

# CHRONIC MORPHINE EXPOSURE AFFECTS VISUAL RESPONSE LATENCY OF THE LATERAL GENICULATE NUCLEUS IN CATS

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## SUMMARY

**1. Chronic morphine exposure results in degradation of the functional properties of cortical cells. However, little evidence has been reported about the effect of morphine on the temporal properties of the visual system.**

**2. We compared the visual response latency of different cells of the lateral geniculate nucleus (LGN) in morphine (10 mg/mL)- and saline-treated cats. Morphine-treated cats were given morphine sulphate by cervical subcutaneous injection twice daily for 10 days, whereas saline-treated cats received injections of 0.9% saline instead. We found that LGN neurons in morphine-treated cats exhibited significantly longer response latency than those in saline-treated cats ( $P < 0.001$ ).**

**3. To investigate whether different types of neurons exhibited similar changes in response to morphine, we classified LGN neurons as on- and off-centre, X and Y, and layer A, A1 and C neurons. There was a tendency for prolonged latency in layer C neurons and a significantly longer latency for the other neurons in morphine-treated cats ( $P < 0.05$ ).**

**4. These findings suggest that chronic morphine administration (10 mg/kg by cervical subcutaneous injection twice daily for 10 days) delays information transfer earlier in the visual pathway.**

**Key words:** lateral geniculate nucleus, morphine, response latency.

## INTRODUCTION

The lateral geniculate nucleus (LGN) receives visual information from retinal ganglion cells and sends most projections to the primary visual cortex. In turn, the LGN receives strong feedback signals from higher brain areas. It is not merely a relay station between the retina and cortical areas, but also a complicated nucleus that integrates neural information and plays an important role in the processing of visual information.<sup>1</sup> Three main layers have been recognized in the cat LGN from the dorsal to ventral sides: A, A1 and C.<sup>2</sup> Layers A and A1 have been widely studied, whereas layer C has been investigated in less detail. According

to linear or non-linear spatial summation, LGN neurons can also be divided into X and Y cells.<sup>3</sup>

A rich distribution of opiate receptors has been observed in visual systems of rats,<sup>4</sup> cats<sup>5</sup> and macaques.<sup>6</sup> This suggests that the visual system is subject to opiate modulation. Previous research has shown that morphine-like drugs can alter visual discrimination performance in rats,<sup>7</sup> evoke cortical potentials in cats<sup>8</sup> and reduce visual sensitivity in humans.<sup>9</sup> Recent studies have also revealed that chronic morphine exposure induces degradation of the receptive field properties of the LGN and primary visual cortex (V1) cells in cats.<sup>10,11</sup> However, it is not known whether chronic morphine exposure affects the temporal properties of LGN cells and whether morphine has similar effects on different types of neurons.

In the present study, we compared the visual response latency of different LGN cells in morphine- and saline-treated cats using extracellular single-unit recording techniques.

## METHODS

### Animals and drug exposure

Experiments were performed in eight healthy adult male cats (2–3 kg): four cats were allocated to the morphine-treated group and four cats were allocated to the saline-treated group, which served as the control. All cats were examined with an ophthalmoscope to avoid obvious optical or retinal problems that could impair visual function. Cats were treated strictly in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (<http://www.nap.edu/readingroom/books/labrats/>). A similar method of morphine administration was used in the present study as reported previously.<sup>12</sup> Cats were given morphine sulphate (10 mg/kg) by cervical subcutaneous injection twice daily at 0900 and 2100 hours for 10 days before the electrophysiological experiments. Control cats were treated similarly but were injected with saline rather than morphine.

