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Novel Optical Method for Visualization of Intracerebral Grafts

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Experiments with intracerebral grafting studying the processes along the graft-host interface sometimes face a technical difficulty – determining of the exact borders of the graft. Processes of cell migration, decay, and gliosis may obscure the outline of the transplanted tissue. The present report aims to describe a new, simple, yet reliable method for optical demonstration of the graft-host interface. We performed oblique light microscopy using a standard bright field microscope with a built-in the condenser lens diffuser. This method demonstrated good contrast between graft and host brain tissue, regardless of the staining of the otherwise standard specimen. This is the first use of oblique light microscopy in the context of intracerebral transplantation. This method is an easy, replicable, and reliable way to delineate intracerebral grafts without additional histological preparations.

Key words: intracerebral transplantation, grafts, oblique light microscopy

Introduction

In the context of intracerebral transplantation, the graft-host interface is known to be a site of crucial processes of integration between transplanted cells and the recipient's brain [1, 7, 9]. Intensive cell contacts, migration, but also cellular decay and reactive gliosis are known to happen along the border between the graft and the host brain [2, 4, 11, 13]. Histological evaluation of the relationship between the transplanted cells and the nervous tissue of the host requires the exact determination of the graft-host interface. Sometimes, however, this poses a significant difficulty for the investigator, due to accumulation of reactive cells [3, 11] and the resulting intensive staining. Therefore, a method for reliable visualization of this interface is needed, in order to facilitate the evaluation of the processes around grafts.

Dark field microscopy has been previously used with CNS histological preparations to demonstrate white and grey matter structures based on their different refractive properties [10]. It was also used with intracerebral grafts, but only to visualize the fluorescent nerve fibres growing out from them, and not for the graft tissue itself [5].

The aim of the present report is to give an account of a method involving oblique light microscopy for visualization of intracerebral grafts.

Materials and Methods

Intracerebral grafting was performed in the standard unilateral 6-hydroxydopamine model of Parkinson's disease, using single cell suspension of E14 ventral mesencephalic tissue. Details regarding the experimental setting are given in detail elsewhere [12].

The resulting 40 μm sections were stained immunohistochemically for GFAP and processed according to a standard protocol. Transparency of the dehydrated sections was achieved with immersion in xylene, and they were subsequently coverslipped with Histofluid (Marienfeld, Germany). Slides were observed using a standard bright field microscope (Leica DX1000). A diffusing element built in the condenser lens was used to direct the light beam in an oblique direction through the slide. A low power objective lens was implemented to observe and photograph the specimen with a standard camera. Same sections were observed using a dark field microscope in order to visually compare the structures demonstrated using both methods. Furthermore, serial sections processed according a different staining protocol (immunohistochemistry for tyrosine hydroxylase, a marker for the grafted neurons) were also observed for confirmation of the exact site of the graft-host interface.

Results

Observation of the histological preparations confirmed the presence of intracerebral grafts in the expected coordinates. The grafts were ovoid in shape, however their exact outlines could not be always demonstrated easily, as seen on **Fig. 1A.** Especially when observing grafts in later time points of the experiment (as the case with the depicted on the figure graft) the astroglial envelope around the transplant was seen as a dense meshwork, which completely obscured the view of the graft-host interface. In cases of TH staining, the graft cores themselves were not contrasted against the background of the host striatum and their outlines could be only roughly traced thanks to the presence of stained cellular elements.

The observation using oblique light microscopy revealed that the graft cores possessed significantly different light scattering properties. The grafts appeared as uniformly darker objects, contrasted against the silvery, lighter but heterogeneous striatal tissue (**Fig. 1B**). The contrast between graft and host tissue was especially superb in sites of

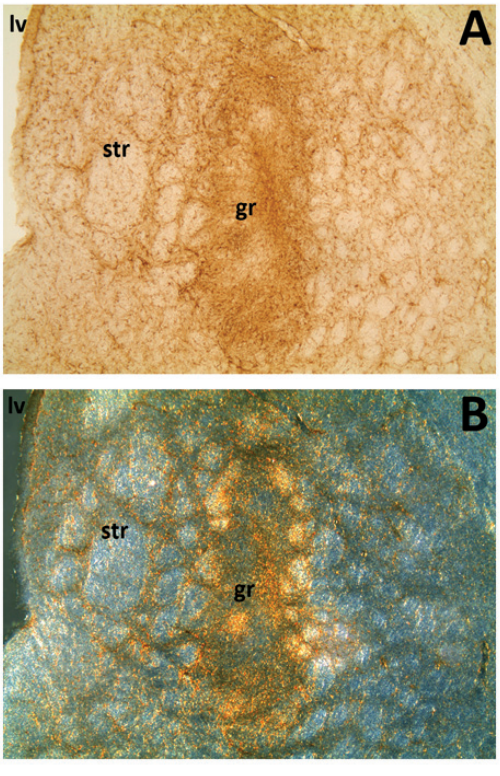

Fig. 1. Low-power microphotographs (4x) of the same GFAP-stained intracerebral graft, observed using bright field microscopy (A) and oblique light microscopy (B). gr – graft, str – striatum, lv – lateral ventricle.

contact between the transplants and the fibers of the internal capsule. In the same time, the DAB-stained (immunopositive) elements did not obscure the picture in any way, merely casting a shadow, as demonstrated on **Fig. 1B**.

