Three-Independent-Compartment Chamber to Study In Vitro Commissural Synapses

ROUSTEM KHAZIPOV, LUC DESFRERES, ILGAM KHALILOV, AND YEHEZKEL BEN-ARI
Centre de Recherches sur l'Epilepsie et l'Ischemie Cerebrale, Institut National de la Sante et de la Recherche Medicale U29, 123 Bd de Port-Royal, Paris 75674, France

Khazipov, Roustem, Luc Desfreres, Ilgam Khalilov, and Yehezkel Ben-Ari. Three-independent-compartment chamber to study in vitro commissural synapses. *J. Neurophysiol.* 81: 921–924, 1999. We describe a novel chamber in which the two intact neonatal rat hippocampi and the commissural fibers are placed in three independent compartments separated by latex membranes and perfused selectively with different solutions. A set of control tests showed that the compartments are well isolated: *1*) methylene blue or eosin applied to one compartment did not diffuse to other compartments when verified via the microscope, and spectrophotometry revealed that <1/10.000th of the dye diffuses to other compartments; *2*) tetrodotoxin (1 μM) applied to the commissural compartment blocked the synaptic responses evoked contralaterally without affecting those evoked on the ipsilateral side. This chamber enables a wide range of experiments that cannot be performed in conventional chambers, e.g., to study the maturation and plasticity of the commissural connections, bilateral synchronization of the rhythmic activities in the limbic system, commissural propagation of the epileptiform activities, etc.

INTRODUCTION

A novel in vitro preparation has been recently described based on the use of the young rat intact hippocampal formation (IHF) (Khalilov et al. 1997). The entire repertoire of electrophysiological, morphological, and imaging techniques can be used in the IHF including field and patch-clamp recordings, infrared and confocal microscopy, labeling, and three-dimensional reconstruction of neurons. One of the principal advantages of this preparation is that it allows one to study the generation and propagation of the neuronal network-driven activities in the intact structure with preserved intrinsic synaptic connections. In addition, the two interconnected hippocampi, the septohippocampal and entorhinohippocampal complexes can be used to study the synaptic connections and coordination of the network-driven activities between connected limbic structures in vitro (Khalilov et al. 1997; Leinekugel et al. 1998). However, to take the entire advantage of these in vitro preparations, a special multicompartment chamber is required to enable a selective application of drugs to interconnected structures. Such a chamber has to meet the two principal requirements: to isolate the compartments and to preserve the connections between structures. Several approaches have been previously used for this purpose in different in vitro models. A petroleum jelly (Vaseline) wall was used to separate two compartments with spinal cord (Cazalets et al. 1996) and neonatal rat hippocampi (Khalilov et al. 1997) preparations. In another study using spinal cord preparation, the compartments were separated by plastic bars glued to the dorsal and ventral midlines of the cord (Kjaerulff and Kiehn 1997). However, these approaches have certain disadvantages: the Vaseline wall is unstable and melts fast at temperatures higher than 30°C, whereas the use of the glue might be toxic for the tissue. In addition, neither of these two approaches allows the application of drugs on the connecting fibers between structures.

Here we propose to isolate the compartments using latex membrane with an aperture adjusted to the size of the connections between structures. The use of two membranes easily allows the introduction of a thin intermediate compartment to selectively perfuse the connections. We have also ameliorated the system of perfusion by bringing the outlets from the compartments to an independent drawing-off chamber so that the pressure in all compartments can be kept constant, thereby reducing the precondition for the leakage between compartments.

METHODS

Experiments were performed on neonatal Wistar rats (*postnatal days 4–8*). The complexes of two hippocampi connected via commissure were prepared as described previously (Khalilov et al. 1997).

Figure 1*A* schematically represents how latex membrane was used to separate the compartments. Latex membranes were cut from the latex gloves (Safeskin). A round aperture of ~0.5 mm diam was made by cutting the membrane stretched on the plastic tip by scissors. Upon stretching the membrane, the aperture dilates to 3–4 mm diam so that the hippocampus can pass through it (Fig. 1*Ab*). As soon as the commissure occurs in the aperture, the tension on the membrane is relaxed. The aperture recuperates its initial diameter and tightens the ring round the commissure (Fig. 1*Ac*). To better adjust the diameter of the aperture according to the size of the commissures, we developed a simple system to stretch and relax the membrane using two bolts (not shown).