### Preparation for extracellular recording

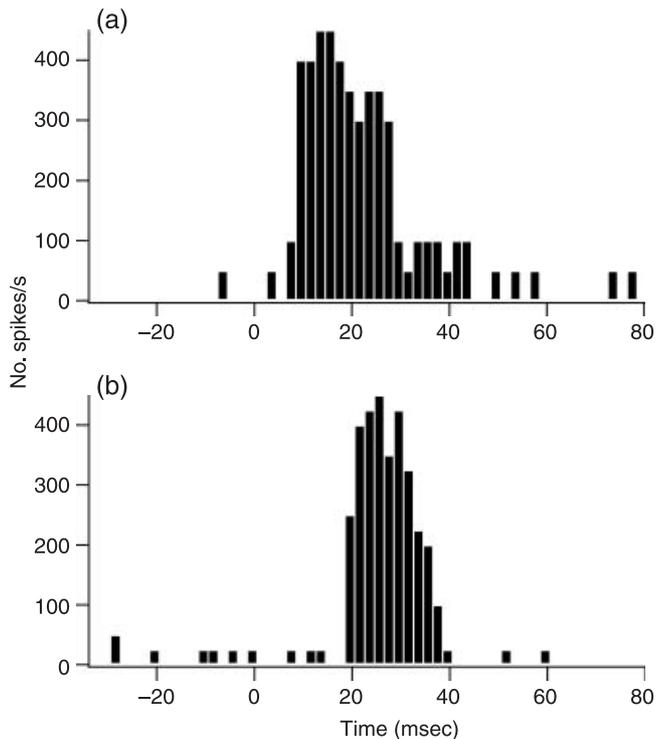
On the 11th day of administration, animals were prepared for extracellular single-unit recording as described previously.<sup>13,14</sup> Briefly, cats were initially anaesthetized with ketamine HCl (20 mg/kg). Lignocaine (1%) was applied to all surgical incisions. After intravenous and tracheal cannulas had been inserted, cats were placed in a stereotaxic apparatus. Pupils were dilated with atropine (1%) and nictitating membranes were retracted with phenylephrine HCl (5%). Eyes were protected with contact lenses and focused at a distance of 57 cm. A mixture of urethane (20 mg/h per kg bodyweight) and gallamine triethiodide (10 mg/h per kg) was infused intravenously to maintain anaesthesia and paralysis. Expired CO<sub>2</sub> was tested by a CO<sub>2</sub> monitor (Multinex Datascope, Montvale, NJ, USA) and maintained at approximately 4% by adjusting the respiratory rate and the inspired volume. Heart rate (approximately

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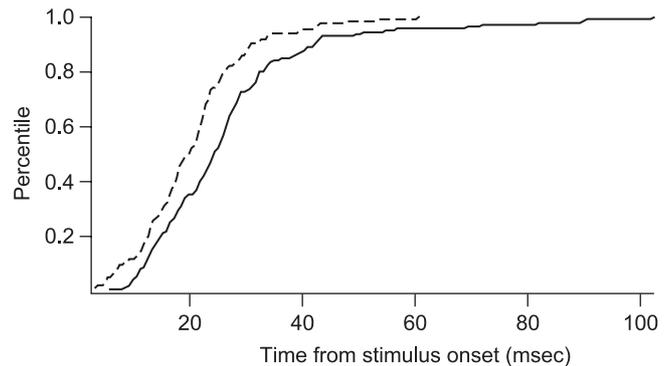


**Fig. 1** Post-stimulus time histograms (PSTHs) of neuronal responses obtained from a representative cell in (a) saline- and (b) morphine-treated cats with a bin width of 2 msec. The latency of the saline- and morphine-treated cells was 16.6 and 24.7 msec, respectively. Visual responses were driven by a flashing spot within the receptive field with an optimal size for each cell. The mean luminance of the display was 19 cd/m<sup>2</sup> and the environmental luminance on the cornea was 0.1 lux.

180–220 b.p.m.), electroencephalogram (EEG) and neural electrical activity were monitored throughout the experiment to evaluate the level of anaesthesia. All animals were studied under similar stable, reliable recording conditions. A small hole was drilled in the skull 6 mm anterior to the ear bar and 8.5 mm lateral to the midline. A tungsten-in-glass microelectrode (with an impedance of 2–3 M $\Omega$ ) was positioned and advanced using a hydraulic microdriver (Narishige, Tokyo, Japan). The small hole was filled with a 4% solution of agar in saline and sealed with wax. The optic discs were projected on a tangent screen situated 114 cm away from the eyes of cats and the position of the area centralis was then determined. Receptive fields were hand-plotted using a flashing spot. Cells had receptive fields located within 8° of the area centralis. We kept recording and a typical recording lasted for 3 days. During recording, morphine or saline was injected in the same way as described above.

