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Yan Zhang
University of Nebraska-Lincoln, yzhang3@unl.edu

You Zhou
University of Nebraska-Lincoln, yzhou2@unl.edu

Ulrich Schweizer
Neurobiology of Selenium, Neuroscience Research Center, Charite-Universitatsmedizin, 10117 Berlin, Germany

Nicolai E. Savaskan
Department of Neuromorphology, Brain Research Institute, ETH & University of Zurich, Winterthurerstrasse 190 CH-8057 Zurich, Switzerland

Deame Hua
University of Nebraska-Lincoln

See next page for additional authors

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Comparative Analysis of Selenocysteine Machinery and Selenoproteome Gene Expression in Mouse Brain Identifies Neurons as Key Functional Sites of Selenium in Mammals*[S]

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Yan Zhang[‡], You Zhou[§], Ulrich Schweizer[¶], Nicolai E. Savaskan[‖], Deame Hua[‡], Jonathan Kipnis[**], Dolph L. Hatfield[‡‡], and Vadim N. Gladyshev[‡1]

From the [‡]Department of Biochemistry, University of Nebraska, Lincoln, Nebraska 68588, [§]Center for Biotechnology, University of Nebraska, Lincoln, Nebraska 68588, [¶]Neurobiology of Selenium, Neuroscience Research Center, Charite-Universitatsmedizin, 10117 Berlin, Germany, [‖]Department of Neuromorphology, Brain Research Institute, ETH & University of Zurich, Winterthurerstrasse 190 CH-8057 Zurich, Switzerland, [**]Department of Neuroscience, University of Virginia, Charlottesville, Virginia 22908, and [‡‡]Molecular Biology of Selenium Section, Laboratory of Cancer Prevention, Center for Cancer Research, NCI, National Institutes of Health, Bethesda, Maryland 20892

Although dietary selenium (Se) deficiency results in phenotypes associated with selenoprotein depletion in various organs, the brain is protected from Se loss. To address the basis for the critical role of Se in brain function, we carried out comparative gene expression analyses for the complete selenoproteome and associated biosynthetic factors. Using the Allen Brain Atlas, we evaluated 159 regions of adult mouse brain and provided experimental analyses of selected selenoproteins. All 24 selenoprotein mRNAs were expressed in the mouse brain. Most strikingly, neurons in olfactory bulb, hippocampus, cerebral cortex, and cerebellar cortex were exceptionally rich in selenoprotein gene expression, in particular in *GPx4*, *SelK*, *SelM*, *SelW*, and *Sep15*. Over half of the selenoprotein genes were also expressed in the choroid plexus. A unique expression pattern was observed for one of the highly expressed selenoprotein genes, *SelP*, which we suggest to provide neurons with Se. Cluster analysis of the expression data linked certain selenoproteins and selenocysteine machinery genes and suggested functional linkages among selenoproteins, such as that between SelM and Sep15. Overall, this study suggests that the main functions of selenium in mammals are confined to certain neurons in the brain.

Selenium (Se)[2] is an essential micronutrient that occurs in proteins in the form of the 21st amino acid, selenocysteine (Sec). In selenoenzymes, Sec is an integral component of the active site (1, 2). The synthesis of Sec and its insertion into polypeptides require a complex molecular machinery that recodes in-frame UGA codons, which normally function as stop signals, to serve as Sec codons (3).

25 and 24 selenoproteins have been identified in humans and mice, respectively (4). The functions of some selenoenzymes are well characterized. For example, glutathione peroxidases (GPxs) and thioredoxin reductases (TRs or Txnrds) regulate thiol-based redox activities in cells and are responsible for much of the antioxidant effect of Se (5, 6), whereas iodothyronine deiodinases (Dios) are involved in thyroid hormone metabolism (7). However, the functions of the majority of selenoproteins are not known.

In recent years, the roles of Se have been examined with emphasis on human health (3, 7–9) and disease (10–12). Se content in the brain is not high but, in contrast to most other organs, remains remarkably stable during Se deficiency, most likely at the expense of other organs (13–15). Recent data from transgenic mice suggest that selenoprotein P (SelP) is the key factor for the privileged Se supply to the brain and Se storage in this organ (16–20). Because of the complexity of the brain structure and function with many interspersed cell types (neurons, astrocytes, oligodendrocytes, microglia, endothelial cells, ependymal cells, etc.), studies on selenoprotein expression and the roles of these proteins in brain function lagged behind those in other organs, such as liver and kidney. With the exception of GPx1 (21), SelW, SelP (22–24), and GPx4 (25), systematic expression data in the brain for selenoproteins are not available. Expression of Sec biosynthesis and insertion machinery, such as Sec synthase, SECIS-binding protein 2 (SBP2), and *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ kinase (PSTK) also has not been examined.

The recently published Allen Brain Atlas (ABA) provides a genome-wide gene expression data base of the young adult mouse brain. The initial atlas data include expression patterns

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental text and references, Tables 1–3, and Figs. 1–6.

[1] To whom correspondence should be addressed: Dept. of Biochemistry, University of Nebraska, Lincoln, NE 68588-0664. Tel.: 402-472-4948; Fax: 402-472-7842; E-mail: vgladyshev1@unl.edu.

[2] The abbreviations used are: Se, selenium; Sec, selenocysteine; GPx, glutathione peroxidase; TR or Txnrd, thioredoxin reductase; Dio, iodothyronine deiodinase; SelP, selenoprotein P; SBP2, SECIS-binding protein 2; PSTK, *O*-phosphoseryl-tRNA$^{[Ser]Sec}$ kinase; ABA, Allen Brain Atlas; ARA, Allen Reference Atlas; ISH, *in situ* hybridization; MOB, main olfactory bulb; CA1-CA3, Ammon's horn CA1–CA3 regions; DG, dentate gyrus; ER, endoplasmic reticulum; ApoER2, apolipoprotein E receptor 2; PBS, phosphate-buffered saline; BisTris, 2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol.

for more than 21,000 genes in adult mouse brain (26). The dataset provides both mRNA *in situ* hybridization (ISH) data and a detailed reference atlas. This resource represents a comprehensive platform for exploring gene expression in the brain and can be used to examine and compare expression of all selenoprotein genes. A bioinformatics algorithm has also been developed that allows semi-automatic searches for genes showing similar hybridization patterns (27).

In this work, we extracted expression information for various brain regions based on the ISH image data provided by ABA for all mammalian selenoprotein genes as well as for the Sec machinery genes (*i.e.* the genes involved in Sec biosynthesis and incorporation). We normalized and quantified the original gene expression data and clustered them by hierarchical cluster analysis to identify linkages among selenoproteins and between selenoproteins and the Sec machinery. Our analysis identified neurons in hippocampus, olfactory area, cerebellar cortex, and isocortex as the sites with increased selenoprotein gene expression. Cluster analysis allowed us to identify functional links among selenoproteins and their biosynthetic machinery. The results of this study open new opportunities for research on Se, an essential trace element, and are important for understanding the relationship between Se and brain function.

EXPERIMENTAL PROCEDURES

The ABA Resources and Query Proteins—The ABA provides an automated platform for high throughput ISH that supports systematic analysis of gene expression in young adult (8-week-old) mouse brain (26). We also used a subsequent software tool, Brain Explorer version 1.3 (downloaded from the ABA website), for viewing ABA gene expression data in the framework of the Allen Reference Atlas (ARA) in three dimensions, comparing expression data for multiple genes and navigating two-dimensional ISH images from the ABA. Both coronal and sagittal datasets were analyzed for each gene of interest if available.

We analyzed expression data for 24 known mouse selenoprotein genes and for genes coding for known Sec machinery components. Except for *SelH*, ISH data were available for all examined genes (each was manually selected and viewed through the publicly accessible ABA application) as follows: (i) selenoproteins (total of 23) GPx1, GPx2, GPx3, GPx4, TR1 (*Txnrd1*), TGR (*Txnrd3*), TR3 (*Txnrd2*), Dio1, Dio2, Dio3, SelI (*D5Wsu178e*), SelK, SelM, SelN (*Sepn1*), SelO (*1300018J18Rik*), SelP (*Sepp1*), SelR (or MsrB1, *Sepx1*), SelS (*H47*), SelT (*2810407C02Rik*), SelV (*BC089491*), SelW (*Sepw1*), SPS2 (*Sephs2*), and Sep15; and (ii) Sec machinery (total of 5): Sec synthase (*D5Ertd135e*), PSTK, Secp43 (*Trspap1*), SBP2 (*Secisbp2*), and EF-Sec (*Eefsec*). It should be noted that SPS2 is both a Sec-containing protein and a component of the Sec insertion machinery. In addition, we also included a recently identified SelP receptor, ApoER2 (28). Several housekeeping genes were also examined, including β-actin (*Actb*), α-tubulin (*Tuba1*), hypoxanthine phosphoribosyltransferase (*Hprt*), ribosomal protein L11 (*Rpl11*), dynein cytoplasmic 1 heavy chain 1 (*Dync1h1*), glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), and cytochrome c_1 (*Cyc1*). These genes served as controls, and for quantification purposes (see below), *Hprt* was used.

Brain Regions of ARA—ARA provides an anatomic framework for brain regions. When selecting an ISH image pane, the relevant ARA automatically updates to the nearest corresponding reference atlas section. In our work, we selected 159 regions, including major parts of the brain: cerebral cortex (25 regions), cerebral nuclei (14 regions), cerebellar cortex (18 regions), cerebellar nuclei (3 regions), interbrain (including thalamus and hypothalamus, 22 and 18 regions, respectively), midbrain (23 regions), hindbrain (33 regions), and non-neuron regions (3 regions). A complete list of these regions is shown in supplemental Table S1.

Definition of Gene Expression Signals in Different Regions of Mouse Brain—To extract the expression information for each gene (including controls) and for different brain regions from the original ISH images, we used both the ABA on-line tool and Brain Explorer to visually examine expression signals based on the relative measurement of gene expression provided by ABA (hereafter RM_{ABA}). RM_{ABA} is labeled on a discrete eight-color scale of increasing level of expression (from low to high: *blue-aqua-turquoise-bright green-yellow-gold-light orange-orange*), as the ISH process is not strongly quantitative in the sense of measuring transcript copy number (26). In addition, the range of expression (R_{ex}, percentage of expressing area in a given region normalized by the whole area) was considered. We used the following tags to manually define the gene expression signal in each region: (i) −, no signal observed in the area; (ii) +/−, RM_{ABA} is *blue*, and $R_{ex} < 5\%$; (iii) +, RM_{ABA} is *blue* to *aqua*, and $R_{ex} < 25\%$; (iv) +∼++, RM_{ABA} is *aqua* to *turquoise*, and $R_{ex} \geq 25\%$; (v) ++, RM_{ABA} is *green* or *yellow*, and $R_{ex} \geq 50\%$; (vi) ++∼+++, RM_{ABA} is *yellow* to *gold,* and $R_{ex} \geq 50\%$; (vii) +++, RM_{ABA} is *light orange* or *orange*, and $R_{ex} \geq 50\%$; (viii) ++++, RM_{ABA} is *orange*, and $R_{ex} \geq 75\%$.

A series of increasing integers from 0 to 7 were then assigned to the above tags, and the initial gene expression signal profiles were obtained for each gene. We repeated this procedure twice for both the ABA on-line tool and Brain Explorer software. Finally, four independent datasets (or replicates) were obtained.

Gene Expression Signal Normalization and Quantification—The gene expression signals defined above could not be directly used to measure the abundance of selenoprotein mRNAs. Instead, we utilized experimental mRNA levels for different selenoprotein genes and Sec machinery genes in the whole mouse brain, which were determined using real time PCR (29), to quantify gene expression. The reported mRNA levels of different selenoproteins and Sec machinery in whole mouse brain were normalized to the *Hprt* gene, whose levels were most consistent, among housekeeping genes, when different tissues and animals were considered (29). This gene was also used to normalize the ABA expression signals within brain regions. Given the observed expression signal $X_{g,i}$ of a query gene g and $X_{h,i}$ of a control gene h in region i, the normalized expression signal could be calculated as shown in Equation 1,

$$E_s(g,i) = \frac{X_{g,i} + q_g}{X_{h,i} + q_g} \qquad \text{(Eq. 1)}$$

where q_g is an "artificial" value (or pseudocount) to avoid zero-

probability expression when no signal of a particular gene was observed in certain region. In this study, we defined $q_g = 0.1$.

The average expression signal $\bar{E}_s(g)$ among 159 brain regions was then calculated as shown in Equation 2,

$$\bar{E}_s(g) = \frac{\sum_i E_s(g,i)}{N_i} \quad \text{(Eq. 2)}$$

where N_i is the number of brain regions (159 in this study). Here, $\bar{E}_s(g)$ was regarded as measure of the mRNA levels for selenoproteins and Sec machinery in the whole mouse brain (designated $E(g)$). Based on the correlation between $\bar{E}_s(g)$ and $E(g)$, the abundance of mRNA (normalized by *Hprt*) in a given brain region $E(g,i)$ could then be inferred as indicated in Equation 3,

$$E(g,i) = E_s(g,i)\frac{E(g)}{\bar{E}_s(g)} \quad \text{(Eq. 3)}$$

The final $E(g,i)$ was calculated as the average of replicates.

Gene Expression Profile Clustering—To investigate the relationship among expression patterns of different genes, normalized expression values were further quantified as shown in Equation 4,

$$M_{g,i} = \frac{\sum_{t_i} \ln\left(\frac{E(g,i,t_i)}{E(g,0,t_i)}\right)}{t_i} \quad \text{(Eq. 4)}$$

where t_i is the number of available replicates for region i, and $(g,0,t_i)$ is the average expression value of gene g in all regions in the t_ith replicate. If the expression value was not available, Equation 5 was used,

$$M_{g,i} = \frac{\sum_{t_i} \ln\left(\frac{E_s(g,i,t_i)}{E_s(g,0,t_i)}\right)}{t_i} \quad \text{(Eq. 5)}$$

where $E_s(g,0,t_i)$ is the average expression signal of gene g in all regions in the t_ith replicate.

Hierarchical cluster analysis was performed with CLUSTER software (30). We chose the complete linkage clustering algorithm in the software for gene clustering, and the final results were represented graphically using the Java TreeView tool (31). Cells with log ratios of 0 (unchanged compared with the average level) were colored black, increasingly positive log ratios with red of increasing intensity, and increasingly negative log ratios with green of increasing intensity, respectively.

In Situ Hybridization of SelM and SelH—The expression of *SelM* and *SelH* genes at different developmental stages and adult rat brain was studied by *in situ* hybridization. For this purpose, three brains per time point were dissected and frozen in the gaseous phase of liquid nitrogen. Frontal and horizontal sections (10–15 μm) were fixed in 4% paraformaldehyde (w/v), washed in 0.1 M phosphate-buffered saline (PBS, pH 7.4), and dehydrated. For *in situ* hybridization, the following antisense (and corresponding sense) oligonucleotides were used: 5′-gag ctt tcg tgg agg gcc ctt ctt aat acc agt cca gag ttc aac ac-3′ (*SelH*, GenBank™ accession number EST235143_R), and 5′-gga ggt gct tca tca cca ggt tgt ggt aca gtt gaa tgt cct gag tga ca-3′ (*SelM*, GenBank™ accession number XM_223554). The chosen oligonucleotides showed no significant cross-matches with other nonredundant and EST nucleotide sequences by BLAST analyses.

