

A molecular characterization of the choroid plexus and stress-induced gene regulation

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The role of the choroid plexus (CP) in brain homeostasis is being increasingly recognized and recent studies suggest that the CP has a more important role in physiological and pathological brain functions than currently appreciated. To obtain additional insight on the CP function, we performed a proteomics and transcriptomics characterization employing a combination of high resolution tandem mass spectrometry and gene expression analyses in normal rodent brain. Using multiple protein fractionation approaches, we identified 1400 CP proteins in adult CP. Microarray-based comparison of CP gene expression with the kidney, cortex and hippocampus showed significant overlap between the CP and the kidney. CP gene profiles were validated by *in situ* hybridization analysis of several target genes including *klotho*, *CLIC 6*, *OATP 14* and *Ezrin*. Immunohistochemical analyses were performed for CP and endpendyma detection of several target proteins including *cytokeratin*, *Rab7*, *klotho*, tissue inhibitor of metalloprotease 1 (*TIMP1*), *MMP9* and glial fibrillary acidic protein (*GFAP*). The molecular functions associated with various proteins of the CP proteome indicate that it is a blood–cerebrospinal fluid (CSF) barrier that exhibits high levels of metabolic activity. We also analyzed the gene expression changes induced by stress, an exacerbating factor for many illnesses, particularly mood disorders. Chronic stress altered the expression of several genes, downregulating *5HT2C*, *glucocorticoid receptor* and the cilia genes *IFT88* and *smoothed* while upregulating *5HT2A*, *BDNF*, *TNF α* and *IL-1b*. The data presented here attach additional significance to the emerging importance of CP function in brain health and CNS disease states.

Translational Psychiatry (2012) 2, e139; doi:10.1038/tp.2012.64; published online 10 July 2012

Introduction

Two major CNS barriers regulate the movement of molecules between blood, brain and cerebrospinal fluid: the blood brain barrier (BBB) defined by cerebral vasculature, and the blood–cerebrospinal fluid (CSF) barrier (BCSFB) defined by the choroid plexus (CP). An increased appreciation of the involvement of the BBB in brain homeostasis has recently increased research efforts in understanding cerebral vasculature in the context of neurodegenerative diseases. In contrast to BBB, the BCSFB has received limited interest and is significantly understudied. Although the CP has been primarily investigated for its role in CSF production and regulation of its composition,¹ recent work has demonstrated that the CP performs several additional functions, including CNS transport of peripheral trophic factors,² thyroid hormone distribution,³ amyloid clearance,⁴ micronutrient and vitamin transport,^{5,6} protein synthesis⁷ and neuroendocrine signaling.⁸

The CP has also been implicated as a target site for brain drug delivery,⁹ hydrocephalus,¹⁰ CNS inflammation¹¹ and CNS lymphocyte entry.¹² Antidepressant treatment has been shown to alter gene¹³ and protein¹⁴ expression in the CP. Despite the simple cellular configuration of a central vascular

core surrounded by an epithelial layer, the CP has an increasingly significant role in numerous functional processes in the brain. Structural analysis of the CP has shown that ageing and Alzheimer's disease cause atrophy of the epithelial layer and alter CP function.^{15,16}

The high density of serotonin receptor expression in the CP has made it a useful system to examine psychotropic drug-induced signaling.^{17–19} We have previously reported the expression of several growth factor genes in the CP and their regulation by antidepressant treatment.¹³ Microarray analysis of the CP gene regulation after recurrent inflammatory stimuli showed that it elevated the expression of leukocyte extravasation pathway genes, suggesting that the CP could constitute an important site of CNS response to chronic peripheral inflammation.²⁰ A recent transcriptomics analysis of the mouse CP reported that the most highly expressed CP genes were the molecules involved in energy metabolism, and also uncovered several genes associated with the interrelated phenomena of angiogenesis and neurogenesis.²¹ To gain additional insight into the functional role of the CP at the gene and protein levels, we performed a molecular characterization employing a combination of transcriptomics and high-resolution mass spectrometry-based proteomics methods.

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Keywords: choroid plexus; cilia; inflammation; microarray; proteome

Received 14 March 2012; revised 8 June 2012; accepted 11 June 2012

Secondary validation at the mRNA and protein level was performed by *in situ* hybridization and immunohistochemistry, respectively. Candidate molecules were chosen so as to represent diverse molecular functions and expression specificity.

We also examined the role of the CP in psychosocial stress response by employing a chronic unpredictable stress paradigm (CUS). Chronic stress is a well-known risk factor for precipitating several mental health illnesses in humans, including anxiety and depression.^{22,23} The rodent CUS paradigm utilizes a series of mild stressors to cause behavioral changes that parallel the symptoms of depression. We examined gene expression changes in the CP after administration of the CUS paradigm.

Materials and methods

Animals. Male Sprague–Dawley rats (250–300 g, Charles River, MA, USA) were housed under a 12-h light/12-h dark cycle at constant temperature (25 °C) with free access to food and water except when animals were subjected to light disturbance or deprivation stressors during the chronic unpredictable stress (CUS) procedure. The molecular characterization of the CP was performed on naive animals only handled for weighing and cage cleaning, and at least a week after their arrival in the vivarium. Animal-use procedures were in accordance with the Yale University Care and Use of laboratory animals (YACUC) guidelines.

