Published in final edited form as:

Anal Biochem. 2012 June 15; 425(2): 120–124. doi:10.1016/j.ab.2012.02.034.

Protein analysis through Western blot of cells excised individually from human brain and muscle tissue

A.O. Koob^{a,*}, L. Bruns^a, C. Prassler^a, E. Masliah^b, T. Klopstock^{a,c}, and A. Bender^{a,d}

^a Department of Neurology, Klinikum Grosshadern, University of Munich, Marchionistr. 23, 81377 Munich, Germany

^b Department of Pathology, 9500 Gilman Drive, University of California, San Diego, La Jolla, CA 92093, USA

^c Friedrich-Baur-Institute, Department of Neurology, University of Munich, Klinikum Innenstadt, Ziemssenstr. 1, 80336 Munich, Germany

^d Therapiezentrum Burgau, Dr.-Friedl-Str. 1, 89331 Burgau, Germany

Abstract

Comparing protein levels from single cells in tissue has not been achieved through Western blot. Laser capture microdissection allows for the ability to excise single cells from sectioned tissue and compile an aggregate of cells in lysis buffer. In this study we analyzed proteins from cells excised individually from brain and muscle tissue through Western blot. After we excised individual neurons from the substantia nigra of the brain, the accumulated surface area of the individual cells was 120,000, 24,000, 360,000, 480,000, 600,000 μm^2 . We used an optimized Western blot protocol to probe for tyrosine hydroxylase in this cell pool. We also took 360,000 μm^2 of astrocytes (1700 cells) and analyzed the specificity of the method. In muscle we were able to analyze the proteins of the five complexes of the electron transport chain through Western blot from 200 human cells. With this method, we demonstrate the ability to compare cell-specific protein levels in the brain and muscle and describe for the first time how to visualize proteins through Western blot from cells captured individually.

Keywords

Laser capture microdissection; Single cell proteomics; Tyrosine hydroxylase; Substantia nigra; Cytochrome oxidase

In this study, we used laser capture microdissection (LCM)¹ to excise individual cells from frozen brain and muscle tissue, accumulated them in lysis buffer, and analyzed protein levels

* Corresponding author. Address: Biology Department, University of Wisconsin – River Falls, 410 South 3rd St., River Falls, WI 54022. Fax: +1 715 425 0738. andrew.koob@uwrf.edu (A.O. Koob).

Publisher's Disclaimer: This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues. Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited. In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit: <http://www.elsevier.com/copyright>

through Western blot. Using LCM to analyze proteins through Western blot has been attempted previously in oncology research with large sections of tissue, but not with individual cells from normal sectioned tissue [1].

LCM is normally used for DNA and RNA expression analysis [2–4]. However, the apparatus has the ability to be effectively utilized in proteomics [5,6]. Studies have used a specific assay for prostate-specific antigen (PSA) to analyze protein amounts through chemiluminescence from individual cells [5]. However, Western blot assays have been the standard method for analyzing differences in protein levels from cells and tissue to compare experimental conditions. After homogenization of tissue, it is currently not possible to perform Western blots to compare protein levels in different cell types from tissue. Yet, especially in neurobiology research, it is important to analyze specific cell populations with distinct characteristics in order to take into account the extreme diversity of brain structure and function.

LCM has also only recently been used in normal brain tissue—not on the single cell level, but to excise the endothelium of the mouse brain [7]. A limitation of LCM in proteomics is the degradation of tissue after immunolabeling. LCM has been used after immunohistochemistry, for RNA analysis to compare gene expression and not to analyze specific protein levels themselves [8]. However, Western blotting after LCM of mass tumor tissue treated immunohistochemically has been shown [9–11], typically by excising a gross area consisting of ~10,000 tumorigenic cells from a frozen section of tissue ~20 µm thick [9,10,12–17]. In this study we use a shortened variation of standard immunohistochemical protocols that was effective for cell discrimination.

Although the LCM apparatus allows for the ability to excise cells out of tissue, the low amount of tissue area that can be captured over time by taking and accumulating individual cells has contributed to the problem of performing Western blot from individual cells [1]. In this method we also employ special blocking solution and sensitive chemiluminescence to achieve protein visualization from cells excised individually from tissue, aggregated in lysis buffer, and analyzed through Western blot.

