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Using differential solubilization and 2-D gel electrophoresis to visualize increased numbers of proteins in the human cortex and caudate nucleus and putamen

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Abstract

The aim of this study was to determine if differential solubilization of human CNS proteins would increase the total number of proteins that could be visualized using 2-D gel electrophoresis. Hence, proteins were solubilized into Tris, CHAPS and SB3–10 before separation across a pH 4–7 IEF gradient and a 12–14% SDS polyacrylamide gel, which could be achieved with a run-to-run variation of 35% in spot intensity. Because Western blot analyses suggested proteins could be in more than one detergent fraction, we completed a conservative analyses of our 2-D gels assuming spots that appeared on multiple gels at the same molecular weight and *pI* were the same protein. These analyses show that we had visualized over 3000 unique protein spots across three 2-D gels generated from each sample of human frontal cortex and caudate-putamen. This represented, at worst, a significant increase in the number of spots visualized in the acidic protein spectrum compared to what has been reported in other studies of human CNS. This study, therefore, supports the proposal that the analysis of the human CNS proteome using 2-D gel electrophoresis, combined with appropriate sample preparation, can be used to expand the studies on the pathologies of neurological and psychiatric diseases.

Keywords

Caudate-putamen; Frontal cortex; Human CNS; Human proteome; Protein solubilization

1 Introduction

The unexpectedly low number of genes that make up the human genome [1] suggests that variation in the translation of the genome into the human proteome is what provides the

biochemical diversity needed to drive diverse and complex cellular functions. Given that protein expression is a key factor in the control of the cellular processes, it is significant that improving technologies allow the screening of the human proteome [2]. The most developed methodology that allows the study of the human proteome is 2-DE [3] and this methodology has proven particularly valuable in visualizing and quantifying proteins in human CNS [4].

The majority of 2-DE studies have focused on identifying altered protein expression in disease cohorts without considering the complexity of protein expression in the human CNS. This is a major failing as there are, as a minimum, significant differences between protein expression in left and right temporal lobes from the same subjects who died from non-CNS related diseases [5]. This hemispheric finding suggests that protein expression varies between CNS regions within a hemisphere; this is significant because the human cortex and caudate nucleus and putamen (CP) have long been implicated in psychiatric diseases such as schizophrenia [6,7], bipolar disorder [8,9] and unipolar depression [10,11]. Therefore, before studying protein expression in these two CNS regions from subjects with psychiatric diseases we have determined how protein expression varies between subjects with no history of a psychiatric or neurological disorder (Controls).

The challenge in fully understanding the diversity of protein expression in the human CNS is to be able to visualize as many proteins as possible in any given tissue sample. This is particularly important when using 2-DE, as, used with crude tissue homogenate, this methodology is constrained by limits in the resolution, isolation and quantification of proteins expressed in any sample. However, as with 1-DE, processing tissue to enrich proteins of interest can overcome some of the technical limitations of 2-DE including the visualization of low-abundance protein. One well-established, simple and reproducible mechanism to fractionate proteins is by differential solubilization [12,13]. Such protein fractionation should increase the abundance of low-abundance proteins relative to total protein load and, hence, allow more proteins to be visualized after 2-DE. With this objective in mind, we have separated proteins in human dorsolateral prefrontal cortex (DLPFC) and CP after fractionation using differential protein solubility, using 2-DE. We have also investigated the ability of tissue solubilization to separate specific proteins using Western blot analyses.

2 Materials and methods

2.1 Materials

Sequencing grade trypsin was obtained from Promega, Sydney, Australia. PlusOne Silver Stain, ExcelGel SDS 12–14% gradient gels, IPG strips, the IPGphor IEF system, the Multiphor II flatbed system, the ImageMaster flat-bed scanner, the ImageMaster 2D Elite image analysis software and the Ettan MALDI-TOF Pro mass spectrometer were obtained from Amersham Biosciences, Sydney, Australia. Broad molecular range protein markers and ReadyPrep Sequential Extraction Kit were obtained from Bio-Rad Laboratories (Hercules, CA). Goat anti-mouse IgG conjugated with HRP was obtained from Dako (Australia) Pty, Botany, Australia. Anti-human 14-3-3- ζ antibody was obtained from IBL *via* Abacus Diagnostics Australia, Brisbane, Australia; anti-human apolipoprotein E (apoE) and neuregulin 1 (Nrg1) antibody was obtained from Abgent *via* BioCore Pty, Alexandria, Australia. Human α -synuclein antibody was obtained from BD Biosciences, North Ryde, Australia whilst anti-human actin, SNAP 25, synaptophysin, syntaxin and VAMP antibodies were obtained from Chemicon Pty, Boronia, Australia. Finally, anti-human GFAP and NCAM antibodies and all other reagents were purchased from Sigma-Aldrich, Sydney, Australia.

