

Prenatal Morphine Exposure Alters the Layer II/III Pyramidal Neurons Morphology in Lateral Secondary Visual Cortex of Juvenile Rats

BIN MEI,¹ LEI NIU,² BIN CAO,² DAKE HUANG,¹ AND YIFENG ZHOU^{2*}

¹Department of Biology, School of Basic Medical Science, Anhui Medical University, Hefei, Anhui 230032, People's Republic of China

²Department of Neurobiology and Biophysics, School of Life Science, University of Science and Technology of China, Hefei, Anhui 230027, People's Republic of China

KEY WORDS basal dendrite, Golgi-Cox; prenatal morphine exposure; pyramidal neurons

ABSTRACT Altered cortical neuronal morphology and juvenile behavior manifestation by prenatal morphine exposure were well documented. However, this developmental morphine exposure affect the lateral secondary visual area (V2L), which may be critically involved in the multisensory of auditory and visual stimulus, remained poorly understood. To clarify the neuronal architecture changes possibly occurring in the V2L, Golgi-Cox staining was used in this study to count dendritic length and the spine density of the layer II/III pyramidal neurons in the V2L of the juvenile rats (postnatal day 25, PND25) prenatally exposed to morphine (gestation days 11–18). Quantitative analysis showed that prenatal morphine exposure decreased the total length, branch number, and spine density of the layer II/III pyramidal neurons in the V2L, and selectively altered the total length of the basal dendrites but not of the apical dendrites. The findings may provide the mechanistic understanding of the behavioral changes in the children whose mothers abuse opiates during pregnancy. **Synapse 63:1154–1161, 2009.** © 2009 Wiley-Liss, Inc.

INTRODUCTION

Prenatal exposure to opiates, such as morphine, that could cross the placenta is known to affect the development of the central nervous system and lead to the behavioral changes including the abnormalities of play behaviors and adult social behaviors in the juvenile offsprings (Hol et al., 1996; Niesink et al., 1999; Slamberova et al., 2001; Sobrian, 1977). These changes may be due to the alterations of the neuronal properties in hippocampus, nucleus accumbens, amygdaloid nuclei, and frontal cortex (Niu et al., in press; Robinson and Kolb, 1999; Vathy et al., 2003; Villarreal et al., 2008; Yang et al., 2000). Cross-modal information intergration, which presumably induces functional connections between different sensory modalities, has been reported to benefit for the behavioral enhancement (Jazayeri, 2008; Lewkowicz and Turkewitz, 1981; Stein et al., 1993). However, whether and how prenatal morphine exposure would affect functioning of sensory system remained to be fully understood.

Previous studies reveal that newborn animals are more sensitive to summed intensity of visual and

audio stimulus (Kenny and Turkewitz, 1986; Lewkowicz and Turkewitz, 1981), for example, the newborns' optimal or preferred amount of stimulation is based on the total amount or intensity of stimulus input (Lawson and Turkewitz, 1980). Additionally, the visual and auditory experience is important not only for the development of other sensory systems such as spatial sensation (Lickliter et al., 1996), but also for the visual or auditory perception development in the infants (Sleigh et al., 1998). The convergence of auditory and visual information is important for the newborns' behavior development (Lickliter et al., 1996). Recently, it was proposed that the lateral secondary

Contract grant sponsor: National Natural Science Foundation of China; Contract grant number: 30470561; Contract grant sponsor: National Basic Research Program; Contract grant number: 2006CB500804; Contract grant sponsor: University Teacher Research Program of Anhui Education Department; Contract grant number: 2006jq101.

*Correspondence to: Yifeng Zhou, School of Life Science, University of Science and Technology of China, Hefei, Anhui 230027, People's Republic of China. E-mail: zhouy@ustc.edu.cn

Received 9 October 2008; Accepted 29 March 2009

DOI 10.1002/syn.20694

Published online in Wiley InterScience (www.interscience.wiley.com).

visual area (V2L) played critical roles in the multisensory facilitation of reaction time in the presence of both auditory and visual stimuli, and that the convergence of auditory and visual information possibly occurred in the V2L (Hirokawa et al., 2008). Meanwhile, the defects in neurons development in this area may also contribute to the juvenile abnormal behavior after prenatal morphine exposure. Thus, it is necessary to know whether prenatal morphine exposure alters the intersensory of visual and audio system.

In this study, we applied the Golgi-Cox staining method to examine the dendritic length, distribution, and the spine density of the layer II/III pyramidal neurons in the V2L to understand the details.

MATERIALS AND METHODS

Animals and treatments

All animal treatments were performed strictly in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Adult female and male Sprague-Dawley rats (Anhui Experimental Animal Center, Hefei, China) were group housed under standard laboratory conditions (lights on from 9:00 AM to 9:00 PM, room temperature 22°C, ad libitum access to food and drink). Animal care and pregnancy tests were carried out as described previously. We examined the estrous cycle phases of the female rats (Marcondes et al., 2002), and the rats were mated during their proestrus or estrus phase. We took the presence of the vaginal plug or sperm cells in vaginal smear the next morning as criteria for successful mating and the subsequent pregnancy. The pregnant rats were then randomly divided into control group and experimental group. On gestation days 11–18, the pregnant rats in experimental group were subcutaneously injected with morphine (morphine sulfate, 10 mg/ml, obtained from the Anhui Food Drug Administration, China) twice daily at 9:00 AM and 9:00 PM, while the rats in control group were injected with saline instead. The dose of the first three morphine injections was 5 mg/kg and, 10 mg/kg for all subsequent injections (Niu et al., in press; Vathy et al., 1985). For the individual rat offspring, the day of birth was designated as postnatal day 0 (PND 0). The genders and weights of pups were then recorded. Apparently, the injection regimen did not alter the litter sizes or the body weights of the offspring, which was consistent with results from previous studies (Niu et al., in press; Riley and Vathy, 2006; Vathy et al., 1985; Velisek et al., 2000). On PND 21, the pups were weaned and housed in groups with free access to food and water.

