Laser microdissection in CNS research

Christine Böhm, Dieter Newrzella and Oliver Sorgenfrei

The complexity of the brain makes the investigation of anatomically defined regions using manual dissection techniques problematic. With these manual dissection techniques, only a mixture of many different cell types can be obtained. This leads to averaging the contents of all the different cell types, making it nearly impossible to observe effects that are specific to one type of cell. Laser microdissection enables individual cell-types to be dissected accurately from the brain for subsequent analysis of the genome, proteome or, most frequently, the transcriptome. Investigating only functionally relevant cells with high specificity provides unambiguous data, resulting in faster identification of potential targets, the elucidation of drug mode-of-action, as well as aiding identification of biomarkers for diagnostics use.

Complexity of the brain

The mammalian brain is one of the most complex tissues, consisting of different histological regions with specialized functions and numerous specialized nuclei and different types of cells. The different cell types can be identified using morphological criteria, electrophysiological properties or the expression of marker genes, and comprize predominantly neuronal subtypes, in addition to glial cells like astrocytes, oligodendrocytes and microglia, as well as vessels. The complexity can be increased further by differences in the repertoire of expressed genes within a single cell type depending on the input to the cell, its projection or functional state. Disease conditions will further modify the characteristics of specific types of cells. Differences in the response to a stimulus are frequently quite small in the individual cells in the brain, and are even more difficult to detect in a mixture of different cells. Reducing the complexity of the input material by isolating specific cell populations will therefore improve the experimental outcome and enables observation of properties that would otherwise remain undetected because the use of whole tissue samples can result in an 'averaging out' of the heterogeneous elements of the sample. Laser microdissection (LMD) has turned out to be a highly versatile technology for obtaining pure samples of cells of interest for downstream applications. This review focuses on recent developments in LMD and its application to neuroscience, with a major emphasis on transcription profiling.

Laser microdissection systems

Laser microdissection enables the efficient isolation of single cells or cell groups with no or extremely low, contamination from surrounding tissue components, simultaneously leaving the intracellular structure and molecules intact. In contrast to fluorescent activated cell sorting (FACS), it does not require the disruption of the tissue, which would influence the physiology of the cells. Thus, LMD is well-suited for the isolation of different cell types from CNS samples. Commercially available LMD

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systems employ different technologies for separating selected target areas from tissue sections. Depending on the technology used, different names for the dissection technologies are common for the individual systems.

In a system offered by Arcturus Engineering, a cap coated with a transparent ethylene vinyl acetate layer is placed over the cells of interest and a low power infrared laser beam is directed at these target cells. Pulsing the laser through the cap causes the thermoplastic film to adhere to the target cells and, on removal of the cap from the slide, the chosen tissue remains adhered to the cap. The cells are removed along with the film and cap, from where they can then be harvested for downstream analysis. This technology is often referred to as laser capture microdissection (LCM) and was jointly developed by the National Cancer Institute and Arcturus in 1996 [1,2].

For cold LMD technology, the laser is used to cut around a selected target area. The laser light is focused through an objective lens of the microscope to a diameter of about a micron or less, and only the material within the small laser focus is ablated, leaving the surrounding tissue intact. This technology requires the sections mainly to be mounted on membranes slides [polyethylene naphthalate (PEN), polyethylene terephthalate (PET) or polyester (POL)] or membrane coated glass slides.

The LMD system offered by PALM Microlaser Technologies removes coherent cell fields by applying a pulsed UV-A laser through an inverted microscope after cells have been marked for dissection, either manually or automatically, by a separate image processing program (Cellenger, Definiens). Working with an inverted microscope this system is also able to dissect tissue mounted glass slides, which might be coated with a membrane. Following LMD, the desired cell population is then catapulted into a microtube positioned above the sample by laser induced propulsion giving this system the name Laser Microdissection and Pressure Catapulting (LMPC) technology [3,4].

The Leica AS LMD platform (Leica Microsystems) also uses a pulsed UV-A laser to microdissect the cells of interest by ablating the tissue surrounding the target cells and leaving those behind intact. However, an upright microscope is used within this system and the dissected tissue is transferred into a microcentrifuge tube cap solely by gravity (i.e. without any mechanical contact and without the application of additional forces). This design requires the sample to be mounted on membrane slides. By offering an objective with a magnification factor of 150 x for cell detection and microdissection, this system is particularly well suited for the precise isolation of single cells from brain tissues. It even enables the isolation of cells with delicate processes, such as astrocytes (Figure 1). The LCM system offered by Arcturus is limited to a magnification factor of 40x, the LMPC system by PALM enables micromanipulations ranging from 5–100x. Leica Microsystems in collaboration with Axaron Bioscience have developed a fully automated LMD system (i.e. automatic cell detection by image analysis of stained cells immediately followed by LMD of the identified cells). Software that enables the automatic detection of cells have also been developed by the other providers of LMD hardware.