Chronic unpredictable stress. Chronic unpredictable stress (CUS) is an experimental procedure in which animals are exposed to a variable sequence of mild and unpredictable stressors. This procedure is thought to be a reliable rodent model of depression with high face, construct and predictive validity.²⁴ The CUS animals were subjected to a similar sequence of 12 randomized stressors (2 per day for 35 days) described in detail in Banasr *et al.*²⁵ The stressors used were cage rotation, light on, light off, cold stress, isolation, crowding, food and water deprivation, stroboscope, cold swim stress, wet bedding, cage tilt and odor exposure. Animals were killed on day 36, 24 h after the last stressor.

RNA isolation. The entire lateral choroid plexus (both hemispheres) from individual male Sprague–Dawley rats was manually dissected under a dissection microscope, pooled and rapidly frozen on dry ice. Frontal cortex, hippocampus and whole kidney were also manually dissected and frozen rapidly. Choroid plexus RNA was isolated using the Ambion RNAqueous (minikit), while cortex, hippocampal and the kidney RNA was isolated using the standard Ambion RNAqueous kit and subjected to quality control measurements.

Microarray analysis of gene expression. RNA (5 µg) was reverse transcribed to complementary DNA (cDNA) as previously described,¹³ and hybridized to 18k rat oligo arrays (Microarrays Inc., Huntsville, AL, USA) under stringent hybridization conditions. In each experimental, comparison CP RNA was hybridized in one channel and another brain region

or the kidney was hybridized in the other channel ($N=4$ for each tissue). Arrays were posthybridized with fluorescent dendrimers (Genisphere, Hatfield, PA, USA). Image analysis was performed using GenePix Pro 6.0 software (Molecular Devices, Sunnyvale, CA, USA). Microarray image files were subjected to statistical analysis as previously described.^{26,27} Only spots with signal intensity at least twofold above background were utilized for analysis. Channels were normalized using intensity-dependent Lowess normalization. Genes with a ratio between 0.7 and 1.3 (yellow spots) were considered to overlap in expression. GeneSpring 7.3 software (Agilent Technologies, Santa Clara, CA, USA) was used for the statistical analysis and comparison of gene expression in target tissues. All expression data was filtered for genes that had a *t*-test *P*-value of 0.01 with the false discovery rate (FDR) as the multiple testing correction. Venn diagrams were generated as previously reported.¹³ Bioinformatics analysis was performed by Panther software (Applied Biosystems, Carlsbad, CA, USA) and public database searches.

***In situ* hybridization analysis.** *In situ* analysis of mRNA expression was performed as previously described.^{13,28} Briefly, radiolabeled riboprobes were generated by PCR amplification using gene-specific primers. A T7 template sequence was included at the 5' end of the reverse primer and an SP6 site on the complementary primer for use as the negative control probe. PCR templates for riboprobe generation were verified for specificity by DNA sequencing. No appreciable signal was detected with negative control probes.

Proteomics. Fractionation methods, enzymatic digestion protocol and mass spectrometry (MS) methodology employed to generate the CP proteome are detailed in Supplement 3.

Immunohistochemistry. Immunohistochemical analyses for detection of target proteins in the CP were performed on cryocut sections by employing our previous protocol,²⁹ with minor modifications for use of fluorescent secondary antibodies. Antibodies to Cytokeratin (Abcam, Cambridge, MA, USA; ab9005), Rab7 (Abcam, ab77993) and klotho (Abcam, ab75023) were used at dilutions of 1:500. TIMP-1 (R & D systems, 1:1000), MMP-9 (Torrey Pines, 1:2000), RECA (Serotec, 1:50), GFAP (Millipore, Billerica, MA, USA; 1:3000).

Results

Comparative analysis of gene expression. In an effort to further understand the function and complexity of the mammalian choroid plexus, we examined global gene expression using an 18k rat microarray and compared the CP gene profile with the cortex, the hippocampus and the kidney. Shown in Figure 1 is a comparative CP gene expression overlap with the kidney, cortex and hippocampus. The results show a strikingly higher similarity of the CP with the kidney than either brain region. Functional classification of CP genes shows that it expresses molecules with diverse cellular functions, including multiple categories of receptors, transporters and carrier proteins.