Histological procedures

On PND 25, all rats offspring (control group, $n = 10$, experiment group, $n = 10$; five males and five

females in each group) were anesthetized with urethane and perfused with 0.9% saline solution. Brains were removed and prepared for Golgi-Cox staining according to procedures described by Gibb and Kolb (1998): the removed brains were stored in the darkness for 14 days in Golgi-Cox solution, then 4 days in 30% sucrose solution. Then brains were sectioned in 200 μm thickness in the coronal plane at the level of the V2L by a vibratome (Leica, VT-1000S, Germany). We collected the sections with cleaned and gelatin-coated microscope slides (four sections per slide) and stained with ammonium hydroxide for 30 min followed by washing. Then the sections were immersed in Kodak Film Fixer solution for another 30 min, rinsed with water, dehydrated, cleared, and mounted, according to standard procedures.

As shown in Figure 1A, at low magnification (40 \times and 100 \times), visual cortex of the V2L regions (Bregma -4.8 to -7.8) were identified as regions outside of the whole hippocampus or subiculum (S) and the white matter associated with the hippocampus from DG (anterior extremity) to S (posterior extremity), and near the olfactory sulcus (Paxinos, 1986). Layer II to III pyramidal cells (the cortical depth is from 10 to 35%) (Hirokawa et al., 2008) in the V2L from each hemisphere were examined (Fig. 1B). Golgi-impregnated pyramidal neurons of visual cortex were readily identified by the criteria described previously (Robinson and Kolb, 1999; Silva-Gomez et al., 2003). At least 10 neurons from each region of per animal were photographed, with each neuron recorded at least in three focal plane at a magnification of 200 \times (Eclipse 80i, Nikon, Japan) by a person blind to the experimental groups. The spines of each neuron were photographed on a length (at least 20- μm long) from the last branch point to the terminal tip of the basal dendrite using a magnification of 1000 \times .

2D reconstruction of neurons and dendrite analysis

Software Image J (National Institute of Health) (Abramoff et al., 2004; Pool et al., 2008) with Neuron J plugin (Meijering et al., 2004) and Sholl analysis were used for the reconstruction of the neurons' morphological structure and for the quantitative analysis, respectively. Unless otherwise stated, at least three images were taken for each plane of the same neurons, stacked and presented in one image with averaged signal (Fig. 2A). Using Neuron J plugin, the main dendrites were traced from the cell body at first, and on this dendrite, from the root to the tip, every branch was traced in order (Figs. 2B–2D). Likewise, we traced all the dendrites subsequently. We clustered all the dendrites of the same neuron into two groups: apical dendrites and basal dendrites. In Neuron J, we exported the total length and total branch

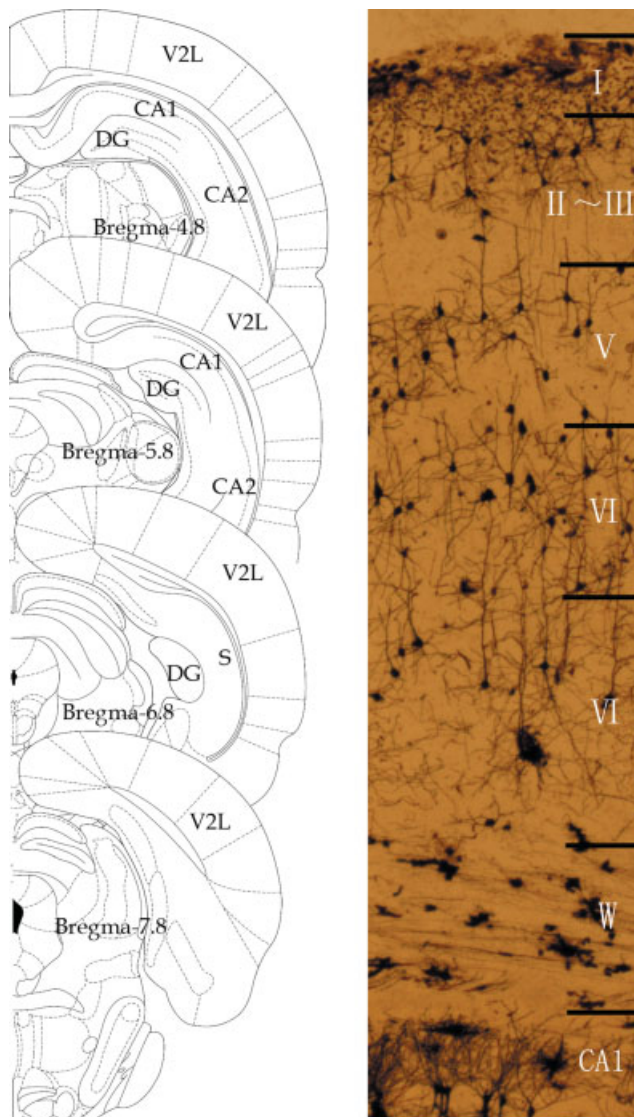


Fig. 1. (A) Coronal diagrams show the location of the V2L, outside of which the whole hippocampus or subiculum (S) can be seen. The white matter is associated with the hippocampus from DG (anterior extremity) to S (posterior extremity). The olfactory sulcus was also considered. (B) shows the location of the layer II~III pyramidal cells, with the cortical depth is from 10 to 35%. The magnification is $\times 40$. W, white matter. (Reproduced with permission from Paxinos and Watson, 1986). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

number of the neuron, as well as the total length of the basal dendrites and the apical dendrite. The traced photograph for Sholl Analysis (Sholl, 1956) was exported with the related plugins in Image J program. The spine density was assessed by measuring a length from the last branch point to the terminal tip of the dendrite (Flores et al., 2007). The exact lengths of the chosen dendrite segments (at least 20 μm long) were calculated, the numbers of spines on each dendrite segment was counted and the density was presented as spines/10 μm .

