A novel form of heterosynaptic depression in the rat auditory cortex

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Introduction

Cortical pyramidal cells exhibit mono- and polysynaptic field excitatory postsynaptic potentials (monoEPSPs and polyEPSPs) each characterized by the complexity of its shape and latency. An *in vivo* study in rat showed that medial geniculate nucleus stimulation that mimics acoustic stimulation triggers both monoEPSPs and polyEPSPs in auditory cortex. Intracortical polysynaptic excitatory connections provide the major, sustained excitation of most cortical neurons. Superficial layer pyramidal neurons exhibit a more marked polysynaptic excitatory phase than deep layer pyramidal neurons, and the excitatory effects on pyramidal neurons, particularly those in the superficial layer, are not generally due to monosynaptic input from the thalamus, but polysynaptic inputs from cortical pyramidal neurons.

There are a lot of reports about homosynaptic plasticity, such as paired-pulse depression (PPD), however, heterosynaptic plasticity (between the poly- and monosynaptic pathways) has not been well characterized. In the present study, we investigated this interaction in the superficial layer of the rat auditory cortex.

Materials and methods

The MED probe is a dish that contains an array of 64 planar electrodes embedded in the center, which are available for both stimulation and recording. Each electrode has a cross sectional area of $50\times50\ \mu m^2$ and the distance between adjacent interpoles is $150\ \mu m$. The MED probe was composed of transparent materials except the electrodes, thereby allowing the localization of the electrodes in the slice under a microscope. Cortical slices were placed directly on the electrodes, and electrical stimuli were delivered and electric activity were recorded via the contacting electrodes (**Fig. 1**), thus it was possible to stimulate and record from any layer (II/III-VI). In the present experiments, layer IV was stimulated every 30 seconds and evoked field potentials were recorded from layer II/III. The intensity of stimulation was adjusted to obtain a submaximal evoked field potential. During electrophysiological recording, the MED probe was perfused continuously at a rate of 1-2 ml/min at 35° C with artificial cerebrospinal fluid (ACSF) (in mM: NaCl 124, KCl 5, $MgSO_4$ 1.3, Na_2HPO_4 1.25, $CaCl_2$ 2.6, $NaHCO_3$ 22, glucose 10) bubbled with 95% O_2 and 5% CO_2.

Results

PPD, which was induced at inter-pulse intervals (IPIs) of 50 to 2000 ms in standard ACSF, was maximal at an IPI of approximately 200-300 ms (**Fig. 3**). Nevertheless, in the rat auditory cortex, PPD was markedly facilitated by perfusion of 20 μM bicuculline, while the amplitude of the monosynaptic component of the first response remained unchanged (**Fig. 2**). The mean ratio (2nd/1st response amplitude) was 0.90±0.05 (n=16) before adding bicuculline, and decreased to 0.30±0.03 (n=19) in the presence of bicuculline.

To examine the effect of $GABA_B$ activity on this depression (bicuculline-facilitated synaptic depression: BFSD), we compared the mean ratios elicited by sequential application of bicuculline and 2-OH-saclofen, a selective $GABA_B$ receptor antagonist. The decreased ratio after the addition of bicuculline was reversed to a moderate degree at an IPI of approximately 250 ms in the presence of 1 mM 2-OH-saclofen (**Fig. 3**).

Blockade of $GABA_A$-receptor-mediated potentials has been reported to promote polysynaptic excitation in the neocortex. We have investigated the aspects of polyEPSPs in the rat auditory cortex that appear in the presence of bicuculline. As shown in Figure 2B, a long-latency negative component of polyEPSPs appeared in the first response of paired responses in the presence of bicuculline. There was no evidence of polyEPSPs in the second response in the pair, and this second response was greatly reduced (**Fig. 2A**), suggesting a link between polyEPSPs in the first response and the synaptic depression of the second response. To examine this possibility, we designed experiments to test the effect of polyEPSPs on BFSD. At first, we compared the responses evoked by two successive stimuli at an IPI between 50 and 2000 ms in the presence of bicuculline. The effect of the first response on the second one was moderate at IPIs longer than 2000 ms (**Fig. 4A1**). When the IPI was shorter than 1000 ms, the polyEPSP component of the second response gradually decreased and was eventually eliminated at IPI shorter than approximately 750 ms. The fast component of the responses, however, did not change at these IPIs.