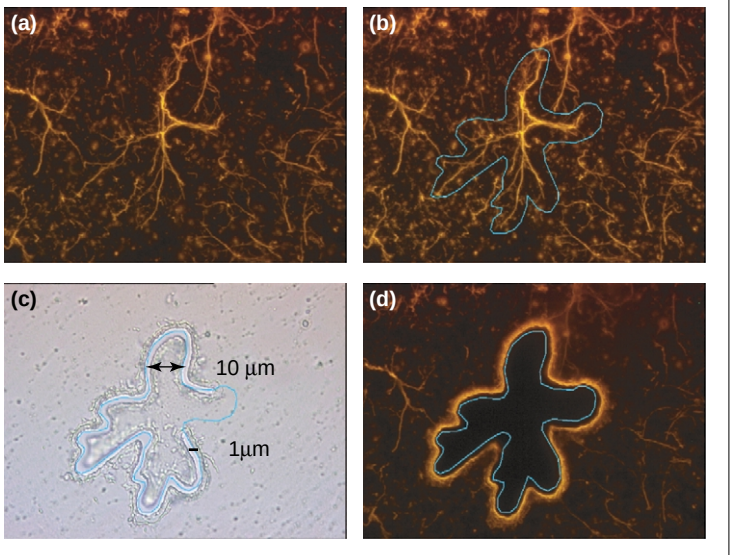

FIGURE 1

Laser microdissection of a single astrocyte. (a) at 150x magnification using the Leica AS LMD; **(b)** Identification of labelled astrocyte by image analysis; **(c)** microdissection of labelled astrocytes, bright field mode; **(d)** following microdissection, fluorescence mode.

Section preparation

Tissue fixation and staining protocols for the section preparation in LMD can vary for different downstream applications and for the different LMD systems being used. The systems require different prerequisites for the dissection process and, thus, it is hard to outline general fixation or staining protocols for all systems. For example, for the thermoplastic film to adhere firmly to the sections using the Arcturus system, all the sections (paraffin and frozen) have to be dehydrated completely using xylene, a step that is not essential for LMD systems. By contrast, if the laser ablative systems are operated with membrane slides and sections from paraffin embedded tissue, the xylene step for dewaxing the sections has to be shortened, compared with standard protocols, because the solvent would affect the membrane.

Fixation

Although the dissection of cells from fresh-frozen as well as from paraffin-embedded tissues is technically possible with all systems, not all fixation and staining procedures are equally well suited for particular downstream applications [2,4,5]. Whereas DNA is quite inert to common fixation and staining procedures, RNA is susceptible to degradation. RNA quality depends on the way the tissue was treated before LMD; this comprizes staining

and fixation procedures as well as the handling of the tissue before fixation (e.g. the time between removal of the tissue and its fixation).

Some groups have investigated the influence of fixation methods on RNA quality. Qin *et al.* compared gene expression patterns of rat dentate granule cells isolated from paraformaldehyde-fixed, paraffin embedded tissue, to frozen and ethanol-fixed tissue and non-fixed, single granule cells [6]. The group found that the RNA extracted from frozen and ethanol-fixed tissue yielded expression profiles similar to those from non-fixed fresh and single cells collected from hippocampal slices through patch clamp electrodes. RNA extracted from paraformaldehyde-fixed, paraffin embedded tissue, however, yielded less reliable results [6].

Van Deerlin *et al.* investigated gene expression in human post-mortem tissue, either flash-frozen or paraffin-embedded with prior fixation in 70% ethanol or 10% formalin. By preparing replicate sets of brain tissue samples from the same autopsy case, they could exclude factors such as clinical variability or post-mortem changes. They were able to demonstrate that longer transcripts of the housekeeping gene β-2-microglobulin could be amplified from flash frozen samples, compared with paraffin-embedded samples, while ethanol-fixed paraffin-embedded tissue displayed better results than formalin-fixed tissue. Although these results were derived from total tissue samples, they could be valid for laser microdissected tissue [7]. These examples show that the RNA quality is generally better preserved in fresh-frozen samples than in paraffin-embedded tissue specimens.

Staining

The issue of suitable staining procedures for LMD, particularly in combination with RNA expression profiling as downstream application, was addressed by several groups. Ginsberg and Che compared the influence of five common stains for brain tissues (cresyl violet, thionin, haematoxylin and eosin, silver stain and acridine orange) on the RNA quality and expression profile of ethanol-fixed and paraffin-embedded sections of hippocampus [8]. Although the results for cresyl violet, thionin and HE staining were comparable, a significant reduction in hybridization signal intensity was observed for both silver- and acridine orange- stained material [8].

