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THE CORRELATIVE STUDY BETWEEN ACUPOINTS AND CORRESPONDING BRAIN CORTICES

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Our previou tudy with functional MRI demon trated that acupuncture timulation on the vi ion-related acupoint, Bl-67, activated the vi ual cortex of human brain. A a further tudy on the effect of Bl-67 acupuncture timulation on the vi ual cortex, we examined c-Fo expre ion in binocularly deprived rat pup . Binocular deprivation ignificantly reduced the number of c-Fo -po itive cell in the primary vi ual cortex, compared with that of normal control rat pup . Intere tingly, acupuncture timulation on Bl-67 re ulted in a ignificant increa e in the number of c-Fo -po itive cell in the primary vi ual cortex wherea acupuncture timulation on other acupoint le important for vi ual function had no ignificant effect on c-Fo expre ion in the primary vi ual cortex. The re ult ugge t the po ibility of the vi ion-related acupoint having an influence over the activity of the primary vi ual cortex.

In oriental medicine, vi ion-related acupoint have been believed to be effective in treating eye-related di order clinically (Hong, 1994; Stux and Pomeranz, 1988). The acupoint Bl-67, located on the lateral ide of the little toe, i known to be one of the vi ion-related acupoint (Fig. 1, Cho *et al*., 1998; Hong, 1994; Stux and Pomeranz, 1998). Our previou functional MRI tudy ha hown imilar pattern of activation ignal in the vi ual cortex of human brain, either after acupuncture timulation on Bl-67 or 8-Hz light-fla h timulation on the eye (Fig. 2, Cho *et al*., 1998). Thi re ult ugge t a po ible correlation between activation of the vi ual cortex and acupuncture timulation on the vi ion-related acupoint. However, there ha been no ba ic tudy to explain how the activation ignal in the vi ual cortex i obtained following acupuncture timulation on the vi ion-related acupoint (Bl-67). In thi tudy, therefore, immunoreactivity for c-Fo protein, known to mediate the rapid phy iological event that occur in the vi ual cortex during early po tnatal life (Beaver *et al*., 1993; Mower, 1994; Yamada *et al*., 1999), wa mea ured for the purpo e of inve tigating the effect of Bl-67 acupuncture timulation on the vi ual cortex.

The neonatal vi ual cortex i highly pla tic, and it development i regulated by vi ual experience during early

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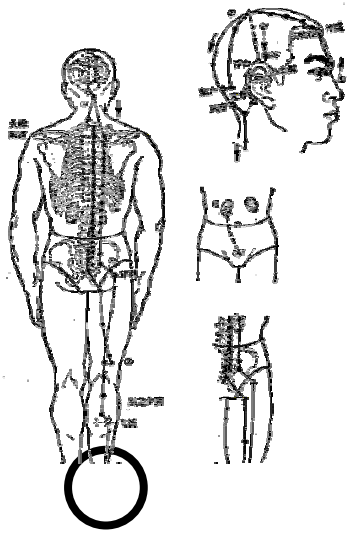

Fig. 1. The vision-related acupoints Bl-67 and acupoints for comparison, Sp-1.

Fig. 2. The fMRI activation maps of the visual cortex resulting from visual stimulation of the eye and acupuncture stimulation at Bl-67, and nonacupoints stimulation, respectively.

po tnatal period (Caleo *et al*., 1999; Kamin ka *et al*., 1996; Mower *et al*., 1983; Ro en *et al*., 1992). Even a brief expo ure to vi ual timuli during a period of dark-rearing re ult in dramatic induction of c-*fos* mRNA and/or c-Fo protein in the vi ual cortex, and thi lead to the rapid development of the vi ual cortex (Beaver *et al*., 1993; Mower, 1994; Ro en *et al*., 1992; Sato *et al*., 2000). A an animal model for the complete exclu ion of light-related phy iological event , rat pup with binocular deprivation before eye opening were employed in the pre ent tudy. Although relatively few re earche have focu ed on the biological effect of manual acupuncture compared to electroacupuncture (Hui *et al*., 2000; Sato *et al*., 1992; Sato *et al*., 1993), manual acupuncture i clinically more often u ed than electroacupuncture. Recent tudie have al o reported that manual acupuncture ha the effect of modulation of the limbic y tem and ubcortical gray tructure of the human brain (Hui *et al*., 2000) and proliferation of newly