Synapse

Statistics

Analyses of the spine densities, dendrite total length, basal and apical dendrite length and Sholl analysis of ring intersections were carried by averaging across cells for each animal. The data of the spine densities, dendrite total length, basal and apical dendrite length were assessed using two-way ANOVA (gender \times group). The data from Sholl analysis of the number of ring intersections were analyzed by using two-tailed Kruskal–Wallis and Mann–Whitney tests ($P < 0.05$ was considered significant).

RESULTS

Morphology of the 200 pyramidal neurons in the layer II/III of the V2L was reconstructed using program Image J with its plugins (Fig. 2). At first, all images of the same neurons at different level were integrated into one (Fig. 2A). Then, using Neuron J plugin, the longest dendrite was traced at first, and then all the offset dendrite on this dendrite was traced (Fig. 2B and Fig. 2D). At last all the dendrites were traced by offsetting one by one and the traced photograph was exported out as a separate photo (Fig. 2C). The Golgi-Cox impregnation procedure clearly filled the dendritic shafts and spines of the pyramidal cells (Fig. 3).

In these experiments, neither main effect of gender nor the gender \times group interaction was significant. Prenatal morphine exposure significantly decreased the total length of the dendrites ($F_{1,19} = 11.88$, $P < 0.01$), suggesting that morphine exposure inhibits the neurons development. We then calculated the branch number of each group, and found a significant difference between the two groups ($F_{1,19} = 5.75$, $P < 0.05$) (Fig. 4).

When the apical dendrites and the basal dendrites were clustered, a significant difference has been observed in total lengths of basal dendrite between the two groups ($F_{1,19} = 7.52$, $P < 0.05$), but not those of apical dendrites ($F_{1,19} = 3.35$, $P > 0.05$) (Fig. 5).

As measured by Sholl analysis, the intersection per radius of shell shows that the prenatally morphine exposed pups had less intersection per shell or less dendritic arborization than the control rats ($P < 0.05$) (Fig. 6). There were significant differences in shells with a radius of 50–80 μm and later than 200 μm ($P < 0.05$).

Because there are more than 90% activated the synapses in pyramidal neurons dendrites, the density of spine has been considered as symbolic of the activation of the pyramidal neuron behavior. In our study, morphine-exposure has obviously resulted in the decrease in spine density ($F_{1,19} = 7.07$, $P < 0.05$) (Fig. 4C).