2.2 Tissue collection

After gaining approval from the Ethics Committee of the Victorian Institute of Forensic Medicine and the North Western Mental Health Program Behavioural and Psychiatric Research and Ethics Committee, samples of gray matter from the DLPFC (Brodmann's area 9: BA 9) and CP were collected from four individuals with no known history of neurological or psychiatric disorders (Table 1). Cortical tissue dissection was completed using gross landmarks to define cytoarchitectonic regions; hence, BA 9 was taken as the region of the CNS on the lateral surface of the frontal lobe and includes the middle frontal gyrus superior to the inferior frontal sulcus. Tissue was taken from a defined area of the caudate nucleus rostral to the anterior commissure. The study of gray matter in the cortex was to avoid the confounding factor of differential myelination between the CP and the white matter of the human cortex. The tissue samples were rapidly frozen and maintained at -80°C until required.

2.3 Sample preparation

Proteins in human BA 9 and CP were separated by differential detergent fractionation using the ReadyPrep Sequential Extraction Kit. In one instance, a sample of BA 9 from a single donor was divided into two aliquots and each aliquot was processed through the process of fractionation and 2-DE with the resulting gels being compared to assess the reproducibility spot intensities on each resulting 2nd dimension gel. For all tissue samples, approximately 200 mg tissue was homogenized by hand (glass-Teflon) into 4×w:v of 40 mM Tris. The homogenate was centrifuged at 20 000×g for 10 min and the supernatant decanted and frozen at -80°C (Extract 1). The pellet was washed twice in 40 mM Tris and then suspended in 0.5×original w:v of 8 M urea, 4% CHAPS, 40 mM Tris, 0.2% ampholytes 3–10, 2 mM tributyl phosphine. The suspension was mixed thoroughly for 5 min, centrifuged at 20 000×g for 10 min and the supernatant decanted and frozen at -80°C (Extract 2). The pellet was washed twice in 8 M urea, 4% CHAPS, 40 mM Tris, 0.2% ampholytes 3–10, 2 mM tributyl phosphine and then the pellet was suspended in an equal volume of 5 M urea, 2 M thiourea, 2% CHAPS, 2% SB3–10, 40 mM Tris, 0.2% ampholytes 3–10, 2 mM tributyl phosphine. The suspension was mixed thoroughly for 5 min, centrifuged at 20 000×g for 10 min and the supernatant decanted and frozen at -80°C (Extract 3).

2.4 1-DE and Western blot analyses

To determine the utility of differential detergent solubilization as a method of protein fractionation, 20 µg protein from each detergent fraction was separated by SDS-PAGE at a constant voltage of 150 V.

The proteins on each gel were transferred overnight onto NC membrane (Hybond-ECL) at constant 40 mA and were visualized using a Ponceau S stain to ensure that the transfer had been effective. The membranes were then probed with a variety of primary antibodies under conditions that were optimized for each antibody. Following processing as detailed, all membranes were washed four times for 5 min in Tris-buffered saline/0.1% Tween 20 (TTBS) at room temperature. For each primary antibody, the secondary antibody was a Dako goat anti-mouse IgG conjugated with HRP. Subsequent to the final wash, the membranes were incubated for 5 mins at room temperature with Pierce Super-signal ECL solution. Each NC was then imaged using a Kodak 440CF image station. The densities of the immunogenic bands for each primary antibody in each detergent fraction was then expressed as a percentage of the summed intensities across all three detergent fractions

2.5 2-DE

Immediately prior to electrophoresis the protein concentrations of each of the three extracts were determined using a modified Bradford protein assay [14].

CNS extracts were diluted in a hydration solution containing 7 M urea, 2 M thiourea, 2% CHAPS, 0.5% ampholytes 4–7 and 18 mM DTT to yield protein concentrations of 100 µg in 350-µL hydration solution (Extracts 1 and 2) or 50 µg in 350-µL hydration solution (Extract 3). Each sample was then loaded onto IPG strips (18 cm, pH gradient 4–7) by passive hydration for 6 h in the 350-µL hydration solution containing the solubilized proteins. Sample loading was completed by active hydration for 6 h at 30 V. First dimension separations were performed on an IPGphor IEFsystem after the in-gel sample application by focusing for 1 h at 200 V, 1 h at 500 V, 1 h at 1000 V, 0.5 h gradient to 8000 V and 12 h at 8000 V [15]. During focusing, the temperature was maintained at 20°C and the current limit was set at 50 µA per strip. The focused strips were stored at –80°C until required for the second dimension (1–2 weeks).