In the following experiments we gather individual cells of specific types from brain and muscle as visualized histologically through immunohistochemistry, cyclooxygenase staining, and Nissl stain and were able to analyze protein level differences through Western blot. In muscle tissue, we were able to visualize proteins in as little as 200 cells excised individually from 25-µm-thick sections. In brain, proteins were visible in as little as 240–280 large dopaminergic neurons from the substantia nigra.

¹ *Abbreviations used:* DAB, diaminobenzene; LCM, laser capture microdissection; PBS, phosphate-buffered solution; COX, cytochrome oxidase; GFAP, glial fibrillary acid protein; PSA, prostate-specific antigen; SDH, succinate dehydrogenase.

Materials and methods

Tissue

A frozen brain specimen of the substantia nigra (SN) was obtained from the German brain bank (Brain-Net) of a normal aged control patient. Additionally, frozen muscle tissue from the quadriceps of a normal aged control patient was acquired from the Friedrich-Baur-Institute, Munich, Germany. The study protocol was approved by the appropriate ethics committee and the study has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. From the substantia nigra and muscle, frozen sections (10 μ m thickness for substantia nigra, 25 μ m for muscle) were obtained with a cryostat on membrane slides, and frozen at -80°C until further processing.

Histology

Large pyramidal neurons were identified by standard neuro-pathological criteria using Nissl staining [18]. Brain sections were stained histologically with a classical Nissl staining procedure using cresyl violet (Merck, Darmstadt, Germany) with shortened protocols. Tissue was placed in -20°C methanol for 3 min, followed by 3 washes in phosphate-buffered solution (PBS) for 1 min each. Tissue sections on slides were then placed in 1% cresyl violet in acetic acid buffer for 5 min, followed by 1 min in acetic acid buffer, 30 s in double-distilled water, 1 min in 70% ethanol, and 1 min in 100% ethanol. Tissue was allowed to dry for 5 min.

Muscle was stained for sequential COX-SDH enzyme histochemistry according to standard protocols [19,20]. Sections were incubated in cytochrome *c* oxidase medium (100 μ M cytochrome *c*, 4 mM diaminobenzidine tetrahydrochloride, and 20 μ g/ml catalase in 0.2 M phosphate buffer, pH 7.0) for 50 min at 37°C . Next, sections were washed in PBS (3×5 min) and incubated in succinate dehydrogenase (SDH) medium (130 mM sodium succinate, 200 μ M phenazine methosulphate, 1 mM sodium azide, 1.5 mM nitroblue tetrazolium in 0.2 M phosphate buffer, pH 7.0) for 40 min at 37°C . Then sections were washed in PBS, pH 7.4 (3×5 min), and dehydrated in an increasing ethanol series (70%, 95%, $2 \times 100\%$)

Immunohistochemistry

Sections from substantia nigra were first immersed in -20 acetone for 3 min and then washed twice for 2 min each in PBS. They were then pretreated with 1% Triton X-100, 3% hydrogen peroxide, proteinase inhibitor in PBS for 5 min, washed again twice for 1 min each in PBS, and blocked in 10% goat serum matching the animal the secondary antibody was raised in for 5 min. Sections were then placed in primary antibody (mouse anti-NeuN, Millipore, MAB377, rabbit anti-S-100, Abcam) (1:40) for 5 min. Sections were washed twice for 1 min each in PBS and then placed in goat biotinylated secondary antibody (1:40) (Vector Laboratories, goat anti-rabbit, AK-6401, goat anti-mouse, AK-6402, Burlingame, CA) for 5 min. After washing again two times in PBS, sections were placed in Vectastain ABC reagent (AK-6000) for 7 min, followed by washing once in PBS and developing with 20% diaminobenzene with nickel (DAB-nickel) (Vector Laboratories, SK-4100) for 20 s–5 min. The reaction with DAB-nickel was halted by immersing the sections in double-distilled water for 30 s. Nickel was used so the stain was a bluish gray color to distinguish it from the

brown melanin in the substantia nigra. Sections were then dehydrated in 70% ethanol for 1 min followed by 100% ethanol for 1 min and allowed to dry for 5 min.