A schematic drawing of the three-compartment chamber is presented in Fig. 1*B*. The chamber is composed of three principal elements: two hippocampal compartments and one commissural compartment. The hippocampal compartments are similar to those described previously (Khalilov et al. 1997). The commissural compartment is made from a 1-mm-thick plastic lamina with a 5-mm square cut. Usually we glue a latex membrane at the lower edge of the lamina and then fold it so that it makes two membranes on both sides of the lamina, and then we make apertures in the mem-

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

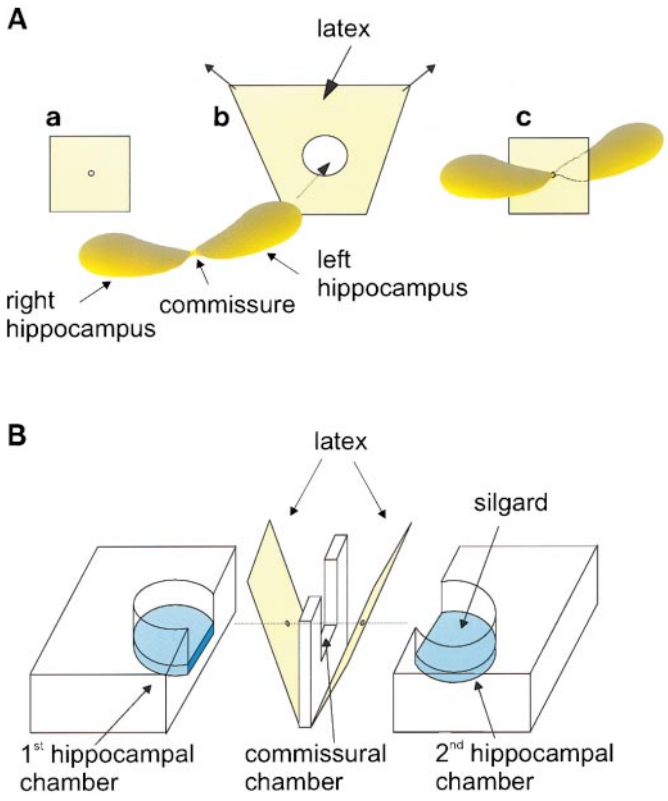

FIG. 1. Scheme of the 3-compartment chamber. *A*: use of the latex membrane to isolate 2 compartments. *Aa*: a small aperture is made in a latex membrane cut from the latex gloves. Diameter of the aperture is adjusted to the size of the commisure. *Ab*: stretching of the membrane dilates the aperture so that a hippocampus can pass through it. *Ac*: upon relaxing tension, the aperture recuperates its initial size, wrings out the commissure and the membrane now isolates the 2 compartments. *B*: schematic drawing of the 3-compartment chamber components. Note 2 latex membranes that will make the borders of the narrow middle commissural compartment when the components of the chamber are setted together. See *methods* for details.

branes as described above. The three components are then set together using two bolts. To better hermetically isolate the compartments, we also put a Rubson mastic glue (Henkel, Paris, France) on the contacting surfaces of the elements of the chamber. The complex of the two hippocampi is positioned so that the commissure appears in the middle compartment, the hippocampi are fixed to the silicone elastomer (Sylgard) layer on the bottom of the compartments by enthomological needles, and the chamber is connected to the perfusion system. The three compartments have independent inlets and outlets (see Fig. 2). The outlets are brought to a common drawing-off compartment so that pressure is kept equal in all three compartments.

Spectrophotometry has been performed using U2001 spectrophotometer (Hitachi). Peak of the methylene blue optical densitiy was measured at a wavelength of 663 nm. Calibration of the system by measuring the optical density in a series of methylene blue diluted solutions revealed that an optical signal of 0.012 optical density can be reliably detected at 0.0001% concentration of the dye.