Immunofluorescence Confocal Microscopy Analysis of SelM—Whole mouse brains were dissected from adult mice immediately after decapitation and fixed in 4% paraformaldehyde in PBS for 2 h (all procedures were carried out at room temperature). After three washes in PBS (15 min each), the samples were dehydrated through an ethanol series and embedded in paraffin. Paraffin sections of the brain samples (~5 μm thick) were processed using standard de-paraffin and re-hydration methods, blocked in 3% bovine serum albumin in PBS for 1 h, and incubated for 2 h in 1% bovine serum albumin in PBS containing 0.05% Tween 20 (PBS-T) and rabbit anti-SelM antibodies (1:100 dilution). Brain sections were then washed three times (15 min each) in PBS-T and incubated in 1% bovine serum albumin in PBS-T containing Cy5-conjugated donkey anti-rabbit IgGs (Jackson ImmunoResearch, 1:100) for 1 h. After three rinses in PBS-T, the samples were stained with 4′,6-diamidino-2-phenylindole, mounted, and examined with an Olympus FV500 confocal system.

Western Blot Analyses—Different brain parts, including the olfactory bulb, hippocampus, hypothalamus, cerebral cortex and cerebellar cortex, were isolated from freshly dissected mouse brains. These brain parts, along with the other tissues from the same mouse (liver and testis), were snap-frozen in liquid nitrogen and then stored at −80 °C. Samples were homogenized in PBS containing complete protease inhibitors (Roche Applied Science), sonicated, and centrifuged at 14,000 × *g* for 15 min. Supernatants were collected, and the lysates were normalized with regard to protein concentration. 10% BisTris NOVEX gels (Invitrogen) were used, and each well was loaded with 25 μg of indicated tissue homogenate. The proteins were separated by SDS-PAGE, transferred onto polyvinylidene difluoride membranes (Invitrogen), and probed with specific antibodies described previously by our laboratories (also shown in the Supplemental Material). Secondary horseradish peroxidase-conjugated antibodies were from Amersham Biosciences, and chemiluminescent peroxidase substrate was from Sigma.

RESULTS

Global Analysis of Selenoprotein and Sec Machinery Gene Expression in Mouse Brain—Original ISH image data could be identified in the ABA dataset for 23 selenoprotein genes and 5 Sec machinery genes. The remaining mouse selenoprotein gene (*SelH*), was not represented in the ABA dataset, but it was detected in mouse brain in previous studies (29), and we experimentally verified its expression during development in rat brain. Thus, all mouse (rodent) selenoprotein genes appear to be expressed in the brain. Whether a full selenoproteome is also expressed in other organs is not known. We further analyzed expression profiles of all detected selenoprotein genes across 159 brain regions. The corresponding expression levels for each selenoprotein gene were then calculated using absolute mRNA levels for whole mouse brains (based on real time PCR data)

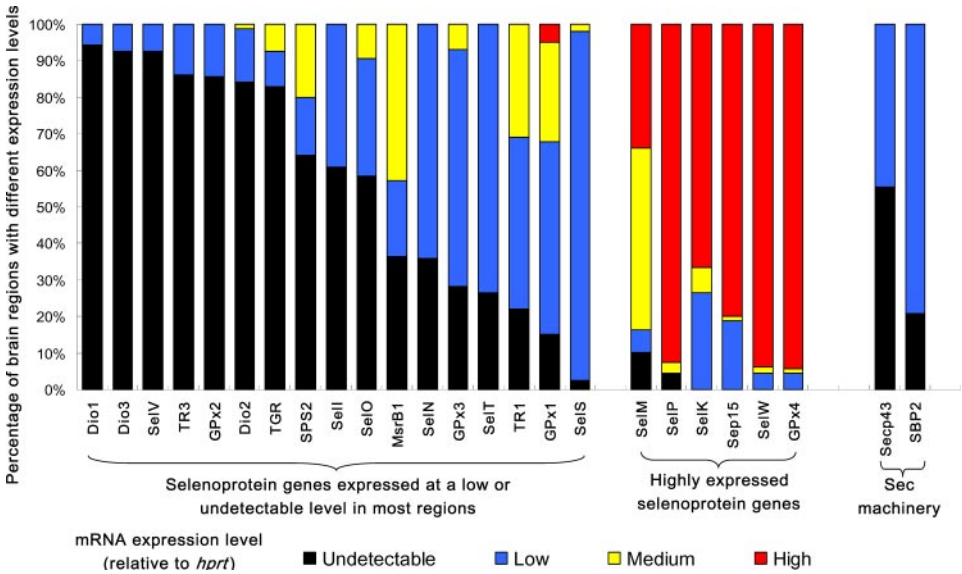

FIGURE 1. **General features of expression of selenoprotein and Se machinery genes in mouse brain.** The actual expression level of each indicated gene in each brain region in the ABA dataset was calculated based on the experimental expression level in whole mouse brain (29). Using *hprt* as a reference gene, expression levels of each examined gene were divided into the following four groups: undetectable (<0.05), low expression (0.05–0.5), medium expression (0.5–2), and high expression (>2). Proportions of brain regions with indicated expression levels are represented in the form of a *histogram*. These data were used to classify selenoproteins into highly expressed selenoprotein genes (at least a medium expression level in more than half, and a high expression level in one-third of the brain regions) and selenoprotein genes expressed at low or undetectable levels in most regions of mouse brain.

that were recently reported by Berry and co-workers (29). We utilized a common control gene (*Hprt*) to link the two datasets, and all data were normalized to this gene. Based on the normalized mRNA expression values, gene expression levels of all examined genes for each brain region were divided into four groups as follows: undetectable (<0.05), low expression (0.05–0.5), medium expression (0.5–2), and high expression (>2).

Distribution and general features of selenoprotein gene expression are shown in Fig. 1. Six (*GPx4, SelK, SelM, SelP, SelW,* and *Sep15*) of 23 selenoprotein genes showed at least a medium expression level in more than half and a high expression level in one-third of brain regions. These genes were designated as highly expressed genes. Of these six genes, *GPx4, SelP,* and *SelW* genes were expressed at a high level in over 90% of brain regions. These observations are consistent with previous studies that found high *SelP* and *SelW* gene expression in rodent brain (15, 20, 21, 23, 29, 32). On the other hand, many selenoprotein genes, including those coding for essential proteins, *e.g.* cytosolic and mitochondrial TRs, and those expressed at high levels in certain organs, *e.g. GPx2, GPx3,* and *TGR,* were expressed at low levels or had undetectable mRNA in most brain regions. Moreover, GPx1 and TR1, the best characterized and most abundant selenoproteins in liver and many other organs, were not among the highly expressed selenoprotein genes. It is also interesting that the three *Dio* genes (*Dio1–3*), which were previously reported to be significantly expressed in the developing brain of mammals (33, 34) and frog (35), were undetectable in more than 85% of the regions of adult mouse brain. However, it has been reported that Dio2 may serve specific roles through regulated expression in specific hypothalamic cells (36–38).

Similar analyses were also carried out for Sec machinery genes. All five examined Sec machinery genes were detected in different regions of mouse brain. We further limited our analysis to *SBP2* and *Secp43*, as real time mRNA levels in the whole brain were not available for other Sec machinery components (29). Although *SBP2* was expressed at low levels in most brain areas, it clearly mimicked expression patterns of five highly expressed selenoprotein genes (*GPx4, SelK, SelM, SelW,* and *Sep15*), suggesting that increased levels of SBP2 were needed to support expression of some or all of these selenoproteins.

Regionally Enriched Selenoprotein Gene Expression—Several selenoprotein genes showed common, yet highly complex expression patterns, whereas some had unique patterns. To identify brain regions with elevated selenoprotein gene expression, we examined each of the 159 brain regions for sets of expressed selenoprotein genes. Representative subdivisions were extracted from these regions based on brain anatomy, function, transmitter systems, and neuron populations affected by major neurodegenerative disorders. Supplemental Table S2 summarizes the data for these regions by showing normalized selenoprotein gene expression levels. A complete expression profile of selenoproteins in 159 brain regions is included in supplemental Table S1.

Main olfactory bulb (MOB, 21 selenoprotein genes), Ammon's horn (CA1–CA3, 20 selenoprotein genes), piriform area (19 selenoprotein genes), anterior olfactory nucleus (19 selenoprotein genes), cortical amygdalar area (19 selenoprotein genes), accessory olfactory bulb (19 selenoprotein genes), isocortex (or neocortex, *e.g.* somatosensory areas, 19 selenoprotein genes), and all folia of vermis and hemispheres of cerebellar cortex (19 selenoprotein genes) were the top regions with regard to the number of expressed selenoprotein genes (supplemental Table S2). Except for a small number of brain stem nuclei, we found that selenoprotein-enriched areas were located in the following four basic brain regions: hippocampus, olfactory area, cerebellar cortex, and isocortex (the part of cerebral cortex with uniform six layers). Olfactory cortex, piriform area, and hippocampus, all belonging to allocortex, showed unique selenoprotein expression patterns. An illustration of this general pattern is given in supplemental Fig. S1 that features elevated SelM gene expression in cerebellum, MOB, CA, and dentate gyrus (DG) structures of hippocampus. We further refer to these four regions as the regions with high selenoprotein gene expression. Except for *SelP* in hippocampus, expression levels of all highly expressed selenoprotein genes were at least medium, and of *GPx4, SelW,* and *Sep15* genes were high in

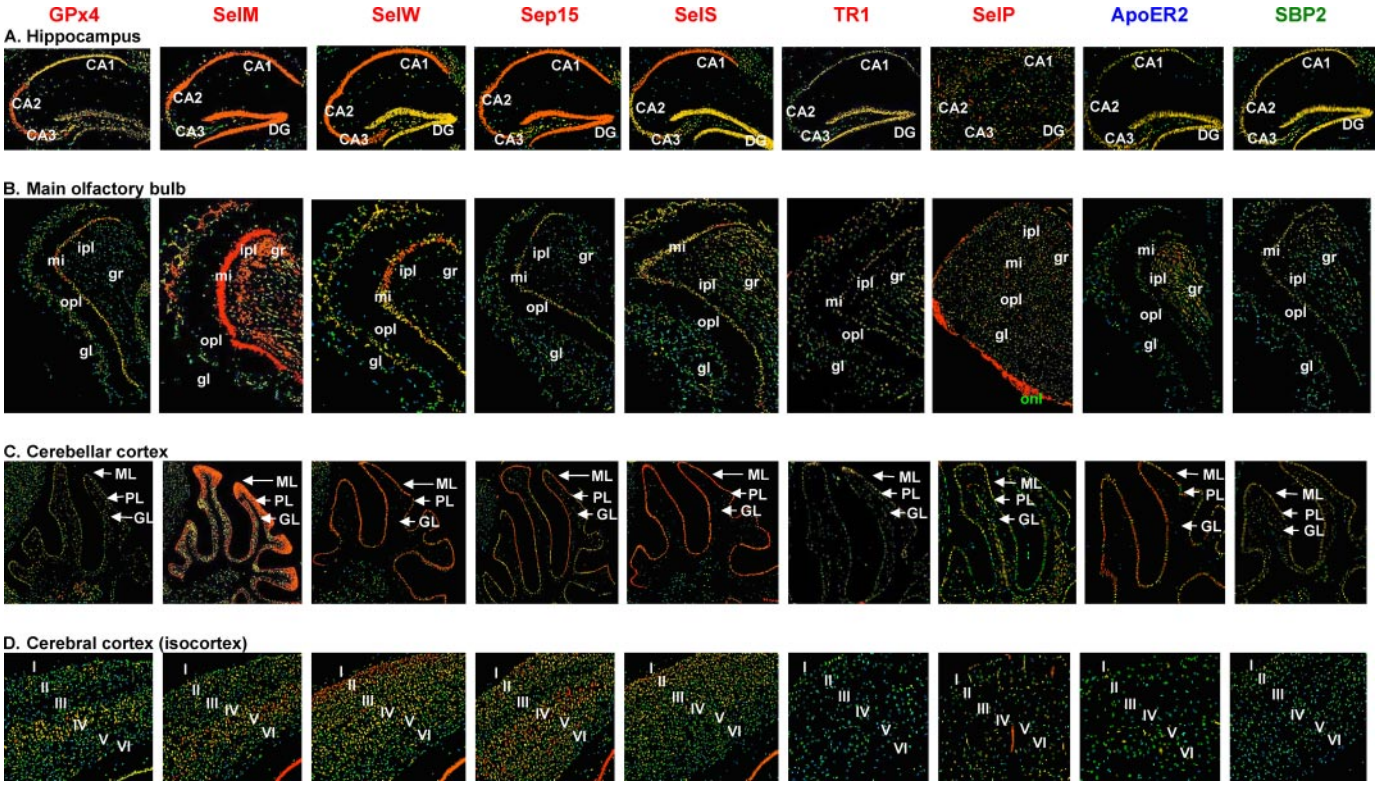

FIGURE 2. **Representative selenoprotein gene expression images in selenoprotein gene-enriched areas.** Each image of the corresponding region was extracted from the same or close plane positions for different genes. Representative selenoprotein genes are shown in *red,* Sec machinery gene, *SBP2* in *green* and the SelP receptor gene, *ApoER2*, in *blue*. *A,* hippocampus. *B,* main olfactory bulb. *gl*, glomerular layer; *opl*, outer plexiform layer; *mi*, mitral cell layer; *ipl*, internal plexiform layer; *gr*, granule cell layer. The highest expression level of SelP in the brain was found in olfactory nerve layer of MOB (*onl*), which is shown in *green*. *C,* cerebellar cortex. *ML*, molecular layer; *PL*, Purkinje layer; *GL*, granular layer. *D,* cerebral cortex (isocortex).

these four regions. Other selenoprotein genes, which were expressed at low or undetectable levels in most brain areas, were also found to be expressed at increased levels in these four regions.

In contrast to the above regions, major parts of the midbrain exhibited a much lower expression of selenoprotein genes. Supplemental Table S2 also summarizes the regions in which the lowest numbers of selenoprotein genes were detected, including oculomotor nucleus, Edinger-Westphal nucleus, nucleus Raphé pontis, anteroventral periventricular nucleus and dorsal premammillary nucleus. Only highly expressed selenoproteins were detected in most of these brain regions. In addition, lower expression of selenoprotein genes was observed in the largest white matter structure in the brain, corpus callosum. The low levels of selenoprotein mRNAs in these structures suggest lower dependence of these structures on selenoproteins and Se.

Cellular Localization of Selenoprotein Expression—Many brain regions show a multilayered neural architecture, a pattern that is also evident in the analysis of selenoprotein gene expression. In contrast, the distribution of glial cells is generally more uniform, except in large fiber tracts devoid of neuronal cell bodies. Analysis of the ABA dataset suggests that certain cell types/layers rather than a distinct anatomical localization are associated with specific expression of selenoprotein genes. To investigate the cytoarchitectonic features of selenoprotein gene expression, we manually examined selenoprotein expression patterns in different cell types and layers that were characterized by significant selenoprotein gene expression (supplemental Table S3). We used expression signal tags to reflect the expression patterns instead of the expression level *per se* to better represent changes in expression between brain regions. However, it should be noted that the same signal tag may represent different mRNA expression levels for different selenoprotein genes, *e.g.* "++" corresponds to low expression level for *SelS*, medium expression level for *SelM*, and high expression level for *GPx4* (see "Experimental Procedures").