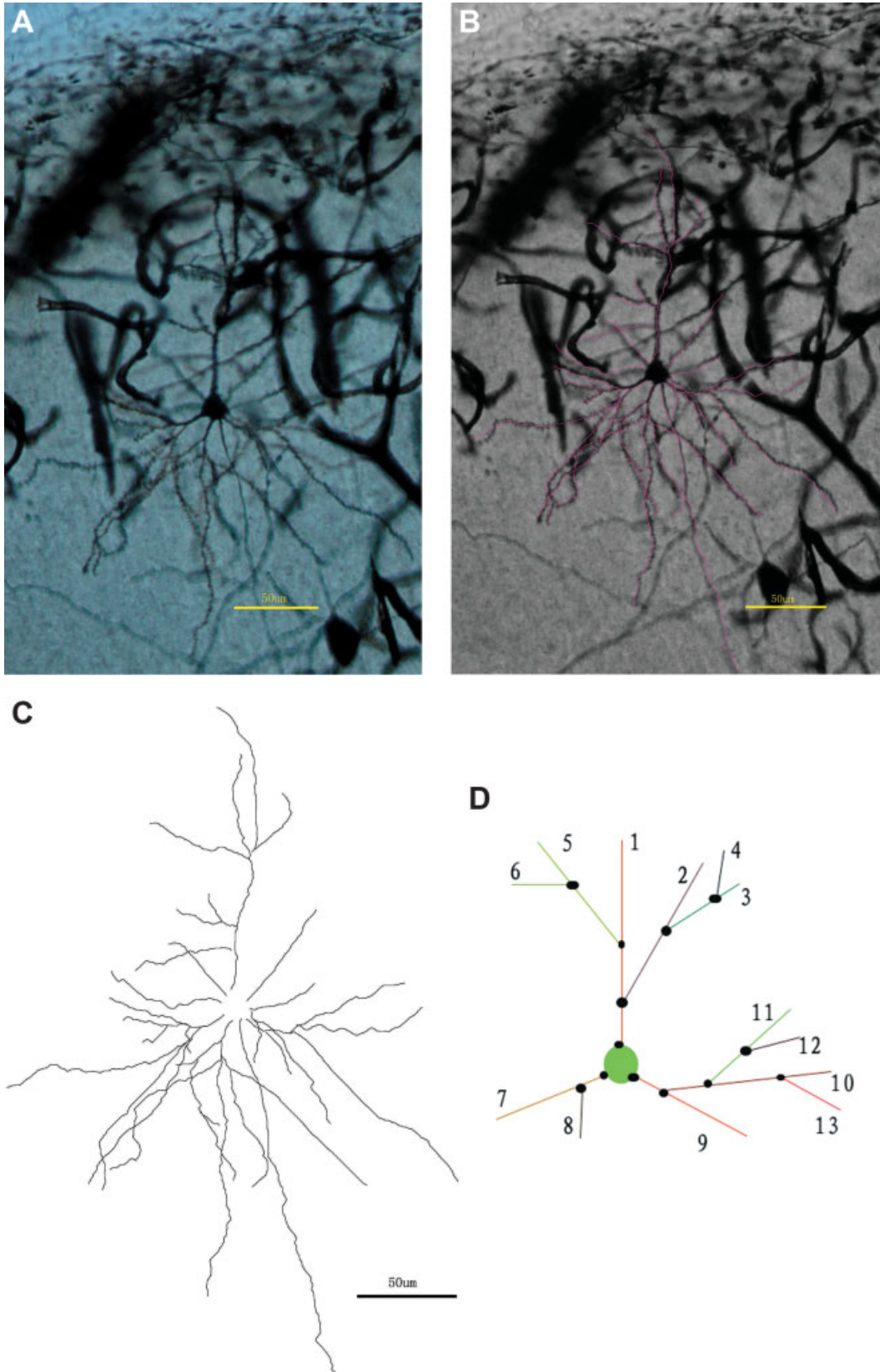


Fig. 2. (A) The images are presented with signal averaged from three photographs (See Materials and Methods for details). (B) Using Neuron J plugin, the longest dendrite was traced at first, and then the longest offset dendrite. (C) The traced photograph exported with Neuron J plugins. (D) Shows the process of tracing and the branch number (or the fork), there are 13 branches or forks in this figure. (Bar = 50 μm).

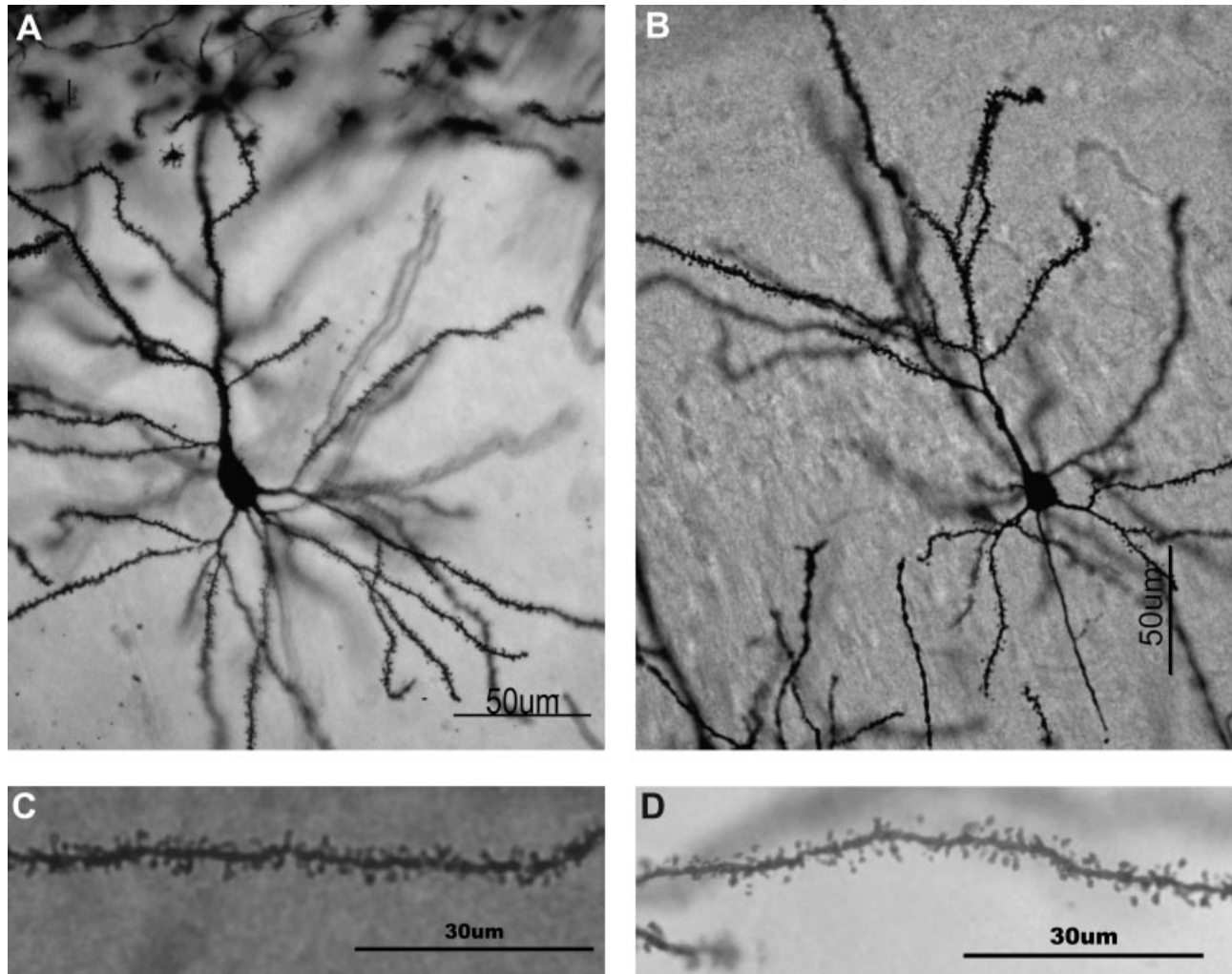


Fig. 3. Photomicrograph showing representative Golgi-Cox impregnated pyramidal neurons of the layer II~III in the V2L from the control and morphine groups at PND25. (A) Pyramidal neuron of control group. (B) Pyramidal neuron of morphine group. (C) The spines of control group. (D) The spines of morphine group. (neuron bar = 50 μ m; spine bar = 30 μ m).

