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Impaired selenoprotein expression in brain triggers striatal neuronal loss leading to coordination defects in mice

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Abstract

Selenocysteine Insertion Sequence (SECIS)-Binding Protein 2 (Secisbp2) binds to SECIS elements located in the 3′-untranslated region of eukaryotic selenoprotein mRNAs. It facilitates incorporation of the rare amino acid selenocysteine in response to UGA codons. Inactivation of *Secisbp2* in hepatocytes greatly reduced selenoprotein levels. Neuron-specific inactivation of *Secisbp2* (*CamK-Cre; Secisbp2*$^{fl/fl}$) reduced cerebral expression of selenoproteins to a lesser extent than inactivation of tRNA$^{[Ser]Sec}$. This allowed us to study the development of cortical parvalbumin-positive (PV+) interneurons, which are completely lost in tRNA$^{[Ser]Sec}$ mutants. PV+ interneuron density was reduced in the somatosensory cortex, hippocampus, and striatum. *In situ*-hybridization for *Gad67* confirmed the reduction of GABAergic interneurons. Because of the obvious movement phenotype involving a broad, dystonic gait, we suspected basal ganglia dysfunction. Tyrosine hydroxylase expression was normal in substantia nigra neurons and their striatal terminals. However the densities of striatal PV+ and *Gad67*+ neurons were decreased by 65% and 49%, respectively. Likewise, the density of striatal cholinergic neurons was reduced by 68%. Our observations demonstrate that several classes of striatal interneurons depend on selenoprotein expression. These findings may offer an explanation for the movement phenotype of selenoprotein P-deficient mice and the movement disorder and mental retardation described in a patient carrying *SECISBP2* mutations.

Keywords

selenium; glutathione peroxidase; neurodegeneration; redox; cholinergic; parvalbumin

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Introduction

Selenoproteins are proteins containing the rare amino acid selenocysteine (Sec). Co-translational incorporation of Sec into proteins is mediated by *cis*- and *trans*-acting factors [1]. An *in-frame* UGA codon is re-coded as a Sec codon, if the respective mRNA contains a selenocysteine insertion sequence (SECIS) element in the 3′-untranslated region. The SECIS forms a kink-turn structure, which is bound by SECIS-binding protein 2 (Secisbp2). Secisbp2 enhances selenoprotein expression and stabilizes selenoprotein mRNAs [2]. Sec is synthesized attached to its $tRNA^{[Ser]Sec}$, which is initially charged with serine, phosphorylated to pSer-$tRNA^{[Ser]Sec}$, and finally converted to Sec-$tRNA^{[Ser]Sec}$ by selenocysteine synthase (SepSecS) [3, 4].

The human and mouse selenoproteomes are encoded by 25 and 24 genes, respectively [5]. Selenoproteins are essential for mammalian development and health. Genetic inactivation in mice of $tRNA^{[Ser]Sec}$ (*Trsp*), glutathione peroxidase 4 (*Gpx4*), thioredoxin reductase 1 (*Txnrd1*), or *Txnrd2* is embryonic lethal [6-9]. Targeted disruption of the selenoprotein P gene (*Sepp*) has demonstrated that selenium is also essential for proper brain function [10-14]. SePP is a plasma selenium transfer protein needed for privileged selenium supply to the brain. Inactivation of its receptor, ApoER2 (Lrp8) leads to a similar phenotype as in $Sepp^{-/-}$ mice with movement disorder, epileptic seizures, and neurodegeneration [15]. Neurons account for most of the selenoprotein expression in the mouse brain [16]. This finding was substantiated when selenoprotein biosynthesis was inactivated selectively in neurons, through the conditional inactivation of $tRNA^{[Ser]Sec}$ [17]. Specifically, parvalbumin (PV)-expressing neurons, a subset of GABAergic interneurons, were completely lacking in the somatosensory cortex and hippocampus of *CamK-Cre;* $Trsp^{fl/fl}$ mice, while the principal neurons and calretinin (CR) expressing interneurons were apparently less affected or only affected later [17]. Interestingly, cortical PV+ interneurons express Lrp8, the Sepp receptor [18]. The critical selenoprotein in the brain is GPx4, since lack of PV+ GABAergic interneurons was also observed in mice deficient in neuronal GPx4 [7, 17]. Similarly, in a related mouse model, which targeted *Gpx4* in cerebellar neurons, Purkinje cells degenerated along parasagittal stripes, and cerebellar hypoplasia was associated with reduced proliferation of granule cells and increased apoptosis [19]. Remarkably, Purkinje cells are PV+ and use the GABA as their neurotransmitter.

Human conditions associated with mutations in genes involved in selenoprotein biosynthesis have been identified in recent years. Children suffering from *Progressive Cerebello-Cerebral Atrophy* (PCCA) carry mutations in the gene encoding selenocysteine synthase (*SEPSECS*) [20]. Neuroimaging revealed progressive cerebellar and cortical atrophy with most patients developing seizures after two years of age [21]. Mutations in *SECISBP2* are mainly associated with defective thyroid hormone signaling [22], but a case with movement disorder and mental retardation has been reported [23]. We report here that neuron-specific inactivation of *Secisbp2* in mice moderately reduces selenoprotein expression in the brain allowing the analysis of older animals than in the previous $tRNA^{[Ser]Sec}$-deficient mouse model. The observed movement phenotype may be caused by reduced densities of PV+/*Gad67*+ GABAergic and ChAT+ cholinergic neurons in the striatum.

Experimental

Nomenclature

Secisbp2 and SECISBP2 refer to rodent and human proteins, respectively. Names in italics indicate the respective genes.

Mouse models

Transgenic Secisbp2$^{tm1a(EUCOMM)Wtsi}$ mice were described recently [24]. The breeding colony was maintained on breeding diets (Ssniff, Soest, Germany) containing on average 0.2-0.3 ppm Se. Animal experiments were approved by the local governmental authorities (Landesamt für Gesundheit und Soziales, LAGeSo Berlin, Germany). *CamK-Cre* and *Trsp* genotyping were done as described [25].

Enzyme assays

GPx assays were carried out with *tert*-butyl hydroperoxide as the substrate [10]. Protein concentrations were determined by the method of Bradford using IgG as a standard.

The insulin-dependent fluorescent Txnrd assay from IMCO (Stockholm, Sweden) was used to determine selenium-dependent Txnrd activities as described [24]. The Txnrd standards were adjusted to be within the range of our samples [in nM]: 0.125, 0.25, 0.375, 0.50, 0.625, 0.75, 0.825, 1.0.