These results led us to test the effects of two patterns of three successive stimuli delivered in the presence of bicuculline. The IPIs between the first and second pulse ranged between 750 and 1000 ms, with a fixed IPI of 50 ms between the second and third pulse (**Fig. 4A2**). The third response was similar to the second response with an IPI of 750 ms between the first and second response (n=3), whereas with an IPI of 1000 ms between the first and second response, the third response exhibited marked depression compared to the second response (n=3). These data suggest that only with the occurrence of the polyEPSP component in the preceding response, was BFSD observed, indicating that there is a close correlation between a preceding polyEPSP and BFSD.

In the next experiment, a single stimulus was delivered to S8 (layer IV) and evoked field potentials were recorded from the horizontal electrode array (1-8; layer II/III) in the absence and the presence of 20 μM bicuculline (**Fig. 4B1**). The propagation of the monoEPSP was tightly limited to the stimulation site, however, polyEPSP components in the presence of 20 μM bicuculline propagated far from the stimulated site (**Fig. 4B1**). We stimulated such that the first response in a pair was composed solely of a polyEPSP. By applying paired-pulse stimulation at an IPI of 50 ms in the stimulation position S1 in the presence of 20 μM bicuculline, the first response in the pair consisted of mono- and polyEPSPs and was recorded at recording site 1 (**Fig. 4B1**), and the second response exhibited a markedly depressed monosynaptic component (**Fig. 4B2**). When S8 was stimulated, however, the polyEPSP propagated to recording site 1, then additional stimulation at the S1 position generated a depressive monoEPSP (**Fig. 4B2**), exactly as was observed with paired-pulse stimulation at S1. This result suggests that BFSD depends on the presence of a polyEPSP in the first response in the pair and is independent of a monoEPSP in the first response in the pair.

Conclusion

We concluded that the activation of polysynaptic excitatory pathways, which are constrained by $GABA_A$ receptor in ACSF, induce depression of the following synaptic transmission in the range of a few seconds.