One of the most obvious regions that showed patches of elevated expression of selenoprotein genes was the hippocampus. However, the selenoprotein expression patterns in this formation were not uniform (Fig. 2*A* and supplemental Fig. S2). A number of selenoprotein genes were strongly expressed in the pyramidal cells of the CA1–3 and granule cells of DG (Fig. 2*A* and supplemental Table S3), but the expression levels of many selenoprotein genes were different among CA1, CA2, and CA3 regions. For example, *SelM* showed highest expression in CA1/CA2, *GPx4* in CA2/CA3, and *SelS* in CA1. On the other hand, *Sep15* and *SelW* showed uniform expression levels in all CA regions. Moreover, the expression pattern of *TR1* was a notable exception with the higher expression signal in the DG than in the CA areas. Expression of *SelP* was more homogeneous in the hippocampus. In contrast to other highly expressed selenoprotein genes, we did not observe *SelP* gene expression in the major neurons of CA and DG structures, suggesting an oligodendroglial or astrocytic expression pattern of this protein. In contrast, selenoprotein biosynthetic machinery genes, *e.g. SBP2*, were generally expressed at the same levels in CA1–3 and DG and

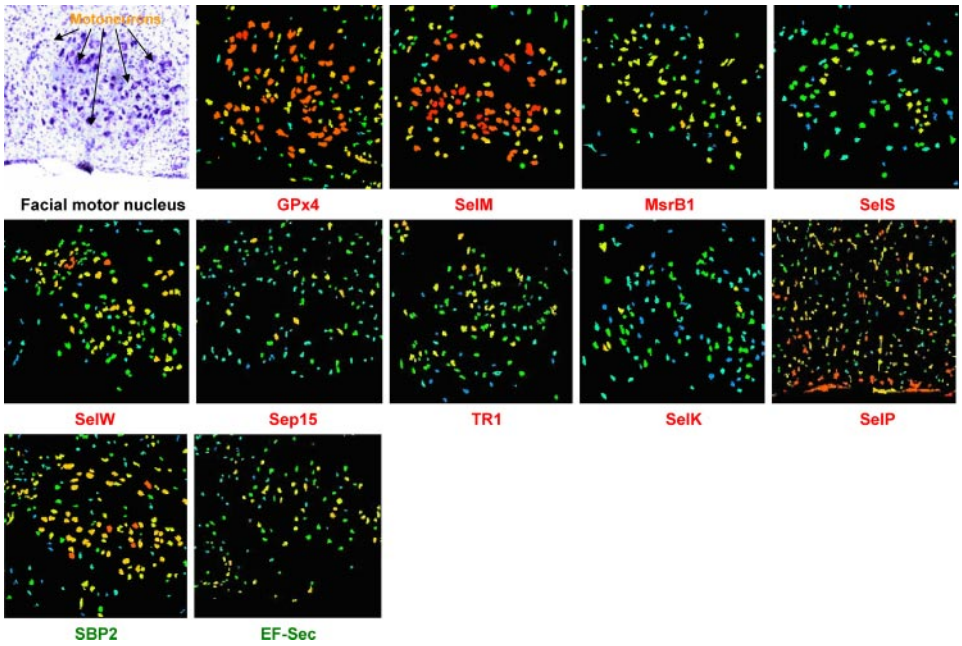

FIGURE 3. **Representative selenoprotein gene expression images in facial motor nucleus.** The motoneurons are indicated with *arrows*. Except for *SelP*, most selenoprotein genes and Sec machinery genes are expressed in a pattern resembling the distribution of motoneurons. Representative selenoproteins are shown in *red*, and Sec machinery genes are shown in *green*.

correlated with expression levels of *GPx4, SelM, SelW,* but not *SelP* genes. The resolution of the data was not sufficient to quantify selenoprotein expression in hippocampal interneurons located in, for example, the strata oriens or radiatum, but signals with a distribution reminiscent of interneurons were observed (*e.g.* for *SelM*).

Olfactory bulb was another region with significant selenoprotein gene expression, but as in the hippocampus, the observed expression pattern was not uniform. Selenoprotein genes and Sec machinery genes in MOB were mainly detected in the glomerular, mitral, and granule cell layers (Fig. 2*B, gl, mi,* and *gr*, and supplemental Fig. S3), with the highest levels in the mitral layer. Again, the *SelP* expression pattern suggested glial expression in white matter. As in CA and DG structures in the hippocampus, *SelP* expression appeared to be excluded from the areas with elevated selenoprotein expression. However, it is interesting that the highest expression of the *SelP* gene was found within the olfactory nerve layer of MOB, which is located on the surface of the bulb. Expression of other selenoprotein genes was not detected in this area.

In the cerebellar cortex, which contains nearly 50% of neurons in the brain, almost all detected selenoprotein genes were expressed in the Purkinje cell layer (Fig. 2*C* and supplemental Fig. S4, and supplemental Table S3). *SelM* had the most significant expression signals covering Purkinje cells, granule cells, and deep cerebellar nuclei. A similar expression pattern was observed for *GPx4, TR1, MsrB1* (supplemental Fig. S4), and the Sec machinery gene *SBP2*, although at a lower signal level. Expression of *SelS, SelW,* and *Sep15* was largely restricted to Purkinje cells. Thus, Purkinje cells apparently express virtually all selenoproteins. *SelP* gene expression was detected in both the monolayer associated with Purkinje cell layer and deep white matter. The monolayer expression was not significantly elevated for *SelP* gene (as was observed for other highly expressed selenoprotein genes), and *SelP*-expressing cells were spaced and had highly variable *SelP* expression levels. In contrast, other selenoprotein genes, such as *SelS, SelW*, and *SelT*, showed uniform expression in Purkinje cells. It is thus possible that *SelP* expression is confined to radial glia (or Bergmann glia), whose cell bodies are located next to the soma of Purkinje cells and the signal may sometimes be misinterpreted as arising from Purkinje cells. Further experiments would be needed to test this possibility, but it fits the idea of separation of high SelP and high selenoprotein expression in different cell types.

We also sampled expression of selenoprotein genes in different layers of the isocortex. For the purpose of presentation, we selected the primary somatosensory area overlying the dorsal hippocampal formation. Interestingly, some selenoprotein genes showed layer-specific expression (Fig. 2*D* and supplemental Fig. S5). For example, the *SelW* gene was highly expressed in layer II compared with other layers, and *GPx4* and *Sep15* gene expressing cells were enriched in layers II and IV. On the other hand, *SelM* gene was enriched in layer V. Analogous patterns were also observed for Sec machinery genes. In contrast, *SelP* showed a uniform distribution in different layers.

Besides the four major selenoprotein-enriched regions, certain brain stem nuclei were detected to express multiple selenoprotein genes. As an example for such nuclei, we selected the facial motor nucleus (VII) that is entirely composed of cholinergic motor neurons. Thirteen selenoprotein genes were detected in this nucleus. Most selenoprotein genes and Sec machinery genes, such as *GPx4, SelK, SelM, SelW,* and *SBP2*, were expressed in a pattern resembling the distribution of motoneurons (Fig. 3).

In addition to neurons and glial cells in various brain regions, we observed significant expression of selenoprotein genes in the choroid plexus of both the lateral ventricle and the fourth ventricle. The choroid plexus is a highly vascularized structure located in the ventricles that produces cerebrospinal fluid and is a key part of the blood-brain barrier. Here, expression of 14 selenoprotein genes was detected. *GPx4* and *Sep15* genes were expressed in particularly high levels (Fig. 4). SelP was detected previously in human cerebrospinal fluid (24). Here, *SelP* expression was also detected at elevated levels, suggesting secretion of SelP into mouse cerebrospinal fluid.

Interestingly, *ApoER2* was observed to have elevated gene expression in the four selenoprotein-enriched areas as well as in the choroid plexus (Fig. 2 and Fig. 4). This finding is consistent with the idea that ApoER2 is important for SelP transport within the brain, especially in selenoprotein-enriched areas.

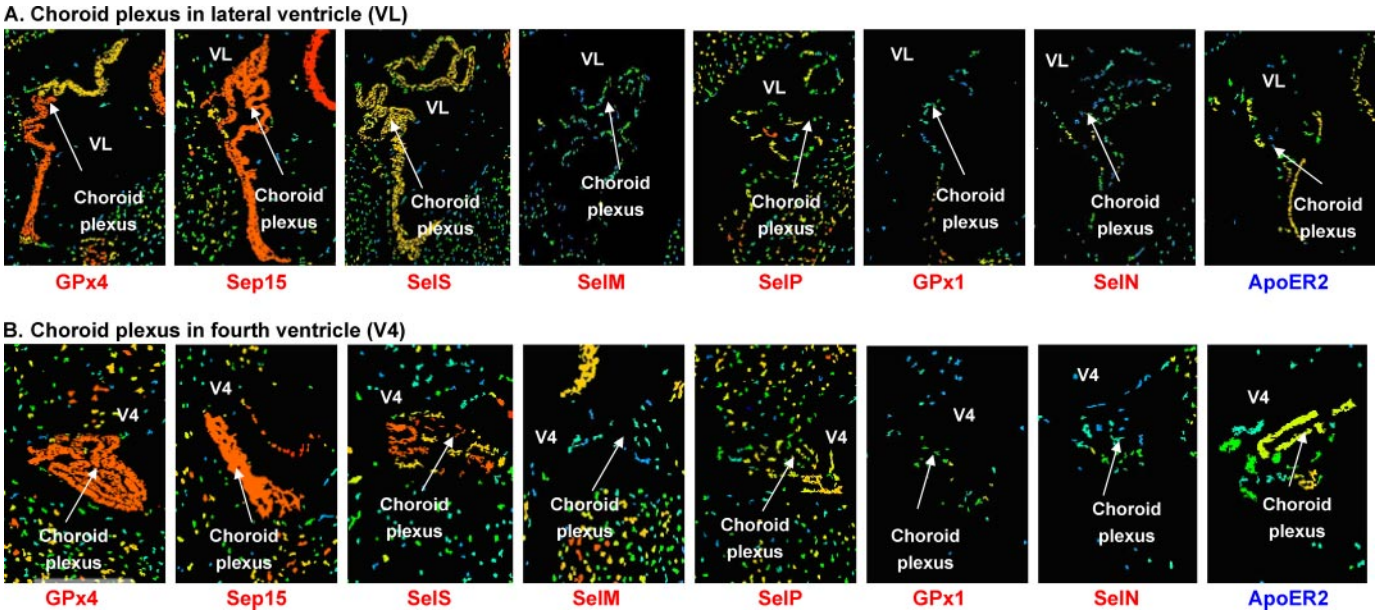

FIGURE 4. **Representative selenoprotein gene expression images in choroid plexus regions in different ventricles.** *A,* choroid plexus in lateral ventricle (*VL*); *B,* choroid plexus in fourth ventricle (*V4*). *GPx4* and Sep15 genes were expressed at particularly high levels. Representative selenoprotein genes are shown in *red* and *ApoER2* in *blue*. *ApoER2* is enriched in choroid plexus, as is *SelP*, suggesting that it could act at the blood-brain barrier as a receptor for SelP.

Verification of Selenoprotein Expression in the Brain—We wanted to extend the expression data of one of the most heavily expressed selenoproteins, SelM, to other rodents, and we thus performed ISH for this gene in the rat hippocampus at different developmental stages. Fig. 5*A* shows that this gene was highly expressed first around birth in hippocampal CA3 and later in all principal divisions of the hippocampus. In parallel, SelM protein was readily detected in mouse brain by immunohistochemistry, which was found to be most prominent in cerebellar Purkinje cells (Fig. 5*B*). In the cerebellar granule cell layer, some unidentified cells stained positive for SelM, possibly interneurons.

Because the mRNA expression data for *SelH* were not available in the ABA dataset, we performed ISH, using rat brain development as a model. Interestingly, *SelH* was highly expressed during development, but it fell after birth and was below detection limit in the adult brain (Fig. 6). Using reverse transcription-PCR, however, *SelH* gene expression was detected in adult mouse brain in a separate study (29). Therefore, all 24 selenoprotein genes were expressed in mouse (or rodent) brain.

Because mRNA expression does not always reflect protein levels, we analyzed expression levels for several selenoproteins in different regions of the mouse brain by Western blot assays (Fig. 7). Liver and testes were used as controls, and as expected, the liver showed high expression of GPx1 and MsrB1, whereas SelS and GPx4 were particularly abundant in mouse testes. We found that TR3 was expressed at similar levels in all analyzed samples, including liver, testes, and various brain regions. This is consistent with a previous observation that gene expression levels of TR3 in liver and brain are not significantly different (39). Consistent with SelM mRNAs levels in whole brain, this selenoprotein showed higher expression levels in brain than in other organs. Within brain, expression of several examined selenoproteins, including GPx4, SelM, MsrB1, SelW and SelS, was particularly high in cerebellar cortex. In contrast, selenoprotein expression was low in hippocampus and olfactory bulb. Here, signal intensity may simply be a consequence of low neuronal density, because neurons, not glial cells, are the major site of selenoprotein expression. Another possibility is that these regions store high levels of selenoprotein transcripts, which can be used for quick selenoprotein expression and subsequent degradation. Such a mechanism would be consistent with the regulation requiring fast protein turnover and may be particularly relevant to olfaction and memory.