Electrophysiological recordings were performed by using two extracellular field amplifiers (WPI). Extracellular electrodes were filled with artificial cerebrospinal fluid and positioned in the stratum radiatum of CA3 or CA1 regions. A stimulating bipolar tungsten electrode was positioned in the septal pool of the hippocampus to evoke field excitatory postsynaptic potentials both on the stimulated (ipsilateral) and contralateral sides. Synaptic responses were acquired and analyzed using Acquis 5.1 program (G. Sadoc, DIPSI, France). Continuous cross-correlation analysis of 30-s records with a 1-ms lag was performed using Origin 5.0 program (Microcal Software).

RESULTS

A multicompartment chamber has to meet two principal requirements: *1*) the solutions in different compartments should not mix, and *2*) the connections between structures should be preserved. To verify whether the present chamber satisfies these requirements, two types of experiments were performed.

First, solutions of methylene blue (1%) and eosin red (1%) were applied to one compartment, and the diffusion of the dye to other compartments was verified visually via the microscope. Even after several hours of perfusion of one hippocampal compartment, the dyes did not diffuse to the contralateral hippocampal, or to the middle commissural chamber (Fig. 2). Similar results were observed when the dye was applied to the middle chamber. By diluting the dye, we found that the threshold concentration for visual detection of methylene blue is ~0.005–0.01%, indicating that <1/100th of the dye diffuses to the neighboring chambers. Spectrophotometry that had a threshold for methylene blue detection at 0.0001% concentration failed to detect any trace of the dye in the neighboring compartments, suggesting that <1/10.000th of the dye does diffuse between chambers.

Second, to block selectively the commissural fibers, the sodium channel antagonist tetrodotoxin (TTX; 1 μM) was applied to the middle compartment. As shown on Fig. 3*A*, this rapidly and fully blocked the synaptic responses evoked contralaterally without affecting those evoked on the ipsilateral side. This effect was reversible as the contralateral response recuperated within 20 min. Similarly, the synchronization of the 4-aminopyridine (200 μM)–induced epileptiform activity in the two hippocampi was efficiently suppressed by application of TTX (1 μM) to the middle commissural compartment (Fig. 3*B*).

Therefore the present three-compartment chamber meets both requirements: the diffusion of the drugs between chambers is minimal, although the synaptic connections between structures are preserved. This procedure enables the disconnection of the two hippocampi transiently and selectively and prevents the propagation of mono- and polysynaptic network-driven activities from one hemisphere to the other.

DISCUSSION

Our results suggest that it is possible to maintain in vitro the two interconnected hippocampi and to study the commissural connections in a chamber with three compartments that can be selectively perfused with different agents. Latex membranes provide good separation between the compartments, and the adjustment of the apertures diameter to the size of the commissural connections prevents the damage of the commissural connections. The major advantage of the use of the latex membranes as separators is that they allow the construction of very thin compartments that are impossible to construct by other means, such as Vaseline wall (Cazalets et al. 1996; Khalilov et al. 1997) or glued plastic bars