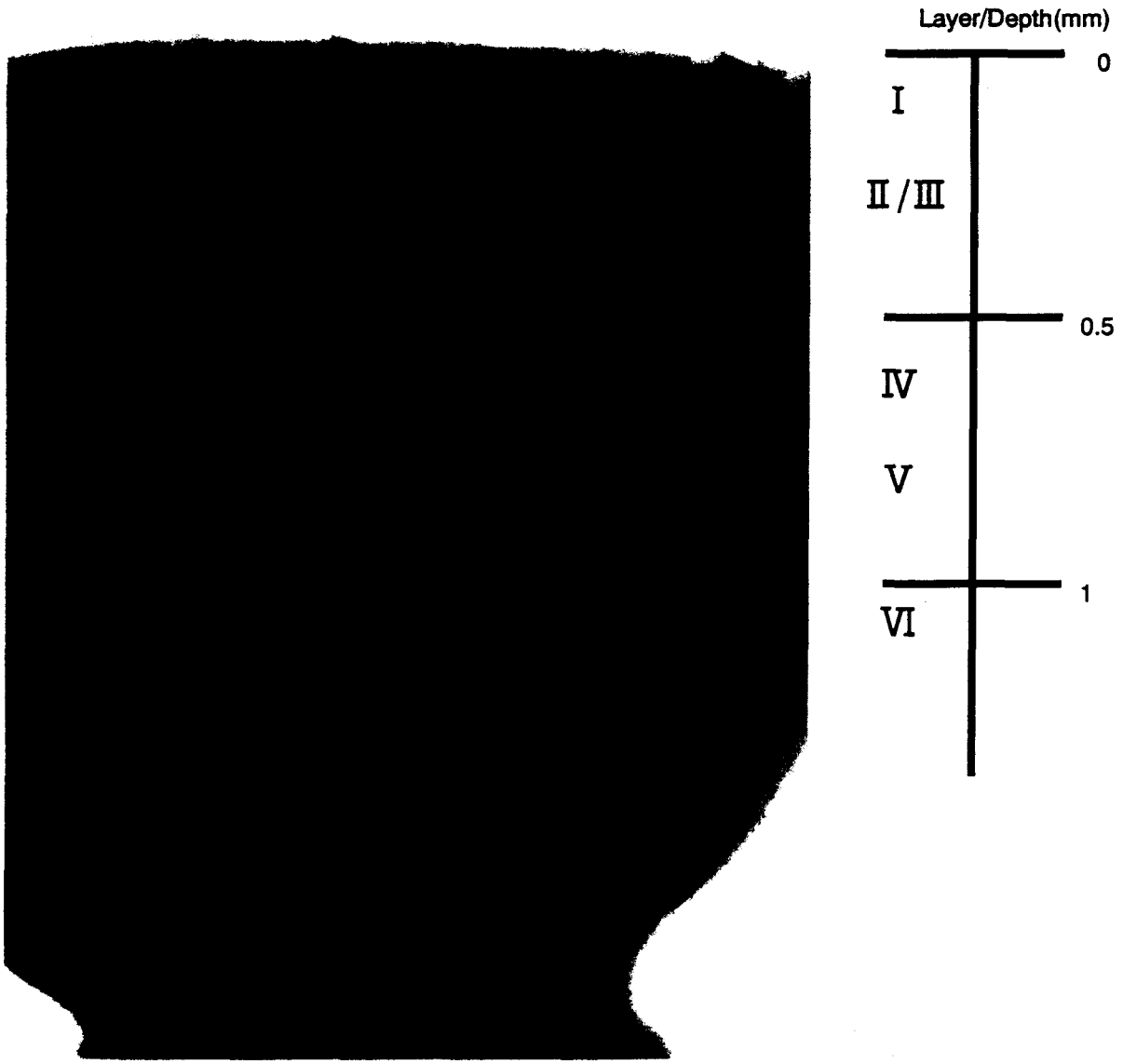


Figure 1. ***Arrangement of an acute slice on the MED probe***
Prior to each experiment, we arranged an acute slice on the electrodes of the MED probe. The vertical line indicates the layers of the primary auditory field and the depth from the pia mater towards the white matter.