generated cell in the dentate gyru (Kim *et al*., 2001). In addition, to further examine whether or not acupuncture timulation on pecific acupoint i more effective than that on non pecific acupoint not clo ely related with corre ponding di order , we performed acupuncture timulation on Bl-54 a well in the binocularly deprived rat pup (Cho *et al*., 1998; Lee *et al*., 1993; Stux and Pomeranz, 1988). In e ence thi tudy wa de igned to tudy the correlation between manual acupuncture timulation on Bl-67 and the activation of the vi ual cortex.

MATERIALS AND METHODS

Animal model

The experimental procedure were carried out in accordance with the animal care guideline of NIH and the Korean Academy of Medical Science . 24 laboratory-born Sprague-Dawley rat pup were divided into three group : eight pup of normal rearing erved a the control (Control), eight pup were binocularly deprived (BD), and eight pup were ubjected to acupuncture timulation on Bl-67 after binocular deprivation (Bl-67+BD). Binocular deprivation wa carefully carried out under ether ane the ia at po tnatal day 10 (P10), ju t prior to eye opening (Caleo *et al*., 1999; Sato *et al*., 2000). Animal were hou ed in cage at controlled temperature (20 ± 2 °C) with a 12:12 h light-dark cycle. Food and water were made available ad libitum. All animal were handled with care to prevent infection and minimize tre .

Acupuncture stimulation

Acupuncture timulation on Bl-67 wa performed for 15 min a day from P14 to P30, during the critical developmental period of the rat' vi ual cortex (Caleo *et al*., 1999). Relevant acupuncture timulation procedure were applied in accordance with previou tudie (Ji *et al*., 1994; Kim *et al*., 2001; Sato *et al*., 1992; Sato *et al*., 1993). Sterilized tainle teel acupuncture needle of 0.1 mm diameter were in erted into the loci of Bl-67 on both feet, located at the lateral proximal corner of the 5th toe, right next to the nail (Stux and Pomeranz, 1988). During the acupuncture timulation, animal were kept in pla tic holder with their tail and hind leg protruding out. Animal in the Control and BD group were imilarly kept in holder without acupuncture timulation in order to rule out variation due to tre cau ed by the re traint.

c-Fos immunohistochemistry

On P30, rat were ane thetized with pentobarbital odium (50 mg/kg, i.p.) to a tate of complete lack of re pon e, and then rapidly perfu ed through the aorta with 0.05 M pho phate-buffered aline (PBS), followed by fre hly prepared 4 % paraformaldehyde in 0.1 M pho phate buffer. Brain were then removed, po t-fixed with the ame fixative for 2 day , and then tran ferred into 30 % ucro e in 0.05 M PBS under torage at 4 °C until ectioning. Section were cut coronally in 30 μm through the area of intere t on a freezing microtome (Leica, Nuβloch,

Germany). The sections were incubated in rabbit anti-c-Fos antibody (Santa Cruz Biotechnology Inc, Santa Cruz, CA) at a dilution of 1:1000 for 24 h at 4 °C. After washing in PBS, they were incubated with the secondary antibody (biotinylated anti-rabbit IgG, Vector Laboratories Inc, Burlingame, CA) for 1 h at room temperature. The sections were reacted with ABC reagent (Vector Laboratories Inc, Burlingame, CA) for 1 h at room temperature, washed in PBS and then incubated for 5 min in 0.1 M Tris-HCl-buffered saline (pH 7.5) containing 0.02 % diaminobenzidine solution and 0.003 % hydrogen peroxide. The sections were mounted on 0.5 % gelatin solution coated glass slides.

