Journal of Neuroscience Methods 194 (2011) 394–401

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

A multi-slice recording system for stable late phase hippocampal long-term potentiation experiments

Katja Sabine Kroker[a,*], Holger Rosenbrock[a], Georg Rast[b]

[a] Boehringer Ingelheim Pharma GmbH & Co. KG, Dept. of CNS Diseases Research, Birkendorfer Strasse 65, 88397 Biberach, Germany
[b] Boehringer Ingelheim Pharma GmbH & Co KG, Dept. of Drug Discovery Support, Birkendorfer Strasse 65, 88397 Biberach, Germany

ARTICLE INFO

Article history:
Received 24 September 2010
Received in revised form 5 November 2010
Accepted 8 November 2010

Keywords:
Long-term potentiation
Brain slices
Hippocampus
Synaptic plasticity
Extracellular recording
fEPSP
Multi-slice recording
Drug screening

ABSTRACT

A major challenge in neuroscience is identifying the cellular and molecular processes underlying learning and memory formation. In the past decades, significant progress has been made in understanding cellular and synaptic mechanisms underlying hippocampal learning and memory using long-term potentiation (LTP) experiments in brain slices as a model system. To expedite LTP measurements it is helpful to further optimize such recording systems. Here, we describe a modification of a multi-slice recording system (SliceMaster, Scientifica Limited, East Sussex, UK) that allows absolutely stable measurements of field excitatory postsynaptic potentials (fEPSPs) for up to 8 h in up to eight slices simultaneously. The software Notocord® was used for on-line data acquisition and to control the digital pattern generator which can generate different patterns for slice stimulation, inducing different types of LTP. Moreover, in contrast to common gravity-driven perfusion systems, a Pumped Perfusion System was employed to recycle drug solutions applied to the hippocampal slice. In addition, slices were positioned on two stacked grids for optimal recording of fEPSPs. These two stacked grids were placed in the measuring chambers allowing recordings for several hours without any perturbances. In summary, this modified slice-recording system improves throughput and allows for better statistical design, increases number of used slices per animal and enables very robust LTP measurements for up to 7 h. Hence, this system is suitable not only to investigate molecular mechanisms underlying the late phase of LTP, but also to screen candidate compounds in the context of drug discovery.

© 2010 Elsevier B.V. All rights reserved.

1. Introduction

One of the most prominent features of the brain is its ability to acquire and store information. Although substantial progress has been made in the past few decades in understanding certain forms of acquisition and recall, the phenomenon of learning and memory is still not fully understood. Mammalian brains are flexible, being able to change their neuronal function and microstructures in response to internal and external stimuli. These forms of neuronal plasticity correspond to long-lasting changes in the strengths of synapses between neurons. Especially the hippocampus, a brain region crucial for the formation of episodic memory, is the preferred system to study changes of synaptic and neuronal plasticity. In vivo, hippocampal learning and memory can be assessed with certain cognition tests, for example the Morris water-maze (Morris, 1984) or the T-maze (Gerlai, 1998). In vitro, cellular and synaptic mechanisms underlying hippocampal learning and memory can be investigated with long-term potentiation (LTP) experiments in brain slices. In the last decades, in vitro studies, especially in hippocampal slices, gained increasing popularity as they allow more detailed investigations of physiology and pharmacology on the cellular and molecular level as compared to in vivo experiments.

LTP is the increase of the strength of synapses between neurons for prolonged periods following brief but intense synaptic activation (Bliss and Gradner-Medwin, 1973; Bliss and Lomo, 1973). Currently, the majority of LTP measurements are performed in the CA1 region of hippocampal slices. Although LTP has been extensively studied in the past years, the major hurdles for establishing LTP measurements are the following. Firstly, LTP experiments are highly time-consuming resulting in a low throughput. Secondly, although researchers are committed to reduce the number of animals, not all prepared brain slices (i.e. about eight hippocampal slices of 400 μm thickness from one rat brain) can be used for experiments, since commonly used recording systems only permit measurements from one slice at a time (Haas et al., 1979). Thirdly, it is difficult to robustly measure LTP over a longer period of time which is required for measuring late phase LTP, an equivalent of synaptic plasticity underlying long-term memory. This difficulty might be due to the decreasing vitality of the slice or, more often, due to movements of the slice or the electrodes caused by mechan-

* Corresponding author. Tel.: +49 7351 547150; fax: +49 7351 5498928.
E-mail address: Katja.Kroker@boehringer-ingelheim.com (K.S. Kroker).