Following separation using IEF, proteins were separated according to molecular weight on ExcelGel SDS 12–14% gradient gels using a Multiphor II flatbed system. Prior to electrophoresis the IPG strips were incubated for 2×15 min in 6 M urea, 30% glycerol, 50 mM Tris, 2% SDS containing 1% DTT and then 2.5% iodoacetamide. Bio-Rad broad molecular range protein markers were added to each ExcelGel at each end of the IPG strip. Electrophoresis proceeded at 20 mA/gel for 45 min and 40 mA/gel for 2 h 45 min.

Proteins on each gel were then visualized by a modified silver stain, which is compatible with subsequent tryptic digestion and analysis using MS [16]. Thus, each gel was sensitized by incubating for 60 min at room temperature in 250 mL of sensitizing solution (25.5 g sodium acetate, 15 mL 5% sodium thiosulfate, 112.5 mL ethanol and 247.5 mL distilled water). The sensitized gel was then washed in distilled water 5×15 min at room temperature. The washed gel stained for 60 min at room temperature in 250 mL of a solution containing 150 mL 2.5% silver nitrate and 1350 mL distilled water. The stained gel was then washed twice for 1 min in distilled water and then developed for 15 min in 250 mL developing agent (37.5 g sodium carbonate, 300 µL for-maldehyde and 1500 mL distilled water). This reaction was stopped by immersing the gel in stopping solution (21.9 g EDTA-Na₂ in 1500 mL of distilled water) prior to two further washes of 30 min at room temperature in distilled water. The resulting stained gels were scanned (see below) and stored until further use moist in a sealed plastic container.

2.6 Image analysis

Stained gels were scanned as 8-bit tagged image file format (TIFF) files on an ImageMaster flatbed scanner and exported to ImageMaster 2D Elite image analysis software. For analyses, gels were grouped into “experiment” folders according to anatomical region and extract number. The same spot detection parameters (sensitivity 8571, operator 51, noise 5 and background 8) were applied to all the gels. A reference gel was created for each CNS regions by including all spots imaged across all gels from that region on computer based “virtual” gel. CNS spots merged into the reference gel after they were identified on individual gel images using computer driven automatic matching protocol followed by manual editing. Normalized spot volumes (total spot normalization) for gels within each experiment group were then exported to Excel spreadsheets.

2.7 Data analyses

All statistical analyses were completed by exporting data to Excel. The inter- and intra-assay variation was taken as the average of the standard error of the mean intensity of all spots on

the gels from the same sample processed on separate days. Highly variably expressed proteins were identified by comparing the fold difference in spot intensity of each spot on every gel from the four subjects study. Proteins that were present in gels from more than two subjects and were shown to vary by more than 150% (*i.e.* 2.5-fold) across tissue from different subjects were selected for analysis by MS.

2.8 Protein identification

Protein spots were manually excised from gels and de-stained by incubating for 2×15 min in 50% methanol and then 2×15 min in 25 mM ammonium bicarbonate/50% ACN at room temperature. Each piece of dissected gel was then washed in distilled water (4×20 min) and then incubated for 20 min at room temperature in 50 mM ammonium bicarbonate/50 % methanol. Each gel fragment was then dried and incubated with 110 µL 20 mM ammonium bicarbonate containing 10 µL trypsin solution (1 µg/10 µL) for 16 hr at 37°C.

After tryptic digestion, resulting protein fragments were co-spotted with CHCA matrix solution (1 mg/mL) onto MALDI sample slides and peptide finger printing performed using an Ettan MALDI-TOF Pro mass spectrometer. Monoisotopic peptide masses were obtained and matched to predicted protein sequences on the Swiss-Prot database (Swiss-Prot. 10.30.2003) using MS-Fit (<http://prospector.ucsf.edu>) [17]. A conservative approach of fitting at least four peptide fragments to a predicted sequence was used to initially identify the protein within each spot of interest. The parameters for each search were: digest: trypsin; maximum # missed cleavages: 1; peptide N terminus: hydrogen; peptide C terminus: free acid; cysteine modification: acrylamide; instrument name: MALDI-TOF; minimum matches: 4; and minimum parent ion matches: 1.