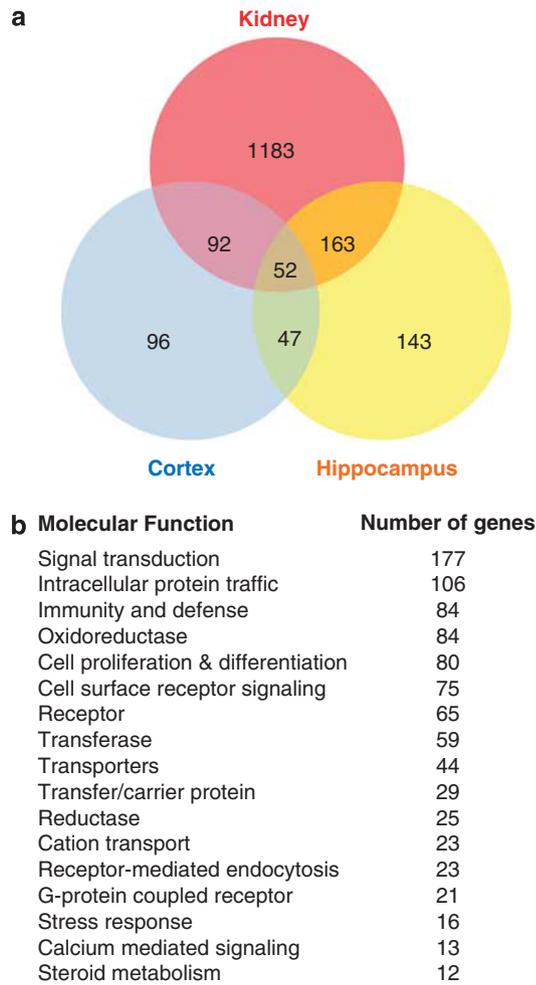


Figure 1 Choroid plexus (CP) gene expression. (a) Venn diagram shows comparative gene expression overlap of the kidney, cortex and hippocampus with CP. Microarray analysis was performed by dual-channel experiments, where CP and other brain-region RNA were simultaneously hybridized. Data shown are from three independent replicates for each region. (b) Major functional categories of genes that were expressed in both the CP and the kidney. Only functional classes that had a minimum of 12 genes are shown.

Secondary validation using *in situ* hybridization. To obtain anatomical expression profiles of several CP genes that were indicated as significantly expressed by the array experiments, we performed *in situ* hybridization (ISH) using radiolabeled riboprobes. We examined a cross section of genes that had a broad range of CP expression levels and diverse molecular function classes (Figure 2): channel protein—chloride intracellular channel (CLIC 6); transporter—organic anion transporting polypeptide (OATP 14); enzymes—Klotho, catechol-*O*-methyltransferase (COMT), superoxide dismutase (SOD1); carrier protein—transthyretin; receptors—insulin-like growth factor receptor (IGFR1), estrogen receptor 1; cytoskeleton protein—ezrin. Transthyretin had the highest level of expression of all the examined targets and showed exclusive CP expression, similar to the klotho expression pattern. Highest brain expression of the klotho expression pattern. Highest brain expression of CLIC6, OATP 14 and ezrin was detected in the CP. COMT was prominently expressed in the CP but was

also detected in the cortex and caudate. Although EGFR1, IGFR1 and SOD 1 were clearly discernible in the CP, expression level was only moderately elevated in comparison to the caudate and cortex.

Protein profiling, identification and genes overlap. Multiple fractionation methods, including fast protein liquid chromatography (FPLC), 2DLC and SDS-polyacrylamide gel electrophoresis, were employed in this study to increase the coverage and confidence of proteins identified. A collective total of 1400 proteins were identified in the CP proteome. Shown in Figure 3 is the FPLC elution profile of CP homogenate and a partial list of proteins identified in particular peaks. It is interesting to note that both dopamine-and cAMP-regulated phosphoprotein-32 (DARPP-32) and its substrate, protein phosphatase 1 (PP1) eluted in B8. This could be due to either shared hydrophobicity or potential protein–protein interactions. Multiple growth factor receptors, insulin-like growth factor (IGF2) and fibroblast growth factor (FGFR2) eluted in B1. Although the peaks were sharp and collected in individual tubes, it is clear from the MS data that each peak contains several different proteins. In the taller peaks, B1 and C2, 80 and 90 proteins were identified, respectively. In peaks B10, B8 and C6, 17, 19 and 20 proteins were identified, respectively. Additional proteins were identified from FPLC separations by individually (40 collection tubes) and collectively (pools of cytosolic and membrane fractions) processing the elution peaks. The pie chart (Figure 4a) shows the proportion of CP proteins associated with various categories of biological processes. The high percentage (33.5%) of the proteins being mapped to metabolism is due to the identification of several enzymes, including 83 hydrolases, 75 oxidoreductases and 67 transferases (Figure 4b). We analyzed the overlap between the identified CP proteins and genes (Figure 4c). To obtain a comparable number of genes and proteins, we utilized the full list of proteins and limited the gene number by using the kidney–CP overlap set. Only genes that had the same annotation as that of the protein were used for comparison. This resulted in an overlap of 211 genes and proteins. The list of proteins that were identified in this are listed in Supplement 1. The list of representative peptides are in Supplement 2 and are hyperlinked to the Uniprot database.

Comparative analysis of cerebellum, lateral ventricle and the kidney. Shown in Table 1 is a comparison of proteome profiles from the CP, the kidney tubules, cerebellum and lateral ventricle glia, analyzed for enriched gene ontology (GO) molecular function terms. Proteomes other than CP were obtained from the human protein atlas database and analyzed by Metacore. Only molecular function classes that exhibited substantial differences in target representation are shown (Table 1). The total numbers of targets in each tissue were CP—1403, kidney tubules—2448, cerebellum—2288 and lateral ventricle—884. Transcription regulators and nucleic acid binding proteins were represented at low levels in the CP, at 3.2% and 6.7%, respectively. In comparison, nucleic acid binding proteins in the kidney, cerebellum and lateral ventricle (LV) were represented at 26.2%, 27.7% and 29%. Electron carrier and transporter proteins exhibited higher representation in the CP, and the most striking difference in transporter