Apart from these classical staining methods, immunohistochemistry is frequently used in combination with LMD. To reduce RNA degradation, several groups have developed rapid staining protocols of 30 min or less, which is considerably shorter than standard immunohistochemical protocols, by drastically shortening the incubation times of the antibodies. Such time savings can be achieved by a concomitant increase in the concentration of primary antibody. An example of such a rapid staining protocol was described by Burbach *et al.* [9]. These authors developed a rapid immunofluorescence protocol for staining astrocytes in brain cryosections using a primary glial fibrillary acidic protein (GFAP) antibody and an Alexa 568 secondary antibody to dissect astrocytes for RT-PCR investigations. Mojsilovic-Petrovic *et al.* showed that RT-PCR from a few hundred laser microdissected brain vessels can be performed following a staining protocol for vessels using fluorescein-tagged lectins [10].

Downstream applications

Within a few years of the invention of laser capture microscopy by Emmert-Buck *et al.* in 1996 [1], LMD became a reliable and popular tool for analyzing defined cell-groups from tissue sections and, from the beginning, LMD was applied to neuroscience [11]. It is now increasingly recognized that to obtain interpretable results from brain tissues, the complexity of the tissue has to be reduced by analyzing only the relevant cell groups out of a tissue section rather than taking a whole tissue approach. This is particularly true for transcription profiling technologies, where thousands of genes can be analyzed simultaneously using microarray hybridization of RNA. However, although most groups combine LMD with expression profiling technologies, either of single genes by RT-PCR or of the whole transcriptome by microarray hybridizations, DNA or proteome analysis can also be combined with LMD. Until now, LMD has been used mainly for research projects such as gene expression studies, target identification, biomarker discovery, pathway finding, mode-of-action studies of drugs and investigation of pathomechanisms in disease models or post-mortem tissue specimens. However, the technology has also found its entry into clinical diagnostics. However, to emerge into this field, further standards have to be defined, in LMD but even more for the applied downstream applications. Standardization will be a necessary prerequisite to obtain reproducible and reliable results and to make the analysis comparable between different laboratories. For the LMD system this could mean that for clinical applications fully automated systems combining cell detection and microdissection might be required, an issue that is currently being recognized by the manufacturers.

In the following section a few examples of applications from research and diagnostics in CNS are described. However, this overview is not intended to be comprehensive.

Laser microdissection in clinical research

Brain tumours

Diagnosis of tumours of the brain, particularly gliomas, is based largely on morphological and histopathological criteria as summarized by the WHO classification [12]. However, brain tumours frequently show considerable histological heterogeneity and because clinical management, particularly of gliomas, currently relies heavily on accurate histopathological diagnosis of grade and subtype for provision of appropriate therapy, molecular classification of gliomas is becoming increasingly important as

an adjunct to histopathological diagnosis. It is hoped that alterations like genetic losses might have prognostic or predictive value and might in future be important for a specialized and individualized therapy [13]. However, less is known about the relationship between histological heterogeneity and genetic alterations. Some groups used LMD to investigate whether detected genetic losses are present throughout the whole tumour tissue or whether only some regions of specific histological phenotype bear the aberration. In addition, the prognostic or predictive value of these markers has to be determined.

Reis *et al*. examined gemistocytic astrocytomas, a histological variant of astrocytomas WHO grade II [14]. They used LMD to dissect two different tumour cell-types, gemistocytes and non-gemistoytic tumour cells, followed by direct DNA sequencing of the *TP53* gene within these cells. The mutation analysis of these two different cell populations revealed that both gemistocytes and non-gemistocytic cells contained an identical TP53 mutation, proving that both cell-types have a common monoclonal origin and that gemistocytes are neoplastic cells, even though they lack proliferative activity [14].

In another study Walker *et al*. investigated allelic imbalances in histological phenotypes of gliomas mainly with an oligodendroglial component [15]. They analyzed allelic imbalances in loci on several chromosomes (1p36, 19q13, 17p13, 10q22–26, 10p11–15) in 42 histological phenotypes from 20 glioma cases with either inter- or intra-tumoural histological heterogeneity. The group applied LMD to isolate the histological heterogeneous regions and performed simultaneous PCR amplification of microsatellite markers to determine the allelic imbalances in the chromosomal loci. They could demonstrate that regions of differing histology showed identical genetic losses. Thus, the gliomas investigated were more homogeneous in their genotype than in their phenotype supporting a monoclonal origin [15].

Also for meningeomas, loss of heterozygosity (LOH) and its biological or clinical predictivity was analyzed. Mihaila *et al*. examined the loss of heterozygosity at individual loci on chromosome 10 in meningeomas [16]. They used LMD to isolate tumour tissue representative of the tumour grade from 208 sporadic and recurrent meningeomas of 173 patients. DNA was isolated and PCR amplified at 11 polymorphic microsatellite markers mapping to chromosome 10. The group observed allelic deletions early in the development of meningeomas. With progression to higher grade the incidence and complexity of allelic loss increased. They further investigated the correlation between the LOH results with respect to tumour location, histology and grade, as well as patient race, age, gender, recurrence and survival. They identified in their cases correlations between individual loci and age, histology and location, suggesting that genetic differences could underlie the genesis of tumours with respect to age of onset, different histologies or different locations [16].