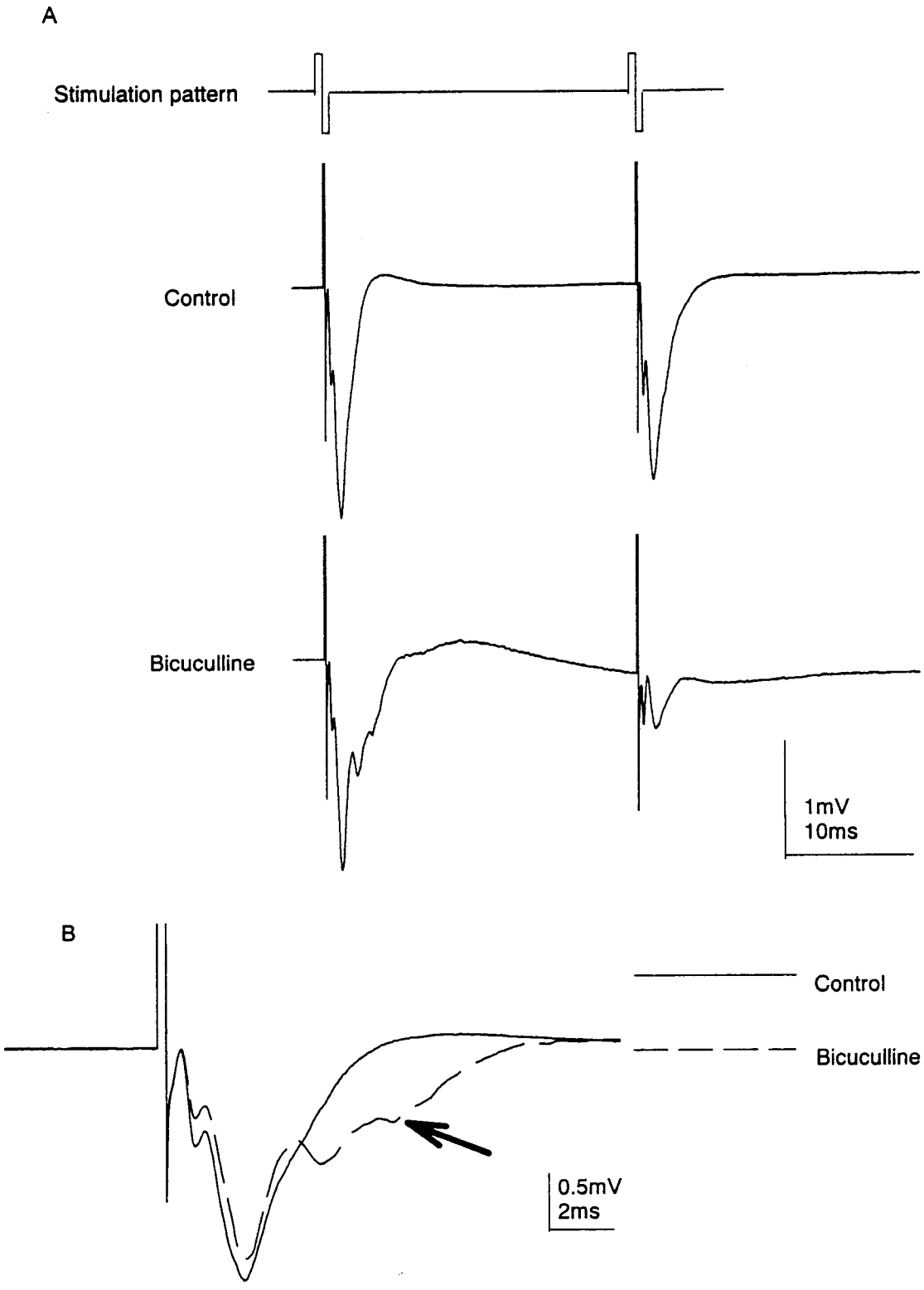


Figure 2. ***Bicuculline-facilitated synaptic depression***
A, Responses were evoked by two successive at interval of 50ms in the absence and presence of 20 μ M bicuculline. The top trace indicates the stimulation pattern. The middle trace shows control response. The lower trace shows paired-pulses evoked in the presence of bicuculline. B, The first responses shown in A, are superimposed. The arrow indicates the polyEPSPs.