Discussion and Conclusions

The present report is the first one to describe the use of oblique light microcopy for demonstration of intracerebral grafts. The differences of the light-scattering properties of gray and white matter of the CNS have been demonstrated before. The property of white matter tracts to appear differently in dark field microscopy depending on their direction relative to the plain of histological section is also known [10]. However, neither dark field microscopy, nor oblique light microscopy have been used to demonstrate the very outlines of intracerebral grafts.

We attribute our observations to the considerable differences of the extracellular matrix in the grafted tissue and in the native brain parenchyma [6]. The preparation of the cell suspension used for transplantation involves intensive washing, which causes considerable dissociation of the tissue and loss of extracellular matter [8]. It is possible that in a later time point following transplantation the consolidation of the graft might lead to a change in the appearance of the transplanted tissue. We, however, could not demonstrate this in the relatively early moment (28 days) following grafting

The proposed method for visualization of intracerebral grafts is fast, easy to apply and reproduce, and does not require any special equipment or histological preparations. This microscopy technique is particularly useful in studies focused on the graft-host interface, because its application allows for uncomplicated determining of its course. A limitation of the method might be the fact that only low-power objective lenses can be used with it; however, we did not find this to be of any practical disadvantage. Validation of the method is needed for studies involving longer follow-ups following grafting, as well as such with different transplantation sites.

References

1. **Barker, R., S. B. Dunnett.** Ibotenic acid lesions of the striatum reduce drug-induced rotation in the 6-hydroxydopamine-lesioned rat. – *Exp. Brain Res.,* **101**, 1994, 365-374.
2. **Barker, R. A., S. B. Dunnett, A. Faissner, J. W. Fawcett**. Time course of loss of dopaminergic neurons and the gliotic reaction surrounding grafts of embryonic mesencephalon to the striatum. – *Exp. Neurol.,* **141**, 1996, 79-93.
3. **Barrett, C. P., L. Guth, E. J. Donati, J. R. Krikorian.** Astroglial reaction in the graymatter of lumbar segments after midthoracic transection of the adult rat spinal cord. – *Exp. Neurol.,* **73**, 1981, 365-377.
4. **Brundin, P., J. Karlsson, M. Emgård, G.S. Schierle, O. Hansson, A. Petersén, R. F. Castilho.** Improving the survival of grafted dopaminergic neurons: a review over current approaches. – *Cell. Transplant*., **9**, 2000, 179-195.
5. **Denham, M., C. L. Parish, B. Leaw, J. Wright, C. A. Reid, S. Petrou, M. Dottori, L. H. Thompson.** Neurons derived from human embryonic stem cells extend long-distance axonal projections through growth along host white matter tracts after intra-cerebral transplantation. – *Front. Cell. Neurosci*., **6**, 2012, 11.http://doi.org/10.3389/fncel.2012.00011
6. **Gates, M. A., E. D. Laywell, H. Fillmore, D. A. Steindler.** Astro cytesandex trace llularma trixfollo wingintra cerebral transplantation of embryonic ventralmesence phalonorlateral ganglioni ceminence. – *Neurosci.*, **74**, 1996, 579-597.
7. **Perlow, M. J., W. J. Freed, B. J. Hoffer, A. Seiger, L. Olson, R. J. Wyatt.** Brain grafts reduce motor abnormalities produced by destruction of nigrostriatal dopamine system. – *Science*, **204**, 1979, 643-647.

8. **Pruszak, J., L. Just, O. Isacson, G. Nikkhah.** Isolation and culture of ventral mesencephalic precursor cells and dopaminergic neurons from rodent brains. – *In: Current protocols in stem cell biology*. New Jersey, Wiley, 2009, 2D.5.1-2D.5.21
9. **Saltykow, S.** Versuche über gehirnplantation, zugleich ein beitrag zur kenntniss der vorgänge an den zelligen gehimeelementen. – *Arch. Psychiatr. Nervenkr*., **40**, 1905, 329-388.
10. **Senatorov, V. V. Dark-field microscopy visualization of unstained axonal pathways using oil of wintergreen. –** *J. Neurosci. Methods*, **113**, 2002, 59-62.
11. **Shinoda, M., J. L. Hudson, I. Strömberg, B. J. Hoffer, J. W. Moorhead, L. Olson.** Microglial cell responses to fetal ventral mesencephalic tissue grafting and to active and adoptive immunizations. – *Exp. Neurol*., **141**, 1999, 173-180.
12. **Tomov, N., L. Surchev, C. Wiedenmann, M. D. Döbrössy, G. Nikkhah.** Astrogliosis has different dynamics after cell transplantation and mechanical impact in the rodent model of Parkinson's disease. – *Balkan Med. J*., **35**, 2018, 141-147. https://dx.doi.org/10.4274%2Fbalkanmedj.2016.1911
13. **Zawada, W. M., D. J. Zastrow, E. D. Clarkson, F. S. Adams, K. P. Bell, C. R. Freed.** Growth factors improve immediate survival of embryonic dopamine neurons after transplantation into rats. – *Brain Res*., **786**, 1998, 96-103.