Western blot analysis

For Secisbp2 (antiserum directed against the C-terminal 349 amino acids of mouse Secisbp2 was generated by Protein Tech Group, Chicago, IL; 1:1000), GPx4 (rabbit antiserum from Abcam, Cambridge, UK; 1:1000), SePM (rabbit antiserum from Sigma, St. Louis, USA; 1:1000), SePT (rabbit antiserum from Sigma; 1:250), SePW (rabbit antiserum from Rockland, Gilbertsville, USA; 1:2000), SePK (rabbit antiserum from Sigma; 1:500), SePS (rabbit antiserum from Sigma; 1:1000), Sod1 (rabbit antiserum from Abcam; 1:2000), Prdx3 (rabbit antiserum from Sigma; 1:3000) and Prdx3 (rabbit antiserum from Abcam; 1:2000). Proteins from cerebral cortex were isolated with RIPA buffer (50 mM Tris-HCl pH 8.0; 150 mM NaCl; 1% NP-40; 0.5% deoxycholic acid; 0.1% SDS, 2x protease inhibitor cocktail; 1 mM DTT). The Secisbp2 Western blot was done on whole cell lysates; frozen tissues were homogenized directly in Laemmli buffer. Protein concentrations were determined with the BCA protein assay kit (ThermoFisher Scientific, Waltham, USA). 100 μg (50 μg for Secisbp2) of whole lysate was separated on SDS/15% polyacrylamide gels (8% for Secisbp2). For 4-HNE (rabbit antiserum from Calbiochem/Merck, Darmstadt, Germany; 1:2500), GPx1 (1:2000; rabbit antiserum from Abcam) and Txnrd1 (mouse monoclonal antibody from Abcam; 1:1000), proteins from cerebral cortex were isolated with homogenization buffer (250 mM Saccharose; 20 mM HEPES; 1 mM EDTA, pH 7.4). 100 μg of protein was used for Western blotting. As loading controls the following antibodies were used: Rabbit polyclonal β-actin antiserum (Rockland) at 1:2000 dilution and mouse monoclonal 6C5 against GAPDH (Abcam) at 1:300.000 dilution. Densitometric quantifications are given in Supplementary Figures S1 and S2.

Real-time PCR

Total RNA was isolated from powdered mouse cerebral cortices or striata according to the TRIzol protocol (Invitrogen, Darmstadt, Germany). Samples were treated with RQ1 RNase-Free DNase (Promega, Madison, USA). cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's protocol. Real-time PCR was performed using SYBR Green from ABgene (ThermoFisher Scientific, Waltham, USA) on an iCycler (Bio-Rad). Primers used for real-time PCR are given in Supplementary Table 1. *18S* rRNA was used as reference gene for mRNA quantification. 5-6 animals per group were analyzed.

Rotarod

Rotarod analysis was carried out as described [25]. Animals were placed on the rotating drum at 4 rounds per min (rpm). After 2 min of accommodation, the rotation was linearly accelerated over 3 min to 40 rpm. The retention time spent on top of the rotating drum was recorded. 5 trials per animals (n = 5-9) were performed.

Immunohistochemistry

Brains from mouse (postnatal day 19-21) were immediately fixed after dissection in 4% paraformaldehyde in 0.1 M phosphate buffer (PB), pH 7.4. Brains were cryoprotected in 30% sucrose in PB and stored at -80 °C till they were cut on a cryostat at 35 μm. Free-floating sections were stained with the indicated antibodies at 4°C overnight. Polyclonal rabbit α-PV (1:6000) and α-CR (1:4000) antibodies were from Swant, Bellinzona, Switzerland. Rabbit polyclonal α-GFAP (1:1500) antibody was from Dako, Hamburg, Germany and polyclonal rabbit α-TH (1:750) was from Abcam, Cambridge, UK. For these antibodies horseradish peroxidase and diaminobezidine substrate were used in conjunction with the Vectastain ABC Rabbit IgG Kit (Vector Laboratories, Burlingame, USA). Polyclonal goat α-ChAT (1:250) was from Millipore, Billerica, USA, and visualized with the DAKO LSAB™+/HRP kit. Photomicrographs were taken at a Zeiss Axioskop II equipped with AxioCam MRc and operated with Axiovision software (Carl Zeiss, Oberkochen, Germany).

Riboprobe synthesis and *in situ* hybridization (ISH)

Rat *Gad67* cDNA was cut from Bluescript transcription vector (kind gift from Thomas Naumann, Institute of Anatomy, Charité, Berlin) with *Apa*I and *Spe*I (New England BioLabs, Ipswich, USA) and subcloned into pGEM-T Easy (Promega, Madison, USA). The plasmid DNA was linearized with *Spe*I, or *Apa*I, for the sense and antisense probes, respectivly. *In vitro* transcription was performed according to the manufacturer's protocol (Roche Biochemicals, Mannheim, Germany) with 1 μg plasmid template DNA, using the DIG RNA labeling kit and appropriate enzymes (T7 polymerase for *Gad67* antisense and SP6 polymerase for *Gad67* sense probe). Brains were sectioned as described for immunohistochemistry. The ISH was performed as described [26].

Neuron counting and statistics

The areas comprising the barrel field cortex (S1BF) and striatum (caudate putamen) were determined using Axiovision software (2-4 mm^2). Neuronal profiles within were counted in several sections and in both hemispheres. PV+, *Gad67*+, ChAT+, and CR+ cells were counted and quantified as cells/area (mm^2). Values are shown as means ± S.E.M.

For all computations, Prism 6 software (for OS X from GraphPad, San Diego, USA) was used for the tests as indicated in the figure legends. Data are expressed as means ± standard error of the mean (S.E.M.). Statistical significance was determined and indicated as *p<0.05, **p<0.01, or ***p<0.001.

Results

Neuron-specific *Secisbp2* inactivation reduces selenoprotein expression

Secisbp2 is essential for embryonic development [24]. In order to investigate its function in neurons, we specifically inactivated the Secisbp2 gene by an *out-of-frame* deletion of exon 5 using a *CamK-Cre* transgene (Fig. 1A). Full-length Secisbp2 protein was significantly reduced in cortical extracts from *CamK-Cre; Secisbp2*$^{fl/fl}$ mice (Fig. 1B) as were *Secisbp2* mRNA levels (Fig. 1C). From the second postnatal week on, mutant mice appeared smaller. On postnatal day 21 (P21) the mean body weights were 8.1g ± 0.1g and 4.2 g± 0.1 g for wild-type and mutants, respectively (n=8-25; p<0.001, Student's *t* test).