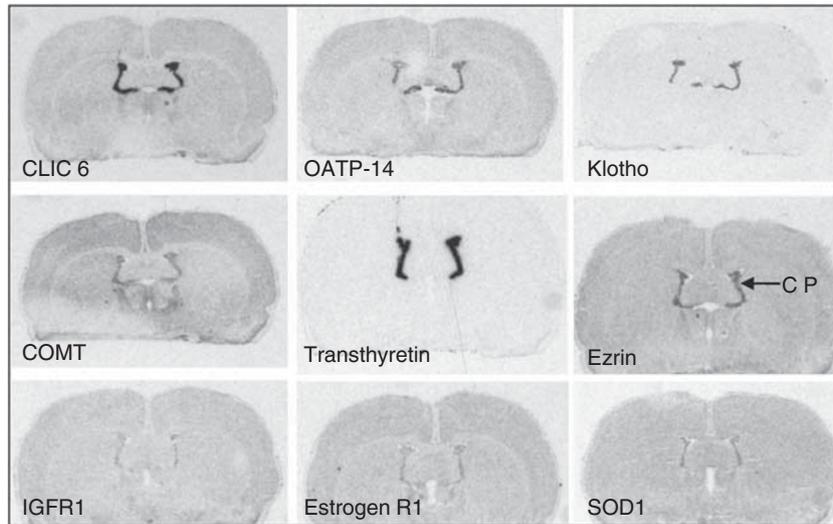


Figure 2 *In situ* hybridization analysis of choroid plexus (CP) gene expression. Radiolabeled riboprobes were used to examine CP expression of a subset of genes from array data. CLIC6, chloride intracellular channel protein 6; COMT, catechol-O-methyl transferase; IGFR1, insulin-like growth factor receptor 1; SOD1, superoxide dismutase; OATP 14, organic anion-transporting polypeptide.

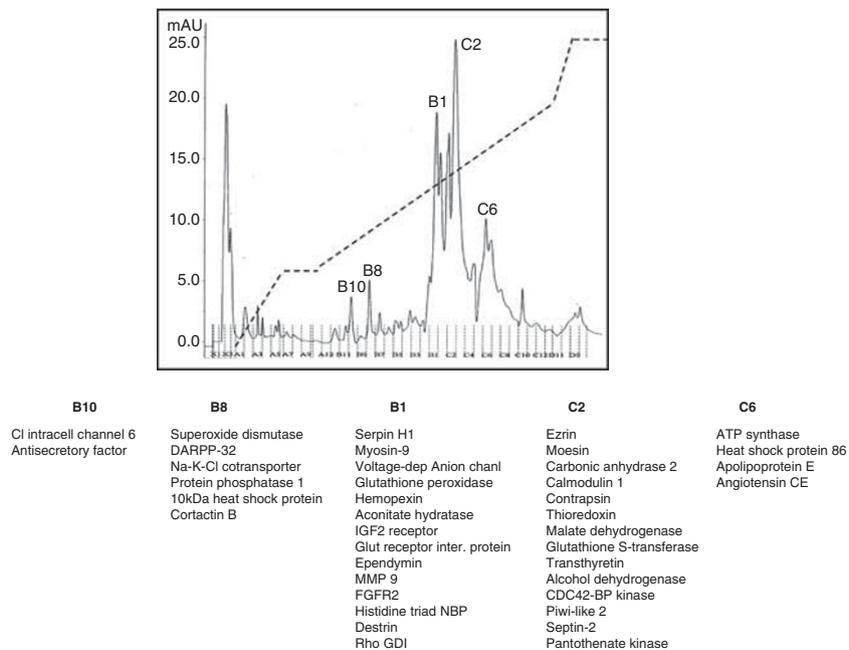


Figure 3 FPLC elution profile of choroid plexus (CP) homogenate. A representative elution profile is shown along with a short list of major proteins that were identified by mass spectrometry from specific numbered peaks. CP tissue ($n=3$) was pooled after rinsing in cold PBS. An elution gradient of increasing acetonitrile concentration is indicated by the background line. DARPP32, dopamine- and cAMP-regulated neuronal phosphoprotein; FGFR2, fibroblast growth factor receptor 2; GDI, GDP dissociation inhibitor; IGF2, insulin-like growth factor; MMP9, matrix metalloprotease; NBP, nucleotide binding protein; PBS, phosphate buffered saline.

expression was between CP and LV. Although represented by only 21 distinct proteins, the percentage of antioxidant molecules (1.6%) identified in the CP is at least twofold higher than the other tissues used in the comparison (Table 1).

Immunohistochemical analysis of CP protein expression. We examined five CP genes for expression at the protein level by immunohistochemistry. Multiple isoforms

of keratin were identified by the MS analysis. We chose an antibody that recognizes keratins 5 and 8 to confirm expression in the CP. Cytokeratin 5/8 was exclusively expressed in the CP (Figure 5a) and no signal was noted in any other brain regions. Very low levels of expression were observed in the ependyma. The cytoskeletal and filamentous expression pattern is discernible at higher magnification (Figure 5d). Klotho was also strongly expressed only in the CP with low expression in the ependyma (Figures 5b and e).