DISCUSSION

Prenatal morphine exposure altered the layer II/III pyramidal neurons morphology of the V2L in juvenile rats

In this study, we found that prenatal morphine exposure caused a significant decrease in total dendrite length and spine density of the lateral secondary visual cortex (V2L) in PND25 rats, whose cerebral cortex were still under development (Taylor and Chapman, 1969; Necdet and Ramazan, 1998). Interestingly, our results showed that prenatal morphine exposure selectively induced morphological rearrangements in the total length of the basal dendrites, but not in the total length of apical dendrites, of the layer II/III pyramidal neurons in V2L of PND 25 rats. These alterations led to an overall remodeling of the dendrites branching pattern, for example, loss of dendrites branches and reduction in total length per neuron.

Prenatal morphine exposure also resulted in the decrease of the dendrites branches in the medial-to-distal portions of the dendrites and, concomitantly, significant reductions in the dendrites disposition at the distal portions. These observations strongly suggested that prenatal morphine exposure seriously impaired the development of neurons in the V2L in juvenile rats.

Alterations of GABAergic system may contribute to the changes of the neuron morphology in V2L

Transcription factors have a profound influence on dendrites arbor size and complexity (Parrish et al., 2007). Prenatal morphine exposure increased the number of opiate receptors in rats of PND1-PND7, but had no effect on those of PND14-PND28 (Bhat

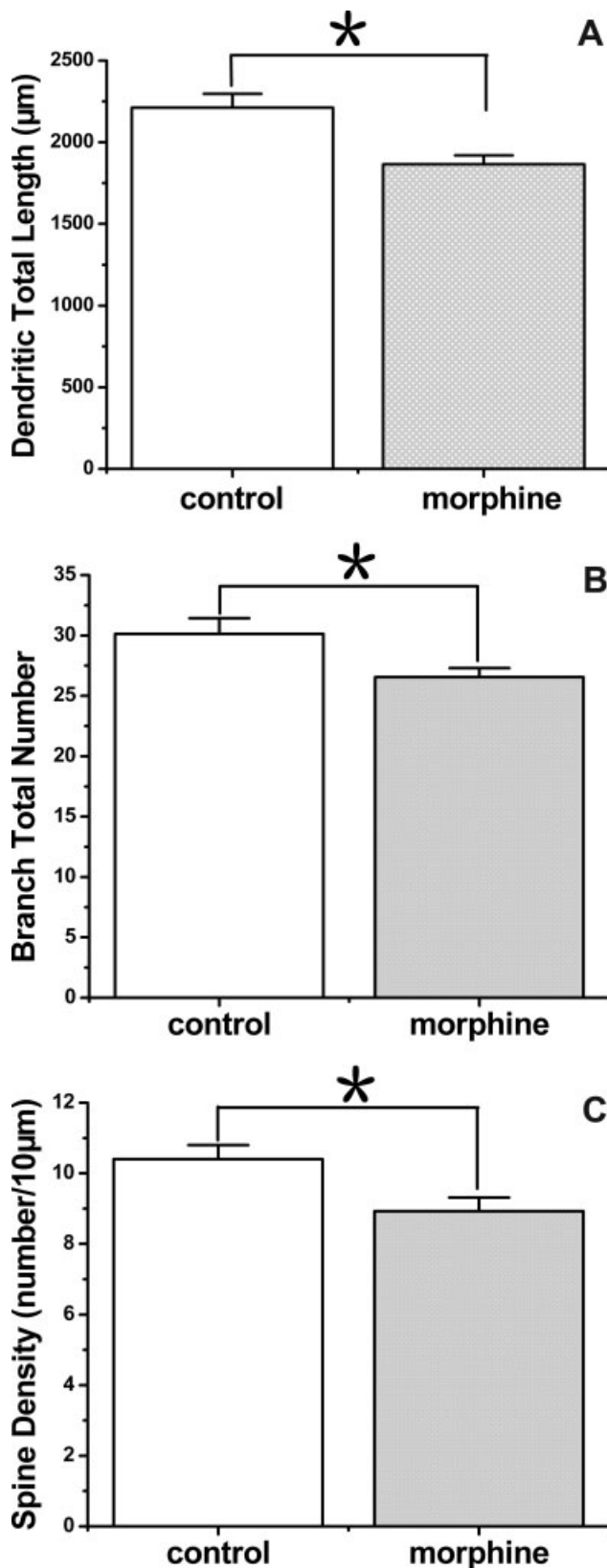


Fig. 4. Analysis of the pyramidal neurons in both the control and the morphine groups at PND 25 ($n = 10$ animals per group). Prenatal morphine exposure decreases the total dendritic length (A), the dendritic branch number (B), and spine density (C). (* $P < 0.05$).