Longitudinal growth was also affected, as tail length was 5.2 cm versus 4.1 cm (n=8-25; p<0.001, Student's *t* test). Selenoprotein expression was analyzed in cortical extracts from *CamK-Cre; Secisbp2*$^{fl/fl}$ mice on P16-18. Western blot analysis showed that selenoproteins GPx1, SePW, SePM, SePT, and GPx4, were significantly reduced in mutant mice (Fig. 2A). SePS and SePK, which carry the Sec codon close to the C-terminus, were not reduced. Txnrd1 protein levels were only reduced in male mutant mice. Residual selenoprotein expression and activity may be due to glial and endothelial cells, which are not targeted by the *CamK-Cre* transgene, or may indicate partial independence from Secisbp2. To distinguish these possibilities, we ran samples from *CamK-Cre; Trsp*$^{fl/fl}$ mice [17] in parallel. As shown in Fig. 2A, inactivation of *Trsp* consistently reduced selenoprotein expression to lower amounts than inactivation of *Secisbp2*. Selenoproteins K and S clearly depend on *Trsp*, but not on *Secisbp2*. The more complete inactivation of selenoproteins in *CamK-Cre; Trsp*$^{fl/fl}$ mice is consistent with their shorter survival (up to P12-P14) compared to P18-P23 in *CamK-Cre; Secisbp2*$^{fl/fl}$ mice. To directly probe for the presence of Sec in the proteins, enzymatic activities were determined where possible. Cytosolic GPx activity was reduced by about 43% (Fig. 2B). Cytosolic Txnrd activity, as determined with the Sec-sensitive insulin assay, was moderately reduced indicating that Txnrd1 was not fully functional despite unchanged protein levels (Fig. 2C).

We have recently demonstrated that selenoprotein mRNA levels were unexpectedly reduced in hepatocytes genetically deficient for Secisbp2 [24]. We therefore studied mRNA levels in cortical extracts from *CamK-Cre; Secisbp2*$^{fl/fl}$ mice to compare these to the changes in selenoprotein levels. Messenger RNA levels of *Gpx1* and *Sepw* were significantly reduced in both sexes and paralleled changes in protein levels (compare Figs. 3A and 2A). Other

selenoprotein mRNAs were significantly changed only in male or female mutants (Fig. 3B), but respective protein levels were also found to be decreased (Fig. 2). The *Txnrd1* and *Gpx4* mRNAs remained unaffected by *Secisbp2* inactivation (Fig. 3C), while the respective proteins were clearly reduced (Fig. 2A).

Markers of oxidative stress are moderately induced

Secisbp2-inactivation in hepatocytes clearly induces Nrf2-dependent genes, albeit at a much lower level than inactivation of $tRNA^{[Ser]Sec}$ [24]. In cortex, only two Nrf2 target genes were significantly induced (*Cd36* and *Nqo1*) and only in male mutants, while *Gsta1*, *Gsta4* and *Gstm1* remained similar to control mice (Fig. 4A). Based on Western blot analysis, there was no change in Sod1, Prdx3 and Prdx6 proteins and no increase in protein modification by the lipid peroxidation product 4-hydroxy-nonenal (4-HNE), which activates the transcription factor Nrf2 (Fig. 4B).

Numbers of parvalbumin-positive interneurons are reduced in somatosensory cortex

We have previously demonstrated that inactivation of selenoprotein biosynthesis in neurons completely abrogates the developmental appearance of parvalbumin-positive (PV+) GABAergic interneurons in the hippocampus and somatosensory cortex [17]. These mice showed a dystonic gait, hyperexcitability when handled, and rarely survived beyond P13. *CamK-Cre; Secisbp2*$^{fl/fl}$ mice almost always survived beyond P16, the time when maximal numbers of PV expressing interneurons are normally reached in the somatosensory cortex. Consistent with the fact that selenoprotein expression is higher than in *CamK-Cre; Trsp*$^{fl/fl}$ mutants (Fig. 2), PV+ interneurons were detected in *CamK-Cre; Secisbp2*$^{fl/fl}$ mice on P21. PV+ interneuron density in the primary somatosensory cortex (S1BF, barrel field cortex) was significantly decreased to 63 % of controls (Fig. 5A,B), while calretinin-positive (CR+) neuron density was not reduced (Fig. 5B). The immediate question was whether PV+ cells are lost or simply do not express PV. We therefore performed *in situ* hybridization with a probe against glutamate decarboxylase 67kD isoform (*Gad67*) to determine the density of GABAergic (*Gad67*+) interneurons in the *Secisbp2*-deficient brain (Fig. 5C). The density of *Gad67*+ interneurons was significantly reduced to 77% of controls in the S1BF (Fig. 5D). PV+ cells account for roughly half of all *Gad67*+ cells depending on the brain region. This number is therefore compatible with a 40% PV+ loss, if PV+ cells represent 60% of all *Gad67*+ cells in this area.

Is the loss of PV+/*Gad67*+ cells a developmental or a degenerative process? We assume that PV+/*Gad67*+ neurons are lost in a degenerative process, since we observed massive reactive astrogliosis in the cerebral cortex and striatum (Fig. 6). While astrocytes around some blood vessels and in white matter tracts normally express GFAP, increased GFAP expression is seen in the mutant striatum and cerebral cortex, particularly in layer 4.

Reduced numbers of PV+, *Gad67*+, and cholinergic striatal neurons

Commencing postnatal days 13-15, mutant mice exhibited a marked movement phenotype with an awkward, broad based, and dystonic gait, sometimes loosing balance resembling *Sepp*-deficient mice [11, 12, 14]. *Secisbp2* mutant mice performed poorly in the rotarod task, a measure of motor coordination, although they were able to walk (Fig. 7A). Since the

CamK-Cre transgene does not recombine in the cerebellum, cerebellar defects are unlikely to be the reason for the observed defect. The supplemental video sequence shows a *CamK-Cre; Secisbp2*$^{fl/fl}$ mouse, which at first appears to suffer from complete hind limb paralysis, but within seconds recovers and properly uses its hind legs again (supplemental video). Axonal degeneration along the pyramidal tract or loss of motor neurons is expected to lead to permanent paralysis. We therefore suspected dysfunction of the basal ganglia. Tyrosine hydroxylase (TH) immunostaining as a probe of the dopaminergic system appeared unaltered in the striatum (Fig. 7B) and substantia nigra (not shown). The density of PV+ neurons was reduced to 35% of control in the caudate putamen (Fig. 7C, D). The density of *Gad67*+ neurons in the caudate putamen was reduced to 51% (Fig. 7 E,F). Finally, the density of cholinergic (choline acetyltransferase-positive; ChAT+) neurons in the caudate putamen was significantly reduced to 32% (Fig. 7 G, H).