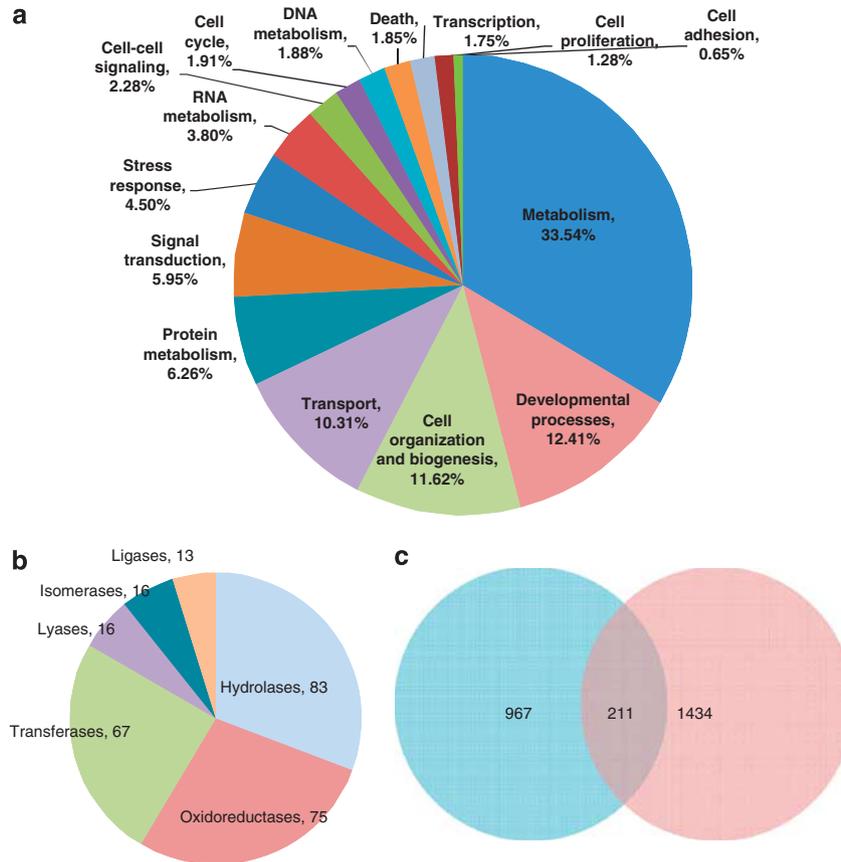


Figure 4 Choroid plexus (CP) proteome. (a) Proteins were identified by the International Protein Index (IPI) identifiers and then converted to gene names and mapped to Gene Ontology (GO) terms. Mouse genome institute (MGI) GO slim 2 schema for biological processes was used to classify the CP proteome based on the biological role of the molecules. Pie chart shows the number of proteins represented in the GO molecular function term categories. (b) The enzymes that were identified by the proteomics analysis were classified using the UniProt database. Pie chart shows the number of proteins represented in the enzymatic function categories. (c) Venn diagram showing overlap between genes from the kidney-CP overlap set (blue circle) and the identified CP proteins (salmon circle).

Table 1 Comparison of molecular functional classes of proteins represented in multiple tissue types

Molecular function	CP	Kidney	Cerebellum	LV
Antioxidant activity (GO:0016209)	21/1.6	15/0.6	15/0.7	5/0.6
Electron carrier activity (GO:0009055)	35/2.8	36/1.6	31/1.5	15/1.9
Ion transmembrane transporter activity (GO:0015075)	84/6.7	105/4.8	83/4.1	22/2.9
Nucleic acid binding (GO:0003676)	220/6.7	570/26.2	555/27.7	221/29
Signal transducer activity (GO:0004871)	83/6.6	192/8.8	174/8.7	57/7.5
Structural molecule activity (GO:0005198)	134/10.7	137/6.3	109/5.4	39/5.1
Transcription regulator activity (GO:0030528)	40/3.2	201/9.2	192/9.5	88/11.5
Transporter activity (GO:0005215)	129/10.3	167/7.6	137/6.8	40/5.2

Abbreviation: CP, choroid plexus.

Proteome profiles from the human proteome atlas database (<http://www.proteinatlas.org/>) were used to compare the representation of gene ontology (GO) categories. The number of distinct proteins in the particular GO category and percentage representation in the total number of identified proteins is shown. CP, choroid plexus; kidney, kidney tubules; LV, lateral ventricle glia. Numbers before the slash denote actual number of proteins and numbers after the slash denote the percentage of proteins in the particular functional class.

However, the pattern of klotho expression was distinctly different from cytokeratin, exhibiting strong signal polarity (Figure 5e). Rab7 was predominantly expressed in the CP (Figure 5c), followed by substantial expression in vasculature and low levels in the ependyma. Matrix metalloproteinase 9 (MMP 9) has emerged as a key molecule in matrix remodeling and regulation of barrier integrity during inflammation. We therefore investigated the expression of

MMP 9 in the CP and colocalization with cellular markers. Shown in Figure 5g is double immunohistochemical staining to detect GFAP and MMP 9 expression. Prominent MMP 9 staining was observed in the CP, whereas GFAP signal was restricted to the ependyma and absent in the CP (Figures 5g and j). MMP 9 was also expressed in the ependyma but signal colocalization with GFAP was not detected (Figure 5j). Rat endothelial cell antigen (RECA) staining was seen only in