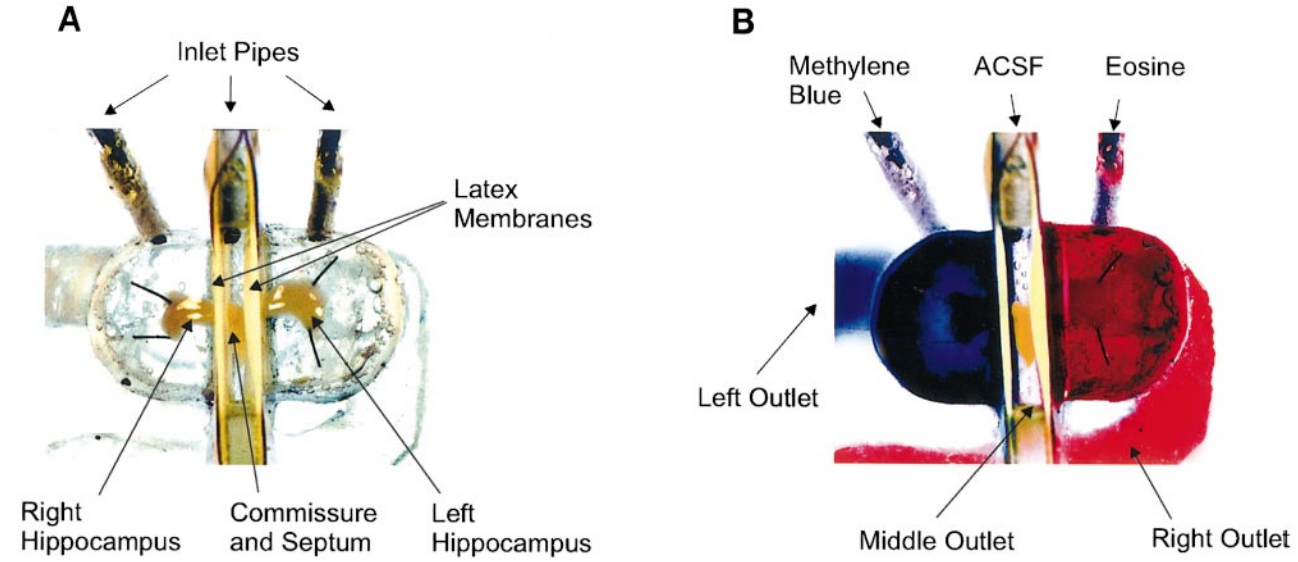

FIG. 2. Photograph of the 3-compartment chamber. *A*: view from above. Two hippocampi and the commissure are placed in the different compartments demarcated by the latex membranes. Each compartment has a separate perfusion with inlet (inlet pipes) and outlet. The 2 lateral outlets pass below (see also *B*) and are brought together in the separate drawing-off chamber (not shown) so that a pressure in all 3 compartments is equal. *B*: dyes methylene blue and eosin red (1%) are applied to the hippocampal chambers, the commissural chamber is perfused with artificial cerebrospinal fluid. Note that solutions do not mix between the compartments. The oulet tubes that pass below are also colored.

