Journal of Neuroscience Methods 285 (2017) 49–57

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

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

Research paper

Optimisation of murine organotypic slice culture preparation for a novel sagittal-frontal co-culture system

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HIGHLIGHTS

- Time-saving optimisation of the preparation process for sagittal slice cultures.
- First murine nigrostriatal organotypic slice co-culture system.
- First nigrostriatal co-culture system using sagittal organotypic slices.

ARTICLE INFO

Article history:
Received 3 March 2017
Received in revised form 21 April 2017
Accepted 3 May 2017
Available online 4 May 2017

Keywords:
Slice culture organotypic co-culture
Nigrostriatal
Embedding
Axonal outgrowth
Astroglia

ABSTRACT

Background: The nigrostriatal pathway is of great importance for the execution of movements, especially in the context of Parkinson's disease. In research, analysis of this pathway often requires the application of severe animal experiments. Organotypic nigrostriatal slice cultures offer a resource-saving alternative to animal experiments for research on the nigrostriatal system.
New method: We have established a time-saving protocol for the preparation of murine sagittal nigrostriatal slice cultures by using a tissue chopper and agarose embedding instead of a vibratome. Furthermore, we developed the first murine co-culture model and the first co-culture utilising sagittal slices for modelling the nigrostriatal pathway.
Results: Sagittal nigrostriatal slice cultures show good overall tissue preservation and a high number of morphologically unimpaired dopaminergic neurons in the substantia nigra. Sagittal-frontal co-culture demonstrates massive outgrowth of dopaminergic fibres from the substantia nigra into co-cultured tissue.
Comparison with existing methods: The use of a tissue chopper instead of a vibratome allows notable time-saving during culture preparation, therefore allowing optimisation of the preparation time. Sagittal co-cultures offer the opportunity to study dopaminergic fibres in their physiological environment and in co-cultured tissue from a different animal in the same culture system.
Conclusion: We here present a possibility to optimise the slice culture preparation process with the simple means of using a tissue chopper and fast agarose embedding. Furthermore, our sagittal-frontal co-culture system is suitable for the observation of dopaminergic outgrowth in both co-cultured tissues.

1. Introduction

The nigrostriatal pathway is a part of the basal ganglia motor loop and therefore of vital importance for the execution of movements. It consists of the dopaminergic neurons of the substantia nigra pars compacta (SN) and their processes running through the medial forebrain bundle (MFB) into the caudate-putamen complex (CPu) (Fuxe et al., 2006). Degeneration of these neurons leads to Parkinson's disease, the second most common neurodegenerative disorder and therefore a challenge for the public health system for today and in the future (Kalia and Lang, 2015).

Analysing and manipulating the nigrostriatal pathway *in vivo* requires large experimental efforts involving severe animal experiments. Regarding the principles of ethical use of animals in testing, the three R's (replacement, reduction, refinement; Russell and Burch, 1959), organotypic slice culture preparations could be a valuable alternative for studying and modelling the nigrostriatal

Abbreviations: SN, substantia nigra pars compacta; CPu, caudate-putamen complex; MFB, medial forebrain; eGFP, enhanced green fluorescent proteinbundle; TH, tyrosine hydroxylase; DIV, days *in vitro*; PBS, phosphate-buffered saline; PFA, paraformaldehyde.

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http://dx.doi.org/10.1016/j.jneumeth.2017.05.003
0165-0270/