Discussion

We propose that the most striking finding of this work is the selective damage of PV+/*Gad67*+ neurons in specific brain areas elicited by reduced selenoprotein expression. Our finding of striatal neuron loss is novel as is the involvement of cholinergic neurons in addition to GABAergic ones. The results suggest that our animal model reflects key features of the movement phenotype and possibly mild mental retardation of a patient carrying mutations in *SECISBP2* [23].

Selenoprotein expression in neurons and Secisbp2

Inactivation of the gene encoding tRNA$^{[Ser]Sec}$ in neurons reduced expression of all tested selenoproteins [17]. Inactivation of *Secisbp2* in the current study likewise reduced selenoprotein expression and activity, namely GPx1, GPx4, SePW, SePM, SePT, and Txnrd1. On the other hand, mRNA levels were only reduced for *Gpx1, Sepw, Sepm,* and *Sept*, while *Txnrd1* and *Gpx4* messages remained unaltered. Thus, Secisbp2 enhances not only translational read-through at the in-frame UGA codon, but also affects mRNA abundance. Interestingly, these effects appear specific for each individual selenoprotein, as shown in hepatocytes [24], and will certainly require a dedicated investigation into potential mechanisms. For example, impaired Sec incorporation into SePS and SePK leads to reduced protein levels in *Trsp*-deficient mice, while *Secisbp2*-deficiency does not alter the amount of either protein. Since the same transgenic *Cre* driver line was used in both the *Trsp* and *Secisbp2* studies, we cannot attribute the moderate mRNA and protein changes in *Secisbp2* mutants to incomplete penetrance. Therefore, while tRNA$^{[Ser]Sec}$ is essential for functional selenoprotein expression, lack of Secisbp2 may allow some selenoproteins to be expressed, albeit at lower levels.

Another finding in favor of a milder effect of *Secisbp2* inactivation in neurons compared to *Trsp* inactivation is the very moderate induction of Nrf2-dependent genes. The smaller induction of Nrf2 target genes compared with the situation in *Secisbp2*-deficient hepatocytes may simply reflect a cell type difference. Alternatively, the moderate reduction of many selenoproteins in *Secisbp2*-deficient neurons may point to differences in selenoprotein biosynthesis and perhaps implies substantial expression of neuronal selenoproteins (e.g.

GPx4), even in the absence of Secisbp2. Accordingly, we did not detect any signs of enhanced 4-HNE protein modification or induction of several antioxidant enzymes. There is nevertheless a substantial tissue response in mice lacking *Secisbp2* expression as demonstrated by enhanced astrogliosis.

Inhibitory interneurons and selenoproteins

A key finding in mice entirely lacking neuronal selenoprotein biosynthesis was the absence of PV+ interneurons in the somatosensory cortex and hippocampus, which mimics inactivation of *Gpx4* alone [17]. The *CamK-Cre; Trsp*$^{fl/fl}$ and *CamK-Cre; Gpx4*$^{fl/fl}$ mouse models, however, had the disadvantage that mutant mice rarely survived beyond P13. The development of PV+ cortical interneurons could thus not be followed up to its peak on P16, and it remained unknown whether PV+ interneuron development was simply retarded or absent. The mouse model developed in the current study, possibly due to low residual GPx4 synthesis, allowed us to show that impaired selenoprotein expression not only reduced PV+ interneuron density at later stages of development, but also reduced the density of *Gad67*+ neurons. The loss of only 37% of the cortical PV+ interneurons on P16 in this model suggests that PV+ neurons are able to mature in *Secisbp2*-deficient mice, but likely degenerate at a later stage of development. In agreement with this hypothesis and with more than 50% of *Gad67*+ cell expressing PV, the decrease of *Gad67*+ cells of 23% is smaller than the loss of PV+ cells.

As a possible explanation for the movement phenotype of *Secisbp2*-deficient mice, we showed that reduced selenoprotein expression also decreases striatal neuron numbers. The density of PV+ striatal neurons is reduced by 65% in agreement with the reduced density of striatal *Gad67*+ neurons. Another novel finding is the loss of cholinergic (ChAT+) striatal neurons, a cell type so far not known to depend on selenoprotein expression. The observed changes appear specific, as dopaminergic innervation emanating from the substantia nigra is obviously not reduced. However, astrogliosis within the striatum clearly points to degenerative processes within this brain region.

What could be the common mechanism involved in the loss of specific groups of cortical, hippocampal, and striatal neurons? Most of these neurons are GABAergic and express the calcium-binding protein PV as a neurochemical marker. Both features are also true for Purkinje cells in the cerebellar cortex, which degenerate if *Trsp* or *Gpx4* are inactivated [19]. Accumulating lipid peroxides that initiate apoptosis may be involved in a potentialmechanism [7], since PV+ interneurons are particularly sensitive to oxidative stress, e.g. upon ketamine exposure [27] or reduced glutathione levels [28]. Another possibility is deregulated signaling due to inactivation of oxidation-sensitive phosphatases [29]. It is, however, not even clear at the moment whether the process of cell death of PV+ cells is cell autonomous. It is known that PV+ cells require signaling by the neurotrophin brain derived neurotrophic factor (BDNF) and its receptor tyrosine kinase TrkB for maturation and PV expression in the cortex and striatum [30, 31]. If glutamatergic principal neurons respond to selenoprotein deficiency by reduced secretion of BDNF, this lack of BDNF could directly reduce PV expression by interneurons. But how are ChAT+ neurons related to GABAergic neurons? Cholinergic signals from the basal forebrain stimulate hippocampal BDNF

expression [32]. Alternatively, PV+ and striatal ChAT+ interneurons share expression of the transcription factor Nkx2.1 during development [33, 34]. It would thus be interesting in future studies to determine striatal neuron numbers in *Sepp*$^{-/-}$ mice, since these share a movement phenotype that may be compatible with striatal dysfunction [11, 12, 14].