Analysis of c-Fos immunoreactivity

Analysis of the rat brain areas was carried out using the rat brain atlas of Paxinos and Watson (Paxinos and Watson, 1997). The boundaries of the cytoarchitecture of the rat primary visual cortex were determined according to the previously described method (Reid and Jurakas, 1991; Zilles *et al.*, 1984). The sections were selected from the comparable regions in the lateral geniculate nucleus (LGN) and the primary visual cortex, and photographed each at ×100 (LGN) and ×40 (primary visual cortex). For the analysis of c-Fos immunoreactivity in the primary visual cortex, the method by Sato *et al.* (2000) was used. To explain the method briefly, a sheet of paper having a rectangular slit (1.98 cm wide and 6 cm long) was placed in the middle of the photomicrograph, with the longer side of the slit perpendicular to the cortical surface, so that all the c-Fos-positive cells from the pia to the white matter of the primary visual cortical area in a hemisphere can be seen at a time. The width of 1.92 cm in photograph corresponded to 400 μm. The number of c-Fos-positive cells per slit was manually counted by experimenter unaware of the animal group assignment.

Statistical analysis

The data were assessed by one-way ANOVA with Tukey's post hoc test. Difference between groups were considered significant for $p < 0.05$.

RESULTS

c-Fos immunoreactivity in the LGN

Binocular deprivation resulted in a clear down regulation of c-Fos expression in the LGN, compared with that of the Control group (Fig. 3 and 5, mean ± s.e.m., 31.04 ± 1.05 vs. 18.79 ± 2.23; $p < 0.001$). In the Bl-67+BD group, c-Fos immunoreactivity in the LGN showed a similar pattern compared with that of the BD group (Fig. 3 and 5, mean ± s.e.m., 18.79 ± 2.23 vs. 24.85 ± 1.63; $p = 0.079$).

c-Fos immunoreactivity in the primary visual cortex

In the primary visual cortex, the number of c-Fos-positive cells per slit in the BD group was significantly smaller

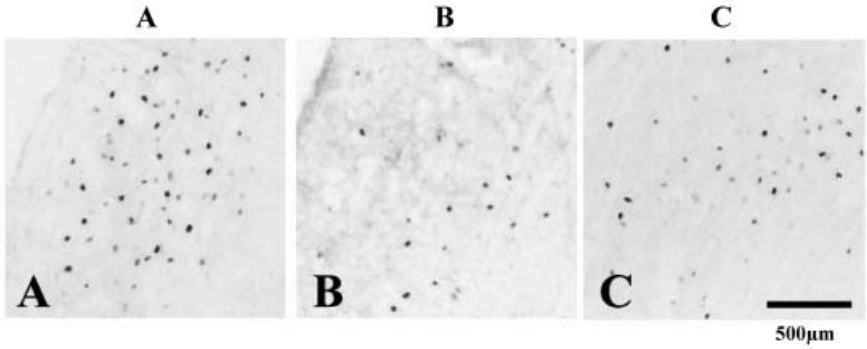

Fig. 3. Bright-field photomicrographs showing c-Fos-positive cells in the LGN at postnatal day 30 (P30). (A), Control group is normally reared rat pups; (B), BD group was binocularly deprived at P10; (C), Bl-67+BD group was binocularly deprived at P10 and acupuncture stimulated on the vision-related acupoint Bl-67 from P14 to P30. Binocular deprivation downregulated c-Fos expression in the LGN (A, B). No significant difference was observed between BD and Bl-67+BD groups (B, C). Scale bar represents 100. BD, binocular deprivation; LGN, lateral geniculate nucleus.

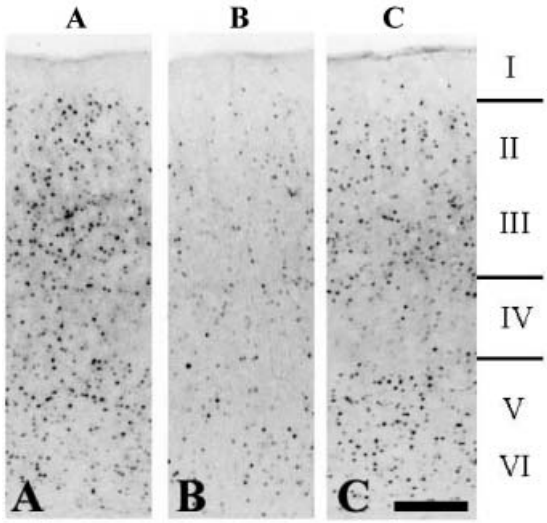

Fig. 4. Bright-field photomicrographs showing c-Fos-positive cells in the primary visual cortex. (A), Control group is normally reared rat pups; (B), BD group was binocularly deprived at P10; (C), Bl-67+BD group was binocularly deprived at P10 and acupuncture stimulated on the vision-related acupoint Bl-67 from P14 to P30. A smaller number of c-Fos-positive cells were observed in BD group than in Control group (A, B). Acupuncture stimulation on Bl-67 clearly increased the number of c-Fos-positive cells compared with that of BD rat pups (B, C). Scale bar represents 125. BD, binocular deprivation.