Laser capture microdissection

From the brain, neurons and astrocytes were dissected by an LCM system (AS LMD, Leica, Germany) [21,22] (Fig. 1). While performing preliminary experiments with the Western blotting technique to maximize the visualization of proteins, it became clear that taking the equivalent in area of the amount of cells was more applicable to analyzing protein levels than to harvest similar cell numbers. We determined that 120,000 μm^2 of accumulated surface area of specific individual cells excised was equivalent to 120–140 neurons. Therefore, in the cell gradient for neurons to discern whether our cells were specific we took 120,000 μm^2 ~ 120–140 cells, 240,000 μm^2 ~ 240–280 cells, 360,000 μm^2 ~ 360–420 cells, 480,000 μm^2 ~ 480–560 cells, and 600,000 μm^2 ~ 600–700 cells. Protein visualization through Western blot was effective at 240,000 μm^2 . Therefore, for the following experiments, to make certain of satisfactory visualization and counter differences in antibody strength, we took cells individually whose surface area accumulated to 360,000 μm^2 after tissue was stained immunohistochemically for s-100 β for astrocytes and NeuN for neurons.

For muscle cells, 200 cells were taken individually in triplicate from cells that were COX– and for cells that were COX+. COX– staining was determined through blue coloring of the cells and COX+ staining was through brown coloration.

Cells were excised with the LCM device and captured in the cap of 500- μl Eppendorf tubes containing 15 μl lysis buffer (Sigma, C3228) and proteinase and phosphatase inhibitors (Fig. 1). After capture, the tubes were snap-frozen in liquid nitrogen for 10 s and stored at -80°C until further processing.

Western blot analysis

The Western blotting procedure was as follows: Cells in the cap were lysed by vortexing the 500 μl tubes upside down for 2 min. The cells were centrifuged for 20 min at 13.2 rpm. The tubes were then sonicated for 5 min in a sonication water bath (Bandelin Sonorex). Samples were mixed with 5 μl sample buffer and 2 μl reducing agent (Invitrogen), boiled at 70°C for 10 min, and separated on 4–12% SDS-PAGE gels (NuPAGE, Invitrogen). They were then transferred onto 0.22 μM PDVF membranes (Invitrogen) using 1X 3-[cyclohexylamino]-1-propanesulfonic acid (CAPS) transfer buffer containing 20% methanol. Membranes were blocked with Superblock T20 (PBS) blocking buffer (Thermo Scientific) for 1 h, followed by incubation in primary antibody (mouse anti-tyrosine hydroxylase, Abcam, rabbit anti-glial fibrillary acidic protein (GFAP), Dako, Z0334, Mouse anti-Oxphos, Mitosciences MS601) (1:5000) in Superblock T20 blocking buffer for 72 h at 4°C . Membranes were washed 3X half hour in PBST (0.5% Tween) with quick washes in PBS between. Membranes were further incubated with goat anti-mouse or anti-rabbit IgG secondary antibodies conjugated to horse-radish peroxidase (1:160,000, Invitrogen, G21040, G21234) for 1 h, washed 3×15 min in PBST with quick PBS washes between, and visualized with SuperSignal West Femto chemiluminescent substrate (Thermo Scientific, 34095) and

imaged with a Fusion Fx 7 imaging system (Vilber Lourmat, Marne-la-Vallée Cedex 1 France).

Results

Brain

On analysis, the immunoblot expression of tyrosine hydroxylase of individually excised neurons stained with Nissl (cresyl violet) preparation taken from LCM (Fig. 1) increased at an equivalent gradient, with 120,000 μm^2 slightly visible, and definitely visibility at 240,000 μm^2 (Fig. 2). The blot increased dramatically when detecting tyrosine hydroxylase from 480,000 μm of aggregated individual cells to 600,000 μm . To determine the cell specificity of excising individual cells with LCM, we noted that 360,000 μm aggregated of individual astrocytes immunohistochemically stained for s-100 (Fig. 3C) contained GFAP when visualized through Western blot, whereas NeuN-positive neurons contained little or no GFAP. However, individually excised melanin-containing dopaminergic neurons also expressed GFAP through Western blot, but at lower levels than s-100-positive astrocytes (Fig. 3A and B). These results indicate that protein analysis of different cell types is possible after immunohistochemistry.

We also analyzed melanin-containing substantia nigra neurons for the 5 complexes of the mitochondria respiratory chain (Fig. 4). We compared cells with little melanin with cells with high amounts of melanin to determine the difference. Although little or no difference is seen in protein levels, the method allowed us to adequately visualize proteins when excising neurons individually.