Phenotypes and relevance for humans

There are a few reports that suggest a direct link between low brain selenoprotein levels in humans and neurological or psychiatric symptoms. Case reports on intractable epilepsy in selenium-deficient children pointed in this direction [35, 36]. These are supported by the more recent identification of mutations in selenocysteine synthase, which result in *Progressive Cerebello Cerebral Atrophy*, an inherited neurodevelopmental and neurodegenerative disorder in children [20, 21]. Milder syndromes caused by moderate deficiency of selenoprotein biosynthesis may be relevant for more patients, if PV+, and possibly ChAT+, neurons are selectively lost or functionally impaired. It is tempting to speculate that analogy with the mouse model would predict that more severe loss of PV+ interneurons may lead to seizures, while a moderate reduction may rather impair motor and cognitive functions with phenotypes resembling schizophrenia and autism [37]. Mutations in *SECISBP2* show large phenotypic variability, whereas all patients show a disrupted thyroid hormone axis and thyroid tissue function [22, 38]. However, some patients have more severe phenotypes [39], but only one individual has been described as mildly mentally retarded with a movement disorder [23].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviation footnote

ChAT	choline acteyltransferase
GABA	γ-amino butyric acid
GAD	glutamic acid decarboxylase
Gpx	glutathione peroxidase
Sec	selenocysteine
SECIS	selenocysteine insertion sequence
Secisbp2	SECIS binding protein 2
SePH	selenoprotein H
Txnrd	Thioredoxin reductase.

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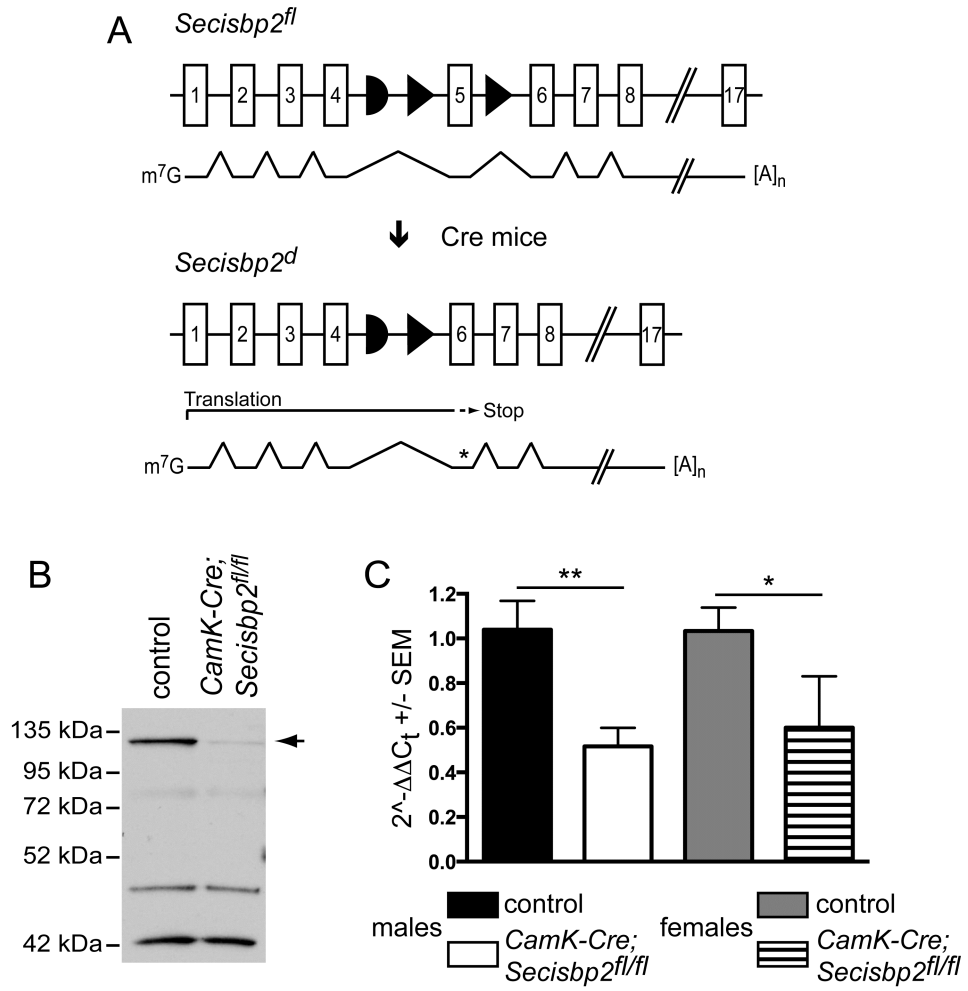

Fig. 1. Neuron-specific deletion of exon 5 in *Secisbp2*

(A) Cre-mediated deletion of exon 5 leads to a frame shift and premature termination codon (*) in exon 6. **(B)** Secisbp2 protein is significantly reduced in cortical homogenate. Western blot using an antibody directed against the C-terminus of the protein. The full-length protein is indicated by an arrowhead. Nonspecific bands of lower molecular weight indicate equal loading. **(C)** *Secisbp2* mRNA is significantly reduced. Real-time PCR using primers spanning exons 2/3 (n=5-6 animals per group; Student's *t*-test; *$p<0.05$; **$p<0.01$).