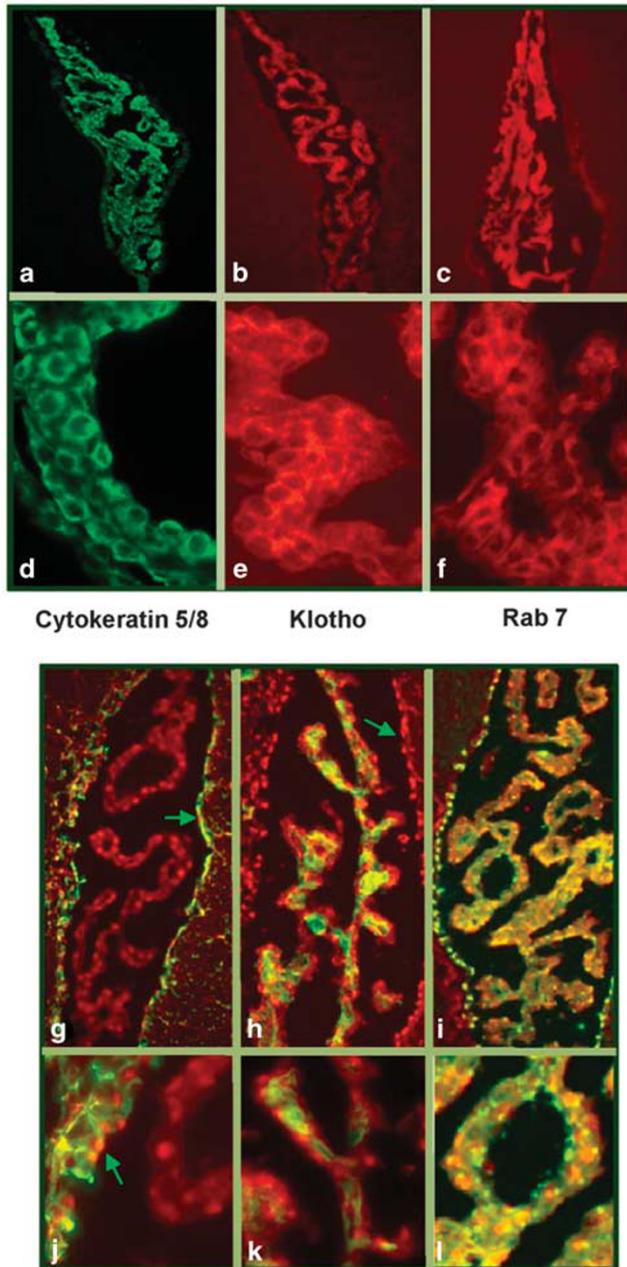


Figure 5 Immunohistochemical analysis of choroid plexus (CP) protein expression. CP expression of cytokeratin, klotho and Rab 7 are shown in **a–c**, respectively ($\times 150$). Higher magnification images of the same proteins are shown in the corresponding lower panel **d–f**. Double immunohistochemical analysis of matrix metalloproteinase 9 (MMP 9) (red) and GFAP (green) is shown in **g** and **j** at low and high magnification, respectively. Arrow in **g** denotes GFAP expression in the ependyma. Arrow in **j** denotes MMP 9 expression in ependymal cells. Double immunohistochemical analysis of rat endothelial cell antigen (RECA) (green) as a vascular marker and MMP 9 (red) is shown in **h** and **k**. Arrow in **h** denotes MMP 9 expression in the ependyma. Double immunohistochemical analysis of MMP 9 (red) and TIMP 1 (green) is shown in **i** and **l**.

the vascular core of the CP and did not appear to colocalize with the epithelial cell expression of MMP 9 (Figures 5h and k). TIMP 1 expression was sparse in the CP and more strongly noted in the ependyma (Figure 5i). Although some TIMP 1 expression was in proximity to MMP 9 positive cells

in the CP, it appeared to be limited to the basement membrane (Figure 5l).

Effects of chronic stress on CP gene regulation. In order to investigate the effects of chronic stress on choroid plexus gene expression, we performed a preliminary microarray analysis of gene expression using a pooled sample ($N=3$) of CP tissue from rats exposed to the chronic unpredictable stress (CUS) paradigm compared with home cage control animals. This analysis indicated that 35 days of CUS influenced CP gene regulation and enabled us to focus our attention on a subset of dysregulated genes. Using an independent cohort of rats ($N=4$), we examined stress-induced CP gene regulation by quantitative PCR analysis. CUS animals showed substantially increased expression of the 5HT_{2A} receptor but reduced expression of 5HT_{2C} and the glucocorticoid receptor (6A). CUS also influenced the CP expression of several trophic factors. Brain-derived neurotrophic factor (BDNF) was prominently elevated by 65% followed by IGF1 (6B). Erythropoietin was significantly decreased, whereas there was no change in vascular endothelial growth factor levels (6B). CUS reduced the expression of intraflagellar transport protein 88 (IFT88), smoothed (smo), platelet-derived growth factor receptor alpha (PDGFR α) and klotho (Figure 6c). IFT88, smo and PDGFR α are known to be expressed by cilia and could represent stress-induced alteration of CP cilia. In contrast to the decrease in expression of cilia genes, CUS elevated the expression of the proinflammatory cytokines interleukin 1b (IL-1b) and tumor necrosis factor α (TNF α). There was a 40% elevation in IL-1b and MMP 9, and a 30% increase in TNF α expression (Figure 6d).