Muscle

In muscle, after taking cells stained for cytochrome oxidase (COX), clear expression was seen for all five proteins of the respiratory chain as well (Fig. 5) in the mitochondria. An obvious increase was visible in complexes I and IV in COX+ cells when compared to CO- cells. Since COX is complex IV of the electron transport chain, cells that were COX- were deficient in complex IV. Since, complex IV was less in COX- cells this also confirmed the specificity of the method. Additionally, mitochondrial deficiencies in COX- cells might also account for less complex I also seen in these cells.

Discussion

In biology, current protein analysis through Western blot occurs after homogenizing of whole tissue, eliminating the ability to study proteins only in a specific subset of cells. This limitation inhibits the ability for researchers to compare protein expression in different cell types in variegated tissue like the brain [1]. It also inhibits researchers from understanding protein level differences in cells which are the same but have slightly different properties, for example, COX- and COX+ muscle cells. With immunostaining techniques that enable researchers to visualize different cells in tissue, it would be beneficial to compare cells further for other immunohistochemical differences to further understand cell mechanisms [23].

Previous attempts have been shown using fluorescent assays for prostate-specific antigen [5]. This study showed the capability for LCM to quantify proteins in individual cells using an established method to visualize PSA. Recent developments with two-dimensional differential gel electrophoresis (2D-DIGE) [24,25] have so far only excised out a mass of carcinogenic tissue and has not been utilized for individually excised cells [15], but have shown promise. Additionally, reverse-phase protein microarrays after excising breast cancer tissue have been previously demonstrated [26]. Other uses of LCM to analyze proteins have included tumor cell mass and labeling for analysis in mass spectrometry (MS) [27–29]. The ability to visualize proteins after excision of tumor mass in the brain following immunohistochemistry has been shown previously, but not when comparing different cell types [9].

Here, we not only exhibit the ability to compare proteins in different cell types taken individually from tissue but also demonstrate the ability to visualize proteins through Western blot from individual cells after immunohistochemistry.

By accumulating individual cells, and analyzing proteins directly through Western blot, it allows for a more specific representation of protein levels. We believe the puissance of this technique would help further the understanding and treatment of not only muscle and brain disease but any disease that may be the result of cell-specific protein mechanisms.

Acknowledgments

We give special thanks to the Bavaria California Technology Center (BaCaTec) and Exchange Program for their support with this project. We would also like to acknowledge Luccia Rosemarie Koob for her help with manuscript preparation.

References

1. Xu BJ. Combining laser capture microdissection and proteomics: methodologies and clinical applications. *Proteomics Clin. Appl.* 2010; 4:116–123. [PubMed: 21137037]
2. Emmert-Buck MR, Bonner RF, Smith PD, Chuaqui RF, Zhuang Z, Goldstein SR, Weiss RA, Liotta LA. Laser capture microdissection. *Science.* 1996; 274:998–1001. [PubMed: 8875945]
3. Bonner RF, Emmert-Buck M, Cole K, Pohida T, Chuaqui R, Goldstein S, Liotta LA. Laser capture microdissection: molecular analysis of tissue. *Science.* 1997; 278:1481–1483. [PubMed: 9411767]
4. Simone NL, Bonner RF, Gillespie JW, Emmert-Buck MR, Liotta LA. Laser-capture microdissection: opening the microscopic frontier to molecular analysis. *Trends Genet.* 1998; 14:272–276. [PubMed: 9676529]
5. Simone NL, Remaley AT, Charboneau L, Petricoin EF 3rd, Glickman JW, Emmert-Buck MR, Fleisher TA, Liotta LA. Sensitive immunoassay of tissue cell proteins procured by laser capture microdissection. *Am. J. Pathol.* 2000; 156:445–452. [PubMed: 10666374]
6. Simone NL, Pawletz CP, Charboneau L, Petricoin EF 3rd, Liotta LA. Laser capture microdissection: beyond functional genomics to proteomics. *Mol. Diagn.* 2000; 5:301–307. [PubMed: 11172494]
7. Murugesan N, Macdonald JA, Lu Q, Wu SL, Hancock WS, Pachter JS. Analysis of mouse brain microvascular endothelium using laser capture microdissection coupled with proteomics. *Methods Mol. Biol.* 2011; 686:297–311. [PubMed: 21082378]
8. Fend F, Emmert-Buck MR, Chuaqui R, Cole K, Lee J, Liotta LA, Raffeld M. Immuno-LCM: laser capture microdissection of immunostained frozen sections for mRNA analysis. *Am. J. Pathol.* 1999; 154:61–66. [PubMed: 9916919]