3 Results

3.1 1-DE and Western blot analyses

Ten proteins were analyzed by Western blot analyses in the different fractions of human cortex following separation using the ReadyPrep Sequential Extraction Kit (Fig. 1). In four out of ten cases, 80% or more of the immunoreactive material was in Extract 1. In three cases, the immunoreactive material was detectable in both Extract 1 and 2. In the final three cases, there was an immunogenic band in all three extracts but there were less than 20% of the immunogenic materials present in Extract 3.

3.2 2-DE

Typical 2-DE gels obtained from the different protein soluble fractions of human BA 9 and CP are shown (Fig. 2). To assess the variability inherent to the detergent extraction process in tandem with 2-DE, two aliquots of the same sample from BA 9 were processed independently on separate days. These data showed that the mean variance in spot density for proteins in Extracts 1 and 2 was 25% whilst the mean variance of protein spots in Extract 3 was 34%.

The total number of spots from each donor ranged from 1564 to 2241 in BA 9 and 1699 to 2203 in the CP (Table 2A). Overall, across the tissue from the four donors, 3817 spots were visualized in BA 9 and 4263 spots were visualized in the CP. In most cases, Extract 1 (Tris soluble proteins) allowed the visualization of the most protein spots in both CNS regions studied (except CP in donor 2). For all extracts, the majority of spots visualized in samples only appeared in tissue from a single individual. However, 709 spots were present in all gels from BA 9 and 680 spots were present in all gels from the CP (Table 2B). In addition, 1859 (48.7%) in BA9 and 1868 (43.8%) in CP were present in two or more donors, representing

the minimum potential proteins that could be sequence and analyzed to give information on 50% or more of the population studied.

Our Western blotting experiments showed that the same protein could be in multiple CNS extracts and hence be present on multiple gels. Thus, to obtain a more conservative estimate of proteins that could be visualized using detergent fractionation, we identified spots across the three second dimension gels generated from each CNS sample that had similar combinations of molecular weight ($\pm 10\%$) and pI ($\pm 10\%$) and assumed these spots represented a single protein. This approach identified 260 spots in BA 9 and 333 spots in the CP that represented proteins that could be present across multiple gels. In BA 9, these were made up of 185 spots in gels of Extract 1 and Extract 2, 33 spots on gels of Extract 1 and Extract 3, 30 spots on gels of Extract 2 and Extract 3 and 12 spots on all three gels (Table 2B). This meant that there were 3285 spots visualized in BA9 that only appeared on a single gel. In the CP, there were 259 spots in Extract 1 and Extract 2, 41 spots in Extract 1 and Extract 3, 33 spots in Extract 2 and Extract 3 with five spots being present on all gels. Hence, these data indicated there were 338 spots present on multiple gels and 3623 spots present on single gels.

An analysis of the variability of normalized spot volumes between individuals in both CNS regions was completed on spots present in two or more gels. Significantly, normalized spot volumes variability for 6.2% of the spots in BA 9 and 7.2% of the spots in the CP exceed 100% across individuals (Fig. 3). There was no clear relationship between variability of normalized spot volumes and the number of cases in which the spot was visualized (*i.e.* where a spot was present in tissue from two, three or four donors) in either BA 9 ($r^2 = 0.009$) or CP ($r^2 = 0.006$). Similarly, the variability of spot intensity across donors was not related to the number of gels on which a spot detected [BA 9 ($n = 4$, $r^2 = 0.015$; $n = 3$, $r^2 = 0.010$; $n = 2$, $r^2 = 0.002$): CP ($n = 4$, $r^2 = 0.020$; $n = 3$, $r^2 = 0.007$; $n = 2$, $r^2 = 0.007$)].

Protein spots that showed highest variability ($>150\%$) between individuals were picked from BA 9 and CP for tryptic digestion. Proteins from BA 9 and CP were successfully identified (Table 3), showing that identifying proteins after solubilization and 2-DE was viable.

4 Discussion

In this study, we have examined whether a commercially available detergent based protein separation methodology was effective in separating individual proteins by completing a Western blot analyses to determine the distribution of ten proteins across each detergent fraction. These data show that the majority of immunoreactivity does differentially fractionate predominantly into a single detergent fraction but, in some cases, a proportion of immunoreactivity is present in more than one detergent fraction. Simplistically, this can be interpreted as showing that resolving multiple detergent fractions on more than one 2-D gel would result in some proteins being visualized on multiple gels. However, caution must be applied in interpreting Western blot data in such a simple manner. For example, proteins such as GFAP [18] and NCAM [19] exist in many different isoforms that may separate into different detergent fractions.