Clustered Gene Expression—Information on selenoprotein gene expression in different regions of mouse brain provided us with an opportunity to identify possible functional linkages among selenoproteins and between specific selenoproteins and components of Sec insertion machinery by comparing their expression patterns. To this end, we subjected the selenoprotein and Sec insertion gene expression dataset for 159 selected brain regions to clustering with CLUSTER (30). Gene expression profiles were classified by hierarchical cluster analysis and displayed in a correlation map (supplemental Fig. S6). A fraction of the map corresponding to the regions with elevated selenoprotein gene expression is shown in Fig. 8. Consistent with significant selenoprotein gene expression in CA1–3 and DG regions of the hippocampus and in cell layers in the olfactory bulb, isocortex, and cerebellar cortex structures, genes for several Sec machinery components were significantly expressed in these regions. In contrast, although other structures that consist of discrete nuclei, such as the hypothalamus, pons, medulla, and midbrain, had locally enriched expression of certain selenoprotein genes, the expression level for most genes was similar to or even lower than the average level in the whole brain. Almost all selenoprotein genes, which were expressed at low levels in most brain regions, clustered in one branch, whereas most highly expressed selenoprotein genes clustered in another

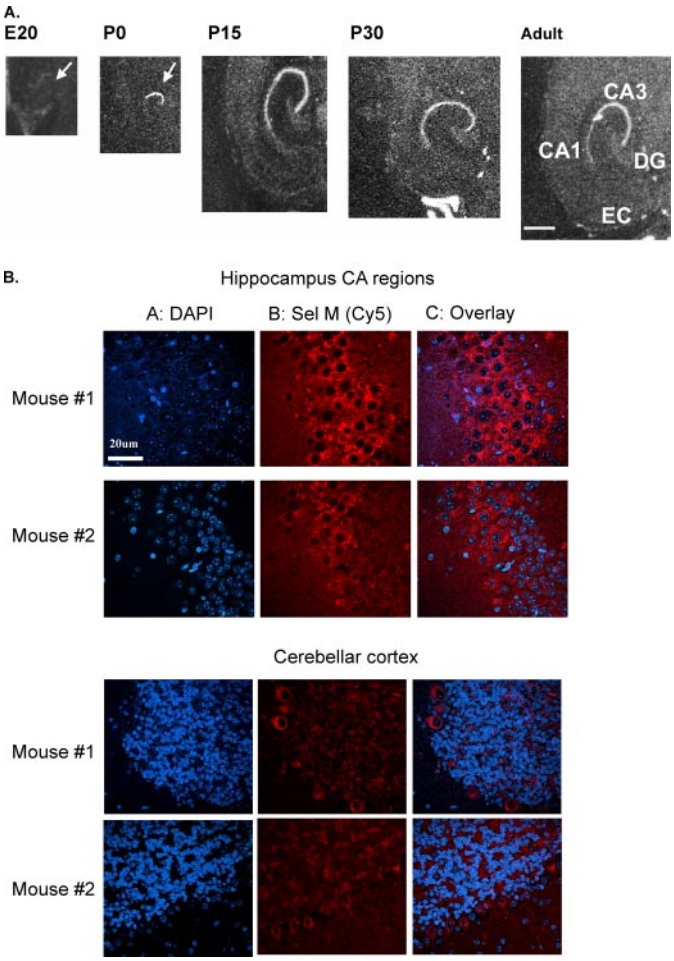

FIGURE 5. **Gene expression and immunohistochemical analysis of SelM in developing and adult rodent hippocampus.** *A, SelM* gene expression images in the rat hippocampus at embryonic, young, and adult stages are shown. High expression of *SelM* is indicated by an *arrow*. *EC*, entorhinal cortex. *B,* immunohistochemical analysis of SelM in adult mouse. SelM was detected in wild type mouse brain slices with antibodies specific for mouse SelM. Expression of SelM is shown in CA neurons in hippocampus, small neurons in granule cell layer, and large Purkinje cells. *Scale bars* = 20 μm.

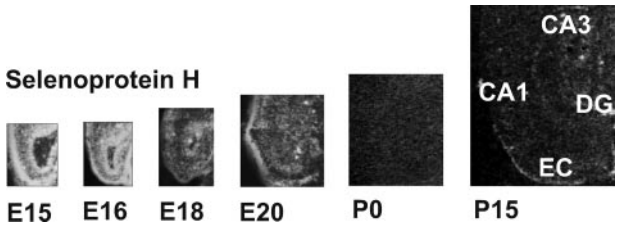

FIGURE 6. **Gene expression of SelH in the developing rat hippocampus.** *SelH* gene expression images in rat hippocampus at different embryonic stages are shown. *EC*, entorhinal cortex.

branch. Consistent with the oligodendroglial expression pattern of *SelP*, it did not cluster with any other examined genes.

Finally, pairs of genes exhibiting a similar expression pattern were identified. For example, *Sep15* and *SelM* clustered together. These are homologous proteins and are both targeted to the endoplasmic reticulum (40). Possibly, these proteins have similar functions *in vivo*. However, differences in expression patterns for these selenoprotein genes were also identified. For example, *SelM* gene expression was relatively low in several brain stem nuclei and choroid plexus, whereas *Sep15* gene was

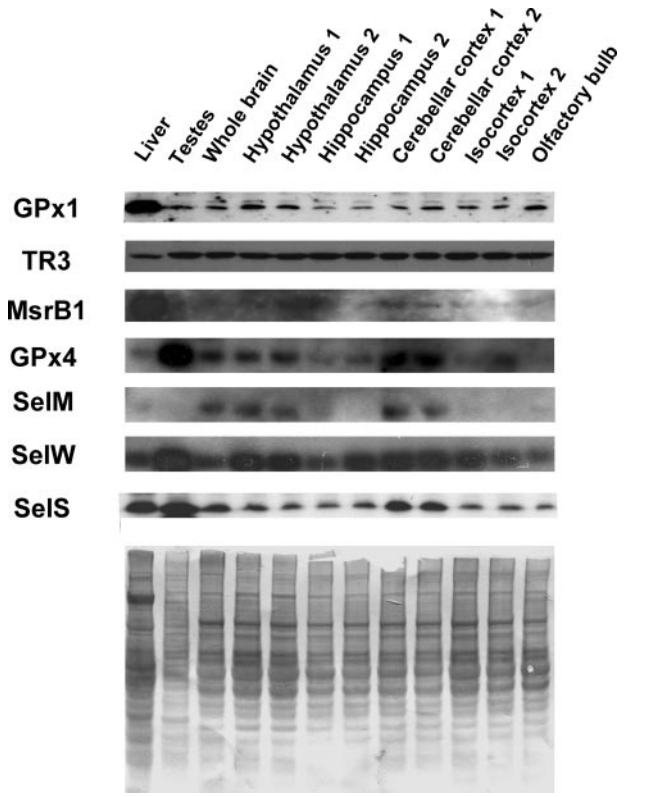

FIGURE 7. **Western blot analysis of selenoprotein expression in different regions of mouse brain.** Expression of GPx1, GPx4, MsrB1, SelM, SelW, SelS, and TR3 selenoproteins was assayed using antibodies specific for these proteins. The *lower panel* shows Coomassie Blue staining pattern and indicates approximately equal loading of protein extracts. Two independent samples (from two different mice) were analyzed for hypothalamus, hippocampus, cerebellar cortex, and isocortex, whereas individual samples were assayed for liver, testis, whole brain, and olfactory bulb.

highly expressed in these structures (supplemental Table S2). In addition, the *SBP2* gene clustered within the group containing highly expressed selenoprotein genes, suggesting that *SBP2* expression is elevated to support increased expression of a select group of selenoprotein genes in certain regions of mouse brain.

DISCUSSION

Our study represents, to date, the most complete analysis of selenoprotein gene expression in the mammalian brain. Expression of mRNA for selenoproteins and Sec machinery genes was analyzed in 159 regions of the adult mouse brain, and cluster analysis revealed coexpression patterns of potential functional significance. To complete the analysis, ISH and Western blot experiments were carried out. These data will aid in the design and interpretation of experiments aimed at elucidating the mechanisms of how Se, through selenoproteins, supports brain function.

Se is a trace element indispensable for mammals and is present in each organ and in body fluid. Numerous studies have examined the levels of Se in human and animal tissues and found an uneven distribution of this trace element (41–45). Se content of human brain (~88 ng/mg wet weight) is much lower than that of kidney and liver (~469 and ~221 ng/mg wet weight, respectively (45). Similarly, Se content of mouse brain is

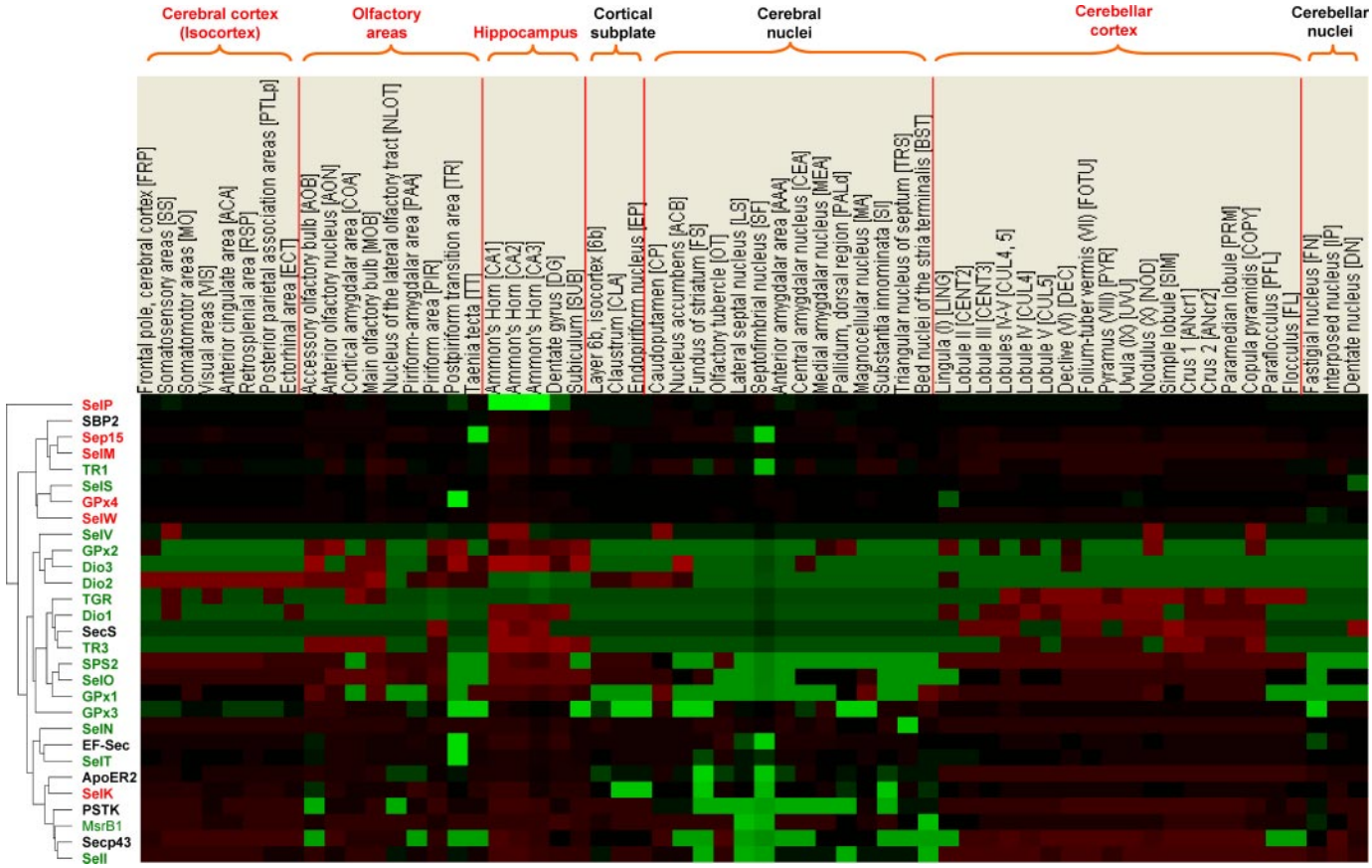

FIGURE 8. **A fraction of the correlation map that shows elevated gene expression for most selenoproteins in the four selenoprotein-enriched areas.** The *dendrogram* and the image were produced as described in the text; the color scale is from saturated *green* (significant negative change) to *black* (no significant change) and then to saturated *red* (significant positive change). *Red* and *green* colors represent increased and decreased expression, respectively, when compared with the average expression level for each gene. Each gene is represented by a single row of *colored boxes*; and each region is represented by a *single column*. Highly expressed selenoprotein genes are shown in *red font*, and selenoprotein genes expressed at a low or undetectable level in most brain regions in *green font*. The four selenoprotein-enriched areas are highlighted in *red*.

lower than that of most other organs (15). However, during Se deficiency, the brain shows an ability to preserve this trace element, whereas other organs readily lose Se (13, 15, 46). In addition, several studies revealed that the regions of human brain enriched for gray matter tend to have higher Se levels, whereas white matter was found to have reduced Se, and Se appeared to concentrate in glandular parts of the brain (41, 44, 47). Similar patterns were observed for animals (48, 49). However, how this information can be translated at the level of selenoproteins, which mediate the biological effects of Se, is not known. Expression patterns and levels for most brain selenoproteins and Sec machinery factors have not been documented, although significant data exist for some selenoproteins, such as GPx1, a selenoenzyme family that removes hydroperoxides and is expressed in all cell types (50–52), and SelP, a selenium transport selenoprotein (16, 17, 22).

Previous studies found that some of the analyzed selenoprotein genes were expressed predominantly in neurons (such as *GPx4*), or in both neuronal and glia cells (such as *GPx1* and *TR*). However, our study suggests that neurons are the primary sites of selenoprotein expression in the brain. This result nicely matches the data on neuron-specific deletion of the $tRNA^{[Ser]Sec}$ where the majority of analyzed selenoproteins were severely reduced or were undetectable in brain as analyzed by Western blot experiments.[3] Thus, whatever the mechanism of neurological damage may be in mice with reduced brain Se content (16, 17, 54, 55), it likely results from impaired neuronal selenoprotein expression.

Another salient finding of this work is that there are four regions in the brain that express the following: (i) the most selenoprotein genes within the same cells, and (ii) the highest levels of selenoprotein mRNAs. These regions were olfactory bulb, cerebral cortex, hippocampus, and cerebellar cortex. Taking into account the equally strong expression of selenoprotein genes in some brain stem nuclei, as shown for the facial nucleus, we can rule out a methodological bias based on the size of the brain structure. Moreover, the striatum expresses only average levels of selenoprotein genes, although it is a comparably homogeneous structure. Thus, homogeneity of cell types also does not introduce a bias for interpretation of ISH data. This indicates that our findings are of biological significance, although we cannot, at present, correlate high selenoprotein gene expression with certain types of neuron (pyramidal *versus*

[3] E. K. Wirth, M. Conrad, S. B. Bharathi, C. Iserhot, B. A. Carlson, S. Roth, D. Schmitz, G. W. Bornkamm, M. Brielmeier, V. Coppola, L. Tessarollo, E. Pohl, L. Schomburg, J. Kohrle, D. L. Hatfield, and U. Schweizer, submitted for publication.

granule) or with certain neurotransmitters (glutamate, γ-aminobutyric acid, or acetylcholine).

An additional significant result of this work is that hierarchical cluster analysis of gene expression data is shown to be a useful method to extract biological information from the ISH data base. To be able to correlate the ABA signals with actual gene expression levels, we took advantage of the data on experimental gene expression for selenoproteins and Sec machinery in the whole mouse brain (29). With this approach, normalized expression levels of selenoprotein genes in individual regions of mouse brain could be inferred, enhancing the primary data provided by the Atlas. Conclusions of biological significance based on the bioinformatics approach included the finding that, among Sec machinery genes, *SBP2* expression most closely correlated with high selenoprotein gene expression, supporting the hypothesis that SBP2 is the limiting factor for selenoprotein translation. In addition, we have previously found that SelM and Sep15 are distantly related proteins of unknown function (40, 56). It is thus significant that these two selenoproteins clustered together in the expression data.

Which selenoprotein genes are most important to brain function? Our study identified six highly enriched selenoprotein genes, including *GPx4, SelK, SelM, SelP, SelW,* and *Sep15*. Particularly abundant were *GPx4* and *SelW* genes, which were detected at high levels in more than 90% regions of mouse brain. Of the six selenoproteins with elevated gene expression levels, the functions of SelM, SelW, Sep15, and SelK are not known. It is interesting, however, that classical gene targeting of *SelP*, but not liver-specific inactivation, leads to neurological dysfunction and motor incoordination (16, 19, 54). Although *SelP*-deficient mice can be rescued phenotypically by increasing dietary Se intake, synaptic function and spatial learning have been reported altered in the knockouts (57). In addition, GPx4 has recently been shown to be essential for neuronal development and survival (25). Thus, high and clustered *SelM, SelK,* and *SelW* gene expression is also consistent with important physiological functions of these proteins in the brain. Unfortunately, little is known about their biochemical functions.