9. Mouldous L, Hunt S, Harcourt R, Harry JL, Williams KL, Gutstein HB. Proteomic analysis of immunostained, laser-capture microdissected brain samples. *Electrophoresis*. 2003; 24:296–302. [PubMed: 12652601]
10. Braakman RB, Luiider TM, Martens JW, Foekens JA, Umar A. Laser capture microdissection applications in breast cancer proteomics. *Methods Mol. Biol.* 2011; 755:143–154. [PubMed: 21761300]
11. He H, Sun G, Ping F. Laser-capture microdissection and protein extraction for protein fingerprint of OSCC and OLK. *Artif. Cells Blood Substit. Immobil. Biotechnol.* 2009; 37:208–213. [PubMed: 19735007]
12. Mouldous L, Hunt S, Harcourt R, Harry J, Williams KL, Gutstein HB. Navigated laser capture microdissection as an alternative to direct histological staining for proteomic analysis of brain samples. *Proteomics*. 2003; 3:610–615. [PubMed: 12748941]
13. Zhang D, Lim SG, Koay ES. Proteomic identification of down-regulation of oncoprotein DJ-1 and proteasome activator subunit 1 in hepatitis B virus-infected well-differentiated hepatocellular carcinoma. *Int. J. Oncol.* 2007; 31:577–584. [PubMed: 17671684]
14. Rodriguez AS, Espina BH, Espina V, Liotta LA. Automated laser capture microdissection for tissue proteomics. *Methods Mol. Biol.* 2008; 441:71–90. [PubMed: 18370312]
15. Zhang D, Koay ES. Analysis of laser capture microdissected cells by 2-dimensional gel electrophoresis. *Methods Mol. Biol.* 2008; 428:77–91. [PubMed: 18287769]
16. Ornstein DK, Gillespie JW, Paweletz CP, Duray PH, Herring J, Vocke CD, Topalian SL, Bostwick DG, Linehan WM, Petricoin EF 3rd, Emmert-Buck MR. Proteomic analysis of laser capture microdissected human prostate cancer and in vitro prostate cell lines. *Electrophoresis*. 2000; 21:2235–2242. [PubMed: 10892734]
17. Martinet W, Abbeloos V, Van Acker N, De Meyer GR, Herman AG, Kockx MM. Western blot analysis of a limited number of cells: a valuable adjunct to proteome analysis of paraffin wax-embedded, alcohol-fixed tissue after laser capture microdissection. *J. Pathol.* 2004; 202:382–388. [PubMed: 14991905]
18. Tatton WG, Greenwood CE. Rescue of dying neurons—a new action for deprenyl in Mptp parkinsonism. *J. Neurosci. Res.* 1991; 30:666–672. [PubMed: 1686284]
19. Taylor RW, Barron MJ, Borthwick GM, Gospel A, Chinnery PF, Samuels DC, Taylor GA, Plusa SM, Needham SJ, Greaves LC, Kirkwood TBL, Turnbull DM. Mitochondrial DNA mutations in human colonic crypt stem cells. *J. Clin. Invest.* 2003; 112:1351–1360. [PubMed: 14597761]
20. Old SL, Johnson MA. Methods of microphotometric assay of succinate dehydrogenase and cytochrome-c oxidase activities for use on human skeletal-muscle. *Histochem. J.* 1989; 21:545–555. [PubMed: 2556354]
21. Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, Jaros E, Hersheson JS, Betts J, Klopstock T, Taylor RW, Turnbull DM. High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nat. Genet.* 2006; 38:515–517. [PubMed: 16604074]
22. Bender A, Schwarzkopf RM, McMillan A, Krishnan KJ, Rieder G, Neumann M, Elstner M, Turnbull DM, Klopstock T. Dopaminergic midbrain neurons are the prime target for mitochondrial DNA deletions. *J. Neurol.* 2008; 255:1231–1235. [PubMed: 18604467]
23. Kunz GM Jr, Chan DW. The use of laser capture microscopy in proteomics research—a review. *Dis. Markers*. 2004; 20:155–160. [PubMed: 15502248]
24. Viswanathan S, Unlu M, Minden JS. Two-dimensional difference gel electrophoresis. *Nat. Protoc.* 2006; 1:1351–1358. [PubMed: 17406422]
25. Kondo T. Tissue proteomics for cancer biomarker development: laser microdissection and 2D-DIGE. *BMB Rep.* 2008; 41:626–634. [PubMed: 18823585]
26. Cowherd SM, Espina VA, Petricoin EF 3rd, Liotta LA. Proteomic analysis of human breast cancer tissue with laser-capture microdissection and reverse-phase protein microarrays. *Clin. Breast Cancer*. 2004; 5:385–392. [PubMed: 15585078]
27. Greengauz-Roberts O, Stoppler H, Nomura S, Yamaguchi H, Goldenring JR, Podolsky RH, Lee JR, Dynan WS. Saturation labeling with cysteine-reactive cyanine fluorescent dyes provides

