Short communication

INCREASE OF GABA RECEPTOR BINDING ACTIVITY AFTER SHORT LASTING MONOCULAR DEPRIVATION IN KITTENS

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Abstract. GABA receptor activity, as measured by $[^3H]$muscimol binding, was investigated in visual and auditory cortex of 5 weeks old kittens with different visual experience. Binocular deprivation since birth did not affect the GABA receptor binding activity. Three days of monocular vision resulted in an increase of $[^3H]$muscimol binding in the visual cortex of both normally reared (144%) and binocularly deprived kittens (149%). Neither 3 days of normal binocular vision in deprived animals nor 3 days of binocular deprivation in normally reared ones produced significant changes in GABA receptor binding activity. In all experimental conditions $[^3H]$muscimol binding in auditory cortex was similar. We suggest that activation of the GABA-ergic system is due to asymmetric visual input, possibly connected with mechanisms of ocular dominance shift.

There are many indications of the presence of GABA-ergic inhibition in the mammalian visual cortex. Both the inhibitory action of GABA upon visual cortical neurons (7) and the presence of GABA accumulating neurons in the striate cortex (4, 6, 22, 23) were demonstrated. The experiments with intracortical iontophoresis of GABA antagonist have shown the involvement of GABA in the formation of specific properties of visual units receptive fields (16, 17). It was found that, along with other effects, GABA-mediated inhibition plays a role in determining their ocular dominance (18). This last feature is known to be
particularly easily modifiable by early monocular deprivation (MD) (26). The possible involvement of GABA-ergic inhibition in the mechanism of ocular dominance changes following MD is still disputed (12). Duffy et al. (5) postulated that the deprived eye input is suppressed by the normal eye exerting the GABA-mediated inhibition. Another suggested possibility is that MD causes a reduction in efficiency of excitatory synaptic transmission in the deprived pathways (2, 3).

We approached this problem by investigating the involvement of the GABA system in short lasting monocular deprivation by measuring the GABA receptor binding activity. The experiments were done on kittens during the critical period of cortical sensitivity, when the reaction to closure of one eye is the strongest. Brief MD was used because it has often been found that biochemical changes involved in plasticity are best visible early in the process (8, 14, 20). The results suggest that short lasting monocular deprivation is accompanied by a significant elevation of GABA-mediated inhibition.

The experiment was performed on 35 kittens. Two groups of monocularly deprived animals were examined. The first (N-MD) consisted of normally reared kittens who had one eye covered with a black occluder on the 35th day of life; the other (BD-MD) — of kittens binocularly deprived by rearing in masks from the day of eye opening, a procedure comparable to lid-suturing (11), who had one eye opened on the 35th day. MD lasted for 3 days for both groups. To test for the effect of the onset of normal visual experience a group of binocularly deprived kittens had both eyes opened at the 35th day of life for 3 days (BD-N). Control groups of normal (N-N) and deprived (BD-BD) littermates were sacrificed together with the experimental animals. Additional control group consisted of normally reared kittens binocularly deprived (N-BD) for 3 days. The kittens from one litter were assigned to different experimental groups. All animals were anesthetized with ether and decapitated on the 38th day of life. The brain was dissected on ice. Visual cortex, area 17 according to maps of Tusa et al. (25), and auditory cortex from right and left hemispheres were analyzed separately. The GABA receptor binding activity was examined in washed, frozen and thawed Triton X-100 treated membrane preparation (24) using [3H]-muscimol (15.5 Ci mmol⁻¹, Amersham) as a ligand (21). The final preparations were suspended in 50 mM Tris-citrate (pH 7.1) and [3H]-muscimol was added at final concentration of 3 nM (21). The incubation was performed at 4°C for 10 min. A final sample volume was 1.0 ml and the amount of protein in the sample was about 0.3–0.5 mg. The incubation was terminated by centrifugation for 20 min at 20,000 g. The pellets were rinsed twice, superficially with ice-cold distilled water,
Fig. 1. Binding of [3H]muscimol to membrane preparation from visual and auditory cortices. The experimental groups are shown in the lower part of the Figure. N-N, normally reared kittens; BD-BD, binocularly deprived; N-MD, normal kittens with one eye covered for 3 days; BD-MD, binocularly deprived kittens with one eye opened for 3 days; N-BD, normal kittens with both eyes closed for 3 days; BD-N, binocularly deprived kittens with both eyes opened for 3 days. Opening or closing of the eyes was done at the 35th day of life. The values given are mean ± standard error. \( n \), number of animals in parenthesis. Each \( n \) is a mean of the left and right side sample from one kitten. No differences between left and right side were found * \( P < 0.01 \).
solubilized in 0.5 ml NCS (Nuclear Chicago) and left for 12 h at room temperature. They were kept for 2 h at 55°C before adding scintillation fluid (0.4% PPO 0.02% POPOP in toluen) and counting.