These examples show how LMD can be applied in molecular identification and classification of brain tumours. However, many more studies with higher number of cases will be needed to reach the following goals in brain oncology:

- to test whether regions of differing histology are genetically identical or different;
- to show that identified markers are representative for the whole tumour, enabling an accurate and reliable molecular classification of the tumour,
- to show that the identified markers have prognostic significance and the potential to support the clinical approach for the treatment of patients.

Further clinical applications

Another application of LMD in clinical research is in the field of the diagnosis of infectious diseases, especially of viral infections, even though most of the studies have so far been performed using post-mortem tissue. Some of the studies confirmed known results proving that LMD does not alter the expression of genes. Trillo-Pazos *et al*. used LMD to examine the presence of HIV-1 DNA in microglia, astrocytes and neurons in paraffin-embedded frontal cortex and basal ganglia post-mortem tissue from four cases with HIV-1 encephalitis and two controls [17]. They identified the different cell-types and infected cells by applying immunohistochemical staining before LCM. They showed that in addition to microglia, infection of neurons and of ~1% of the astrocytes may contribute to the development of HIV-1 disease in the brain and might facilitate HIV-associated dementia [17].

The group of Cermelli *et al*. was interested in whether the plaques of multiple sclerosis patients were significantly more infected by HHV6 (human herpesvirus 6) than normal-appearing white matter. Plaques and normal-appearing white matter from post-mortem were isolated via LMD and both investigated for HHV6-DNA by PCR. The authors found that plaques were significantly more infected by HHV6 than normal white matter [18].

The investigation of post-mortem tissue from patients suffering from neurodegenerative disease might help to decipher the pathomechanism of these diseases. Another issue for such investigations is the identification of markers for clinical diagnostics. One example for such investigations is the work by Liao *et al*. who used LMD to isolate ~2000 thioflavin-S stained amyloid plaques from frontal and temporal cortex of two post-mortem Alzheimer disease (AD) brains [19]. They analyzed the protein components by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS). Among the total 488 proteins identified in the plaques, 26 were enriched in the plaques of both cases compared to the surrounding non-plaque tissues enabling for further cellular pathway and disease biomarker identification in AD [19].

However, LMD might also be applied for the investigation of less common diseases. Connor *et al*. used LMD to

BOX 1

Principle of linear RNA amplification

Linear RNA amplification increases the amount of RNA present in a sample by directly amplifying the original template of cDNA (cDNA). The basic antisense RNA (aRNA) amplification protocol involves the incorporation of a specialized oligo-dT primer containing the sequence of a T7 RNA polymerase promoter into the cDNA of the sample mRNAs.