Discussion

We performed a molecular characterization of the adult rat CP by conducting a proteomics and transcriptomics analysis. Utilizing multiple fractionation approaches and mass spectrometry, we identified several categories of proteins associated with diverse biological processes. A microarray based comparison of CP gene profiles revealed significant overlap between CP and kidney gene expression. Our data sheds further light on the physiological parallels between the kidney and CP, revealing overlap in gene and protein expression. The expression of genes associated with functions such as transport, absorption, secretion and enzymatic activity underscores the molecular and functional similarity. The overlap is also due in part to the cell types that compose the CP, which consists of a monolayer of epithelial cells and an endothelial core. A recent transcriptome study of the CP³⁰ reported several genes, such as opioid receptors and netrins that were for the first time detected in the CP. However, the comparison of their gene profile with database profiles of previous mouse gene expression studies³¹ revealed maximal overlap only with endothelial cells. Stress is being recognized as a key contributor to several major diseases including depression, cardiovascular disease, immunodeficiency virus and cancer,³² making it necessary to identify mechanisms and target regions that connect stress with disease states.

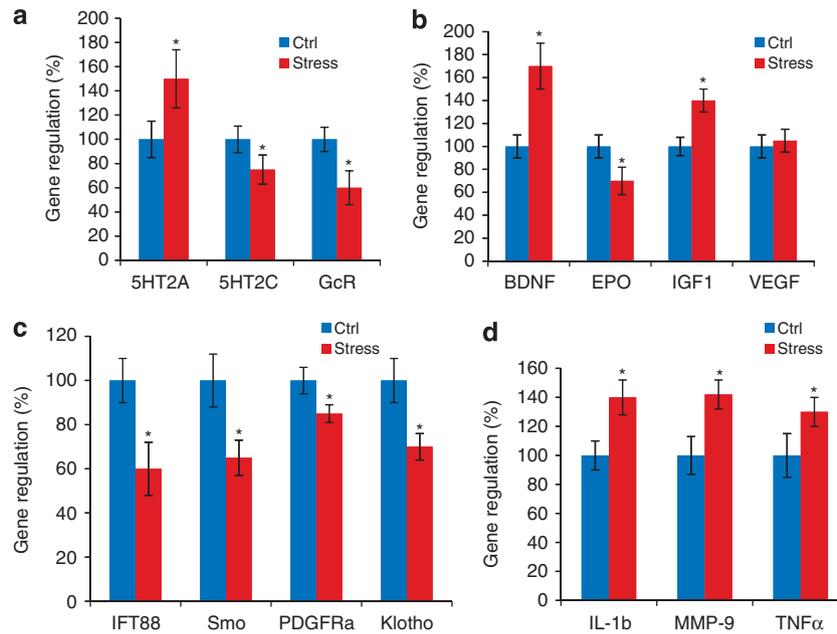


Figure 6 Stress-induced gene regulation in the choroid plexus (CP). (a) Real-time PCR quantitation of CP gene regulation after chronic unpredictable stress. (b) BDNF, brain-derived neurotrophic factor; EPO, erythropoietin; IGF1, insulin-like growth factor 1; VEGF, vascular endothelial growth factor. (c) IFT88, intraflagellar transport; Smo, smoothed; PDGFRa, platelet-derived growth factor receptor. (d) IL-1b, interleukin; TNF α , tumor necrosis factor. * $P \leq 0.05$, error bars represent s.e.m. from $n = 5$.

CP, the ‘kidney’ of the brain. It is interesting to note that the CP has been previously referred to as the ‘kidney’ of the brain based on its physiological role in maintaining the chemical balance of CSF, analogous to the kidney regulating the chemical balance of blood.³³ Some of the genes identified in our study (for example, *klotho*, chloride intracellular channel and Na-K-Cl cotransporter) are almost exclusively expressed in the CP and the kidney. The high level of *klotho* expression in the CP is particularly interesting as mice with a mutant loss of function *klotho* gene knockout exhibit a premature aging phenotype,³⁴ whereas *klotho*-overexpressing mice have an increased life span.³⁵ Studies have predominantly attributed *klotho* phenotypes to disturbances of renal and cardiovascular function. However, cognitive dysfunction and hippocampal apoptosis have also been reported. The physiological actions of *klotho* have been associated with calcium and phosphate homeostasis. It is conceivable that similar functions are performed by CP-expressed *klotho*, and failure of its actions could lead to ionic imbalance in CSF. Targeted studies that specifically delete *klotho* in the CP are needed to elucidate the role of CP *klotho* in modulating brain function.

Although DARPP-32 and PP-1 have been primarily investigated in the context of dopamine signaling, expression in CP serves an important homeostatic function by regulating phosphorylation of Na⁺/K⁺-ATPase,³⁶ a key enzyme associated with CSF secretion. Interestingly, DARPP-32 and Na⁺/K⁺-ATPase also colocalize in renal tubular cells, where they mediate the natriuretic effect of dopamine.³⁷

CP integrity and brain function. The CP proteome contains a substantial number of mitochondrial proteins, suggesting high mitochondrial density. Previous volumetric studies have indicated that mitochondria constitute 10–14%

of the CP cytoplasm.³⁸ This is necessary to support the energy demands of metabolic work such as CSF production, transport and secretion carried out by the CP.³⁹ The elevated metabolic activity of the CP is also indicated by blood flow rate, which is five fold higher than other brain regions.⁴⁰ Mitochondrial disorders frequently exhibit structural malformations in CP cells and altered CSF composition.^{41–43} Given the recent association of mitochondrial dysfunction and neurodegenerative diseases, it is interesting to note that CP-specific knockout of the electrogenic sodium bicarbonate transporter resulted in abnormal mitochondrial localization, CSF electrolyte imbalance and neurological impairment.⁴⁴ Structural CP damage and breakdown in the blood–CSF barrier has been reported after blast waves and traumatic brain injury,⁴⁵ shedding light on the CP as an understudied locus of brain injury. CP damage can adversely affect CSF production, intracranial pressure, clearance of brain metabolites and can potentiate CNS inflammation.