Given the limitations of differential detergent solubilization to separate individual proteins completely into separate fractions we analyzed our data to identify the best (every spot on each gel represents a different protein) and worst (every spot with the same molecular weight and pI that appeared on more than one gel was the same protein) outcomes to give a measure of the number spots visualized by combining protein fractionation and 2-DE. Thus, in the best-case scenario, more than 3800 proteins spots were visualized in both CNS regions. Even our most conservative analyses showed we have visualized 3623 unique spots

in the CP and 3285 unique spots BA 9. Given that our analyses is limited to acidic proteins this is a significant improvements when compared to other studies that looked at all proteins on a single gel and stated visualized less than 2000 proteins (*i.e.* spots) [4,5,20–27].

A significant factor imposed on 2-DE, presumably due to the use of data analyses software to log transform data, is a restriction on analyses to protein spots that appear on every gel in every sample of tissue analyzed. Importantly we have shown, both in BA 9 and CP, that normalized spot volumes do not show increased variance with a decrease in number of individuals in which the spot was detected. This would suggest that there is no overriding methodological reason to only focus on spots present on every gel from every tissue donor [20]. Indeed, ignoring so much data would significantly weaken the likelihood of identifying protein expression affected by the pathology of disorders of the human CNS.

Finally, we have shown that the intra-run variation in spot intensity when the same sample is processed through detergent solubilization and 2-DE was 35%. This meant that variation of spot intensity between donors was above inter-assay variation for close to 2000 spots even though this analysis did not include the 55% of spots that were visualized in only one sample. Thus, our data show that using differential protein solubilization as a step in increasing selective protein load on a gel is useful in extending the number of proteins that can be visualized in a human CNS sample. Therefore differential tissue solubilization alone, or in combining this approach with other sample preparation steps, will allow the study of a much greater proportion of the proteome in the human CNS and significantly extend existing studies on the human CNS proteome in neurological and psychiatric disorders.

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Abbreviations

BA	Brodmann's area
CP	caudate nucleus and putamen

5 References

- [1]. Harrison PM, Kumar A, Lang N, Snyder M, et al. A question of size: the eukaryotic proteome and the problems in defining it. *Nucleic Acids Res* 2002;30:1083–1090. [PubMed: 11861898]
- [2]. Righetti PG, Castagna A, Antonucci F, Piubelli C, et al. Proteome analysis in the clinical chemistry laboratory: myth or reality? *Clin. Chim. Acta* 2005;357:123–139. [PubMed: 15970281]
- [3]. Choudhary J, Grant SG. Proteomics in postgenomic neuroscience: the end of the beginning. *Nat. Neurosci* 2004;7:440–445. [PubMed: 15114355]
- [4]. Edgar PF, Douglas JE, Knight C, Cooper GJ, et al. Proteome map of the human hippocampus. *Hippocampus* 1999;9:644–650. [PubMed: 10641757]
- [5]. Chen W, Ji J, Zhao R, Ru B. Comparative proteome analysis of human temporal cortex lobes by two-dimensional electrophoresis and identification of selected common proteins. *Neurochem. Res* 2002;27:871–881. [PubMed: 12396097]
- [6]. Weinberger DR. On localizing schizophrenic neuropathology. *Schizophr. Bull* 1997;23:537–540. [PubMed: 9327520]