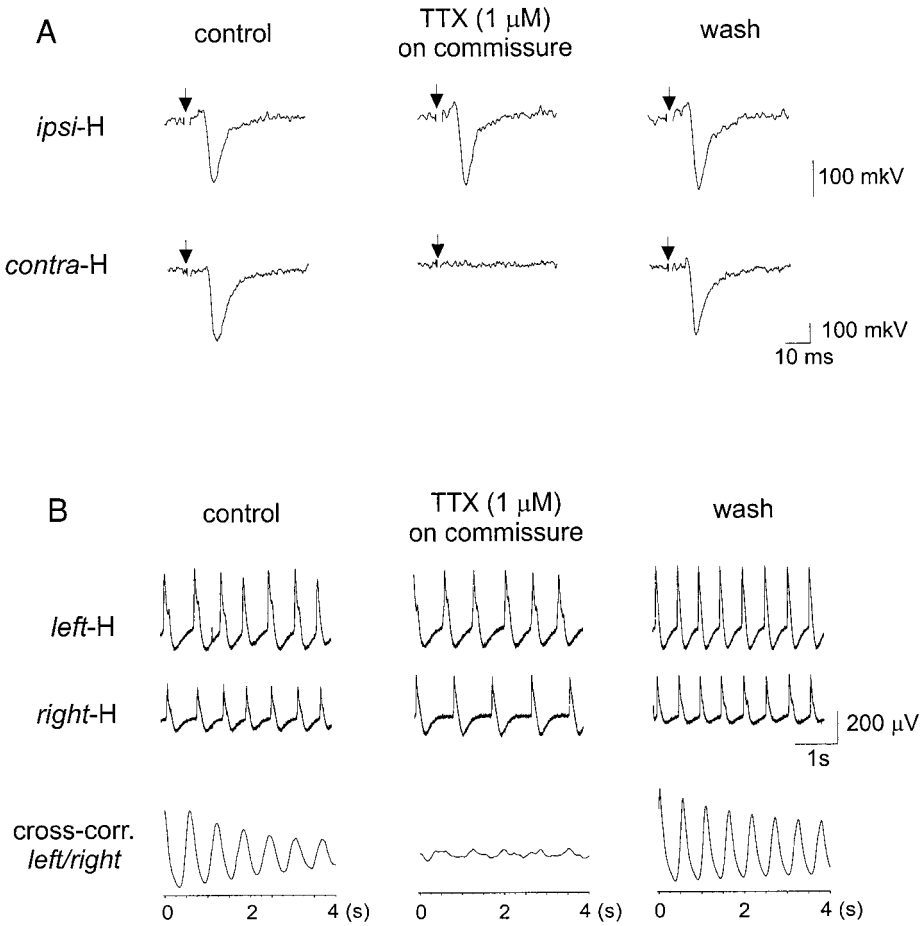

FIG. 3. Reversible blockade of the commissural propagation in the 3-compartment chamber. *A*: field-excitatory postsynaptic potentials (EPSPs) evoked by electrical stimulation are recorded on both ipsi- and contralateral sides in control conditions. Bath-application of tetrodotoxin (TTX; 1 μM) to the commissural compartment blocks the field-EPSPs on the contralateral side without affecting EPSPs on the ipsilateral side. Propagation to the contralateral side is restored after 20 min of washing out of TTX. Stimulation artifact is erased for clarity. *B*: periodic interictal-like activity has been induced by exposure of both hippocampi to 4-aminopyridine (200 μM). In control conditions the epileptiform discharges are highly synchronized in the left and right hippocampi. Application of TTX (1 μM) on the commissure desynchronizes the epileptiform activities. The synchronization was restored after wash of TTX from the commissural compartment. Normalized cross-correlograms of the activities in the 2 hippocampi are shown on the traces below.

(Kjaerulff and Kiehn 1997), and to apply drugs selectively to the commissural connections.

The three-compartment chamber offers a wide range of possibilities and considerably extends the advantages offered by in vitro preparations notably to study: *1*) the generation and commissural propagation of synchronized neuronal network activities, *2*) the maturation of functional commissural connections and the characterization of the receptors involved, and *3*) the dissociation of the local effects of a drug and their distal consequences, and the evaluation of the consequences of neuronal hyperactivity independently from the actions of the drug. This chamber will be particularly useful to study the propagation of the epileptiform activites and formation of the epileptogenic mirror focus.

We thank Dr. H. Gozlan for help in spectrophotometry study and J.-P. Panac and S. Weiller for photography assistance.

Present address of I. Khalilov: Dept. Biophysics of Cell, Institute of Biology of RAN, 2-31, Lobachevski str., Kazan, Russia.

Address for reprint requests: Y. Ben-Ari, INSERM U29, 123 Bd de Port-Royal, Paris 75014, France.

Received 29 June 1998; accepted in final form 21 September 1998.

REFERENCES

CAZALETS, J. R., BORDE, M., AND CLARAC, F. The synaptic drive from the spinal locomotor network to motoneurons in the newborn rat. *J. Neurosci.* 16: 298–306, 1996.

KHALILOV, I., ESCLAPEZ, M., MEDINA, I., AGGOUN, D., LAMSA, K., LEINEKUGEL, X., KHAZIPOV, R., AND BEN-ARI, Y. A novel in vitro preparation: the intact hippocampal formation. *Neuron* 19: 743–749, 1997.

KJAERULFF, O. AND KIEHN, O. Crossed rhythmic synaptic input to motoneurons during selective activation of the contralateral spinal locomotor network. *J. Neurosci.* 17: 9433–9447, 1997.

LEINEKUGEL, X., KHALILOV, I., BEN-ARI, Y., AND KHAZIPOV R. Giant depolarizing potentials: the septal pole of the hippocampus paces the activity of the developing intact septo-hippocampal complex in vitro. *J. Neurosci.* 78: 6349–6357, 1998.