On the other hand, expression levels of several well characterized selenoprotein genes were lower. Some of these, such as Dio1–3, are involved in fine-tuning of thyroid hormone signaling by local activation or inactivation of thyroid hormones. Although Dio2 has been implicated in hypothalamic orexigenic signaling (36), gene targeting of *Dio2* yielded only mild effects on brain development and neurological function in mice (58). Genetic inactivation of *Dio1* had no reported effect on the brain (59). Gene targeting of *Dio3* disrupted normal thyroid hormone signaling in the hypothalamus, but is probably not required for neuronal function in general (60). The best studied selenoprotein in the brain is probably GPx1 (61). Genetic inactivation of *GPx1* increased susceptibility of rodents to neurodegenerative disease, and overexpression of GPx1 mitigated resulting damage (19). However, no spontaneous neurological damage was reported in mice lacking GPx1.

An additional interesting finding of our investigation is the cell type-specific expression of selenoproteins within brain structures. Such a distribution suggests physiological associations between selenoprotein expression and specific functions exerted by these specific neurons. Thus, there is at present no apparent explanation as to why, *e.g.* within the Ammon's horn, selenoprotein genes are differentially expressed between CA1, CA2, and CA3. In addition, layer-specific expression of selenoprotein genes has not been reported previously for cerebral cortex. What could be the functions of selenoproteins in the brain? With regard to GPxs, TRs, and MsrB1, the enzymes with known functions, it appears clear that redox regulation or protection from reactive oxygen species may be the main functions (54). It may well be that neuronal signaling or synaptic transmission are modulated by the (transient) redox potential around neurotransmitter receptors, and at least for one K^+ channel regulation through reversible methionine oxidation has been demonstrated (62). Many selenoproteins with still unknown function have a common thioredoxin fold, suggesting catalysis of redox reactions. Several selenoproteins are associated with the endoplasmic reticulum (ER), including Sep15 and SelM, which may be involved in oxidative protein folding, and SelS, a component of the retrotranslocon. Thus, all these selenoproteins may be involved in protein quality control in the ER. SelN is also located in the ER, but mutations in humans lead to rigid-spine muscular dystrophy or multiple minicore disease (63). Direct neurological defects have not been reported in these patients.

One highly expressed selenoprotein gene, *SelP*, had a unique expression pattern among selenoprotein and Sec machinery genes. It appeared that its expression and secretion mainly from astrocytes or oligodendrocytes supported selenoprotein synthesis in brain structures with high selenoprotein gene expression. In an unbiased approach, the original publication of the ABA classified *SelP* as marker for oligodendroglial cell or white matter tracts. Previously, it was found to be highly expressed in olfactory bulb and cerebellum (22, 23). SelP-like immunoreactivity was detected on neurons and some white matter tracts (24). Given its role as a Se carrier, its expression in glial cells would help store Se in the brain and also provide it to neurons for selenoprotein biosynthesis. Accordingly, many neurons express the SelP receptor, ApoER2 (28, 55), although ApoER2 is also a receptor component for Reelin, a secreted protein involved in brain development and synaptic transmission (53). Thus, we also examined the expression pattern of *ApoER2* in 159 brain regions and found elevated expression in the four selenoprotein-enriched areas and in the choroid plexus (Fig. 2, Fig. 4, Fig. 8, and supplemental Fig. S6). This is consistent with the idea that ApoER2 is important for SelP-mediated Se transport into and within the brain. Considering its mRNA expression pattern, it may thus be that SelP immunoreactivity on neurons, *e.g.* Purkinje cells (24), arises from SelP binding to the ApoER2 on the cell surface rather than reflecting endogenous expression by the neurons. Nevertheless, we recently reported the presence of SelP in human cerebrospinal fluid and high immunoreactivity in ependymal cells lining the ventricles of the human brain (24), also supporting the above idea. Why other selenoproteins are also enriched in the choroid plexus, the source of cerebrospinal fluid, is not clear, but one may speculate that GPx4 and Sep15 may be

involved in the control of massive protein secretion by choroid plexus cells.

Sec machinery genes had specific gene expression patterns in different regions of mouse brain. The correlation of elevated *SBP2* expression with selenoprotein gene-enriched structures has been noted above. *EFsec* mostly followed *SBP2* expression. Interestingly, both *PSTK* and *SecP43* had rather specific expression in cortical layers II and IV/V, the two cortical layers that had the highest selenoprotein expression (supplemental Fig. S5).

In the future, the methods described here could be extended to the entire ABA dataset and identify functional linkages between selenoproteins and other proteins expressed in mouse brain, *e.g.* other proteins involved in redox control, or could be extended to developmental studies. More specifically, these studies suggest candidate selenoproteins with presumed roles in brain function. Therefore, gene expression profiles identified in this study provide informative modality to investigate diversity of selenoprotein functions in the brain.

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[Supplemental/Supporting Materials follow.]

Supplemental information

Antibodies used in microscopy and Western blot analyses.

Polyclonal antibodies against GPx4, MsrB1, SelM and SelW, as described previously (1, 2), were produced by immunization of rabbits with purified recombinant proteins. Dilution used: 1:1000.

Rabbit monoclonal antibodies against GPx1 were obtained from Santa Cruz Biotechnology Inc. (1). Dilution used: 1:1000.

Rabbit polyclonal antibodies against TR3 were from Atlas, Stockholm, Sweden. Dilution used: 1:2000.

Rabbit polyclonal antibodies against SelS were prepared by standard peptide-KHL hemocyanin technology using a peptide corresponding to 15 C-terminal amino acids of mouse SelS (3). Dilution used: 1:2,500.

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Figure legends for supplemental figures

Fig. S1. An example of a SelM image in the ABA dataset. The sagittal plane # 2650 of SelM was used to prepare this figure. Regions with elevated ISH signals are labeled as follows: MOB, main olfactory bulb; PIR, piriform area; CA1-CA3, Ammon's horn field CA1-CA3; DG, dentate gyrus; SUB, subiculum; CTX, cerebral cortex; CBX, cerebellar cortex. Color scales for expression signal are also shown.

Fig. S2. Additional selenoprotein gene expression images in hippocampus. Each image of the hippocampal region was extracted from the same or close plane positions for different genes. A. Selenoprotein genes; B. Sec machinery genes. CA1-CA3, Ammon's horn CA1-CA3; DG, dentate gyrus.

Fig. S3. Additional selenoprotein gene expression images in main olfactory bulb (MOB). A. Selenoprotein genes; B. Sec machinery genes. gl, glomerular layer; opl, outer plexiform layer; mi, mitral cell layer; ipl, internal plexiform layer; gr, granule cell layer. The highest expression level of SelP in the brain was found in olfactory nerve layer of MOB (onl) which is shown in green.

Fig. S4. Additional selenoprotein gene expression images in cerebellar cortex. A. Selenoprotein genes; B. Sec machinery genes. ML, molecular layer; PL, Purkinje layer; GL, granular layer.

Fig. S5. Additional selenoprotein gene expression images in cerebral cortex (isocortex). A. Selenoprotein genes; B. Sec machinery genes. Layers I-IV are shown.

Fig. S6. Correlation map of selenoprotein and Sec machinery gene expression in 159 brain regions. The dendrogram and the image were produced as described in the text; the color scale is from saturated green (significant negative change) to black (no significant change) and then to saturated red (significant positive change). Red and green colors represent increased and decreased expression, respectively, when compared to the average expression level for each gene. Each gene is represented by a single row of colored boxes; and each region is represented by a single column. Highly expressed selenoprotein genes are shown in red font, and selenoprotein genes expressed at a low or undetectable level in most brain regions in green font.

Fig. S1

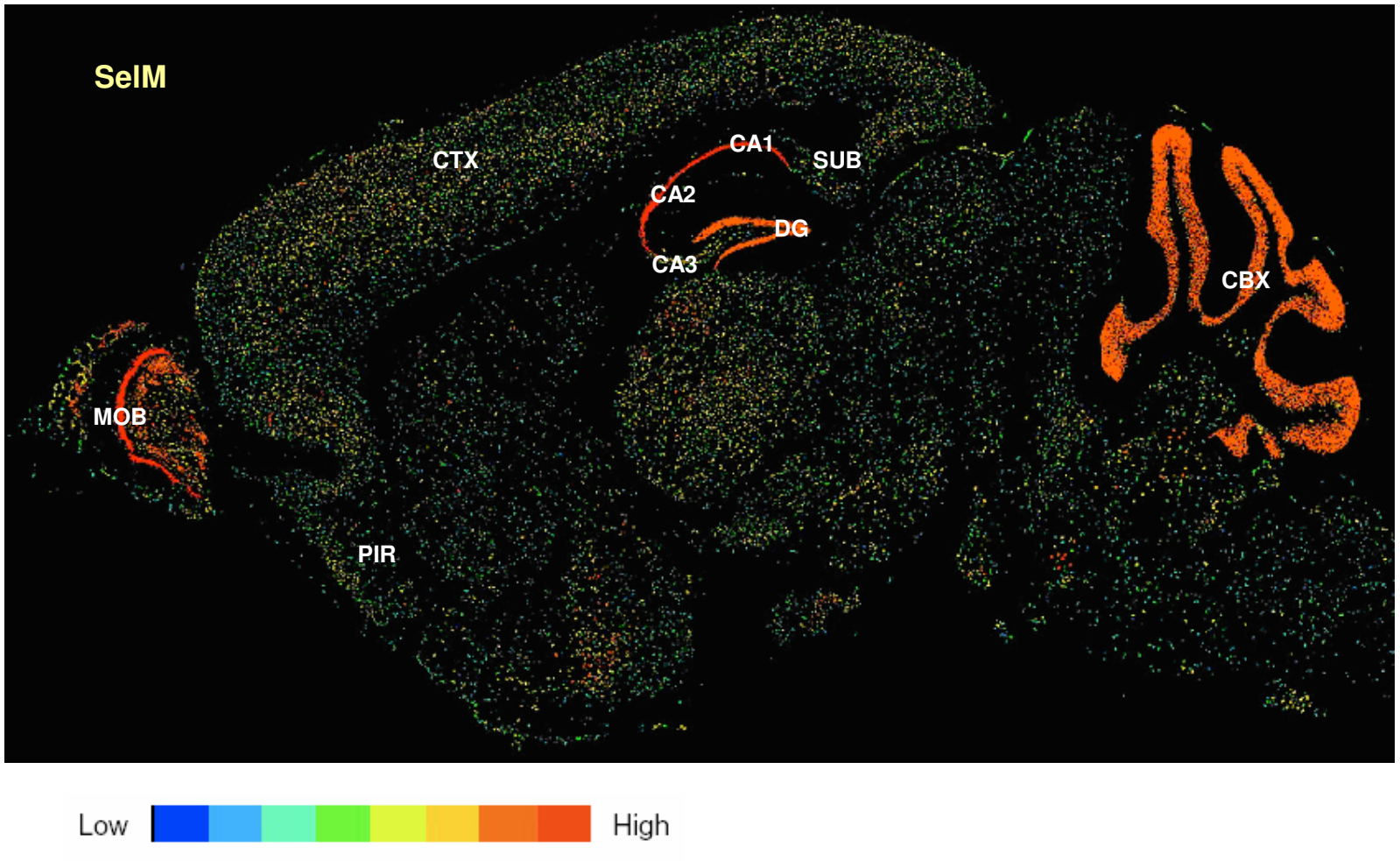
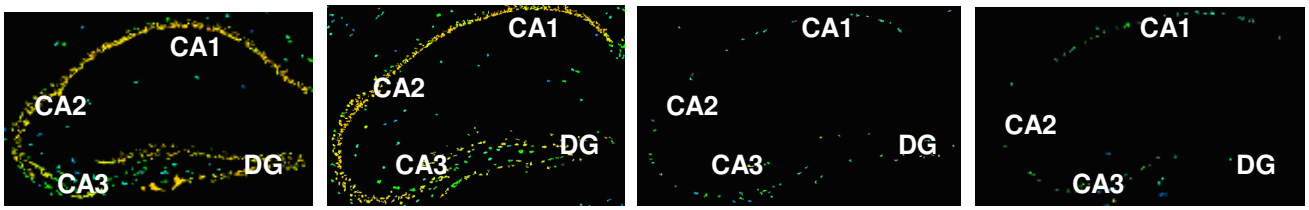