- [7]. Kreczmanski P, Heinsen H, Mantua V, Woltersdorf F, et al. Volume, neuron density and total neuron number in five subcortical regions in schizophrenia. *Brain* 2007;130:678–692. [PubMed: 17303593]
- [8]. Buchsbaum MS, Wu J, DeLisi LE, Holcomb H, et al. Frontal cortex and basal ganglia metabolic rates assessed by positron emission tomography with [18F]2-deoxyglucose in affective illness. *J. Affect. Disord* 1986;10:137–152. [PubMed: 2941470]
- [9]. Hwang J, Lyoo IK, Dager SR, Friedman SD, et al. Basal ganglia shape alterations in bipolar disorder. *Am. J. Psychiatry* 2006;163:276–285. [PubMed: 16449482]
- [10]. Matsuo K, Glahn DC, Peluso MA, Hatch JP, et al. Prefrontal hyperactivation during working memory task in untreated individuals with major depressive disorder. *Mol. Psychiatry* 2007;12:158–166. [PubMed: 16983390]
- [11]. Bragulat V, Paillere-Martinot ML, Artiges E, Frouin V, et al. Dopaminergic function in depressed patients with affective flattening or with impulsivity: [18F]fluoro-L-dopa positron emission tomography study with voxel-based analysis. *Psychiatry Res* 2007;154:115–124. [PubMed: 17306513]
- [12]. Molloy MP, Herbert BR, Walsh BJ, Tyler MI, et al. Extraction of membrane proteins by differential solubilization for separation using two-dimensional gel electrophoresis. *Electrophoresis* 1998;19:837–844. [PubMed: 9629924]
- [13]. Jefferies JR, Brophy PM, Barrett J. Investigation of *Fasciola hepatica* sample preparation for two-dimensional electrophoresis. *Electrophoresis* 2000;21:3724–3729. [PubMed: 11271492]
- [14]. Ramagli LS. Quantifying protein in 2-D PAGE solubilization buffers. *Methods Mol. Biol* 1999;112:99–103. [PubMed: 10027233]
- [15]. Gorg A, Obermaier C, Boguth G, Harder A, et al. The current state of two-dimensional electrophoresis with immobilized pH gradients. *Electrophoresis* 2000;21:1037–1053. [PubMed: 10786879]
- [16]. Shevchenko A, Wilm M, Vorm O, Mann M. Mass spectrometric sequencing of proteins silver-stained polyacrylamide gels. *Anal. Chem* 1996;68:850–858. [PubMed: 8779443]
- [17]. Clauser KR, Baker P, Burlingame AL. Role of accurate mass measurement (+/- 10 ppm) in protein identification strategies employing MS or MS/MS and database searching. *Anal. Chem* 1999;71:2871–2882. [PubMed: 10424174]
- [18]. Blechinger J, Holm IE, Nielsen KB, Jensen TH, et al. Identification and characterization of GFAPkappa, a novel glial fibrillary acidic protein isoform. *Glia* 2007;55:497–507. [PubMed: 17203480]
- [19]. Mukasa R, Umeda M, Endo T, Kobata A, et al. Characterization of glycosylphosphatidylinositol (GPI)-anchored NCAM on mouse skeletal muscle cell line C2C12: the structure of the GPI glycan and release during myogenesis. *Arch. Biochem. Biophys* 1995;318:182–190. [PubMed: 7726560]
- [20]. Edgar PF, Schonberger SJ, Dean B, Faull RL, et al. A comparative proteome analysis of hippocampal tissue from schizophrenic and Alzheimer's disease individuals. *Mol. Psychiatry* 1999;4:173–178. [PubMed: 10208449]
- [21]. Korolainen MA, Goldsteins G, Alafuzoff I, Koistinaho J, et al. Proteomic analysis of protein oxidation in Alzheimer's disease brain. *Electrophoresis* 2002;23:3428–3433. [PubMed: 12373773]
- [22]. Swatton JE, Prabakaran S, Karp NA, Lilley KS, et al. Protein profiling of human postmortem brain using 2-dimensional fluorescence difference gel electrophoresis (2-D DIGE). *Mol. Psychiatry* 2004;9:128–143. [PubMed: 14708031]
- [23]. Alexander-Kaufman K, Dedova I, Harper C, Matsumoto I. Proteome analysis of the dorsolateral prefrontal region from healthy individuals. *Neurochem. Int* 2007;51:433–439. [PubMed: 17590479]
- [24]. Alexander-Kaufman K, James G, Sheedy D, Harper C, et al. Differential protein expression in the prefrontal white matter of human alcoholics: a proteomics study. *Mol. Psychiatry* 2006;11:56–65. [PubMed: 16172612]
- [25]. Clark D, Dedova I, Cordwell S, Matsumoto I. A proteome analysis of the anterior cingulate cortex gray matter in schizophrenia. *Mol. Psychiatry* 2006;11:459–470. [PubMed: 16491132]

- [26]. Beasley CL, Pennington K, Behan A, Wait R, et al. Proteomic analysis of the anterior cingulate cortex in the major psychiatric disorders: Evidence for disease-associated changes. *Proteomics* 2006;6:3414–3425. [PubMed: 16637010]
- [27]. Tsuji T, Shiozaki A, Kohno R, Yoshizato K, et al. Proteomic profiling and neurodegeneration in Alzheimer's disease. *Neurochem. Res* 2002;27:1245–1253. [PubMed: 12462422]

Figure 1.