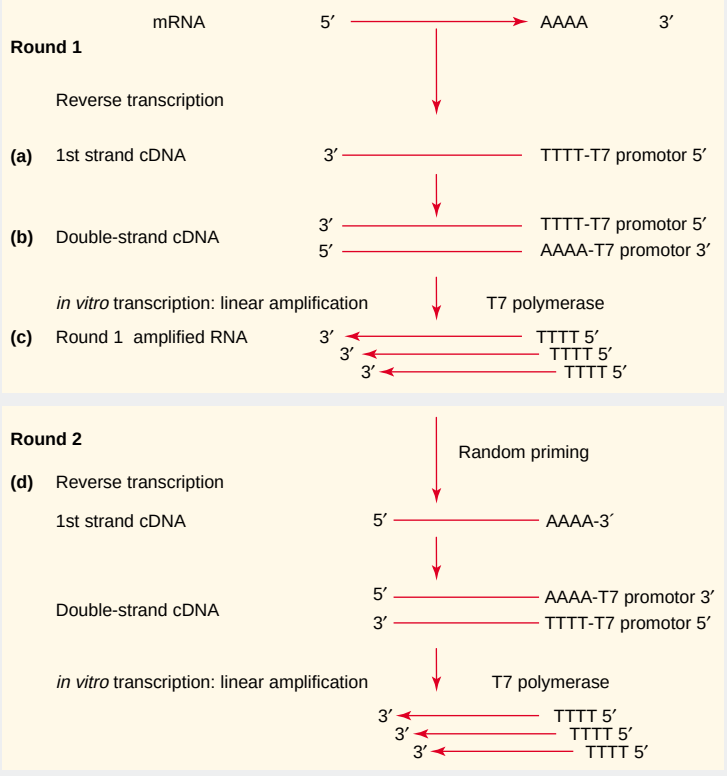

FIGURE I

(a) After second-strand formation, **(b)** highly concentrated T7 RNA polymerase transcribes the double stranded template into amplified antisense RNA **(c)** that can again be reverse-transcribed (e.g. by random priming) starting a second round of amplification. **(d)** Many copies can be generated from each original cDNA and yields of up to 1,000,000-fold can be achieved after as few as two rounds of *in vitro* transcription. The relative abundance of individual mRNA sequences of the original RNA sample is essentially maintained throughout this linear amplification procedure because the activity of the T7 RNA polymerase is generally unaffected by individual template concentrations or the sequences of the transcribed templates. A potential problem with an increasing 3′ bias of the amplified RNA samples does not influence the quality of the microarray data because most microarray probe sets are 3′ biased. Several modifications of the original amplification method have been published [22–24]. Variation in the amplification protocol might be necessary depending on the microarray used. For reviews on the combination of linear RNA amplification and microarray analysis, see references [25,26].

isolate 1000–5000 neuromelanin-containing cells from the substantia nigra from autopsy brains of four patients who had primary restless legs syndrome (RLS). RLS is a sensory-movement disorder characterized by dysesthesias or paresthesias mainly of the legs of unknown aetiology with iron deficiency in the substantia nigra and putamen assumed to play a role. In this study, a quantitative immunoblot assay of the proteins isolated from the captured neuromelanin containing cells revealed that ferritin, divalent metal transporter 1, ferroprotein and transferritin receptor were decreased in neuromelanin cells, and transferrin was increased; this supports the hypothesis that iron deficiency contributes to RLS [20].

Laser microdissection in basic research

Target choices in drug discovery are supported by detailed knowledge of disease pathways. Target discovery and validation has already benefited from molecular profiling because it provides a broad view of the state of cells and tissues. Disease-related genes and functions are hoped to be determined by comparing expression levels of genes in normal and diseased tissue or by studying responses to cellular perturbations [21]. However, pathway identification and target choices are very delicate in neuroscience due to the complexity of the brain, where even individual neighbouring cells frequently have different functions. Moreover, the extent of contribution of different cell types to a certain disease is largely unknown. This makes it particularly difficult to set-up disease models, either in cell culture or on the level of whole tissues for pathway finding. Laser microdissection facilitates pathway identification from individual cell types out of intact tissue. This way, the contribution of defined cell types to pathological conditions can be investigated.

Information on the expression of genes in defined brain regions is crucial to the understanding of its function in normal and diseased states. Several groups have used LMD to identify characteristic genes or gene expression patterns to define specific brain regions like different nuclei or subnuclei. In many cases, they have combined LMD and microarray hybridizations in their approaches in conjunction with a linear RNA amplification (Box 1) because the RNA amount isolated from the microdissected specimens (usually in the pg to low ng range) is not sufficient for microarray analysis (several µg).

The amplification has to be able to multiply the starting material in an efficient and highly reproducible manner and must not change the relative amounts of transcripts in different samples. Table 1 lists service providers and kit suppliers for RNA amplification.

Characterizing brain subregions and cell types

Zirlinger and Anderson used the approach of LMD, followed by RNA isolation, subsequent linear RNA amplification and hybridization of the amplified samples to oligonucleotide microarrays to identify genes enriched in three amygdala subnuclei regions of mice. Several amygdala-enriched genes were identified, which were not identified as amygdala-enriched in a previous study using manually dissected whole amygdala tissue because their level of expression was too low and diluted by other transcripts from the whole tissue. Thereby, the importance of the laser-microdissection approach was validated [22]. The

TABLE 1

Service providers and kit suppliers for RNA amplification

Company	Product	Website
Service providers		
Axaron Bioscience AG	Service using Axaminer Technology	www.axaminer.com
Genus Biosystems	Service with Arcturus RiboAmp Kit	www.genusbiosystems.com
Icoria Bioscience	Service using Arcturus technology	www.icoria.com
Kit suppliers		
Agilent Technologies	Low RNA Input Linear Amplification Kit	www.chem.agilent.com
Ambion	Message Amp aRNA Kits, 5X MegaScript® T7 kit (offers also service)	www.ambion.com
Arcturus Bioscience	RiboAmp RNA Amplification Kits	www.arctur.com
Artus	ExpressArt	www.artus-biotech.com
Enzo Life Sciences	BioArrayTM RNA Amplification and Labeling Kit	www.enzolifesciences.com
Genisphere	SenseAMP TM RNA Amplification Kit	www.genisphere.com
Invitrogen	SuperScriptTM RNA Amplification System	www.invitrogen.com
NuGEN	OvationTM System	www.nugeninc.com
Roche Applied Science	Microarray Target Amplification Kit	www.roche-applied-science.com
SuperArrayBioscience	TrueLabeling-Amp Linear RNA Amplification Kit	www.superarray.com