Specific binding was determined by subtraction of the radioactivity remaining in the pellets when 1 mM of unlabeled GABA was included in otherwise identical incubation. The protein content of the sample was estimated according to Lowry et al. (10). The results were expressed as pmoles of bound [3H]muscimol per mg of protein. Statistical differences between groups were calculated by analysis of variance followed by Duncan test. No difference in GABA receptor binding activity was found between normally reared (N-N) and binocularly deprived kittens (BD-BD), neither in visual nor in auditory cortex (Fig. 1). Three days of monocular input produced a significant elevation of GABA binding ($P < 0.01$) in both normally reared (N-MD) and binocularly deprived (BD-MD) kittens. The elevation was similar in both groups, being 149% ($P < 0.01$) for the BD-MD group and 144% ($P < 0.01$) for the N-MD one, as compared to their respective controls. No changes were found in GABA receptor binding in the auditory cortex of these groups, the levels amounting respectively to 109% and 95% of the control values.

In the groups with 3 days of binocular change in visual input a tendency (14% BD-N and 12% N-BD) for elevation of GABA receptor binding activity was observed, but it was not statistically significant. Again, no changes were found in the auditory cortex. The protein content was similar in all groups.

The results suggest that observed changes in GABA binding levels in the visual cortex are associated mainly with assymetric visual input irrespective of the kitten's previous visual experience. Binocular deprivation since birth (BD-BD) had no effect on the activity of GABA receptors; neither did binocular manipulation of visual input (BD-N, N-BD) produce significant changes.

The elevation of GABA receptor binding activity may be considered as an indicator of activation of the GABA-ergic system. In a few cases when glutamic acid decarboxylase assay was made on the same tissue, concomitant rises in GABA receptor binding and GAD activity were observed. At this stage of investigations we can not yet explain if the changes in receptor binding are due to an altered affinity of receptor for ligand or to an increase in the number of binding sites.

The elevation of GABA receptor binding was observed after 3 days of MD. According to electrophysiological studies considerable changes in the ocular dominance of cortical neurons occur after MD of that duration instituted during the critical period in both normal and BD
kittens (2, 13). It is known from transneuronal transport autoradiography studies that with longer period of MD in cats and monkeys, the deprived eye loses many of its connections to cortical cells in layer IV, while the majority of layer IV is taken up by axons from the normal eye (9, 15). It is not yet known how fast the anatomically observable redistribution of geniculocortical axons occurs (the shortest period of MD studied with this method was 9 days), but such changes could be either accompanied or preceded by functional suppression (1, 15). All of the studies testing directly for the participation of GABA-ergic inhibition (i.e., using GABA inhibitor bicuculline) were done on cats with MD lasting for about 6 months or more (16, 19). In view of our results, which indicate that GABA-ergic mechanisms are activated during the period of development of ocular dominance changes, it would be useful to see what results would be produced by application of GABA inhibitors early in the process of monocular deprivation.

The question arises here as to the interpretation of biochemical indices in terms of physiological mechanisms. It is conceivable that even if regulation of inhibitory mechanisms played a considerable part in some process, no changes of total strength of inhibition would be observable if the distribution of inhibitory synapses rather than the number would be the important factor. In the case of 3 days long monocular deprivation, however, we did observe a significant increase of the GABA receptor binding activity. This effect could be representing the active suppression of the deprived eye input brought about by the increase of activation of existing inhibitory synapses or growth of new ones. Further studies are needed to determine if this is a permanent of transient effect and what is the inhibition exerted upon.

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