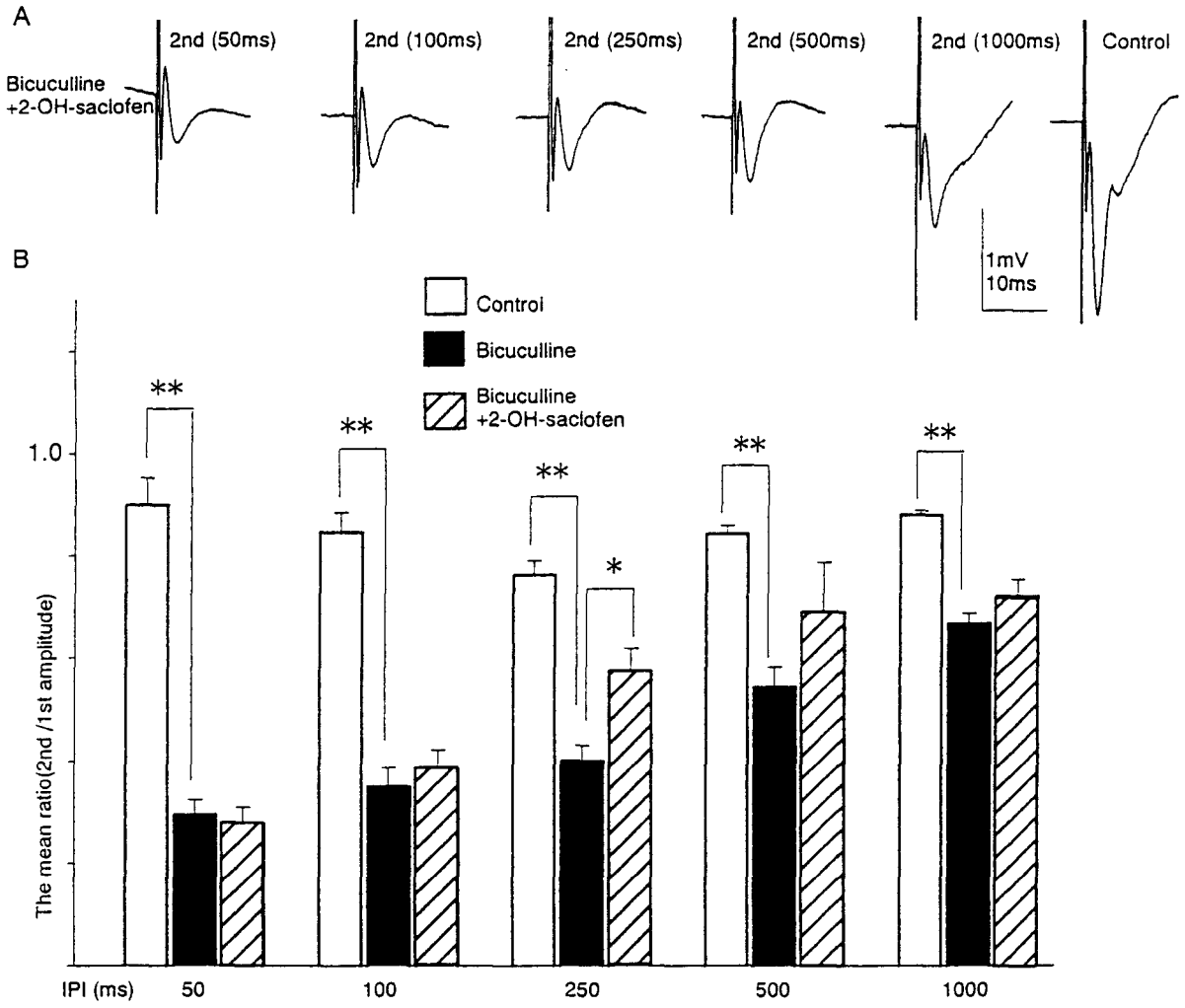


Figure 3. ***The effect of IPIs on BFSD***
A. Representative EPSP trace in the presence of both bicuculline and 2-OH-saclofen, with variable duration of IPIs. B. The histogram shows the mean ratios(2nd/1st response ampulitude) elicited by two successive stimulli at intervals of 50, 100, 250, 500, and 1000 ms in standard ACSF(control, open bars; n=16), in the presence of bicuculline (20 μ M) (close bars, n=19) and in the presence of 2-OH-saclofen (1 mM) bicuculline (20 μ M) (hatched bars, n=7). * and **, indicate $P<.05$ and $P<.01$, respectively (Student' t test).