Relative levels, expressed as a percent of total immunoreactive protein across all extracts, of ten proteins in detergent extracts of BA 9 visualized and quantified using Western blot analyses. Proteins in Extract 1 were solubilized in 40 mM Tris, proteins in Extract 2 were soluble in 8 M urea, 4% CHAPS, 40 mM Tris, 0.2% ampholytes 3–10, 2 mM tributyl phosphine whilst protein in Extract 3 were in 5 M urea, 2 M thiourea, 2% CHAPS, 2% SB3–10, 40 mM Tris, 0.2% ampholytes 3–10, 2 mM tributyl phosphine.

Figure 2.

Typical second dimension gels showing the resolution of protein extracts from human BA 9 (A, B, C) and caudate nucleus and putamen (D, E, F). Proteins were extracted into either 40 mM Tris (Extract 1: A, D), 8 M urea, 4% CHAPS, 40 mM Tris, 0.2% ampholytes 3–10, 2 mM tributyl phosphine (Extract 2: B, E) or 5 M urea, 2 M thiourea, 2% CHAPS, 2% SB3–10, 40 mM Tris, 0.2% ampholytes 3–10, 2 mM tributyl phosphine (Extract 3: C, F).

Figure 3.
The relationship between the variance in protein spot intensity and protein spot frequency in BA 9 and CP.

Table 1

Demographic and tissue collection data for the four tissue donors

Donor	Age (yr)	Sex	PMI (h)	CNS pH	Cause of death
1	84	F	60	6.65	Coronary artery atheroma
2	79	F	50	6.65	Coronary artery atheroma
3	71	M	50	6.59	Coronary artery atheroma
4	59	M	60	6.31	Ischemic heart disease

A: Number of spots identified in each of the three protein fractions isolated from frontal cortex and caudate nucleus and putamen obtained postmortem B: Frequency of spots within CNS region between individuals

Table 2

A														
Donor	Frontal cortex	Extract	1			2			3			4		
			1	2	3	1	2	3	1	2	3	1	2	3
		Spot number %	644	456	484	718	678	517	979	688	574	833	609	325
			41	29	31	38	35	27	44	31	26	47	34	18
		Spot number %	745	511	905	1104	1481	470	737	594	649	1365	324	675
			44	28	28	27	38	35	40	26	34	50	29	20
B														
		Frequency	Extract 1 n (%)	Extract 2 n (%)	Extract 3 n (%)	Totals n (%)								
Frontal cortex	4		325 (23)	342 (34)	42 (3)	709 (19)								
	3		263 (18)	149 (15)	83 (6)	495 (13)								
	2		237 (16)	173 (17)	245 (17)	655 (17)								
Totals	1		615 (43)	334 (33)	1009 (51)	1958 (51)								
			1440 (38)	998 (26)	1379 (36)	3817								
Caudate putamen	4		330 (22)	228 (17)	122 (9)	680 (16)								
	3		236 (16)	122 (9)	119 (8)	477 (11)								
	2		310 (21)	191 (14)	210 (17)	711 (17)								
Totals	1		605 (41)	824 (60)	966 (56)	2395 (56)								
			1481 (35)	1365 (32)	1417 (33)	4263								

Table 3

Proteins showing high variability in postmortem CNS tissue from four subjects with no evidence of neurological or psychiatric disorders