Involvement of CP in stress-related illnesses. A large body of evidence has demonstrated the detrimental effects of stress on the brain, and animal stress models are used to model depressive disorders and test the efficacy of antidepressant drugs. The role of prolonged and involuntary stress in the etiology of depression is widely recognized. The CUS model utilizes this framework to induce anhedonia through exposure to a series of mild stressors presented in random and unpredictable sequence. This stress exposure results in the progressive development of anhedonia, which is observed as a decrease in reward responsiveness, quantified as the degree of preference for weak sucrose solutions over water. The stress-induced decrease in sucrose intake is independent of food-consumption behavior.⁴⁶ CUS thus exhibits good face validity as a

model of depression. Antidepressants reverse the sucrose intake deficits upon chronic but not acute administration, and this model therefore shows good predictive validity for the time course of efficacy of antidepressant treatment. The majority of CUS studies have predominantly implicated the hippocampus, amygdala and frontal cortex as stress-vulnerable regions. We believe our findings of stress-induced gene expression changes are the first to implicate the involvement of the CP in stress effects. The striking increase in brain-derived neurotrophic factor is likely to be related to the elevation in 5HT_{2A} receptor as previous studies have shown that direct activation of 5HT_{2A} increases brain-derived neurotrophic factor in C6 glioma cells,⁴⁷ and the rat frontal cortex.⁴⁸ The reduction in glucocorticoid receptor expression can be inferred as arising from high circulating levels of glucocorticoids, an expected occurrence of the CUS paradigm.^{49,50} However, it is currently unclear whether this is a function of blood or CSF levels of glucocorticoids. The induction of IGF1 in the CP could be a compensatory neuroprotective response to stress. Earlier work in the field has demonstrated that CNS injuries such as ischemia and trauma induced multiple trophic factors in the CP, which are then secreted into the CSF.⁵¹

The induction of proinflammatory cytokines is interesting as IL-1 β and TNF α have been previously shown to independently precipitate depressive behavior.^{52,53} It is possible that chronic stress acts as an inflammatory stimulus and the effects are reflected in the CP. The decrease in erythropoietin, which has antiinflammatory properties in the CNS,⁵⁴ may be due to the action of proinflammatory cytokines as they are known to antagonize erythropoietin expression.⁵⁵ Previous reports have shown that peripheral inflammation via administration of lipopolysaccharide (LPS) increased the expression of IL-1 β and TNF α in the CP.^{56,57} However, in comparison to LPS-induced elevation of inflammatory cytokines, the effects of CUS are modest. Human and rodent studies have demonstrated that chronic stress elevates inflammatory cytokines in the periphery and the CNS. Interestingly, CSF analysis in depressed patients showed that higher levels of proinflammatory cytokines correlated with disease severity.⁵⁸ It is, however, not clear how peripheral levels of these cytokines can influence CNS function. The high level of matrix metalloproteinase expression in the CP and the stress-induced increase could potentially compromise the blood–CSF barrier and allow the passage of these cytokines. The upregulation of TNF α and IL-1 β in the CP draws attention to the CP as a transit point, whereby inflammatory cytokines can directly access the brain via CSF. As the CP can have a critical role in transporting peripheral proteins into the brain,² the integrity of this CNS barrier is crucial for brain homeostasis. The elevation of inflammatory molecules in the CP is not surprising as it has been shown to be a site of active CNS immune surveillance.^{12,59} Improved understanding of CP transport mechanisms and molecules can lead to interventions whereby the CNS entry of harmful proinflammatory cytokines is blocked at the level of the blood–CSF barrier.

IFT88, smoothed and PDGFR α , which were down-regulated by CUS are well-known cilia genes.⁶⁰ CP cilia are classified as nonmotile, primary cilia, with each epithelial cell possessing multiple cilia. These cilia most likely perform

functions that are different from the motile cilia of ependymal cells, which regulate CSF flow. Employing a deciliation model, Narita *et al.*⁶¹ show that CP cilia function as biosensors and have a critical role in regulating CSF production. Recent progress in understanding cilia function has highlighted their role in human diseases and resulted in their categorization as ciliopathies.^{62,63} However, only few studies have focused on understanding the role of CP cilia. Single-gene knockout models have been useful in simulating important aspects of human ciliopathies, but affect cilia in multiple organs, and only limited insight can be obtained on CP-specific effects. It is currently unclear how stress influences cilia gene expression. Stress-induced changes in specific trophic factor signaling cascades could be involved, as a reduction in fibroblast growth factor signaling has been shown to downregulate IFT88 and control the expression of other ciliogenic genes.⁶⁴

Further advances in CP biology and function will require the development of tools that can specifically manipulate the CP. These tools are beginning to emerge in the form of transgenic mouse lines,⁶⁵ targeting ligands⁶⁶ and viral vectors.⁶⁷ These approaches hold much promise toward the development of novel therapeutic strategies in the diagnosis and treatment of CNS disorders.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements. This work was supported by funding from the National Institute of Health (MH 072894), Neuroproteomics Center grant (5P30DA018343), NARSAD Young Investigator award to Mounira Banasr, the Connecticut Mental Health Center and the Department of Mental Health and Addiction Services.

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