### Visual stimulation

Computer-controlled visual stimuli consisting of flicker were presented on a cathode ray tube monitor (1024 × 768, 85 Hz; SONY, Tokyo, Japan), placed 57 cm away from animals' eyes. The program to generate the stimulus was written in MATLAB (MathWorks, Natick, MA, USA), using the extensions provided by the high-level Psychophysics Toolbox<sup>15</sup> and the low-level Video Toolbox.<sup>16</sup> The visual stimulus was a flashing spot within a receptive field with an optimal size for each cell, which has often been used in related studies.<sup>17</sup> The mean luminance of the display was 19 cd/m<sup>2</sup> and the environment luminance on the cornea was 0.1 lux. For each cell, we presented the same visual stimulus 50 times, with an 'on' period of 0.5 s and an 'off' period of 3 s. The phase flicker stimulus was also given to each neuron determine whether it was of the X or Y type.



**Fig. 2** Cumulative distribution plots show the percentage of lateral geniculate nucleus cells with any given peak latency from morphine- (—) and saline-treated (---) cats ( $n = 4$  for both). In total, 147 neurons were examined from morphine-treated cats and 136 neurons were examined from saline-treated cats. It is clear that the morphine group showed a significantly longer response latency than the saline group.

### Data collection and analysis

After the neuronal signals were amplified with a microelectrode amplifier (Nihon Kohden, Tokyo, Japan) and a differential amplifier (FHC, Bowdoinham, ME, USA), action potentials were fed into a window discriminator with an audio monitor attached. The original voltage traces were digitized using an acquisition board (National Instruments, Austin, TX, USA) controlled by IGOR software (WaveMetrics, Portland, OR, USA). The original data were saved for online and offline analysis. Post-stimulus time histograms (PSTHs) of neuronal responses with a bin width of 1 msec were obtained. Then, the rising phase of the first peak in the PSTH with an amplitude greater than or equal to three times the spontaneous activity was fitted by a Gaussian curve:

$$y = y_0 + A \exp(-((x - x_0)/\sigma)^2)$$

whose time offset ( $x_0$ ) was taken as the response latency (peak latency).<sup>18</sup> Briefly, the Gaussian filter smoothed the part of the PSTH between when the stimulus started and the immediate several milliseconds after the neurons fired most. On- and off-centre cells were classified according to their on or off responses to flashing spots. All neurons were classified as X or Y using a modified 'null position test'<sup>3,19</sup> and this test was performed with a stationary alternating phase sinusoidal grating (phase flicker) as a stimulus and Fourier analysis of the responses. Layer A, A1 and C cells had contralateral, ipsilateral and contralateral responses, respectively, in keeping with the eye stimulated. Neuron layers were decided on the basis of this quality. Statistical results are presented as the mean  $\pm$  SEM.

## RESULTS

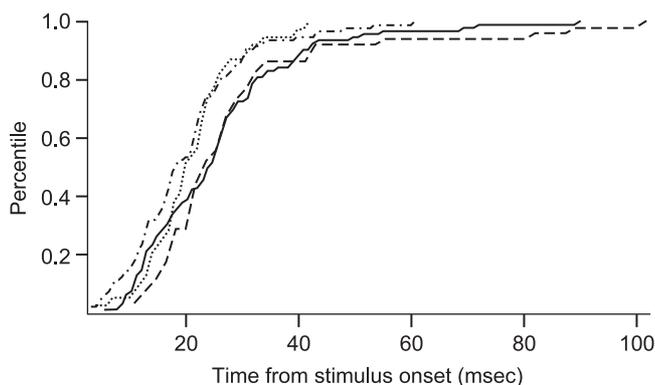
Data were obtained for 147 LGN cells from morphine-treated cats and 136 cells from saline-treated cats (controls). Figure 1 shows representative PSTHs for LGN neurons from control and morphine-treated cats. We found that chronic morphine exposure resulted in a delay of the visual-evoked response of LGN cells. Figure 2 shows cumulative distribution plots of morphine-treated and control cells with any given peak latency. Cells from morphine-treated cats had a significantly longer response latency than did control cells ( $27.3 \pm 1.3$  vs  $20.7 \pm 0.8$  msec, respectively;  $P < 0.001$ ,  $t$ -test). Table 1 gives the visual response latency of LGN cells in morphine-treated and control cats.