gene expression profile of seven rat brain nuclei, the locus coeruleus, dorsal raphe nucleus, parvocellular division and magnocellular division of the paraventricular nucleus and CA1, CA3 and dentate gyrus divisions of the hippocampus were examined by Bonaventure *et al.* who isolated 100 cells by LMD from each rat brain nucleus and used T7-based RNA amplification and cDNA microarrays for gene expression analysis. Employing hierarchical clustering of the expression data they identified several genes with elevated expression within each nucleus, which exhibited a lower expression in the other nuclei. They validated their results by RT-PCR and *in situ* hybridization [23]. Other groups used LMD and microarray hybridization to investigate the expression profiles of hippocampal subregions. These studies were partly performed as technological feasibility studies for the combination of LMD with RNA amplification and microarray hybridization. The hippocampal subregions are particularly suited for such studies because this brain region is well studied [23,24,25]. Some examples for such feasibility studies are given in the next section. The work by Torres-Munoz *et al.* describes the comparison of the CA1 and CA3 subregions in human post-mortem tissue [24]. Datson *et al.* compared the CA3 subregion versus dentate gyrus from rat slices [25] and Kamme *et al.* analyzed gene expression in single cells from the CA1 subregion of an adult rat [11]. The latter group already examined in a proof-of-concept study large- and small-sized neurons in the dorsal root ganglia [11].

Further examples using LMD to examine gene expression of different cell-types in the brain comprize the investigation of stem cells [26], vessels [10,27] or pituitary gland cells [28,29]. Ye *et al.* showed that it is possible to isolate pure cell populations of oligodendrocyte precursor cells, expressing the surface glycoprotein NG2, which was identified immunohistochemically before LMD. They verified by RT-PCR that this cell population did not contain traces of other cell-types like astrocytes or neurons [26].

Disease models

Laser microdissection has not only been applied for the investigation of the pathomechanism of diseases in human tissue but has also been used in animal models. In the field of neuroscience, the neurodegenerative diseases and the modifications of the aged brain are of particular interest. One example for the investigation of age-related gene expression changes is the work by Shimamura *et al.* [30]. They investigated the age-related vulnerability of hippocampal subregions and compared the gene expression of several age-related genes in CA1, CA3 and dentate gyrus subfields in young and aged rat hippocampus. They combined LMD, linear RNA amplification and northern blot analysis and ribonuclease protection assay to study the increased vulnerability of aged brain in a traumatic brain injury model. They confirmed the selective vulnerability of the different hippocampal subregions because they found that p21 and brain derived neurotrophic factor (BDNF) were expressed in CA1, CA3 and dentate gyrus subregions of aged rats. Following traumatic brain injury, these genes exhibited elevated expression in all three subregions analyzed. By contrast, these genes were not expressed in young animals. After traumatic brain injury in young animals an increased expression could only be observed in the CA3 subregion [30].

Mode-of-action studies of drugs

Laser microdissection is also increasingly being used to study drug effects in defined brain regions. Backes and Hemby examined gene profiles of the ventral tegmental dopamine neurons in rats after acute and chronic cocaine

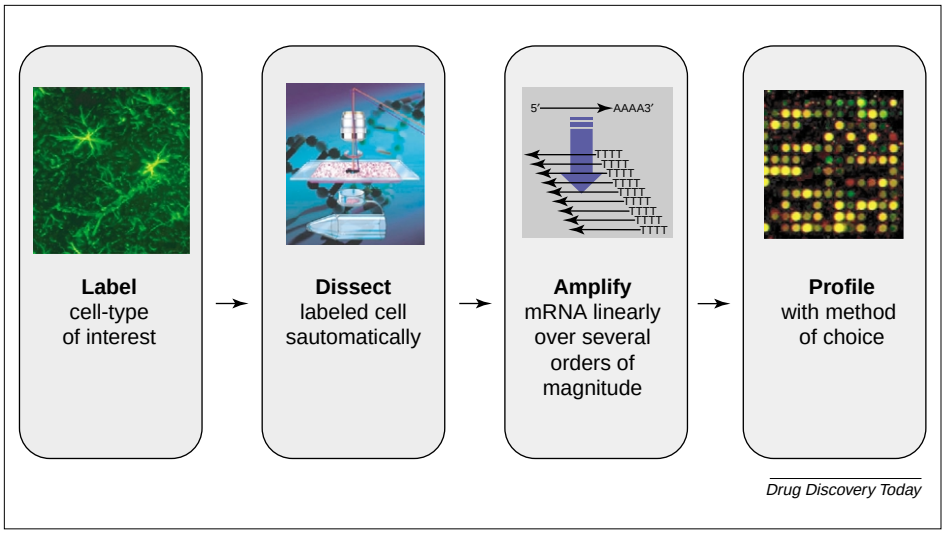

FIGURE 2

The Axaminer technology for cell-type specific transcription profiling. The cell-type of interest is visualized before laser microdissection by immunohistochemistry. Labelled cells are automatically identified by the laser microdissection software, cut out and collected. RNA is isolated and linearly amplified, providing enough material for microarray hybridization. Genome-wide transcription analysis is performed using standard microarray formats.