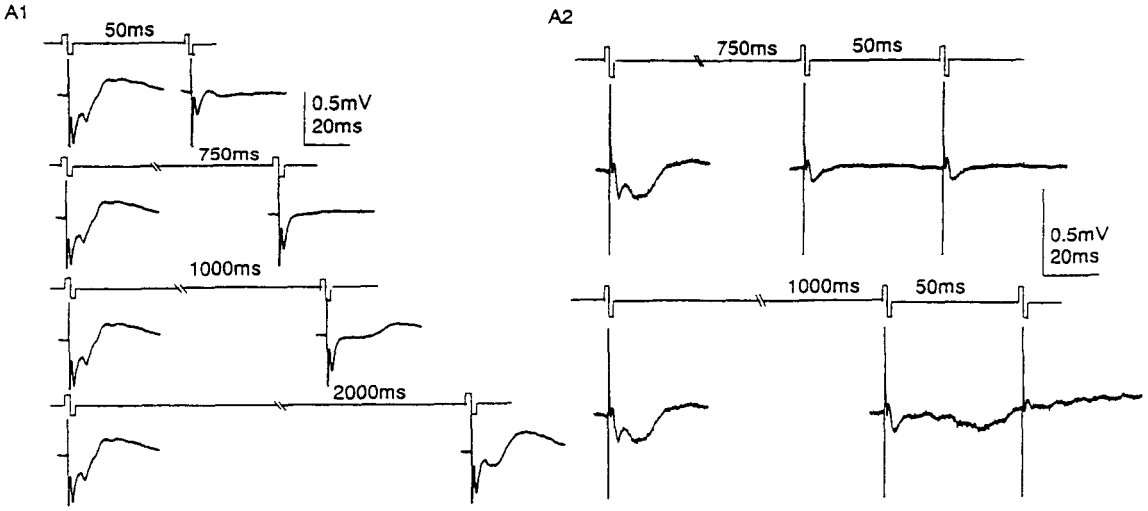
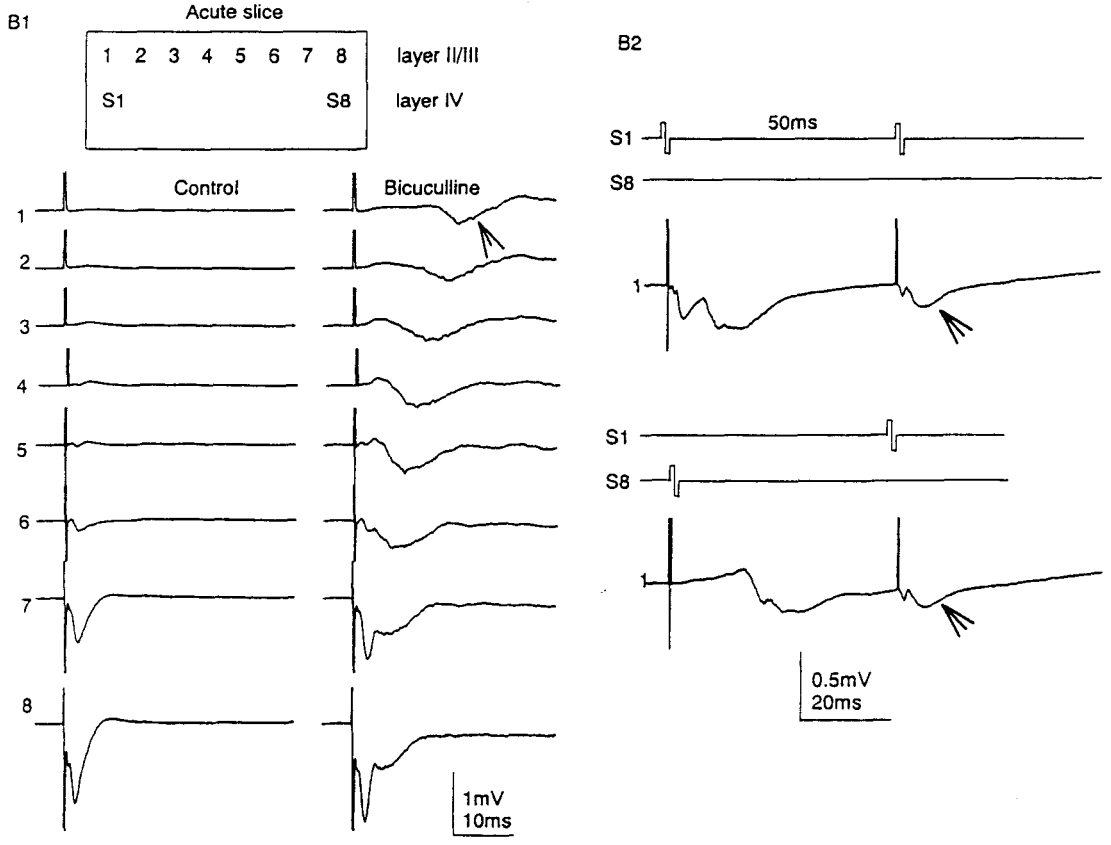


Figure 4. ***Contribution of polyEPSPs on BFSD***
A1. Representive traces indicate synaptic responses evoked by two successive stimuli at interval of 50, 750, 1000, and 2000 ms in the presence of 20 μ M bicuculline. A2. A train responses evoked by three successive stimuli. The interval between the first and second pulse were 750 and 1000ms. The interval between the second and third was fixed at 50ms. B1. The location of the electrodes for recording and stimulation are illustratd at the upper panel. Each responses from layer II/III (1-8) is displayed in the absence (left panel) and the presence of 20 μ M bicucuclline (right panel). An arrow indicates the polyEPSPs. Note that polyEPSP was not evident in the control recording. B2. Typical field EPSPs evoked by two successive stimuli generated at different sites an interval of 50ms in the presence of bicuculline. Stimuli were applied at S1:S1 (top) and at S8:S1 (bottom). Note that the second response in the pair were similar (arrows) and BFSD was observed even when there was no monoEPSP in the first response (bottom). Stimulation protocols are indicated in the first and third panel.



Flugre 4. (continued.)