by GABAergic inhibition in MTCs and STCs. These findings suggest that there is a decrease in GABA-mediated inhibition in area V1 of MTCs and that this decrease may contribute strongly to the apparent degradation of neuronal function observed in animals exposed chronically to morphine.

### Chronic morphine exposure and functional degradation of visual cortex

Given the well-known analgesic effect but the development of tolerance and physical dependence associated with opiates (Koob and Le Moal, 1997), most of previous studies that have investigated physiological changes induced by opiates, or chronic opiate exposure, have been conducted in the midbrain periaqueductal gray matter (Vaughan et al., 2000, 2003), ventral tegmental area (Cruz et al., 2004; Laviolette et al., 2004; Margolis et al., 2005), and hippocampus (Daumas et al., 2007; Niu et al., 2009). In contrast, less is known about the influence of opiates on the visual cortex.

Recently, several studies in our laboratory have investigated this issue. It has been indicated that the dendritic length and spine density of both pyramidal and spiny stellate neurons are decreased as a result of chronic morphine exposure (Li et al., 2007b). The visual response properties of both LGN and V1 neurons, including spontaneous activity (He et al., 2005a,b), visually evoked response (He et al., 2005a,b), signal-to-noise ratio (He et al., 2005a,b) and contrast response function (Song et al., 2012), have also been found to be greatly degraded in cats chronically exposed to morphine. Our results in the present study indicate that orientation selectivity of V1 neurons is also significantly affected by chronic morphine exposure. All these findings support the idea that the neural functions are substantially influenced by morphine exposure in the visual system.

### Chronic morphine exposure and excitatory and inhibitory synaptic transmission

Martin and his colleagues found chronic morphine treatment significantly alters NMDA receptor-mediated synaptic transmission in the accumbens (Martin et al., 1999a,b). Pu and his colleagues investigated the effects of chronic opiate treatment on long-term potentiation (LTP) at CA1 synapses in the rat hippocampus and found significant reduction in the capacity of hippocampal CA1 LTP during the period of drug withdrawal (Pu et al., 2002). Using whole-cell voltage-clamp recording, Zeng and his colleagues found that lamina I cells in spinal slices from opiate-tolerant neonatal rats show an increase in miniature, spontaneous, and primary afferent-evoked excitatory postsynaptic currents (EPSCs) when compared with those from opiate-naive rat spinal slices. This increased excitation can be blocked by the NMDA receptor (NMDAR) antagonist. In addition, they found an increased incidence of NMDARs in substance P-containing spinal dorsal horn primary afferent terminals in opiate-tolerant rats. These findings suggested that excitatory neurotransmission can be changed by opiate exposure. Consistent with this idea, our previous studies have shown that chronic morphine exposure results in morphological changes in pyramidal cells of V1 (Li et al., 2007c; Hu et al., 2008) and impacts the neurons in LGN (Long et al., 2008), which suggests that excitatory inputs to V1 and excitatory V1 neurons themselves are directly influenced by morphine.

It has also been suggested that opiate exposure alters the inhibitory neurotransmission, such as the GABAergic system. Vaughan and his colleagues found that GABAergic synaptic transmission were inhibited by opiates in the midbrain periaqueductal gray matter and other brain regions by reducing the probability of

presynaptic neurotransmitter release (Vaughan and Christie, 1997; Vaughan et al., 1997). Madhavan and his colleagues found that opiates inhibit GABA postsynaptic currents in the ventral tegmental area in naive mice (Madhavan et al., 2010). The previous study of our laboratory has also found that GABAergic system is affected by chronic morphine exposure in the visual cortex (Li et al., 2007a). Our findings in the present study thatV1 cells in MTCs showed increased spontaneous, visually driven activity and decreased signal-to-noise ratios may be particularly consistent with the idea.

Based on all described above, it is reasonable to speculate that changes of excitatory and inhibitory neurotransmission systems may both underlie the opiate-induced degradation of neuronal function in the visual system.

### Functional degradation of visual cortex and decreased GABA-mediated intra-cortical inhibition

Up to now, there is little direct evidence concerning the contribution of excitatory and inhibitory neurotransmission to functional degradation of visual cortex. On the one hand, some studies have suggested that the changes of inhibitory neurotransmission may play a more important role. For example, neurons receiving weak inhibition in normal (Li et al., 2008) and aging visual cortex (Leventhal et al., 2003) can significantly improve their response properties by GABA administration, and stimulus-driven and spontaneous activity increased as a result of the application of $GABA_A$ receptor antagonists (Thiele et al., 2004). Our results provide a direct support to this idea. The administration of GABA not only improved the neuronal function in MTCs significantly, which led to similar levels of function to those in STCs, but also exerted much greater effects on neurons in MTCs than those in STCs. This is in line with the findings (Li et al., 2008) that GABA induced a greater improvement in orientation selectivity in neurons that receive weak intra-cortical inhibition than in those receiving strong intra-cortical inhibition. The results from the application of the $GABA_A$ antagonist bicuculline are also consistent. All these findings suggest that reductions in GABA-mediated intra-cortical inhibition may contribute strongly to the opiate-induced degradation of neuronal function in the visual pathway.

### Reasons for the decrease in GABA-mediated intra-cortical inhibition in MTCs

Decreased intra-cortical inhibition could result from many causes, including abnormal transmitter production, diminished release of transmitter, a degradation of transmitter receptors, membrane changes, and so on. Although we cannot pinpoint the particular reason for this finding in MTCs, our results provide some evidence.

Our results showed that the application of GABA and bicuculline did help to diminish the differences in the level of performance between MTCs and STCs. This may suggest that degradation of transmitter receptors is not the explanation for the decrease in inhibition observed because significant differences in levels of performance between the two groups are also expected even after the application of GABA and bicuculline if the number of $GABA_A$ receptors is really substantially influenced by chronic morphine exposure.

On the other hand, many studies (Capogna et al., 1993; Vaughan and Christie, 1997; Vaughan et al., 1997, 2000, 2003) have shown that opiates affect the GABAergic inhibition system by reducing the probability of pre-synaptic neurotransmitter release. Additionally, GABA release is found to be increased during morphine withdrawal, as a result of maladaptive physiological changes induced by opiate exposure (Ingram et al., 1998; Hack et al., 2003). This is believed to contribute to the withdrawal syndrome that occurs after abrupt cessation of treatment or administration of an opioid receptor antagonist. Therefore, it seems likely that diminished release of transmitter is the potential mechanism behind the decrease in GABA-mediated intra-cortical inhibition in MTCs. However, further evidence is required to support this assumption.

## CONCLUSIONS

In summary, our results indicate that GABA may improve the function of the neurons in V1 of cats chronically exposed to morphine. Furthermore, they suggest that a decrease in GABA-mediated intra-cortical inhibition may contribute to the degraded neuronal function seen in the V1 of cats subjected to chronic morphine exposure.

## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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