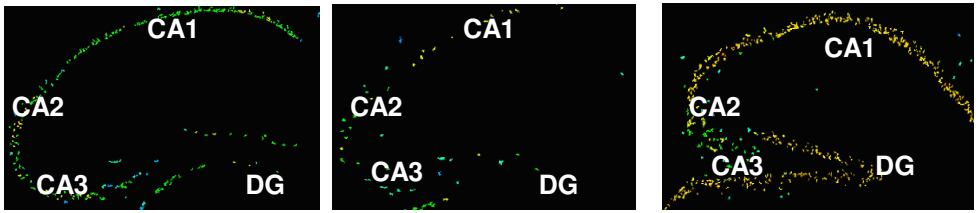
Fig. S2

A. Other selenoprotein genes



MsrB1 SelT TGR GPx1

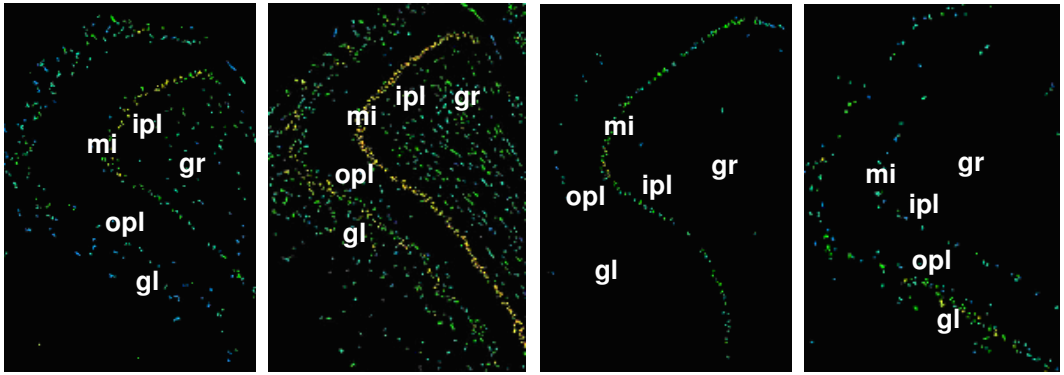
B. Other Sec machinery genes



PSTK Secp43 EF-Sec

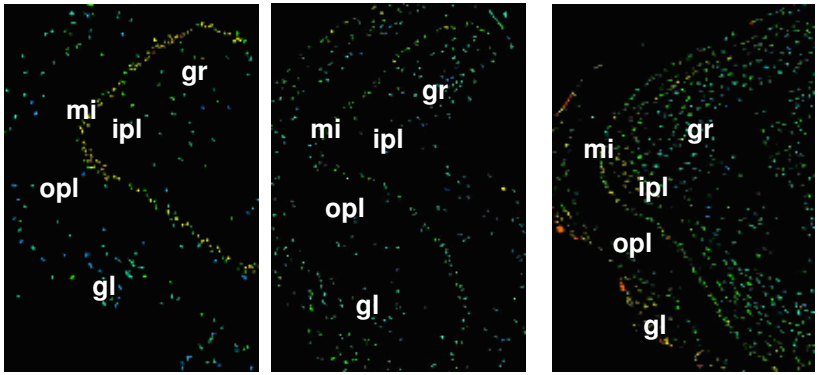
Fig. S3

A. Other selenoprotein genes



MsrB1 SelT SelK TGR

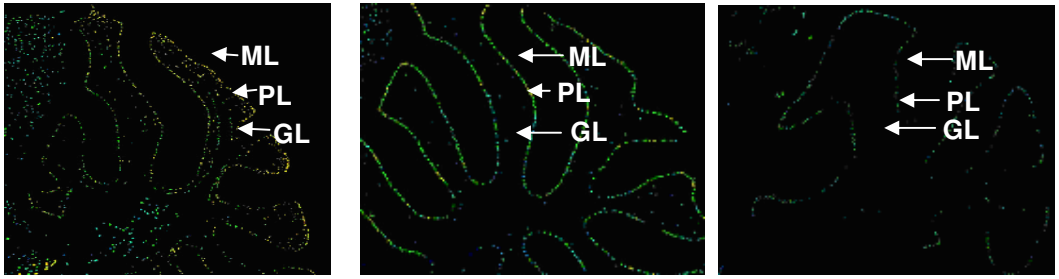
B. Other Sec machinery genes



PSTK Secp43 EF-Sec

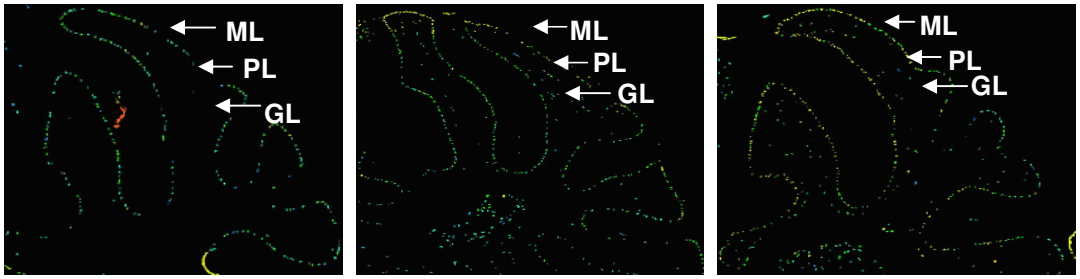
Fig. S4

A. Other selenoprotein genes



MsrB1 SelT SelK

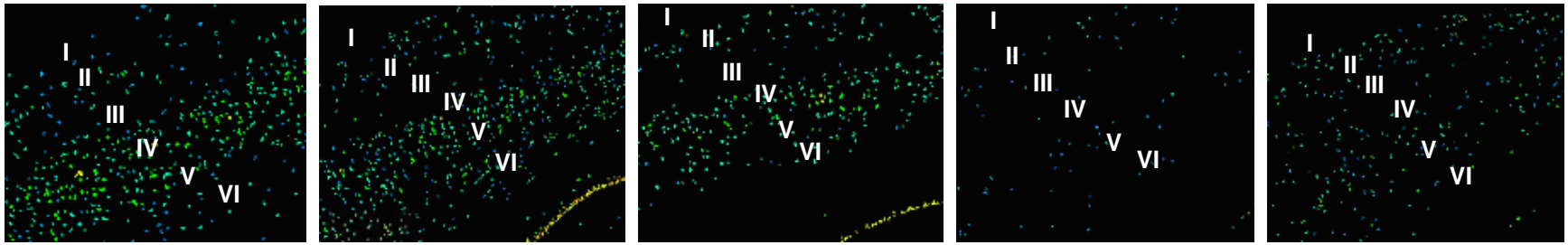
B. Other Sec machinery genes



PSTK Secp43 EF-Sec

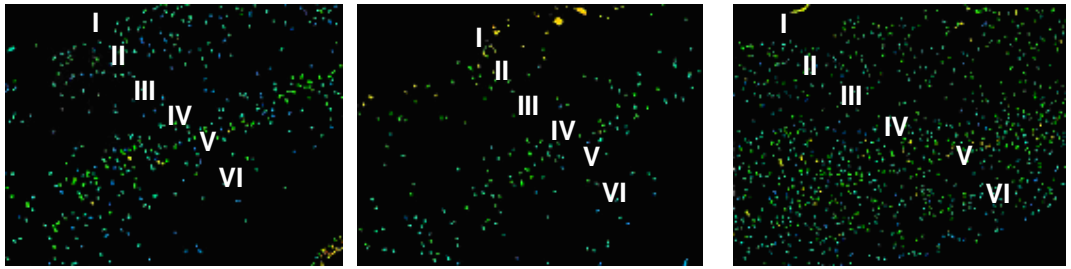
Fig. S5

A. Other selenoprotein genes



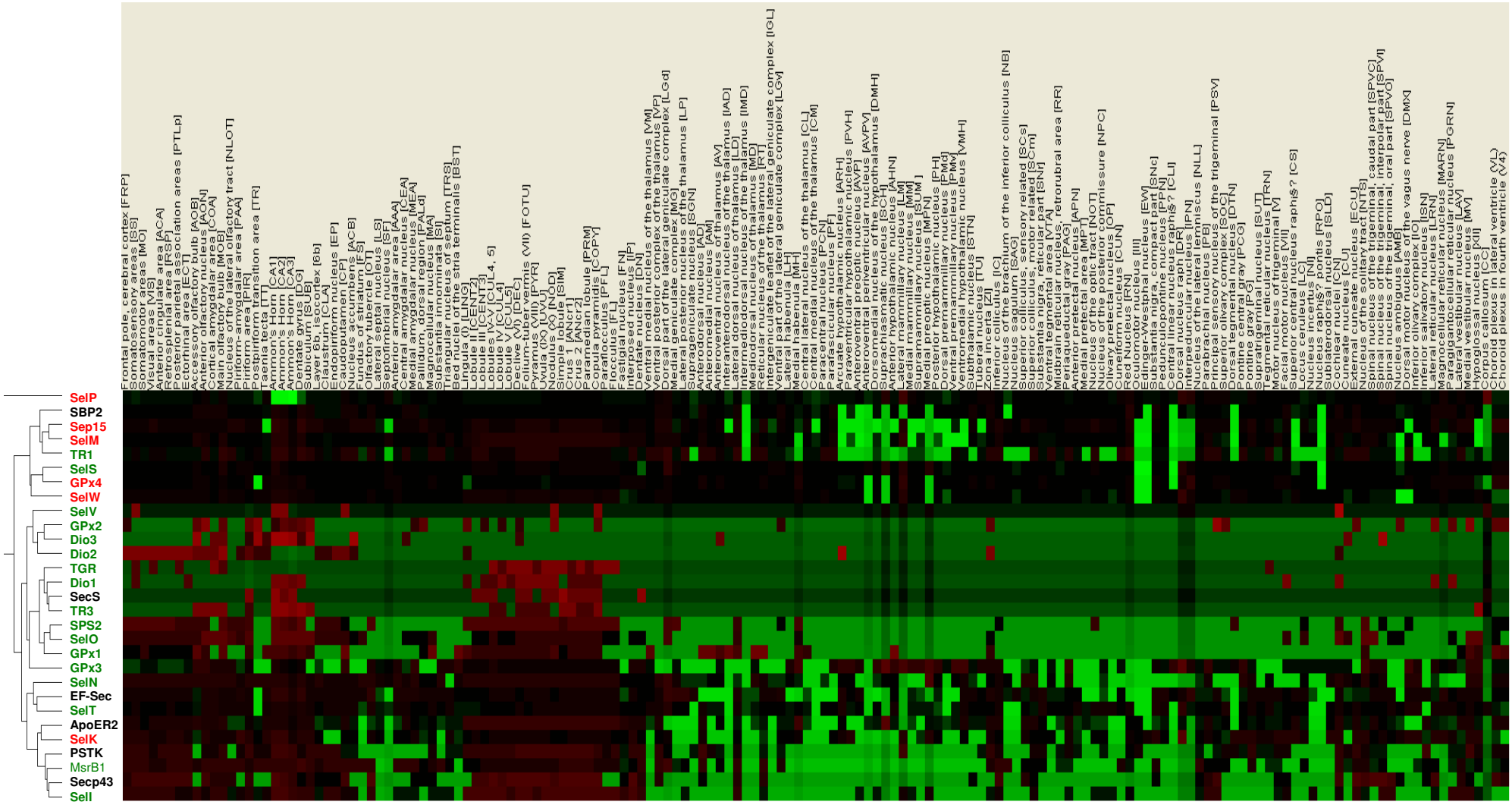
MsrB1 SelT SelK SelO SelN

B. Other Sec machinery genes



PSTK Secp43 EF-Sec

Fig. S6


SelP
SBP2
Sep15
SelM
TR1
SelS
GPx4
SelW
SelV
GPx2
Dio3
Dio2
TGR
Dio1
SecS
TR3
SPS2
SelO
GPx1
GPx3
SelN
EF-Sec
SelT
ApoER2
SelK
PSTK
MsrB1
Secp43
SelI
Frontal pole, cerebral cortex [FRP]
Somatosensory areas [SS]
Somatomotor areas [MO]
Visual areas [VIS]
Anterior cingulate area [ACA]
Retrosplenial area [RSP]
Ectorhinal area [ECT]
Postpiriform transition areas [PTLp]
Accessory olfactory bulb [AOB]
Anterior olfactory nucleus [AON]
Cortical amygdalar area [COA]
Main olfactory bulb [MOB]
Nucleus of the lateral olfactory tract [NLOT]
Piriform-amygdalar area [PAA]
Piriform area [PIR]
Postpiriform transition area [TR]
Taenia tecta [TT]
Ammon's Horn [CA1]
Ammon's Horn [CA2]
Ammon's Horn [CA3]
Dentate gyrus [DG]
Subiculum [SUB]
Layer 6b, Isocortex [6b]
Cerebellum [CBX]
Endopiriform nucleus [EP]
Caudoputamen [CP]
Nucleus accumbens [ACB]
Fundus of striatum [FS]
Olfactory tubercle [OT]
Lateral septal nucleus [LS]
Septofimbrial nucleus [SF]
Anterior amygdalar area [AAA]
Central amygdalar nucleus [CEA]
Medial amygdalar nucleus [MEA]
Basal nuclei of Meynert [ALd]
Magnocellular nucleus [MA]
Substantia innominata [SI]
Triangular nucleus of septum [TRS]
Bed nuclei of the stria terminalis [BST]
Lingula (I) [LING]
Lobule II [CENT2]
Lobule III [CENT3]
Lobules IV-V [CUL4, 5]
Lobule IV [CUL4]
Lobule V [CUL5]
Declive (VI) [DEC]
Folium-tuber vermis (VII) [FOTU]
Pyramus (VIII) [PYR]
Uvula (IX) [UVU]
Nodulus (X) [NOD]
Simple lobule [SIM]
Crus 1 [ANcr1]
Crus 2 [ANcr2]
Paramedian lobule [PRM]
Copula pyramidis [COPY]
Paraflocculus [PFL]
Flocculus [FL]
Fastigial nucleus [FN]
Interposed nucleus [IP]
Dentate nucleus [DN]
Ventral medial nucleus of the thalamus [VM]
Ventral posterior complex of the thalamus [VP]
Ventral anterior-lateral complex [VAL]
Medial geniculate complex [MG]
Lateral posterior nucleus of the thalamus [LP]
Suprageniculate nucleus [SGN]
Anterodorsal nucleus [AD]
Anteromedial nucleus [AM]
Anteroventral nucleus of thalamus [AV]
Interanteodorsal nucleus of the thalamus [IAD]
Lateral dorsal nucleus of thalamus [LD]
Intermediodorsal nucleus of the thalamus [IMD]
Mediodorsal nucleus of thalamus [MD]
Reticular nucleus of the thalamus [RT]
Intergeniculate leaflet of the lateral geniculate complex [IGL]
Ventral part of the lateral geniculate complex [LGv]
Lateral habenula [LH]
Medial habenula [MH]
Central lateral nucleus of the thalamus [CL]
Paracentral nucleus of the thalamus [CM]
Parafascicular nucleus [PF]
Parafascicular nucleus [PF]
Arcuate hypothalamic nucleus [ARH]
Paraventricular hypothalamic nucleus [PVH]
Anterodorsal preoptic nucleus [ADP]
Anteroventral periventricular nucleus [AVPV]
Dorsomedial nucleus of the hypothalamus [DMH]
Suprachiasmatic nucleus [SCH]
Anterior hypothalamic nucleus [AHN]
Lateral mammillary nucleus [LM]
Medial mammillary nucleus [MM]
Supramammillary nucleus [SUM]
Medial preoptic nucleus [MPN]
Posterior hypothalamic nucleus [PH]
Dorsal premammillary nucleus [PMd]
Ventral premammillary nucleus [PMv]
Tuberomammillary nucleus [TMv]
Subthalamic nucleus [STN]
Tuberal nucleus [TU]
Zona incerta [ZI]
Inferior colliculus [IC]
Nucleus of the brachium of the inferior colliculus [NB]
Nucleus sagulum [SAG]
Superior colliculus, sensory related [SCs]
Superior colliculus, motor related [SCm]
Substantia nigra, reticular part [SNr]
Ventral tegmental area [VTA]
Retrorubral area, retrorubral nucleus [RR]
Periaqueductal gray [PAG]
Anterior pretectal nucleus [APN]
Medial pretectal area [MPT]
Nucleus of the optic tract [NOT]
Olivary pretectal nucleus [OP]
Cuneiform nucleus [CUN]
Red Nucleus [RN]
Oculomotor nucleus [III]
Edinger-Westphal nucleus [EW]
Substantia nigra, compact part [SNc]
Pedunculopontine nucleus [PPN]
Central linear nucleus raphe§? [CLI]
Dorsal nucleus raphe [DR]
Interpeduncular nucleus [IPN]
Parabrachial nucleus, lateral [PBL]
Principal sensory nucleus of the trigeminal [PSV]
Superior olivary complex [SOC]
Dorsal tegmental nucleus [DTN]
Pontine central gray [PCG]
Supragenual nucleus [SUT]
Tegmental reticular nucleus [TRN]
Motor nucleus of trigeminal [V]
Facial motor nucleus [VII]
Superior central nucleus raphe§? [CS]
Nucleus raphe§? pontis [RPO]
Nucleus incertus [NI]
Nucleus raphe§? pontis [RPO]
Sublaterodorsal nucleus [SLD]
Cochlear nuclei [CN]
Cochlear nuclei [CN]
External cuneate nucleus [ECU]
Nucleus of the solitary tract [NTS]
Spinal nucleus of the trigeminal, caudal part [SPVC]
Spinal nucleus of the trigeminal, interpolar part [SPVI]
Spinal nucleus of the trigeminal, oral part [SPVO]
Vestibular nuclei [VNC]
Dorsal motor nucleus of the vagus nerve [DMX]
Inferior olivary complex [IO]
Inferior salivatory nucleus [ISN]
Lateral reticular nucleus [LRN]
Magnocellular reticular nucleus [MARN]
Parvicellular reticular nucleus [PGRN]
Lateral vestibular nucleus [LAV]
Medial vestibular nucleus [MV]
Hypoglossal nucleus [xii]
Corpus callosum [CC]
Lateral ventricle (VL)
Choroid plexus in lateral ventricle (VL)
Choroid plexus in fourth ventricle (V4)