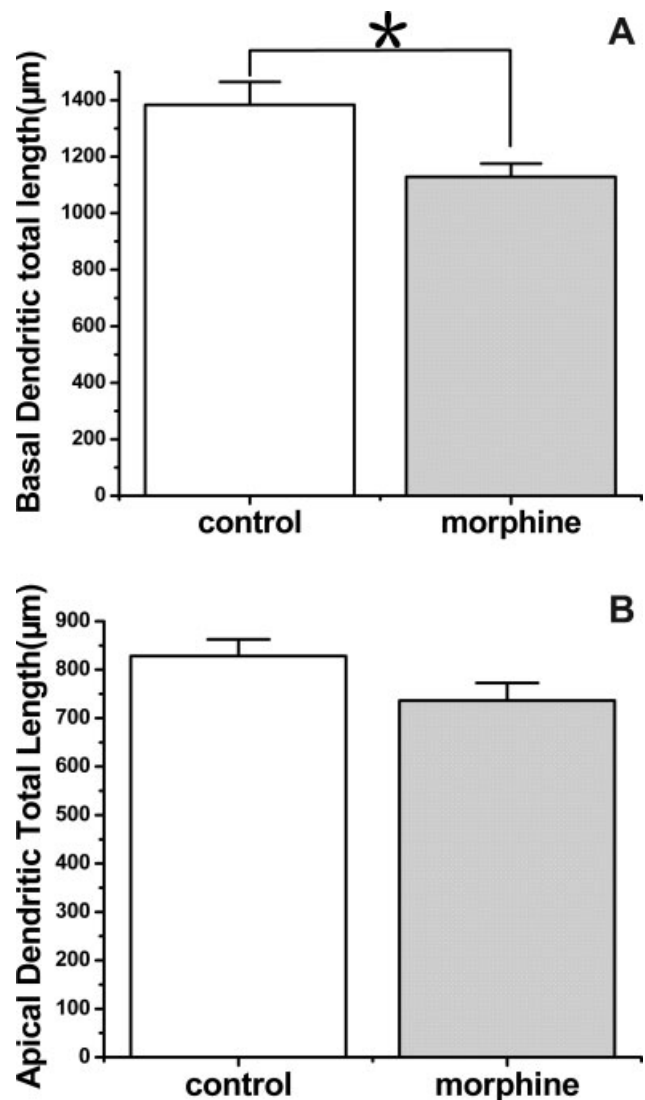


Fig. 5. Cluster analysis. Prenatal morphine exposure significantly decreased the basal dendritic length (A), but not the apical dendritic length (B) (* $P < 0.05$).

et al., 2006). It was reported that, by activating ubiquitously clustered μ -opioid receptor in excitatory synapses, morphine caused the collapse of preexisting dendrites spines (Liao et al., 2005). Hence, it is speculated that morphine exposure may induce changes in the expression of opiate receptors at early developing stages, which possibly causes the alteration of the neuronal morphology in the visual cortex.

It has been of wide interest in understanding how the opiate receptor modulates the neuron development in the past years (Robinson and Kolb, 2004). Our previous studies suggest that GABAergic inhibitory synaptic is crucial for the development and maintenance of visual cortical function (Leventhal et al., 2003; Li et al., 2007a,b, 2008). In addition, GABAergic synaptic transmission in adult animal could be significantly influenced by opiate (Laviolette et al., 2004; Li

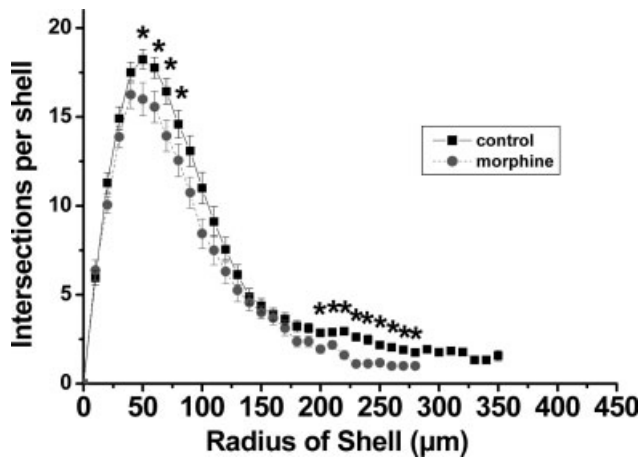


Fig. 6. Sholl analysis of intersections per shell. Prenatal morphine exposure led to decrease of dendrites branching in the medial portions and, concomitantly, significant reductions of dendrites arborization in the middle to distal portions ($*P < 0.05$).

et al., 2007a; Vaughan et al., 1997). Decreased number of GABA-containing neurons in the DG area by prenatal morphine exposure demonstrates a dysfunctional GABA system (Niu et al., in press). Excitatory GABA actions (Ben-Ari, 2002) in the embryonic period plays critical roles in morphological maturation of layer II/III of the somatosensory cortical neurons (Cancedda et al., 2007), loss of function will cause severe deficit in neuronal architecture. The decreased total length of dendrites and spine density by prenatal morphine exposure observed in this study very likely attribute to the weakened GABA-mediated (GABAergic) synaptic transmission, which may consequently impair the information integration between the audio and visual system.

Prenatal morphine exposure probably changes the excitability of neurons in V2L

Communication between neurons is important for the dendrites growth and the synapse maturation on the dendrites (Leite et al., 2005; Matsuzaki, 2007). Therefore, the changes in the neurons morphology are somewhat indicative of alterations in pyramidal neurons excitability. In our study, morphine exposure was found to have the effect of decreasing total length, spine density, and branch number of the dendrites, suggesting that prenatal morphine exposure alters the neurons excitability in the V2L. Decrease in the density of dendritic spines may further exacerbate this alteration. Although the dendritic spines can form and retract quickly in vivo and the shape and size of spines also change over time, there is consensus that most spines from cortical pyramidal neurons are stable, and that spine stability is developmentally regulated in visual cortex (Alvarez and Sabatini, 2007; Holtmaat et al., 2005; Majewska et al., 2006). It is estimated that more

than 90% of excitatory synapses are on dendritic spines (Kolb and Whishaw, 1998). Taken together with our result that the morphine exposure decreased the spine density of the neurons, it is natural to hypothesize that prenatal morphine exposure can also affect the excitability of input in V2L.