All cells were divided into 95 on- and 52 off-centre cells in morphine-treated cats and into 97 on- and 39 off-centre cells in

**Table 1** Response latency of cells recorded in lateral geniculate nucleus of morphine- and saline-treated (control) cats

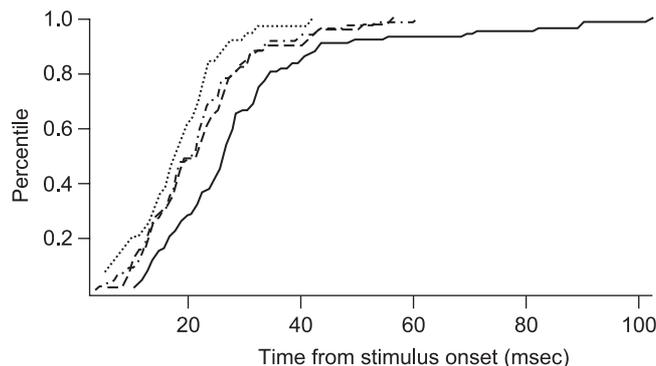
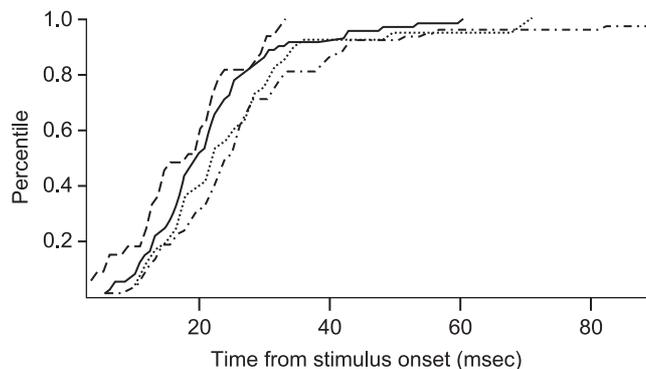
Latency	Morphine	Control	<i>P</i> ( <i>t</i> -test)	<i>t</i>
All cells	27.3 ± 1.3	20.7 ± 0.8	<0.001	4.18
X cells	30.1 ± 1.8	21.6 ± 2.3	<0.001	3.93
Y cells	22.7 ± 1.5	18.3 ± 2.9	<0.05	2.12
Layer A cells	28.2 ± 1.8	21.8 ± 1.2	<0.01	-2.90
Layer A1 cells	26.2 ± 2.1	18.2 ± 1.5	<0.01	-2.98
Layer C cells	26.3 ± 4.3	21.6 ± 1.8	0.298	-1.05
On-centre cells	26.1 ± 1.5	20.3 ± 1.1	<0.001	-3.24
Off-centre cells	29.5 ± 2.5	21.6 ± 1.2	<0.05	2.54

Data are presented as the mean ± SEM. Three group comparisons of latency according to different classifications were performed between morphine- and saline-treated cats using an independent sample *t*-test.

**Fig. 3** Cumulative distribution plots showing the percentage of on- and off-centre cells from morphine- and saline-treated cats, with any given peak latency. The neurons included 95 on-centre (—) and 52 off-centre (----) cells for the morphine group and 97 on-centre (· · · ·) and 39 (— · —) off-centre cells in control group. Morphine-treated cats had a consistently longer latency in on- and off-centre cells than did the saline-treated (control) cats.

