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Rapid communication

# Bicuculline induces ictal seizures in the intact hippocampus recorded in vitro

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**Abstract**

The effects of the γ-aminobutyric acid (GABA) receptor antagonist bicuculline on rat hippocampal neurons recorded in slices and in the intact hippocampi kept in vitro were studied using whole-cell patch-clamp recordings. Bicuculline (10 μM) evoked ictal discharges in the intact hippocampus but only interictal discharges in conventional slices. Recording from the intact hippocampus in vitro is an alternative preparation to study the organization of the hippocampal neuronal network.

*Keywords:* Hippocampus; GABA (γ-aminobutyric acid); Network

Studies using acute hippocampal slices kept in vitro have enormously contributed to our understanding of brain mechanisms and pathologies. However, conventional slices eliminate the contribution of intrinsic connections that originate from the remaining of the structure. We now report that neonatal and young intact structures such as the hippocampus can be kept in vitro in a conventional chamber for several hours and the entire repertoire of physiological, morphological and pharmacological techniques can be used. Neonatal (P0–P3) or young (P4–P12) male Wistar rats were used. In brief (Ben-Ari et al., 1989), after decapitation, both hippocampi were rapidly removed in ice-cold Krebs phosphate buffer. They were then transferred to a beaker containing a traditional oxygenated (95% $O_2$/5% $CO_2$) artificial cerebrospinal fluid (ACSF) and kept at room temperature for 1–2 h before use. The hippocampus was then transferred to a fully submerged slice chamber and superfused at a rate of 5–6 ml/min with oxygenated ACSF (30–32°C). Blind whole-cell recordings were performed as described earlier (Khazipov et al., 1995). Biocytin (0.5%) and/or lucifer yellow (0.3%) were routinely added to the patch pipette for morphological identification of the recorded cell.

In neonatal hippocampus kept in vitro, network driven giant depolarizing potentials (Ben-Ari et al., 1989) were routinely observed ($n = 25$ neurons in 10 hippocampi); they were also evoked by electrical stimuli. In pyramidal cells or interneurons of the CA3 subfield synaptic currents had the typical features of $GABA_A$ and glutamate receptor mediated events, including their reversal potentials, kinetics and sensitivity to antagonists. At P6–P8, bath application of the $GABA_A$ receptor antagonist bicuculline (10 μM) evoked an ictal discharge with a peak amplitude of $2.4 \pm 0.6$ nA and duration of $56 \pm 6$ s ($n = 13$) (Fig. 1A); these were fully blocked by 6-cyano-7-nitroquinoxaline (CNQX) (10 μM and D,L-2-amino-5-phosphonovaleric acid (APV) (100 μM) ($n = 3$). In contrast, in conventional submerged slices (400–500 μm thick) obtained from either the contralateral hippocampus of the same animal or from the same hippocampus bicuculline (10 μM) evoked in agreement with earlier studies (Ben-Ari et al., 1989) interictal discharges with an amplitude of $0.57 \pm 0.15$ pA and duration of $0.20 \pm 0.04$ s ($n = 6$) (Fig. 1B). This difference may be due to the better preservation of the recurrent excitatory network and to more efficient nonsynaptic mechanisms of neuronal interactions (Jefferys, 1995) in the intact hippocampus.

Thus, neonatal and young hippocampi can be kept in vitro for several hours and conventional methodologies used to characterize the properties of the synaptic currents. This preparation combines the advantages of in vitro manipulation (control of temperatures, pH, drug concentra-

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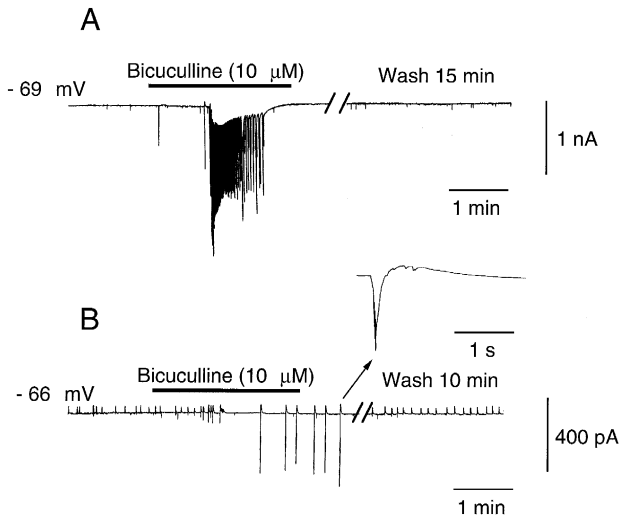


Fig. 1. Effects of bicuculline on the spontaneous synaptic activity in the intact hippocampus (A) and hippocampal slices (B) in vitro. Notice that bicuculline evokes ictal discharges in the intact hippocampus and interictal discharges in slices. Recordings with K-gluconate solution ($[Cl^-]_{in}$ = 4.2 mM) in whole-cell voltage-clamp mode.

tion, etc.) with the use of intact brain structures. In several domains, it may replace conventional slices.

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