Regions of mouse brain	Selenoproteins																							Sec Machinery	
	Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
Cerebrum [CH]																									
Cerebral cortex [CTX]																									
Cortical plate [CTXpl]																									
Frontal pole, cerebral cortex [FRP]	-	L	-	M	-	L	H	L	H	M	L	L	H	M	L	L	-	H	H	M	L	-	-	L	L
Somatosensory areas [SS]	-	L	-	M	-	L	H	L	H	M	L	L	H	M	L	L	L	H	H	M	L	L	-	L	L
Somatomotor areas [MO]	-	L	-	M	-	L	H	L	H	M	L	L	H	M	L	L	-	H	H	M	L	-	-	L	L
Visual areas [VIS]	-	L	-	M	-	L	H	L	H	M	L	L	H	M	L	L	-	H	H	M	L	L	-	L	L
Anterior cingulate area [ACA]	-	L	-	M	-	L	H	L	H	M	L	L	H	M	L	L	-	H	H	M	L	-	-	L	L
Retrosplenial area [RSP]	-	L	-	M	-	L	H	L	H	M	L	L	H	M	L	L	-	H	H	M	L	-	-	L	L
Posterior parietal association areas [PTLp]	-	L	-	M	-	L	H	L	H	M	L	L	H	M	L	L	-	H	H	L	L	L	-	L	L
Ectorhinal area [ECT]	-	L	-	M	-	L	H	L	H	M	L	L	H	M	L	L	-	H	H	L	L	-	-	L	L
Olfactory areas [OLF]																									
Accessory olfactory bulb [AOB]	-	L	L	M	L	L	H	L	M	H	L	L	H	M	L	-	-	H	H	L	L	-	L	-	L
Anterior olfactory nucleus [AON]	-	L	-	M	L	L	H	L	H	H	L	M	H	M	L	L	-	H	H	L	M	-	L	L	L

Regions of mouse brain	Selenoproteins																						Sec Machinery	
	Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
Cortical amygdalar area [COA]	-	L	L	L	-	L	H	L	H	M	L	M	H	M	L	L	-	H	H	-	L	M	L	L	L
Main olfactory bulb [MOB]	-	M	L	M	L	L	H	L	H	H	L	M	H	M	M	L	-	H	H	M	M	L	L	L	L
Nucleus of the lateral olfactory tract [NLOT]	-	-	-	L	-	L	H	-	M	M	L	M	H	M	L	L	-	H	H	L	M	-	-	L	L
Piriform-amygdalar area [PAA]	-	L	-	L	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	L	L	-	-	-	L
Piriform area [PIR]	-	L	-	M	L	L	H	L	H	M	L	M	H	M	L	L	-	H	H	L	L	-	L	L	L
Postpiriform transition area [TR]	-	L	L	L	L	-	L	-	H	M	L	-	H	M	L	-	-	H	H	-	L	-	-	-	L
Taenia tecta [TT]	-	L	-	M	-	-	H	L	H	M	L	-	H	M	L	L	-	H	L	-	L	-	-	-	-
Hippocampal formation [HPF]																									
Hippocampal region [HIP]																									
Ammon's Horn [CA1]	L	-	L	M	-	L	H	L	H	H	L	M	-	M	M	L	L	H	H	M	M	-	L	L	L
Ammon's Horn [CA2]	L	-	L	M	L	L	H	L	H	H	L	M	-	M	L	L	L	H	H	M	M	-	L	L	L
Ammon's Horn [CA3]	L	-	L	M	L	L	H	L	H	H	L	M	-	M	L	L	-	H	H	L	L	-	L	L	L

Regions of mouse brain	Selenoproteins																						Sec Machinery	
	Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
Dentate gyrus [DG]	-	-	-	M	L	L	H	L	H	H	L	M	-	M	L	L	-	H	H	M	M	-	L	L	L
Retrohippocampal region [RHP]																									
Subiculum [SUB]	-	-	L	M	L	-	H	L	H	M	L	M	H	M	L	L	-	H	H	-	M	-	L	L	L
Cortical subplate [CTXsp]																									
Layer 6b, isocortex [6b]	-	L	-	L	-	L	H	-	H	M	L	L	H	M	L	L	-	H	H	L	L	-	-	L	-
Claustrum [CLA]	-	L	-	L	-	-	H	L	L	M	L	L	H	M	L	L	-	H	H	L	L	-	-	L	-
Endopiriform nucleus [EP]	-	L	-	L	-	-	H	L	L	M	L	L	H	M	L	L	-	H	H	L	L	-	-	L	-
Cerebral nuclei [CNU]																									
Striatum [STR]																									
Striatum dorsal region [STRd]																									
Caudoputamen [CP]	-	L	-	H	-	L	H	L	H	M	L	L	H	M	L	L	-	H	H	L	M	-	-	L	L
Striatum ventral region [STRv]																									
Nucleus accumbens [ACB]	-	L	L	L	-	-	H	L	H	M	L	L	H	M	L	L	-	H	H	-	M	-	-	-	L
Fundus of striatum [FS]	-	-	-	L	-	-	H	-	L	M	L	L	H	M	L	-	-	H	H	-	L	-	-	-	-
Olfactory tubercle [OT]	-	-	-	-	-	L	H	L	M	M	L	-	H	M	L	L	-	H	H	M	M	-	-	L	L

Regions of mouse brain	Selenoproteins																							Sec Machinery	
	Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
Lateral septal complex [LSX]																									
Lateral septal nucleus [LS]	-	-	-	L	-	L	H	-	H	M	L	-	H	-	L	-	-	H	H	-	L	-	-	-	L
Septofimbrial nucleus [SF]	-	-	-	L	-	L	H	-	L	H	L	-	H	-	L	-	-	H	L	-	-	-	-	-	L
Striatum-like amygdalar nuclei [sAMY]																									
Anterior amygdalar area [AAA]	-	-	-	L	-	L	H	-	H	M	L	-	-	M	L	L	-	H	H	-	L	-	-	-	L
Central amygdalar nucleus [CEA]	-	-	-	L	-	L	H	-	H	M	L	L	H	M	L	L	-	H	H	-	M	-	-	-	L
Medial amygdalar nucleus [MEA]	-	-	-	L	-	L	H	-	H	H	L	L	H	M	L	L	-	H	H	-	L	-	-	L	L
Pallidum [PAL]																									
Pallidum, dorsal region [PALd]	-	-	-	M	L	-	H	-	M	M	L	L	H	L	L	L	-	H	H	-	L	-	-	L	-
Pallidum, ventral region [PALv]																									
Magnocellular nucleus [MA]	-	-	-	H	-	-	H	-	H	H	L	L	H	M	L	L	-	H	H	-	M	-	-	L	L
Substantia innominata [SI]	-	-	-	L	-	L	H	-	L	M	L	-	H	M	L	L	-	H	H	-	M	-	-	-	L
Triangular nucleus of septum [TRS]	-	-	-	-	-	L	H	-	H	H	-	-	H	L	L	L	-	H	H	-	L	-	-	-	L

Regions of mouse brain	Selenoproteins																						Sec Machinery	
	Dio1	**Dio2**	**Dio3**	**GPx1**	**GPx2**	**GPx3**	**GPx4**	**SelI**	**SelK**	**SelM**	**SelN**	**SelO**	**SelP**	**SelR (MsrB1)**	**SelS**	**SelT**	**SelV**	**SelW**	**Sep15**	**SPS2**	**TR1**	**TGR**	**TR3**	**Secp43**	**SBP2**
Pallidum, caudal region [PALc]																								
Bed nuclei of the stria terminalis [BST]	-	-	-	H	-	L	H	-	M	M	L	-	H	-	L	-	-	H	H	-	L	-	-	-	L
Cerebellum [CB]																								
Cerebellar cortex [CBX]																								
Vermal regions [VERM]																								
Lingula (I) [LING]	-	L	-	M	L	L	H	L	H	H	L	-	H	L	L	-	-	H	H	M	L	-	-	L
Central lobule [CENT]																								
Lobule II [CENT2]	-	-	-	M	-	L	H	L	H	H	L	L	H	L	L	-	-	H	H	M	M	-	L	L
Lobule III [CENT3]	L	-	-	M	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	M	M	-	L	L
Culmen [CUL]																								
Lobules IV-V [CUL4, 5]	-	-	-	M	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	M	L	M	L	L	L
Lobule IV [CUL4]	-	-	-	M	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	M	L	L	L	L	L
Lobule V [CUL5]	-	-	-	M	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	M	L	M	-	L	L
Declive (VI) [DEC]	L	-	-	L	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	M	M	M	L	L	L
Folium-tuber vermis (VII) [FOTU]	L	-	-	M	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	M	M	M	L	L	L
Pyramus (VIII) [PYR]	L	-	-	M	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	M	M	L	L	L	L
Uvula (IX) [UVU]	L	-	-	M	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	M	M	M	L	L	L

Regions of mouse brain	Selenoproteins																						Sec Machinery	
	Dio1	**Dio2**	**Dio3**	**GPx1**	**GPx2**	**GPx3**	**GPx4**	**SelI**	**SelK**	**SelM**	**SelN**	**SelO**	**SelP**	**SelR (MsrB1)**	**SelS**	**SelT**	**SelV**	**SelW**	**Sep15**	**SPS2**	**TR1**	**TGR**	**TR3**	**Secp43**	**SBP2**
Nodulus (X) [NOD]	L	-	-	L	L	L	H	L	H	H	L	L	H	M	L	L	L	H	H	M	M	M	-	L	L
Hemispheric regions [HEM]																									
Simple lobule [SIM]	-	-	-	M	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	L	L	M	L	L	L
Ansiform lobule [AN]																									
Crus 1 [ANcr1]	-	-	-	M	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	M	L	L	L	L	L
Crus 2 [ANcr2]	-	-	-	M	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	M	L	M	L	L	L
Paramedian lobule [PRM]	-	-	-	M	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	M	L	L	L	L	L
Copula pyramidis [COPY]	-	-	-	M	L	L	H	L	H	H	L	L	H	M	L	L	L	H	H	M	L	M	L	L	L
Paraflocculus [PFL]	-	-	-	L	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	M	L	M	-	-	L
Flocculus [FL]	-	-	-	L	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	M	L	M	-	-	L
Cerebellar nuclei [CBN]																									
Fastigial nucleus [FN]	-	-	-	-	-	-	H	L	H	H	L	-	H	L	L	L	-	H	H	-	L	-	-	L	L
Interposed nucleus [IP]	-	-	-	L	-	L	H	L	H	H	L	L	H	L	L	L	-	H	H	-	M	-	-	L	L
Dentate nucleus [DN]	-	-	-	-	-	L	H	L	H	H	L	L	H	M	L	L	-	H	H	-	L	-	-	L	L
Brain stem [BS]																									

Regions of mouse brain	Selenoproteins																						Sec Machinery	
	Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
Interbrain [IB]																								
Thalamus [TH]																								
Thalamus, sensory-motor cortex related [DORsm]																								
Ventral group of the dorsal thalamus [VENT]																								
Ventral medial nucleus of the thalamus [VM]	-	-	-	M	-	L	H	-	L	M	L	-	H	-	L	L	-	H	H	-	M	-	-	-	L
Ventral posterior complex of the thalamus [VP]	-	-	-	L	-	-	H	-	H	H	L	-	H	M	L	L	-	H	H	-	M	-	-	L	L
Geniculate group, dorsal thalamus [GENd]																								
Dorsal part of the lateral geniculate complex [LGd]	-	-	-	L	-	-	H	-	H	H	L	L	H	M	M	L	-	H	H	M	M	-	-	-	L

Regions of mouse brain	Selenoproteins																						Sec Machinery	
	Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
Medial geniculate complex [MG]	-	-	-	L	-	L	H	-	L	H	L	-	H	L	L	L	-	H	H	M	L	-	-	-	L
Thalamus, polymodal association cortex related [DORpm]																									
Lateral group of the dorsal thalamus [LAT]																									
Lateral posterior nucleus of the thalamus [LP]	-	-	-	L	-	-	H	-	L	M	-	-	H	L	L	L	-	H	H	-	M	-	-	-	L
Supragenicu late nucleus [SGN]	-	-	-	-	-	-	H	-	L	M	-	-	H	-	L	-	-	H	H	-	-	-	-	-	-
Anterior group of the dorsal thalamus [ATN]																									
Anterodorsa l nucleus [AD]	-	-	-	M	-	-	H	-	H	H	-	-	H	M	L	L	-	H	H	-	M	-	-	L	L
Anteromedi al nucleus [AM]	-	-	-	H	-	-	H	-	L	M	L	-	H	-	L	-	-	H	H	-	M	-	-	-	L
Anteroventr al nucleus of thalamus [AV]	-	-	L	M	-	L	H	-	L	M	L	-	H	L	L	-	-	H	H	-	L	-	-	-	L

Regions of mouse brain		Selenoproteins																						Sec Machinery	
		Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
Interanterodorsal nucleus of the thalamus [IAD]		-	-	-	H	-	-	H	-	L	H	-	-	-	-	L	-	-	H	L	-	L	-	-	-	-
Lateral dorsal nucleus of thalamus [LD]		-	-	-	L	-	-	H	L	H	H	-	L	H	M	L	L	-	H	H	L	M	-	-	L	L
Medial group of the dorsal thalamus [MED]																										
Intermediodorsal nucleus of the thalamus [IMD]		-	-	-	L	-	-	H	-	L	-	-	-	-	-	L	-	-	H	L	-	-	-	-	-	-
Mediodorsal nucleus of thalamus [MD]		-	-	-	H	-	L	H	-	L	H	L	-	H	-	L	-	-	H	H	-	M	-	-	-	L
Reticular nucleus of the thalamus [RT]		-	-	-	H	-	-	H	-	M	M	L	-	H	M	L	L	-	H	H	-	M	-	-	L	L
Geniculate group, ventral thalamus [GENv]																										

Regions of mouse brain	Selenoproteins																						Sec Machinery	
	Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
Intergeniculate leaflet of the lateral geniculate complex [IGL]	-	-	-	-	-	-	H	-	L	M	-	-	H	-	L	L	-	H	M	-	L	-	-	-	L
Ventral part of the lateral geniculate complex [LGv]	-	-	-	L	-	-	H	-	H	M	-	-	H	M	L	L	-	H	H	L	L	-	-	-	L
Epithalamus [EPI]																									
Lateral habenula [LH]	-	-	-	L	-	L	H	-	H	M	L	-	H	-	L	-	-	H	H	-	L	-	-	-	L
Medial habenula [MH]	-	-	-	M	-	M	H	-	H	L	L	L	H	-	L	L	-	H	M	M	-	-	-	-	-
Intralaminar nuclei of the dorsal thalamus [ILM]																									
Central lateral nucleus of the thalamus [CL]	-	-	-	M	-	-	H	-	L	M	-	-	H	-	L	-	-	H	H	-	L	-	-	-	L