- increased sensitivity for protein expression profiling of laser-microdissected clinical specimens. *Proteomics*. 2005; 5:1746–1757. [PubMed: 15761955]
28. Baker H, Patel V, Molinolo AA, Shillitoe EJ, Ensley JF, Yoo GH, Meneses-Garcia A, Myers JN, El-Naggar AK, Gutkind JS, Hancock WS. Proteome-wide analysis of head and neck squamous cell carcinomas using laser-capture microdissection and tandem mass spectrometry. *Oral Oncol*. 2005; 41:183–199. [PubMed: 15695121]
29. Palmer-Toy DE, Sarracino DA, Sgroi D, LeVangie R, Leopold PE. Direct acquisition of matrix-assisted laser desorption/ionization time-of-flight mass spectra from laser capture microdissected tissues. *Clin. Chem*. 2000; 46:1513–1516. [PubMed: 10973907]

Fig.1.

Neurons prepared and captured with LCM. Cresyl violet stained neurons with brown melanin pigment visible inside the cell body. From top left, (A) the tissue without Eppendorf cap underneath, (B) the cell with Eppendorf cap underneath ready for capture of excised cells, (C) cell outlined in red for selection, (D) cell dissected out with laser, (E) red selection line removed (scale bar A–E, 50 μ m), (F) and the cell in the Eppendorf cap in lysis buffer with proteinase and phosphatase inhibitors (green arrow). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig.2.

Tyrosine hydroxylase protein expression in single neurons. (A) Western blot of single neurons of the substantia nigra for tyrosine hydroxylase (TH). Cells were lasered out of the tissue as described in Fig. 1. When the areas of the single cells reached the totals listed, the Western blot was performed. In B, quantification of the pixel density revealed a gradient increase; a large increase was seen in the blot with the most cells.

Fig.3.

Western blot of single cells after immunohistochemistry. (A) Western blot for glial cells (astrocytes) dissected out after immunohistochemistry for s-100 β , melanin, and neurons after immunohistochemistry for NeuN. The total accumulated surface area of the individual cells taken for each lane was 360,000 μm^2 . Western blot was for glial fibrillary acid protein (GFAP), an astrocyte marker. (B) Very little GFAP is seen in the lane for NeuN-expressing neurons but it is seen in the lane for s-100 β -expressing astrocytes. In C, astrocytes visualized through immunohistochemistry and dissected with LCM as described in Fig. 1 (D, E) (scale bar, 25 μm).

Fig.4.

Western blot from single cells for mitochondrial complexes in the brain. Western blot for an antibody recognizing the 5 complexes of the electron transport chain in the mitochondria, indicating the proficiency with this technique with another antibody. Melanin-expressing cells were excised from the substantia nigra as shown in Fig. 1.

Fig.5.

Single cell Western blot from muscle. (A) Cytochrome oxidase (COX)-labeled muscle cells were dissected out (200 each for COX– in blue, and COX+ in brown) and a Western blot was performed for the 5 complexes of the respiratory chain (I–V). In B, COX+ cells that have been excised from quadriceps tissue are shown in lysis buffer in the Eppendorf cap. Western blot analysis in C showed higher expression of the enzyme in complexes II and IV in cells that were COX+, indicating that the specificity of the method. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)