Name	Swiss-Prot accession	Ext	p(Obs/Th ^a)	Mwht Obs/Th	Gene symbol	Chromosome	Function
Brodmann's area 9							
Chorionic somatomotropin hormone-like 1	Q14406	1	5.78/5.56	30.9/22.6	CSHL1	17q24.2	Growth control
Hepatoma derived growth factor like-1	Q5TGJ6	1	5.14/4.50	30.4/27.2	HDGFL1	6p22.3	Growth control
Interleukin-32 [Precursor]	P24001	1	5.40/5.14	27.3/26.7	IL32	16p13.3	Pro-inflammatory pathways
Toll-interacting protein	Q9H0E2	1	5.40/5.68	27.3/30.3	TOLLIP	11p15.5	Ubiquitin pathways
Spindlin (Ovarian cancer-related protein)	Q9Y657	1	5.40/6.46	27.3/29.6	SPIN1	9q22.1-q22.3	Cell division
Chromatin modifying protein 4B	Q9H444	1	4.73/4.76	32.8/25.0	CHMP4B	20q11.22	Chromatin-modifying protein
Rab-9B (Rab-9L) (RAB9-like protein)	Q9NP90	1	4.73/4.76	32.8/22.7	RAB9B	Xq22.1-q22.3	GTP Binding
Potassium inwardly-rectifying channel, subfamily J, member 5	P48544	1	4.95/5.19	49.2/47.7	KCNJ5	11q24	Potassium channel
Mutated in colorectal cancers	P23508	2	5.23/5.40	88.2/93.0	MCC	5q21-q22	Candidate for the putative colorectal tumor suppressor gene
MCM6 minichromosome maintenance deficient 6	Q14566	2	5.72/5.29	88.3/92.9	MCM6	2q21	Initiation of eukaryotic genome replication
Heat shock 70kDa protein 8	P11142	2	5.34/5.37	78.4/70.9	HSPA8	11q24.1	Protein folding and ATPase
Filensin	Q12934	2	5.56/5.09	78.4/74.5	BFSP1	20p11.23-p12.1	Intermediate filament-like protein
α1-microglobulin/bikunin precursor	P02760	2	5.69/5.95	38.1/39.0	AMBIP	9q32-q33	Protein transport enhancer
Protein arginine N-methyltransferase 2	P55345	2	4.95/5.03	49.2/49.0	PRMT2	21q22.3	Signal transduction
Fractalkine precursor	P78423	2	5.69/6.08	43.1/42.2	CX3CL1	16q13	Pro-inflammatory pathways
HLA class I histocompatibility antigen, B-35 alpha chain precursor	P30685	2	5.69/6.02	43.1/40.4	HLA-B	6p21.3	Immune system
Eukaryotic translation initiation factor 3 subunit 2	Q13347	2	5.04/5.38	40.7/36.5	EIF3I	1p34.1	Protein synthesis
NF-κappaB inhibitor beta	Q15653	2	5.04/4.70	40.7/37.8	NFKBIB	19q13.1	Signal transduction
Probable serine carboxypeptidase CPVL precursor	Q9H3G5	2	5.42/5.39	52.5/54.2	CPVL	7p15-p14	Protease
Vacuolar ATP synthase subunit B, brain isoform	P21281	2	5.42/5.57	52.5/56.5	ATP6V1B2	8p22-p21	Energy and metabolism
Caudate-putamen							
Secretagogin, EF-hand calcium binding protein	O76038	1	5.88/5.25	24.1/32.0	SCGN	6p22.3-p22.1	Cell proliferation
Thiopurine S-methyltransferase	P51580	1	5.90/5.85	27.9/28.2	TPMT	6p22.3	Drug metabolism

Name	Swiss-Prot accession	Ext	p(Obs/Th) ^{a)}	Mw/ht Obs/Th	Gene symbol	Chromosome	Function
Capping protein (actin filament) muscle Z-line, β	P47756	1	5.05/5.36	33.6/31.3	CAPZB	1p36.1	Regulates growth of actin filaments
Keratin 8	P05787	1	5.24/5.52	52.3/53.7	KRT8	12q13	Cytoskeleton
Glial fibrillary acidic protein	P14136	1	5.65/5.42	51.7/49.9	GFAP	17q21	Intermediate filament protein of mature astrocytes
Glutathione S-transferase Mu 3	P21266	1	5.16/5.37	29.5/26.6	GSTM3	1p13.3	Oxidative stress
Microtubule-associated protein RP/EB family member 1	Q15691	1	5.16/5.02	29.5/30.0	MAPRE1	20q11.1-q11.23	Regulation of microtubule structures and chromosome stability
Thioredoxin-like protein 1	O43396	1	4.85/4.84	38.7/32.3	TXNL1	18q21.31	Transcriptional repressor
Melanoma-associated antigen 6	P43360	1	4.85/4.57	38.7/34.9	MAGEA6	Xq28	Immune system
Haptoglobin-1 precursor	P00737	1	6.05/6.13	43.0/45.2	HP	16q22.1	Anti-inflammatory
Cholinephosphate cytidylyltransferase β	Q9Y5K3	1 & 2	6.05/5.99	43.0/41.9	PCYT1B	Xp22.11	Energy and Metabolism
Leukotriene A-4 hydrolase	P09960	2	5.36/5.80	69.3/69.3	LTA4H	12q22	Pro-inflammatory lipid mediators
26S proteasome non-ATPase regulatory subunit 9	O00233	2	6.52/6.46	25.4/24.7	PSMD9	12q24.31-q24.32	Peptide cleavage
HLA class I histocompatibility antigen, A-80 alpha chain precursor	Q09160	2	6.07/5.90	43.3/40.8	HLA-A	6p21.3	Immune system
F-box WD-repeat protein 8	Q8N3Y1	2	5.36/5.32	69.3/67.3	FBXW8	12q24.22	Phosphorylation-dependent ubiquitination

^{a)} Abbreviations: Ext, extract, Obs, observed, Th, theoretical.