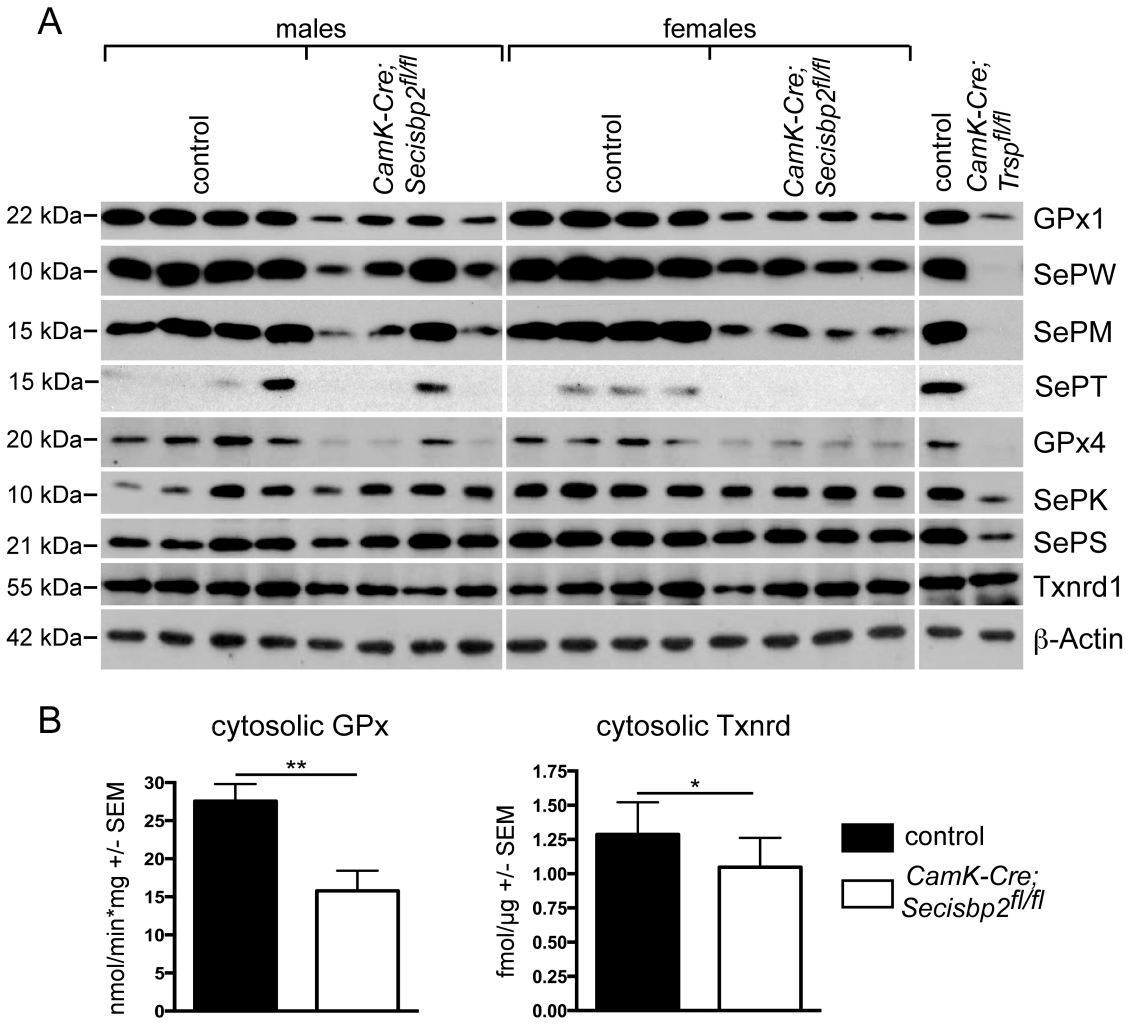

Fig. 2. Selenoprotein expression in *Secisbp2*- deficient cortex

(A) Inactivation of *Secisbp2* in neurons significantly reduces the levels of many selenoproteins as judged by Western blot. Densitometric quantifications are given in Supplementary Figure S1. One male mutant showed incomplete selenoprotein inactivation and was censored in enzymatic assays. **(B)** Activity of cytosolic glutathione peroxidase (GPx) in cortical homogenates determined with *tert*-BuOOH as substrate (n=11 animals per group; Student's *t*-test; $^{**}p<0.01$). **(C)** Activity of cytosolic thioredoxin reductase (Txnrd) determined with insulin as substrate (n=12 animals per group; Student's *t*-test; $^{*}p<0.05$).