Furthermore, our results showed that morphine exposure significantly altered the total length of basal dendrites without significantly affecting the apical dendrites distribution. Indeed, functional differences between apical and basal dendrite have long been illustrated (Arai et al., 1994; Yuste et al., 1994). It was reported that LTP in basal dendritic branches of hippocampus CA1 pyramidal cells led to stronger enhancement of cellular activity than that in the apical (Papp et al., 2004). In layer V pyramidal neurons from slices of rat somatosensory cortex, the apical band may serve as a dendritic trigger zone for regenerative Ca^{2+} spikes or as a current amplifier for distal synaptic events (Yuste et al., 1994). Although it is yet unclear whether there is a difference between basal dendrite and apical dendrite of pyramidal neurons in the layer II/III of V2L, the fact that morphine exposure selectively alters their arborization strongly suggests apical dendrites and basal dendrites have different function and different excitability in V2L. The different effect on basal and apical dendrite in visual cortex maybe also suggests that morphine exposure changes the neurons excitability.

CONCLUSION

Here we have observed that prenatal morphine exposure altered the pyramidal neurons morphology of the layer II/III of the V2L in juvenile rats. The total length, the branch number, and the spine density were both decreased in young rats after prenatal morphine exposure. This treatment selectively decreased the basal dendritic length. These may be result in the impairment of the integration of input from visual and audio primary cortex and thus the impairment of the behavior of juvenile rats.

REFERENCES

- Abràmoff MD, Magalhães PJ, Ram SJ. 2004. Image Processing with ImageJ. *Biophotonics International* 11:36–42.
- Alvarez VA, Sabatini BL. 2007. Anatomical and physiological plasticity of dendritic spines. *Annu Rev Neurosci* 30:79–97.
- Arai A, Black J, Lynch G. 1994. Origins of the variations in long-term potentiation between synapses in the basal versus apical dendrites of hippocampal neurons. *Hippocampus* 4:1–9.
- Ben-Ari Y. 2002. Excitatory actions of gaba during development: The nature of the nurture. *Nat Rev Neurosci* 3:728–739.
- Bhat R, Chari G, Rao R. 2006. Effects of prenatal cocaine, morphine, or both on postnatal opioid (μ) receptor development. *Life Sci* 78:1478–1482.
- Cancedda L, Fiumelli H, Chen K, Poo MM. 2007. Excitatory GABA action is essential for morphological maturation of cortical neurons in vivo. *J Neurosci* 27:5224–5235.
- Flores C, Wen X, Labelle-Dumais C, Kolb B. 2007. Chronic phencyclidine treatment increases dendritic spine density in prefrontal cortex and nucleus accumbens neurons. *Synapse* 61:978–984.