administration by microdissecting tyrosine hydroxylase immunopositive cells from the ventral tegmental area out of paraffin embedded tissue slices [31]. The expression of 95 genes was assessed on nylon membrane cDNA macroarrays. They found a statistically significant up- and down-regulation of specific GABA-A receptor subunits, $\alpha 1$ and $\gamma 2$, respectively, after acute and chronic cocaine administration. This potentially mirrors specific neuroadaptions associated with cocaine and determining functional properties of the GABA A receptor [31].

We investigated the effect of the antidepressant amitriptyline on region specific and cell-type specific gene expression in mice using the Axaminer™ technology. This technology consists of an optimized workflow for cell-type specific expression profiling comprizing RNA-friendly immunohistochemistry, LMD, linear RNA amplification and microarray hybridization (Figure 2). A time-dependent upregulation of specific receptors and ion channels in subregions like the nucleus accumbens, the raphe nucleus and the hippocampal subregions CA1 and CA3 in the treated animals compared to the sham group was found. Approximately one hundred genes were detected as regulated in an LMD approach applying the Axaminer technology, whereas in a whole tissue approach only a few genes were found to be regulated [32].

Conclusion

Laser microdissection has become a versatile tool to examine complex cell populations of the brain at the DNA, RNA or protein level. Applications range from basic research studying cell populations, to the analysis of disease models and drug discovery related research, as well as clinical research. Tissue samples include sections from fresh-frozen as well as fixed and paraffin-embedded specimens. LMD itself has become a mature and reliable technology. However, further standardization is required in several upstream and downstream applications to obtain results that can be compared between different laboratories. This is particular evident if complex downstream applications, such as microarray or proteome analysis, are used and when the technology in applied clinical diagnostics.

Successful drug discovery in neuroscience will depend upon knowledge about the specific contribution of defined cell types and brain regions to the pathomechanism of neurological disorders. More specific drugs with fewer side effects and better defined mode-of-actions can be designed by understanding the relevant pathways in the molecular pathomechanism of neurological disorders. The investigation of laser microdissected cell populations and brain regions reduces the complexity of the brain tissue, which is an important prerequisite for the identification of relevant pathways and targets already at an early stage in the drug discovery process. Although LMD certainly holds great promise further research is required to promote its results into clinical development.

References

1 Emmert-Buck, M.R. *et al.* (1996) Laser capture microdissection. *Science* 274, 998–1001
2 Taylor, T.B. *et al.* (2004) Microgenomics: Identification of new expression profiles via small and single-cell sample analyses. *Cytometry A* 59, 254–261
3 Schütze, K. *et al.* (2003) Laser pressure catapulting. In *DNA Microarrays. A Molecular Cloning Manual* (S.J. Bowtell D., ed.), pp. 331–356, Spring Harbor Laboratory Press
4 Nafe, R. *et al.* (2003) Laser microdissection and pressure catapulting (LMPC) in paraffin sections mounted on glass slides. A methodological report. *Pathol. Res. Pract.* 199, 411–414
5 van Dijk, M.C. *et al.* (2003) Improved resolution by mounting of tissue sections for laser microdissection. *Mol. Pathol.* 56, 240–243
6 Qin, Y. *et al.* (2003) Gene expression patterns in rat dentate granule cells: comparison between fresh and fixed tissue. *J. Neurosci. Methods* 131, 205–211
7 Van Deerlin, V.M. *et al.* (2002) Optimizing gene expression analysis in archival brain tissue. *Neurochem. Res.* 27, 993–1003
8 Ginsberg, S.D. and Che, S. (2004) Combined histochemical staining, RNA amplification, regional, and single cell cDNA analysis within the hippocampus. *Lab. Invest.* 84, 952–962
9 Burbach, G.J. *et al.* (2004) Laser microdissection of immunolabeled astrocytes allows quantification of astrocytic gene expression. *J. Neurosci. Methods* 138, 141–148
10 Mojsilovic-Petrovic, J. *et al.* (2004) Development of rapid staining protocols for laser-capture microdissection of brain vessels from human and rat coupled to gene expression analyses. *J. Neurosci. Methods* 133, 39–48
11 Luo, L. *et al.* (1999) Gene expression profiles of laser-captured adjacent neuronal subtypes. *Nat. Med.* 5, 117–122
12 Kleihues, P. and Cavenee, W.K. (2000) Pathology and genetics of tumors of the nervous system. In *WHO Classification of Tumours*, Lyon, France, IARC Press
13 Carpentier, A.F. (2005) Neuro-oncology: the growing role of chemotherapy in glioma. *Lancet Neurol.* 4, 4–5
14 Reis, R.M. *et al.* (2001) Genetic evidence of the