than that in the Control group (Fig. 4 and 5, mean ± .e.m., 62.52 ± 4.56 v . 31.31 ± 1.27 per lit; $p < 0.001$). Intere tingly, acupuncture timulation on Bl-67 re ulted in a ignificant increa e in the number of c-Fo -po itive cell in the primary vi ual cortex but not in the LGN. The Bl-67+BD group howed higher c-Fo expre ion in the primary vi ual cortex than the BD group (Fig. 4 and 5, mean ± .e.m., 31.31 ± 1.27 v . 37.05 ± 1.57 per lit; $p < 0.05$). In each layer of the primary vi ual cortex, the difference in c-Fo immunoreactivity were pre ent, but not tati tically

Fig. 5. The quantification of c-Fos expression

consistent with our previous functional MRI experiment, which showed activation of the visual cortex by acupuncture stimulation on Bl-67, suggesting that a specific acupoint may have a correlation with a specific cortical area of the brain (Fig. 2, Cho *et al*., 1998). The present study additionally showed that acupuncture on the vision-related acupoint Bl-67 induced c-Fos-positive cells while Bl-54 acupuncture stimulation had little effect. It suggest that Bl-67 may specifically induce c-Fos positive cells in the primary visual cortex in the BD rat pups.

It has been reported that changes in immediate early gene mRNA levels including c-*fos* in the LGN of dark-reared kittens are not induced by brief visual stimuli (Rosen *et al*., 1992). The absence of significant difference of c-Fos expression between the LGN area of BD and Bl-67+BD groups suggest the possibility of difference in the induction of c-Fos by acupuncture stimulation on the vision-related acupoint at the LGN and the primary visual cortex.

From the viewpoint of Western medicine, it is difficult to believe that certain acupoints are related to corresponding organs or clinically effective for specific organ-related disorders via corresponding meridians. However, there are studies that have demonstrated that acupuncture stimulation may affect the corresponding organs or the related brain area (Cho *et al*., 1998; Hui *et al*., 2000; Kim *et al*., 2001; Sato *et al*., 1992; Sato *et al*., 1993). Our present experiment suggest another possibility of correlation between the acupuncture stimulation and related brain area. In conclusion, this study suggest acupuncture stimulation on Bl-67 may have a correlation with activation of the primary visual cortex.

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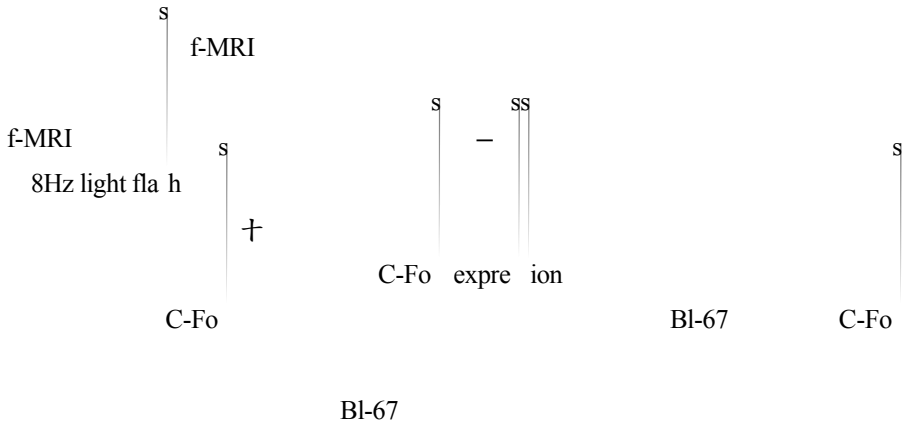

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