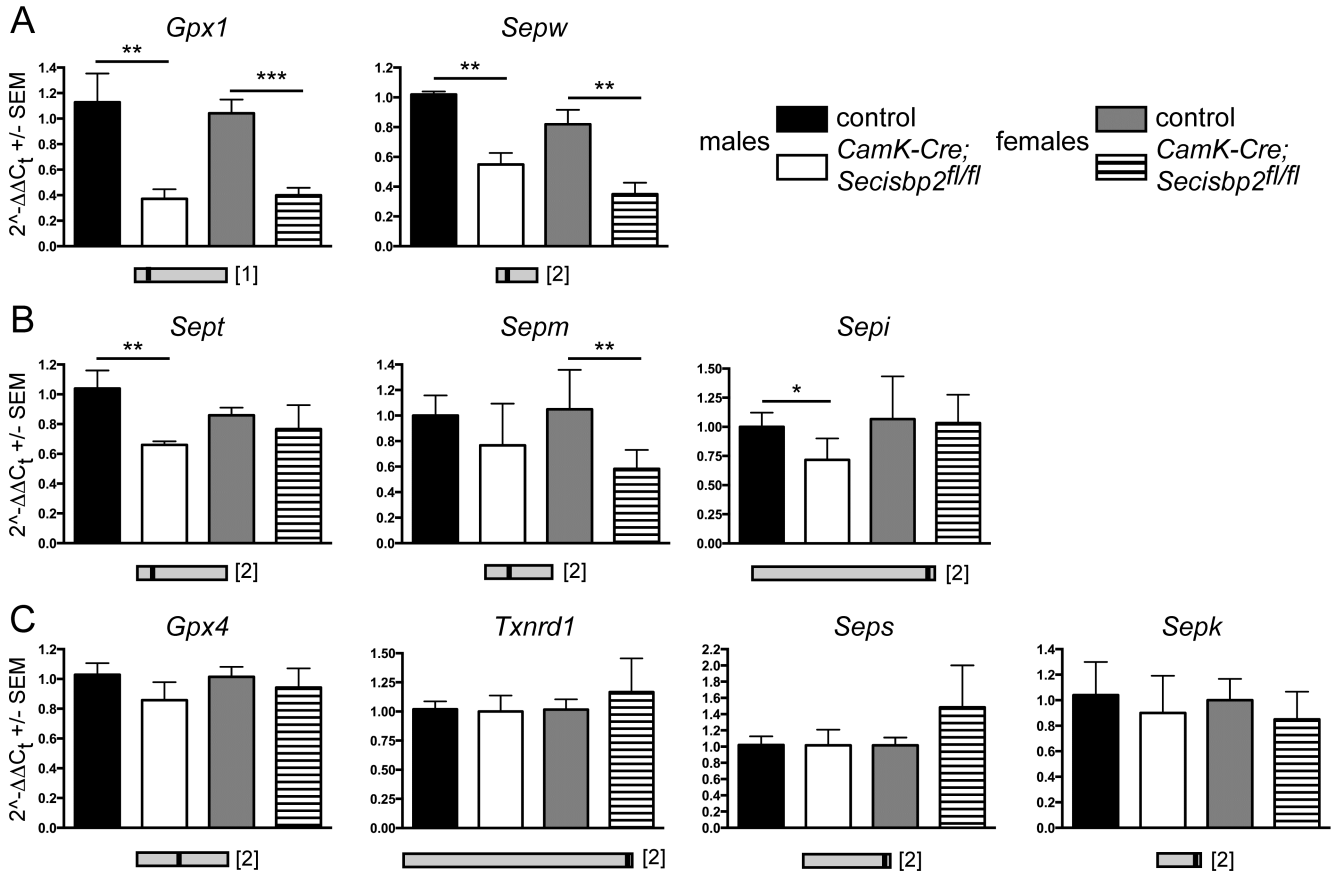

Fig. 3. Selenoprotein mRNA expression in *Secisbp2*-deficient cortex

Selenoprotein mRNA levels were determined by real-time PCR (n=5-6 animals per group; Student's *t*-test; **p*<0.05; ***p*<0.01; ****p*<0.001) from cerebral cortex. The selenoproteins are schematically shown as gray bars with the position of Sec as black bars. The type of SECIS element is shown in brackets and does not correlate with the response. **(A)** Selenoprotein mRNAs that are decreased in both genders, when *Secisbp2* is deleted. **(B)** Transcripts of selenoproteins that are reduced only in male or in female mutants. **(C)** Selenoprotein mRNAs that are not affected by *Secisbp2* inactivation.

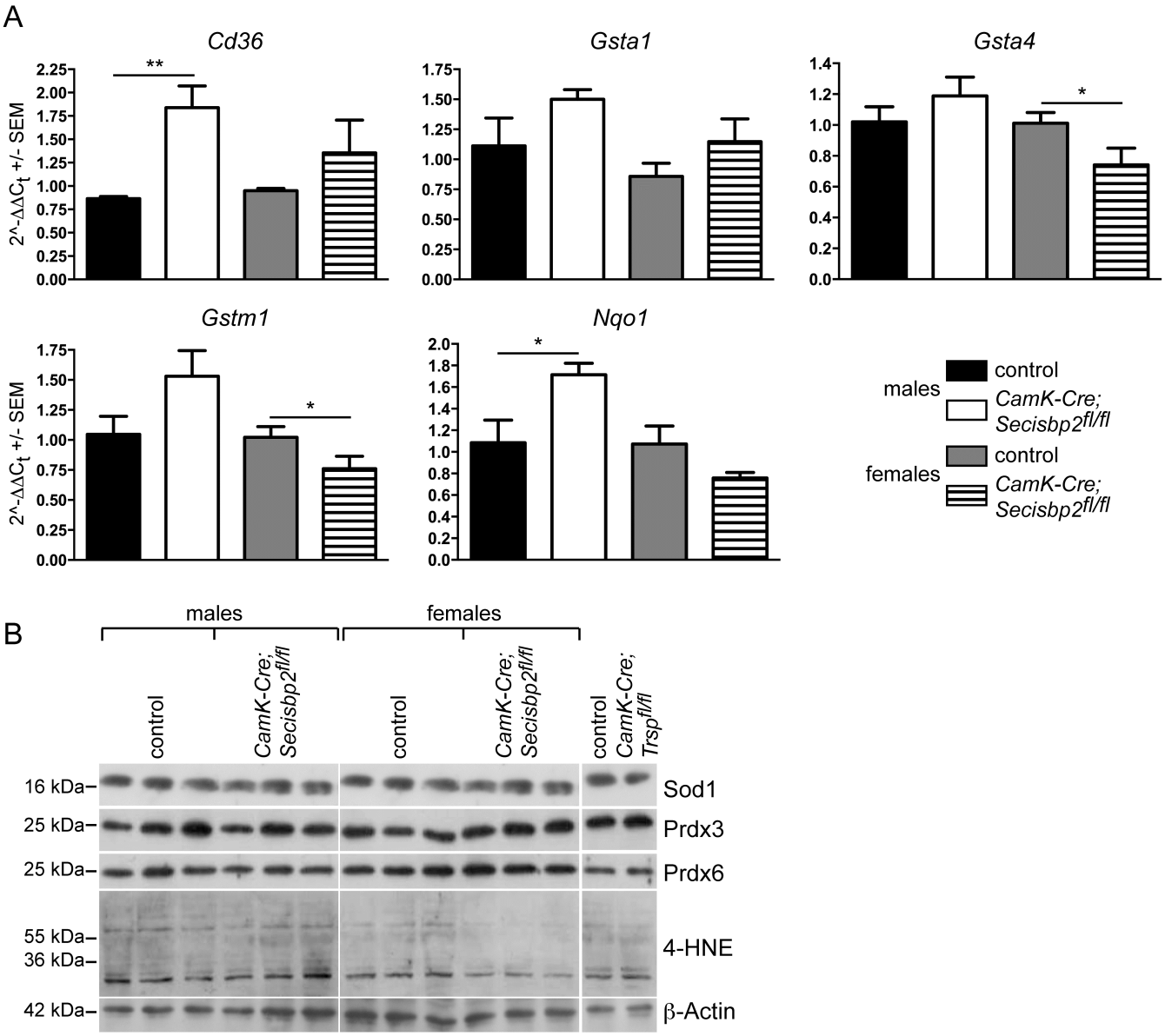

Fig. 4. Nrf2-dependent gene expression and oxidative stress in *Secisbp2*-deficient cortex

(A) Transcript levels of Nrf2-dependent genes were determined by real-time PCR (n=5-6 animals per group; Student's *t*-test; **p*<0.05; ***p*<0.01). **(B)** Oxidative stress markers were determined by Western blotting. Neither Sod1, Prdx3 nor Prdx6 expression was increased. Elevated 4-HNE protein modification, as an indicator for increased lipid oxidation, was not detected. Protein quantification is given in Supplementary Figure S2.