neoplastic nature of gemistocytes in astrocytomas. *Acta Neuropathol. (Berl.)* 102, 422–425
15 Walker, C. *et al.* (2003) Phenotype versus genotype in gliomas displaying inter- or intratumoral histological heterogeneity. *Clin. Cancer Res.* 9, 4841–4851
16 Mihaila, D. *et al.* (2003) Meningiomas: analysis of loss of heterozygosity on chromosome 10 in tumor progression and the delineation of four regions of chromosomal deletion in common with other cancers. *Clin. Cancer Res.* 9, 4435–4442
17 Trillo-Pazos, G. *et al.* (2003) Detection of HIV-1 DNA in microglia/macrophages, astrocytes and neurons isolated from brain tissue with HIV-1 encephalitis by laser capture microdissection. *Brain Pathol.* 13, 144–154
18 Cermelli, C. *et al.* (2003) High frequency of human herpesvirus 6 DNA in multiple sclerosis plaques isolated by laser microdissection. *J. Infect. Dis.* 187, 1377–1387
19 Liao, L. *et al.* (2004) Proteomic characterization of postmortem amyloid plaques isolated by laser capture microdissection. *J. Biol. Chem.* 279, 37061–37068
20 Connor, J.R. *et al.* (2004) Decreased transferrin receptor expression by neuromelanin cells in restless legs syndrome. *Neurology* 62, 1563–1567
21 Stoughton, R.B. *et al.* (2005) How molecular profiling could revolutionize drug discovery. *Nat. Rev. Drug Discov.* 4, 345–350
22 Zirlinger, M. and Anderson, D. (2003) Molecular dissection of the amygdala and its relevance to autism. *Genes Brain Behav.* 2, 282–294
23 Bonaventure, P. *et al.* (2002) Nuclei and subnuclei gene expression profiling in mammalian brain. *Brain Res.* 943, 38–47
24 Torres-Munoz, J.E. *et al.* (2004) Gene expression profiles in microdissected neurons from human hippocampal subregions. *Brain Res. Mol. Brain Res.* 127, 105–114
25 Datson, N.A. *et al.* (2004) Expression profiling in laser-microdissected hippocampal subregions in rat brain reveals large subregion-specific differences in expression. *Eur. J. Neurosci.* 20, 2541–2554
26 Ye, P. *et al.* (2003) Mouse NG2+ oligodendrocyte precursors express mRNA for proteolipid protein but not its DM-20 variant: a study of laser microdissection-captured NG2+ cells. *J. Neurosci.* 23, 4401–4405
27 Ball, H.J. *et al.* (2002) Isolating vessels from the mouse brain for gene expression analysis using laser capture microdissection. *Brain Res. Brain Res. Protoc.* 9, 206–213
28 Jin, L. *et al.* (2001) Analysis of homogeneous populations of anterior pituitary folliculostellate cells by laser capture microdissection and reverse transcription-polymerase chain reaction. *Endocrinology* 142, 1703–1709
29 Gamel-Didelon, K. *et al.* (2003) Molecular and physiological evidence for functional gamma-aminobutyric acid(GABA)-C receptors in growth hormone-secreting cells. *J. Biol. Chem.* 278, 20192–20195
30 Shimamura, M. *et al.* (2004) Laser capture microdissection and analysis of amplified antisense RNA from distinct cell populations of the young and aged rat brain: effect of traumatic brain injury on hippocampal gene expression. *Brain Res. Mol. Brain Res.* 122, 47–61
31 Backes, E. and Hemby, S.E. (2003) Discrete cell gene profiling of ventral tegmental dopamine neurons after acute and chronic cocaine self-administration. *J. Pharmacol. Exp. Ther.* 307, 450–459
32 Boehm, C. *et al.* (2004) Cell type specific gene expression profiling. *Genet. Eng. News* 24, 38–39
33 Baugh, L.R. *et al.* (2001) Quantitative analysis of mRNA amplification by *in vitro* transcription. *Nucleic Acids Res.* 29, E29
34 Kamme, F. *et al.* (2003) Single-cell microarray analysis in hippocampus CA1: demonstration and validation of cellular heterogeneity. *J. Neurosci.* 23, 3607–3615
35 Van Gelder, R.N. *et al.* (1990) Amplified RNA synthesized from limited quantities of heterogeneous cDNA. *Proc. Natl. Acad. Sci. U. S. A.* 87, 1663–1667
36 Buesa, C. *et al.* (2004) DNA chip technology in brain banks: confronting a degrading world. *J. Neuropathol. Exp. Neurol.* 63, 1003–1014
37 Mirnics, K. and Pevsner, J. (2004) Progress in the use of microarray technology to study the neurobiology of disease. *Nat. Neurosci.* 7, 434–439