- Gibb R, Kolb B. 1998. A method for vibratome sectioning of Golgi-Cox stained whole rat brain. *J Neurosci Methods* 79:1-4.
- Hirokawa J, Bosch M, Sakata S, Sakurai Y, Yamamori T. 2008. Functional role of the secondary visual cortex in multisensory facilitation in rats. *Neuroscience* 153:1402-1417.
- Hol T, Niesink M, van Ree JM, Spruijt BM. 1996. Prenatal exposure to morphine affects juvenile play behavior and adult social behavior in rats. *Pharmacol Biochem Behav* 55:615-618.
- Holtmaat AJ, Trachtenberg JT, Wilbrecht L, Shepherd GM, Zhang X, Knott GW, Svoboda K. 2005. Transient and persistent dendritic spines in the neocortex in vivo. *Neuron* 45:279-291.
- Jazayeri M. 2008. Probabilistic sensory recoding. *Curr Opin Neurobiol* 18:431-437.
- Kenny PA, Turkewitz G. 1986. Effects of unusually early visual stimulation on the development of homing behavior in the rat pup. *Dev Psychobiol* 19:57-66.
- Kolb B, Whishaw IQ. 1998. Brain plasticity and behavior. *Annu Rev Psychol* 49:43-64.
- Lavolette SR, Gallegos RA, Henriksen SJ, van der Kooy D. 2004. Opiate state controls bi-directional reward signaling via GABAA receptors in the ventral tegmental area. *Nat Neurosci* 7:160-169.
- Lawson KR, Turkewitz G. 1980. Intersensory function in newborns: Effect of sound on visual preferences. *Child Dev* 51:1295-1298.
- Leite JP, Neder L, Arisi GM, Carlotti CG Jr, Assirati JA, Moreira JE. 2005. Plasticity, synaptic strength, and epilepsy: What can we learn from ultrastructural data? *Epilepsia* 46 (Suppl 5):134-141.
- Leventhal AG, Wang Y, Pu M, Zhou Y, Ma Y. 2003. GABA and its agonists improved visual cortical function in senescent monkeys. *Science* 300:812-815.
- Lewkowicz DJ, Turkewitz G. 1981. Intersensory interaction in newborns: Modification of visual preferences following exposure to sound. *Child Dev* 52:827-832.
- Li Y, He L, Chen Q, Zhou Y. 2007a. Changes of micro-opioid receptors and GABA in visual cortex of chronic morphine treated rats. *Neurosci Lett* 428:11-16.
- Li Y, Wang H, Niu L, Zhou Y. 2007b. Chronic morphine exposure alters the dendritic morphology of pyramidal neurons in visual cortex of rats. *Neurosci Lett* 418:227-231.
- Li G, Yang Y, Liang Z, Xia J, Yang Y, Zhou Y. 2008. GABA-mediated inhibition correlates with orientation selectivity in primary visual cortex of cat. *Neuroscience* 155:914-922.
- Liao D, Lin H, Law PY, Loh HH. 2005. Mu-opioid receptors modulate the stability of dendritic spines. *Proc Natl Acad Sci USA* 102:1725-1730.
- Lickliter R, Lewkowicz DJ, Columbus RF. 1996. Intersensory experience and early perceptual development: The role of spatial contiguity in Bobwhite quail chicks' responsiveness to multimodal maternal cues. *Dev Psychobiol* 29:403-416.
- Majewska AK, Newton JR, Sur M. 2006. Remodeling of synaptic structure in sensory cortical areas in vivo. *J Neurosci* 26:3021-3029.
- Marcondes FK, Bianchi FJ, Tanno AP. 2002. Determination of the estrous cycle phases of rats: Some helpful considerations. *Braz J Biol* 62:609-614.
- Matsuzaki M. 2007. Factors critical for the plasticity of dendritic spines and memory storage. *Neurosci Res* 57:1-9.
- Meijering E, Jacob M, Sarría JC, Steiner P, Hirling H, Unser M. 2004. Design and validation of a tool for neurite tracing and analysis in fluorescence microscopy images. *Cytometry A* 58:167-176.
- Necdet D, Ramazan D. 1998. Neuronal differentiation in the cerebral cortex of the rat: A Golgi-Cox study. *Tr J Med Sci* 28:481-490.
- Niesink RJ, van Buren-van Duinkerken L, van Ree JM. 1999. Social behavior of juvenile rats after in utero exposure to morphine: Dose-time-effect relationship. *Neuropharmacology* 38:1207-1223.
- Niu L, Cao B, Zhu H, Mei B, Wang M, Yang Y, Zhou Y. 2008. Impaired in vivo synaptic plasticity in dentate gyrus and spatial memory in juvenile rats induced by prenatal morphine exposure. *Hippocampus*. DOI 10.1002/hipo.20540.
- Papp G, Huhn Z, Lengyel M, Erdi P. 2004. Effect of dendritic location and different components of LTP expression on the bursting activity of hippocampal CA1 pyramidal cells. *Neurocomputing* 58-60:691-697.
- Parrish JZ, Emoto K, Kim MD, Jan YN. 2007. Mechanisms that regulate establishment, maintenance, and remodeling of dendritic fields. *Annu Rev Neurosci* 30:399-423.
- Paxinos G, Watson C. 1986. *The Rat Brain in Stereotaxic Coordinates*. New York: Academic Press.
- Pool M, Thiemann J, Bar-Or A, Fournier AE. 2008. NeuriteTracer: A novel ImageJ plugin for automated quantification of neurite outgrowth. *J Neurosci Methods* 168:134-139.
- Riley MA, Vathy I. 2006. Mid- to late gestational morphine exposure does not alter the rewarding properties of morphine in adult male rats. *Neuropharmacology* 51:295-304.
- Robinson TE, Kolb B. 1999. Morphine alters the structure of neurons in the nucleus accumbens and neocortex of rats. *Synapse* 33:160-162.
- Robinson TE, Kolb B. 2004. Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology* 47:33-46.
- Sholl DA. 1956. The measurable parameters of the cerebral cortex and their significance in its organization. *Prog Neurobiol* 2:324-333.
- Silva-Gomez AB, Rojas D, Juarez I, Flores G. 2003. Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in postweaning social isolation rats. *Brain Res* 983:128-136.
- Slamberova R, Schindler CJ, Pometlova M, Urkuti C, Purow-Sokol JA, Vathy I. 2001. Prenatal morphine exposure differentially alters learning and memory in male and female rats. *Physiol Behav* 73:93-103.
- Sleigh MJ, Columbus RF, Lickliter R. 1998. Intersensory experience and early perceptual development: Postnatal experience with multimodal maternal cues affects intersensory responsiveness in bobwhite quail chicks. *Dev Psychol* 34:215-223.
- Sobrian SK. 1977. Prenatal morphine administration alters behavioral development in the rat. *Pharmacol Biochem Behav* 7:285-288.
- Stein B, Meredith MA, Wallace MT. 1993. The visually responsive neuron and beyond: Multisensory integration in cat and monkey. *Prog Brain Res* 95:79-90.
- Taylor BA, Chapman AB. 1969. Genetic effects of spermatogonial irradiation on growth and age at sexual maturity in rats. *Genetics* 63:441-454.
- Vathy IU, Etgen AM, Barfield RJ. 1985. Effects of prenatal exposure to morphine on the development of sexual behavior in rats. *Pharmacol Biochem Behav* 22:227-232.
- Vathy I, Slamberova R, Rimanoczy A, Riley MA, Bar N. 2003. Autoradiographic evidence that prenatal morphine exposure sex-dependently alters mu-opioid receptor densities in brain regions that are involved in the control of drug abuse and other motivated behaviors. *Prog Neuropsychopharmacol Biol Psychiatry* 27:381-393.
- Vaughan CW, Ingram SL, Connor MA, Christie MJ. 1997. How opioids inhibit GABA-mediated neurotransmission. *Nature* 390:611-614.
- Velisek L, Stanton PK, Moshe SL, Vathy I. 2000. Prenatal morphine exposure enhances seizure susceptibility but suppresses long-term potentiation in the limbic system of adult male rats. *Brain Res* 869:186-193.
- Villarreal DM, Derrick B, Vathy I. 2008. Prenatal morphine exposure attenuates the maintenance of late LTP in lateral perforant path projections to the dentate gyrus and the CA3 region in vivo. *J Neurophysiol* 99:1235-1242.
- Yang SN, Yang JM, Wu JN, Kao YH, Hsieh WY, Chao CC, Tao PL. 2000. Prenatal exposure to morphine alters kinetic properties of NMDA receptor-mediated synaptic currents in the hippocampus of rat offspring. *Hippocampus* 10:654-662.
- Yuste R, Gutnick MJ, Saar D, Delaney KR, Tank DW. 1994. Ca²⁺ accumulations in dendrites of neocortical pyramidal neurons: An apical band and evidence for two functional compartments. *Neuron* 13:23-43.