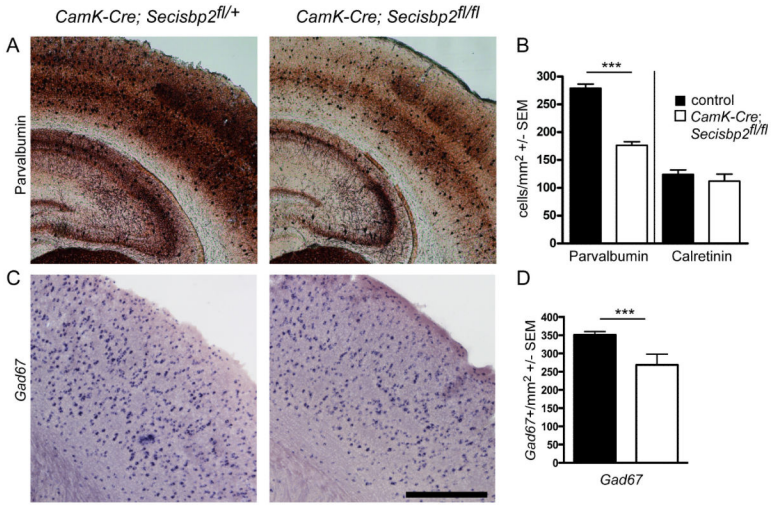

Fig. 5. Specific lack of PV+ interneurons in somatosensory cortex

A) PV staining in S1BF and hippocampus of *Secisbp2*-deficient mice is weaker than in controls. B) PV+ neuron density is significantly lower in S1BF of *Secisbp2*-deficient mice, while CR+ neuron density is not changed. Student's *t* test, ***p<0.001. C) *In situ* hybridization for *Gad67* in S1BF. D) *Gad67*+ neuron density is significantly reduced in S1BF of *Secisbp2*-deficient mice. Student's *t* test, ***p<0.001. Scale bar 500 μm.

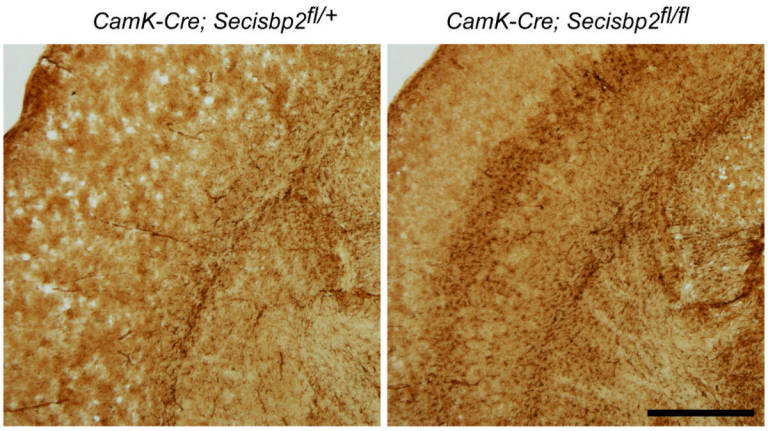

Fig. 6. Immunohistochemical staining for GFAP reveals widespread astrogliosis

Note the stronger GFAP staining in cortical layer 4. Astrogliosis is also seen in the striatum. Scale bar 500 μm.

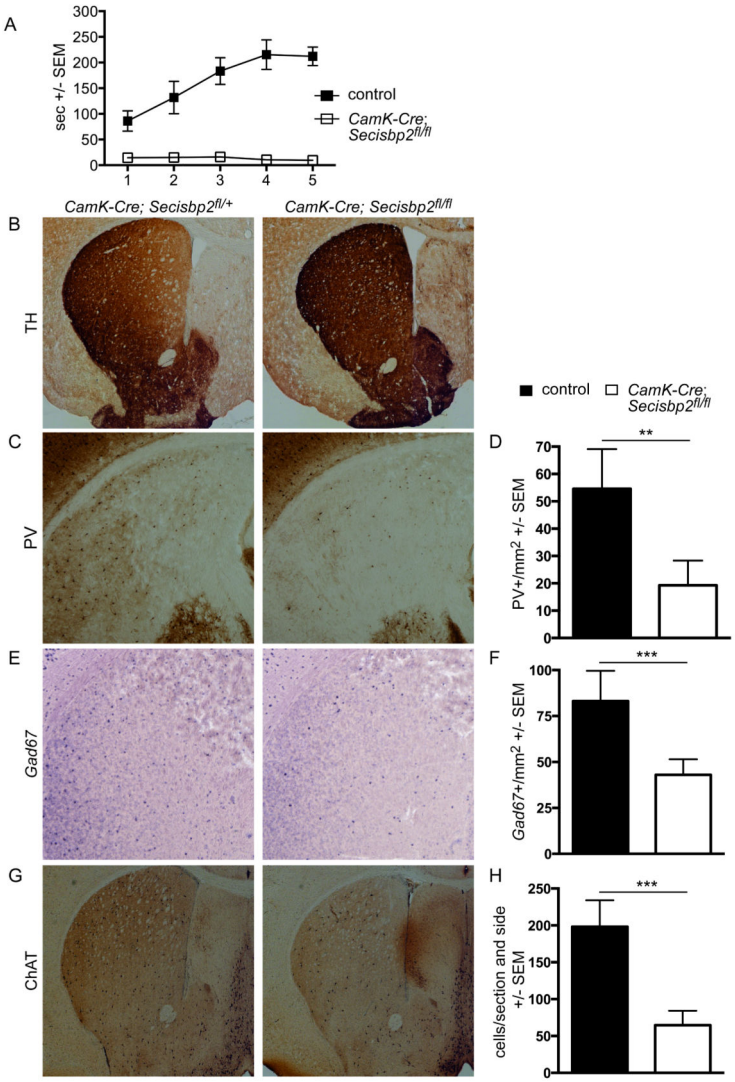

Fig. 7. Movement phenotype and neurochemical markers in the striatum of *CamK-Cre; Secisbp2$^{fl/fl}$* mice

A) Rotarod analysis reveals a movement phenotype. While control litter mates performed well and showed a learning response, *Secisbp2*-deficient mice performed very badly without signs of learning. B) Immunohistochemical staining for tyrosine hydroxylase (TH) shows no difference between mutants and controls. C) Immunohistochemical staining for parvalbumin (PV). D) Decreased density of PV+ cells in the striatum of *Secisbp2*-deficient mice. **p<0.01. E) *In situ* hybridization against glutamate decarboxylase mRNA (*Gad67*) in striatum. F) Decreased density of *Gad67*+ cells in the striatum of *Secisbp2*-deficient mice. ***p<0.001. G) Immunohistochemical staining for choline acetyltransferase (ChAT). H) Decreased density of ChAT+ cells in the striatum of *Secisbp2*-deficient mice. ***p<0.001.Student's *t* test. Scale bars 500 μm.