Regions of mouse brain		Selenoproteins																						Sec Machinery	
		Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
	Central medial nucleus of the thalamus [CM]	-	-	-	L	-	-	H	-	L	H	-	-	H	-	L	-	-	H	L	-	L	-	-	-	-
	Paracentral nucleus [PCN]	-	-	-	L	-	-	H	-	L	M	-	-	H	-	L	L	-	H	H	-	M	-	-	-	L
	Parafascicular nucleus [PF]	-	-	-	-	-	L	H	-	H	H	-	-	H	-	L	L	-	H	H	-	M	-	-	-	L
Hypothalamus [HY]																										
Periventricular zone [PVZ]																										
	Arcuate hypothalamic nucleus [ARH]	-	M	-	-	-	L	H	-	H	-	L	-	H	-	L	L	-	H	L	-	-	-	-	-	-
	Paraventricular hypothalamic nucleus [PVH]	-	-	-	L	-	L	H	-	H	L	L	-	H	-	L	-	-	H	L	-	-	-	-	-	-
Periventricular region [PVR]																										
	Anteroventral preoptic nucleus [AVP]	-	-	-	L	-	L	H	-	L	-	-	-	H	-	L	L	-	H	L	-	-	-	-	-	-
	Anteroventral periventricular nucleus [AVPV]	-	-	-	L	-	-	M	-	L	L	-	-	H	-	L	-	-	L	L	-	-	-	-	-	-

Regions of mouse brain		Selenoproteins																						Sec Machinery	
		Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
Dorsomedial nucleus of the hypothalamus [DMH]		-	-	-	L	-	L	H	-	L	M	-	-	H	-	L	L	-	H	L	-	L	-	-	-	-
Suprachiasmatic nucleus [SCH]		-	-	-	L	-	M	L	-	L	L	L	-	H	-	L	-	L	M	L	-	-	L	-	-	-
Hypothalamic medial zone [MEZ]																										
Anterior hypothalamic nucleus [AHN]		-	-	-	H	-	L	H	-	L	M	L	-	H	-	L	L	-	H	L	-	-	-	-	-	L
Mammillary body [MBO]																										
	Lateral mammillary nucleus [LM]	-	-	-	L	-	M	H	L	H	H	-	-	H	-	L	-	L	H	H	-	-	L	-	-	-
	Medial mammillary nucleus [MM]	-	-	-	L	-	L	H	-	L	-	-	-	H	-	L	-	-	H	L	-	L	-	-	-	L
	Supramammillary nucleus [SUM]	-	-	-	L	-	L	H	-	M	H	-	-	H	-	L	-	-	H	L	-	L	-	-	-	L
Medial preoptic nucleus [MPN]		-	-	-	-	-	M	L	L	H	L	L	-	H	-	L	-	L	M	L	-	-	L	-	-	-

Regions of mouse brain	Selenoproteins																						Sec Machinery	
	Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
Posterior hypothalamic nucleus [PH]	-	-	-	-	-	L	H	-	H	L	L	-	H	-	L	-	-	H	L	-	-	-	-	-	L
Dorsal premammillary nucleus [PMd]	-	-	-	-	-	L	H	-	L	-	-	-	H	-	L	-	-	H	L	-	-	-	-	-	L
Ventral premammillary nucleus [PMv]	-	-	-	L	-	M	H	-	L	-	L	-	H	-	L	-	-	H	H	-	L	-	-	-	L
Ventromedial hypothalamic nucleus [VMH]	-	-	-	L	-	M	H	-	H	-	L	-	H	-	L	L	-	H	L	-	L	-	-	-	L
Hypothalamic lateral zone [LZ]																									
Subthalamic nucleus [STN]	-	-	-	L	-	L	H	-	L	M	-	-	H	-	L	L	-	H	H	-	-	-	-	L	L
Tuberal nucleus [TU]	-	-	-	L	-	L	H	-	L	M	-	-	H	-	L	L	-	H	L	-	L	-	-	-	L
Zona incerta [ZI]	-	L	-	L	L	L	H	-	H	M	L	L	H	M	L	L	-	H	H	-	M	-	-	-	L
Midbrain [MB]																									
Midbrain, sensory related [MBsen]																									

Regions of mouse brain	Selenoproteins																							Sec Machinery	
	Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
Inferior colliculus [IC]	-	-	-	-	-	L	H	L	H	H	L	-	H	M	L	L	-	H	H	L	M	-	-	L	L
Nucleus of the brachium of the inferior colliculus [NB]	-	-	-	L	-	-	H	-	L	M	L	-	H	M	L	-	-	H	H	-	-	-	-	-	-
Nucleus sagulum [SAG]	-	-	-	-	-	-	H	-	L	M	-	-	H	-	L	-	-	H	H	-	-	-	-	-	L
Superior colliculus, sensory related [SCs]	-	-	-	L	-	L	H	-	H	M	L	-	H	L	L	L	-	H	H	-	-	-	-	L	L
Midbrain, motor related [MBmot]																									
Superior colliculus, motor related [SCm]	-	-	-	L	-	L	H	L	H	M	L	-	H	L	L	-	-	H	H	-	M	-	-	L	L
Substantia nigra, reticular part [SNr]	-	-	-	-	-	-	H	-	H	M	-	-	H	L	L	L	-	H	H	-	L	-	-	-	L
Ventral tegmental area [VTA]	-	-	-	L	-	L	H	-	H	M	L	-	H	-	L	L	-	H	H	-	M	-	-	-	L
Midbrain reticular nucleus, retrorubral area [RR]	-	-	-	L	-	-	H	-	L	M	-	-	H	L	L	L	-	H	H	-	L	-	-	-	L
Periaqueductal gray [PAG]	-	-	-	L	-	M	H	-	H	M	L	-	H	L	L	L	-	H	H	-	M	-	-	-	L
Pretectal region [PRT]																									
Anterior pretectal nucleus [APN]	-	-	-	L	-	-	H	-	M	M	-	-	H	-	L	L	-	H	H	-	L	-	-	-	L
Medial pretectal area [MPT]	-	-	-	L	-	-	H	-	L	M	-	-	H	-	L	-	-	H	H	-	-	-	-	-	L

Regions of mouse brain	Selenoproteins																						Sec Machinery	
	Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
Nucleus of the optic tract [NOT]	-	-	-	L	-	-	H	-	H	M	-	-	H	-	L	L	-	H	H	L	M	-	-	-	L
Nucleus of the posterior commissure [NPC]	-	-	-	L	-	L	H	-	H	M	-	-	H	-	L	L	-	H	H	-	-	-	-	-	L
Olivary pretectal nucleus [OP]	-	-	-	L	-	-	H	-	L	M	-	-	H	-	L	L	-	H	H	-	-	-	-	-	L
Cuneiform nucleus [CUN]	-	-	-	-	-	L	H	-	L	M	-	-	H	M	L	L	-	H	H	-	M	-	-	-	L
Red Nucleus [RN]	-	-	-	-	-	-	H	-	H	H	-	-	H	L	L	L	-	H	H	-	M	-	-	-	L
Oculomotor nucleus [III]	-	-	-	-	-	-	L	-	H	-	-	-	H	-	-	-	-	L	L	-	-	-	-	-	L
Edinger-Westphal nucleus [EW]	-	-	-	-	-	-	L	-	H	-	-	-	H	-	-	-	-	L	L	-	-	-	-	-	-
Midbrain, behavioral state related [MBsta]																									
Substantia nigra, compact part [SNc]	-	-	-	-	-	L	H	-	H	M	-	-	H	M	L	L	-	H	H	-	L	-	-	L	L
Pedunculopontine nucleus [PPN]	-	-	-	L	-	L	H	-	H	M	-	-	H	-	L	L	-	H	H	-	L	-	-	-	L
Midbrain raphé nuclei [RAmb]																									
Central linear nucleus raphé [CLI]	-	-	-	L	-	L	L	-	L	-	-	-	H	-	-	-	-	H	L	-	-	-	-	-	-

Regions of mouse brain		Selenoproteins																						Sec Machinery	
		Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
	Dorsal nucleus raphé [DR]	-	-	-	L	-	M	H	-	H	L	-	-	H	-	L	L	L	H	L	-	-	L	-	-	L
	Interpeduncular nucleus [IPN]	-	-	-	L	-	L	H	-	L	L	-	-	M	-	L	-	L	H	L	-	-	L	-	-	-
Hindbrain [HB]																										
Pons [P]																										
Pons, sensory related [P-sen]																										
	Nucleus of the lateral lemniscus [NLL]	-	-	-	L	-	L	H	-	H	M	-	-	H	M	L	L	-	H	H	-	L	-	-	L	L
	Parabrachial nucleus [PB]	-	-	-	L	-	M	H	L	H	H	L	-	H	L	L	L	-	H	H	-	M	-	-	L	L
	Principal sensory nucleus of the trigeminal [PSV]	-	-	-	L	L	L	H	L	H	M	-	-	H	L	L	L	-	H	H	-	M	-	-	L	L
	Superior olivary complex [SOC]	-	-	-	L	L	L	H	-	M	M	-	-	H	L	L	L	-	H	H	-	L	-	-	-	-
Pons, motor related [P-mot]																										
	Dorsal tegmental nucleus [DTN]	-	-	-	L	-	L	H	-	H	-	-	-	H	-	L	-	-	H	L	L	-	-	-	-	-
	Pontine central gray [PCG]	-	-	-	-	-	L	H	-	H	M	L	-	H	-	L	L	-	H	H	-	M	-	-	-	L

Regions of mouse brain	Selenoproteins																						Sec Machinery	
	Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
Pontine gray [PG]	-	-	-	L	-	L	H	-	H	H	L	-	H	M	L	L	-	H	H	-	L	-	-	-	L
Supratrigeminal nucleus [SUT]	-	-	-	L	-	-	H	-	H	M	-	-	H	-	L	L	-	H	H	-	L	-	-	-	L
Tegmental reticular nucleus [TRN]	-	-	-	-	-	-	H	-	H	H	L	-	H	L	L	L	-	H	H	-	M	L	-	-	L
Motor nucleus of trigeminal [V]	-	-	-	L	-	-	H	-	H	H	-	-	H	L	L	-	-	H	H	-	L	-	-	L	L
Facial motor nucleus [VII]	-	-	-	-	-	L	H	L	H	M	-	L	H	-	L	L	-	H	H	-	L	-	-	L	L
Pons, behavioral state related [P-sat]																									
Superior central nucleus raphé [CS]	-	-	-	L	-	L	H	-	H	-	-	-	H	-	L	L	-	H	L	-	-	-	-	-	-
Locus ceruleus [LC]	-	-	-	-	-	L	H	-	H	M	L	L	H	L	L	L	-	H	H	L	-	-	-	-	-
Nucleus incertus [NI]	-	-	-	L	-	L	H	L	L	M	-	-	H	-	L	-	-	H	H	-	-	-	-	-	L
Nucleus raphé pontis [RPO]	-	-	-	-	-	L	M	-	L	-	-	-	H	-	-	-	-	H	L	-	-	-	-	-	-

Regions of mouse brain		Selenoproteins																							Sec Machinery	
		Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
	Sublaterodorsal nucleus [SLD]	-	-	-	L	-	L	H	-	H	H	L	-	H	-	L	L	-	H	H	-	L	-	-	-	L
Medulla [MY]																										
Medulla, sensory related [MY-sen]																										
	Cochlear nuclei [CN]	-	L	-	L	-	L	H	L	H	H	L	M	H	M	L	L	L	H	H	L	L	-	-	L	L
	Dorsal column nuclei [DCN]																									
	Cuneate nucleus [CU]	-	-	L	L	-	L	H	-	L	M	L	L	H	L	L	L	-	H	H	-	M	-	-	-	L
	External cuneate nucleus [ECU]	-	-	-	L	L	-	H	L	H	M	L	-	M	M	L	L	-	H	H	-	M	-	-	L	L
	Nucleus of the solitary tract [NTS]	-	-	-	M	-	M	H	-	H	H	L	M	H	M	L	L	-	H	H	M	M	-	-	L	L
	Spinal nucleus of the trigeminal, caudal part [SPVC]	-	-	-	L	-	L	H	-	H	H	L	M	M	L	L	L	-	H	H	-	L	-	-	L	L
	Spinal nucleus of the trigeminal, interpolar part [SPVI]	-	-	L	L	-	L	H	L	H	M	L	-	H	L	L	L	-	H	H	L	L	-	-	L	L

Regions of mouse brain		Selenoproteins																						Sec Machinery	
		Dio1	Dio2	Dio3	GPx1	GPx2	GPx3	GPx4	SelI	SelK	SelM	SelN	SelO	SelP	SelR (MsrB1)	SelS	SelT	SelV	SelW	Sep15	SPS2	TR1	TGR	TR3	Secp43	SBP2
	Spinal nucleus of the trigeminal, oral part [SPVO]	-	-	-	L	-	-	H	L	H	M	-	L	H	-	L	L	-	H	H	-	L	-	-	L	L
Medulla, motor related [MY-mot]																										
	Nucleus ambiguus [AMB]	-	-	-	L	L	-	H	-	H	-	-	L	H	-	L	L	-	L	H	-	-	-	-	L	-
	Dorsal motor nucleus of the vagus nerve [DMX]	-	-	-	L	-	M	H	-	H	M	L	-	H	-	L	L	-	L	L	L	M	-	-	-	-
	Inferior olivary complex [IO]	-	-	-	L	-	L	H	-	H	-	L	-	H	L	L	L	-	H	H	-	-	-	-	-	L
	Inferior salivatory nucleus [ISN]	-	-	-	L	-	-	H	-	M	M	-	-	H	L	L	-	-	H	H	-	-	-	-	L	L
	Lateral reticular nucleus [LRN]	-	-	-	L	L	L	H	-	H	M	-	-	H	L	L	L	-	H	H	-	L	-	-	L	L
	Magnocellular reticular nucleus [MARN]	-	-	-	L	-	L	H	-	H	L	-	-	M	-	L	L	-	H	H	-	L	-	-	-	L
	Paragigantocellular reticular nucleus [PGRN]	-	-	-	L	L	L	H	L	H	M	-	L	H	L	L	L	-	H	H	-	L	-	-	-	L

Regions of mouse brain		Selenoproteins																						Sec Machinery	
		Dio1	**Dio2**	**Dio3**	**GPx1**	**GPx2**	**GPx3**	**GPx4**	**SelI**	**SelK**	**SelM**	**SelN**	**SelO**	**SelP**	**SelR (MsrB1)**	**SelS**	**SelT**	**SelV**	**SelW**	**Sep15**	**SPS2**	**TR1**	**TGR**	**TR3**	**Secp43**	**SBP2**
Vestibular nuclei [VNC]																									
	Lateral vestibular nucleus [LAV]	-	-	-	L	-	L	H	L	M	M	-	-	H	M	L	L	-	H	H	-	M	-	-	L	L
	Medial vestibular nucleus [MV]	-	-	-	M	L	L	H	L	H	M	L	M	H	L	L	L	-	H	H	-	L	-	-	L	L
	Hypoglossal nucleus [XII]	-	-	-	M	-	L	H	-	H	-	L	M	M	L	L	L	-	H	L	-	L	-	L	-	L
Others (non-neuron)																									
Corpus callosum [CC]		-	-	-	L	-	-	L	-	L	L	-	L	H	L	L	L	L	M	L	L	-	L	-	-	-
Choroid plexus in lateral ventricle (VL)		-	-	-	M	L	L	H	-	L	M	L	L	H	L	L	-	-	L	H	L	L	-	-	L	-
Choroid plexus in fourth ventricle (V4)		-	-	-	M	L	L	H	-	L	M	L	-	H	L	L	-	-	L	H	L	L	-	-	L	-

Note: Gene expression level is relative to hprt housekeeping gene