0165-0270/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2010.11.006

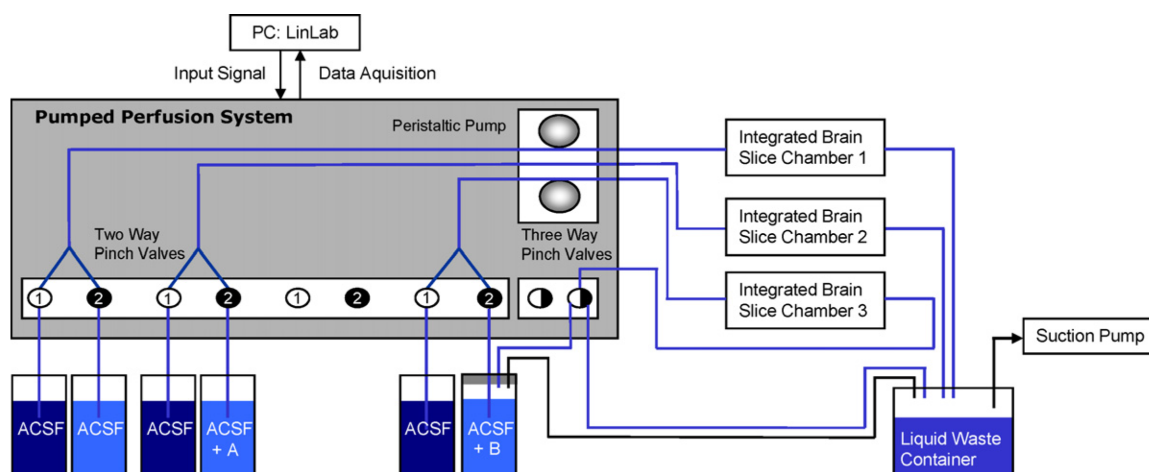

Fig. 1. Pumped Perfusion System. Schematic drawing showing the flow of ACSF and drug solutions (ACSF + A and ACSF + B) from the independent reservoirs (ACSF shown in dark blue, control and drug solutions shown in light blue) via the two-way pinch valves and the peristaltic pump to the single integrated brain slice chambers. After passing the integrated brain slice chambers the fluid is discarded by a suction pump (shown for the integrated slice chambers 1 and 2) or recycled via a three-way pinch valve (shown in black–white, shown for the integrated slice chamber 3). Opening of valve set 1, consisting of 4 two-way pinch valves (shown in white), results in application of ACSF. Two different drug solutions and ACSF, as control, are applied to the individual integrated brain slice chambers as soon as valve set 2 is opened, which consists of 4 two-way pinch valves (shown in black). The eight two-way pinch valves, the two three-way pinch valves and the peristaltic pump are controlled logged by the software LinLab. This figure shows the perfusion of 3 chambers as in this study 3 chambers were used. However, this Pumped Perfusion System can perfuse up to 5 chambers without any dismounting as one two-way pinch and one three-way pinch valves are free to use.

ical instability of the recording system. The first two points were already addressed by Stopps et al. (2004) who established a system allowing electrophysiological recordings from up to eight brain slices simultaneously and hence increasing throughput and optimizing tissue use from animals. In this study, mechanical instability compromising recording quality especially over a longer period of time is addressed.

Here, we describe the improvement of the measuring chambers of an already existing multi-slice recording system with two stacked grids enabling absolutely stable measurements of LTP for up to 7 h from several slices simultaneously. Further modification includes the software Notocord® used for on-line data acquisition and a Pumped Perfusion System allowing recycling of drug solutions applied to the slice.

2. Materials and methods

2.1. Multi brain slice system

The SliceMaster multi-slice recording system (Scientifica Limited, East Sussex, UK) was used to record field excitatory postsynaptic potentials (fEPSPs). The system is semi-automated allowing one operator to record simultaneously from an assembly of up to eight brain slices. The system has been described in detail by Stopps et al. (2004). Briefly, the SliceMaster used for this report consists of three temperature-controlled integrated brain slice chambers and can be upgraded with five more. Each chamber is equipped with one stimulation electrode, one recording electrode and a camera system allowing a visualisation of each brain slice on a shared video monitor. A central control panel makes the positioning of all electrodes and cameras possible, allowing a single operator to run all experiments simultaneously.

2.2. Perfusion

A separate inflow and outflow for each integrated brain slice chamber allows individual perfusion of each brain slice. Each integrated brain slice chamber has independent reservoirs for buffer and drug solutions. These independent reservoirs allow random assignment of treatments across slices. The tube lengths are minimal and identical for each integrated brain slice chamber. A suction pump discards the used liquid via the outflow into a waste box or recycles it back to the original reservoir. Unlike the gravity-fed system described previously (Stopps et al., 2004), a Pumped Perfusion System (PPS; Scientifica Limited, East Sussex, UK) was used in this study. Gassing and recirculation of solutions is possible and a flow rate between 0.1 and 20 mL min^{-1} can be chosen. The PPS combines full computer control of eight two-way, two three-way pinch valves and a peristaltic pump. It allows easy configuration of a series of valve functions and was modified to enable four two-way valves being controlled simultaneously (Fig. 1). There are two sets of valves, each equipped with four two-way valves: valve set one and valve set two. All tubes connected with valve set one arise from the buffer reservoirs, whereas all tubes coming from the different drug solutions are linked to valve set two. As soon as an experiment is started, valve set one is opened and automatically valve set two is closed. Hence, from three different buffer reservoirs artificial cerebrospinal fluid (ACSF) passes the open valve set one, the peristaltic pump and is applied directly to the individual integrated brain slice chamber. The valve set two can be opened by software control, simultaneously closing valve set one, applying the different drug solutions to each of the individual integrated brain slice chambers. The remaining two three-way pinch valves can be used to recycle drug solutions at two individual chambers, with 20 mL being the minimum recycle volume. For documentation, the system can be configured to send a TTL pulse for each opening event. Furthermore, for each experiment a data log is created for all parameters of the valves and the peristaltic pump.

2.3. Minimization of fluidic turbulences

As all reservoirs for buffer and drug solutions have to be saturated with 95% O_2 and 5% CO_2, they need to be constantly bubbled with carbogen. This causes small bubbles being transported via the PPS to the integrated brain slice chambers. Bubbles in the PPS cause movements of the grid supporting the slice and result in unstable recordings. To minimize the movement of the slice, stacked grids were integrated. The original single grid was replaced by a very

Download English Version:

https://daneshyari.com/en/article/6269895

Download Persian Version:

https://daneshyari.com/article/6269895

Daneshyari.com