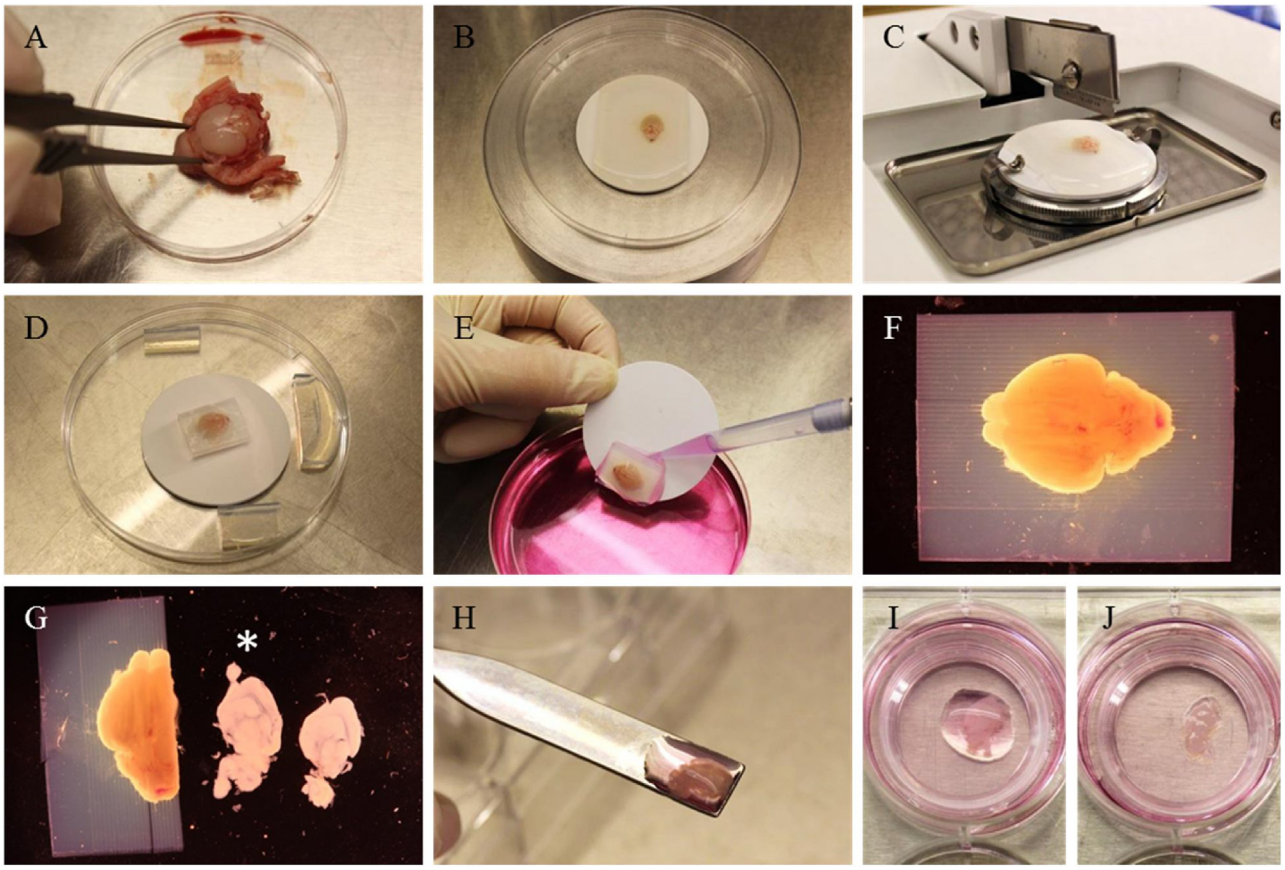

Fig. 1. Preparation of sagittal organotypic slice cultures. (A) The brain of a five day old mouse pup is rapidly dissected and (B) embedded in 4% agarose on the plastic cutting dish of a McIlwain tissue chopper. (C) The embedded brain is sagittally cut at 350 μm with the tissue chopper. (D) Excess agarose is cut off with a razor blade and (E) the sliced brain is carefully flushed from the plastic disc. (F) Under the dissection microscope, the sliced brain can be separated into sagittal slices with the aid of microspatulas. (G) Slices containing all components of the nigrostriatal pathway are chosen for culture (the slice marked with an asterisk is suitable for culture). (H) Slices can be transferred on a spatula in a drop of medium. (I) The liquid allows the slice to slip off the spatula onto the semiporous membrane. (J) Excess medium on the membrane will be soaked in after a few minutes and the slice is ready for culture.

pathway. They allow to observe neurons of the nigrostriatal pathway in their physiological three-dimensional environment but do not require the execution of surgical procedures or other interventions on the living animal. Furthermore, they are easily accessible for analysis as well as pharmacological or mechanical lesioning of the dopaminergic system (Daviaud et al., 2013). For organotypic culture, brain tissue is sliced into 300–500 μm thick sections which are cultured in rollertubes (Gähwiler, 1981) or on semipermeable membranes following the Stoppini method (Stoppini et al., 1991). The latter technique is less effortful and therefore usually the method of choice for present studies. A broad range of brain regions can be cultured by this technique, e.g. hippocampus, cortex, striatum, SN, locus coeruleus, the basal forebrain, hypothalamus or the olfactory system (Humpel, 2015). Organotypic slices can be kept in culture for up to several weeks and therefore allow short- and long-term observations of the cultured tissue (Gogolla et al., 2006).

Concerning slice cultures of the nigrostriatal system, there are basically two approaches: single slice cultures with sagittal slices or co-culture systems composed of frontal slices. In co-culture systems of the nigrostriatal pathway, frontal slices containing SN, CPu and cortex are apposed and dopaminergic fibre outgrowth from the SN towards the CPu can be analysed (Franke et al., 2003). Sagittal slices, on the other hand, contain all components of the nigrostriatal system in one slice and offer a model of the almost complete physiological architecture of the whole pathway. They are especially suited to study effects of lesioning experiments on the dopaminergic system (Kearns et al., 2006; McCaughey-Chapman and Connor, 2016; Ullrich et al., 2011). However, preparing, handling and culture of these large slices is considerably more challenging than the use of smaller frontal slices.

The comparably large sagittal slices are usually prepared with the aid of a vibratome, which is a rather time-consuming procedure. In order to minimise the duration of the preparation procedure, we established a cutting technique for murine sagittal organotypic slices by using a McIllwain Tissue Chopper in combination with agarose embedding.

Furthermore, for a detailed study of dopaminergic outgrowth we established a co-culture system of murine sagittal and striatal frontal slices. To our knowledge, this is the first description of a murine co-culture model of the nigrostriatal pathway and the first co-culture utilising sagittal slices.

As a further refinement of the method, we used tissue from transgenic mice expressing enhanced green fluorescent protein (eGFP) under the control of the tyrosine hydroxylase (TH) promotor (Sawamoto et al., 2001). Therefore, in our model system, dopaminergic neurons and fibres are labelled by endogenous eGFP expression and can be observed throughout the whole culture period.

2. Materials and methods

2.1. Animals

For this study, wildtype C57/BL6 mice or transgenic mice with a C57/BL6 background expressing eGFP under control of the rat TH promotor (Sawamoto et al., 2001) were used. The animals were