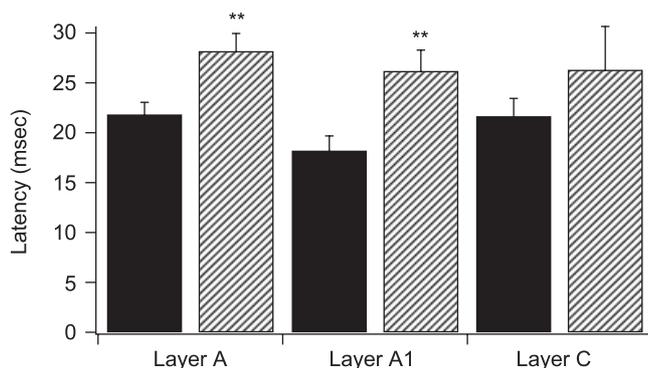
control cats on the basis of their different responses to the flashing spot stimulus. Comparisons between morphine- and saline-treated groups showed that on- and off-centre cells exhibited similar responses: in both groups of cells, the response latency after chronic morphine exposure was significantly prolonged. On-centre cells had longer mean latency in morphine-treated cats compared with controls ( $26.1 \pm 1.5$  vs  $20.3 \pm 1.1$  msec, respectively;  $P < 0.001$ , *t*-test), as did off-centre cells ( $29.5 \pm 2.5$  vs  $21.6 \pm 1.2$  msec, respectively;  $P < 0.05$ , *t*-test). Figure 3 shows the percentages of on- and off-centre cells with any given peak latency from the morphine- and saline-treated groups.

We analysed 93 X and 51 Y cells in morphine-treated cats and 88 X and 39 Y cells in control cats. Three morphine-treated and nine saline-treated cells were excluded from analysis because their responses to the flicker phase were not measured and therefore their type could not be identified accurately. Both X and Y cells from morphine-treated cats had a delayed response latency compared with control cats, with the average latency of X cells being  $30.1 \pm 1.8$  and  $21.6 \pm 2.3$  msec, respectively ( $P < 0.001$ , *t*-test), and the average latency of Y cells being  $22.7 \pm 1.5$  and  $18.3 \pm 2.9$  msec, respectively ( $P < 0.001$ , *t*-test). Figure 4 shows the cumulative distribution of the

**Fig. 4** Cumulative distribution plots showing the percentage of X and Y cells from morphine- and saline-treated cats, with any given peak latency. The neurons included 93 X (—) and 51 Y (----) cells for the morphine group and 88 X (· · · ·) and 39 Y (— · —) cells in the control group. Morphine-treated cats had a consistently longer latency for X and Y cells than did the saline-treated (control) cats.**Fig. 5** Cumulative distribution plots showing the percentage of layer A and A1 neurons from morphine- and saline-treated cats, with any given peak latency. Latency was significantly prolonged in layer A (—) and A1 (----) neurons in morphine-treated cats compared with layer A (· · · ·) and A1 (— · —) neurons in the saline-treated (control) cats.

percentage of X and Y cells with any given peak latency from morphine-treated and control groups.

To explore the changes in response latency in different LGN layers, we classified neurons into layers A, A1 or C according to homolateral or contralateral eye responses. There were 80 layer A, 41 layer A1 and 22 layer C neurons in morphine-treated cats and 73 layer A, 33 layer A1 and 26 layer C neurons in control cats. Four morphine-treated and four saline-treated neurons were excluded from analysis because they had bilateral responses. Latency was significantly prolonged in layer A and A1 neurons in morphine-treated cats ( $28.2 \pm 1.8$  and  $26.2 \pm 2.1$  msec, respectively) compared with layer A and A1 neurons in control cats ( $21.8 \pm 1.2$  and  $18.2 \pm 1.5$  msec, respectively; both  $P < 0.01$ , *t*-test). The percentage of on- and off-centre cells with any given peak latency from morphine- and saline-treated groups is shown in Fig. 5. There was a tendency for prolonged latency in layer C cells in morphine-treated compared with control cats, but the difference was not significant ( $26.3 \pm 4.3$  vs  $21.6 \pm 1.8$  msec;  $P > 0.05$ , *t*-test). A summary of the response latencies of different LGN layer neurons in morphine-treated and control cats is shown in Fig. 6.



**Fig. 6** Different response latency of different lateral geniculate nucleus layer neurons in morphine- (▨) and saline-treated (■) cats. There were 80 layer A, 41 layer A1 and 22 layer C neurons in the morphine group and 73 layer A, 33 layer A1 and 26 layer C neurons in the control (saline-treated) group. Latency was significantly prolonged in layer A and A1 neurons in morphine-treated compared with control cats. Although there was a tendency for latency prolongation in layer C cells in morphine-treated cats, the difference was not significant.

In general, the average latency of all LGN neurons among different populations in morphine-treated cats increased by 31.9% compared with that in controls. After chronic morphine exposure, the average latency of on- and off-centre neurons increased by 28.6 and 36.6%, respectively, that of X and Y cells increased by 35.1 and 34.4%, respectively, and that of layer A and A1 cells increased by 29.4 and 44.0%, respectively.

## DISCUSSION

In the present study, we systematically examined the effect of chronic morphine exposure on the visual response latency of LGN cells and found that LGN neurons in morphine-treated cats exhibited significantly longer response latency than those in saline-treated cats. This suggests that visual information transfer is delayed in LGN cells by morphine administration.

Chronic morphine exposure induces adaptation of the neural system to opiates. It is well known that drug abuse changes both inhibitory and excitatory neurotransmission in many brain areas.<sup>20–23</sup> Visual latency is affected by many of these inhibitory and excitatory mechanisms.<sup>24</sup> It has been reported that spontaneous activity is higher and that the signal-to-noise ratio is lower in LGN cells from morphine-treated cats, which may be due to a decline in GABAergic inhibition.<sup>11</sup> We also observed these changes in response properties. Our estimation of response latency was determined by the neuronal peak responses that achieved the greatest statistical significance. Decreased signal-to-noise ratios caused by morphine may have accounted for latency prolongation, because a visual response only reached a significant background level when it was strong enough.

Every retinal ganglion cell diverges onto a small pool of LGN relay neurons, so there usually is a fast and strong synchrony in the LGN for common input.<sup>25–27</sup> However, the synchronization of visual information inputs from ganglion cells may become worse after chronic morphine exposure. We have observed that visual response trial-by-trial variability was found to increase in the LGN of morphine-treated cats (Z Long *et al.*, unpubl. obs., 2007). Higher variability affects the temporal integration in synaptic transmission.<sup>28</sup> This could result in prolonged latency of LGN cells. Once it has appeared, this information

delay may pass along the visual pathway.<sup>24</sup> The responses of LGN neurons are less affected than those of cortical neurons, because cortical neurons have more varied inputs. For example, Kara and Reid have reported that the relative timing of retinal spike pairs affects the efficacy of visual information processing in cortical neurons more than in LGN neurons.<sup>27</sup> Thus, the prolonged latency of LGN neurons may act as a relatively pure representation of the effect of morphine and will impact on the information processing of higher-area neurons in morphine-treated cats.

It has been suggested that there is a tendency for a lack of  $\mu$ -opioid receptors from the dorsal to the ventral side in the cat LGN.<sup>5</sup> The lower number of  $\mu$ -opioid receptors may explain the different results in layer C cells. A lesser effect of morphine mediated through  $\mu$ -opioid receptors may cause layer C neurons to exhibit a longer, albeit not significantly so, response latency compared with other layers in morphine-treated cats.

The effects of anaesthesia on neuronal function may have influenced our results. Nakai *et al.* have reported that the latency of the evoked potential of neurons in cat inferior colliculus is not affected by any doses of urethane.<sup>29</sup> In addition, Wang *et al.* have demonstrated that latency is not changed in monkey primary visual cortex by varying levels of anaesthesia or paralysis.<sup>24</sup> Thus, we conclude that anaesthesia is not a concern and is unlikely to have affected our results. However, it is not easy to exclude a combined effect of morphine and urethane in our *in vivo* experiment using visual stimuli. If there is a synergistic effect between morphine and urethane on response latency, it is worth investigating.

In summary, the results of the present study suggest that visual response latency is significantly prolonged after chronic morphine exposure. Different types of neurons (on- and off-centre, X and Y and layer A and A1) all have significantly longer latency, whereas layer C neurons show a tendency for latency prolongation. These findings suggest that chronic morphine exposure results in delayed visual information transfer in cats. As a result of this delay, LGN and higher visual cortical areas may exhibit functional deficits. However, it is unknown whether this information delay happens in retinal ganglion cells, in the retina–thalamus pathway or in the LGN. These issues remain to be addressed.

## ACKNOWLEDGEMENTS

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