The deletion of the STOP gene, a microtubule stabilizing factor, leads only to discrete cerebral metabolic changes in mice

Ryosuke Hanaya, MD, PhD*, Estelle Koning*, Arielle Ferrandon*, Annie Schweitzer!, Annie Andrieux, PhD!, Astrid Nehlig, PhD*

*INSERM U 666, 67085 Strasbourg, France and !INSERM U 366, CEA-Grenoble, 38054 Grenoble, France

Address for correspondence: Astrid Nehlig, INSERM U 666, Faculty of Medicine, 11 rue Humann, 67085 Strasbourg Cedex, France

Tel: (33) 390.24.32.43, Fax: (33) 390.24.32.56

E-mail: nehlig@neurochem.u-strasbg.fr

Number of pages: 20

Number of words in abstract: 199

Number of tables: 1

Number of figures: 2 (including one colour figure)

Running title: Cerebral metabolism in STOP KO mice

Abstract

In mice, the deletion of the STOP protein leads to subtle anatomic changes but induces depleted synaptic vesicle pools, impaired synaptic plasticity, hyperdopaminergy and major behavioral disorders that are alleviated by neuroleptics, hence leading to a “schizophrenic-like” phenotype. In this study, we applied the quantitative autoradiographic [^{14}C]2-deoxyglucose technique to study to which extent basal rates of cerebral glucose utilization in STOP KO mice occur in the regions where metabolic changes have been reported in schizophrenic patients. Studies were performed on wild type, heterozygous and homozygous STOP KO mice (7-8 per group). Mice were implanted with femoral artery and vein catheters and cerebral glucose utilization was quantified over 45 min. Compared to wild type mice, glucose utilization in STOP KO mice was significantly increased in olfactory cortex, ventromedian and anterolateral hypothalamus, ventral tegmental area and substantia nigra pars compacta. Non significant increases, ranging between 9 and 19%, were recorded in the whole auditory system, CA1 pyramidal cell layer and dorsal raphe. Glucose utilization was also significantly increased in heterozygous compared to wild type mice in olfactory cortex. These data might reflect hyperdopaminergic activity, olfactory deficits and disturbances in sleep in STOP KO mice that are also reported in schizophrenic patients.

Key words: cerebral glucose metabolism, cytoskeleton, microtubules, STOP protein, schizophrenia, transgenic mice

Introduction

Microtubules play a pivotal role in many vital cell functions, including cell divison, morphogenesis and vesicle trafficking (Dustin, 1984). They are particularly abundant in neurons and tubulin itself accounts for 20% of neuronal proteins. Neuronal microtubules are mandatory for dendritic and axonal trafficking, and the disturbance of these transports by drugs aimed at microtubules as the members of the taxol family used in cancer therapy induces neuropathies (Pazdur et al., 1993). Microtubules act as dynamic polymers, exhibiting both large spontaneous length fluctuations and treadmill-type behavior (Margolis and Wilson, 1978; Mitchison and Kirschner, 1984). Many cell types, including neurons and glial cells contain microtubule-stabilizing factors that can block microtubule dynamics and prevent microtubules assemblies from depolymerizing, as for example in the cold (Baas et al., 1994; Bosc et al., 2003). The microtubule-associated proteins STOPs are responsible for the high degree of stabilization displayed by neuronal and non-neuronal microtubules (Bosc et al., 1996; Denarier et al., 1998; Galiano et al., 2004). More recently it has been shown that STOP is phosphorylated by the multifunctional enzyme calcium/calmodulin-dependent protein kinase II (CaMKII). Phosphorylated forms of STOP do not bind microtubules but co-localize with actin assemblies and with clusters of synaptic proteins, in differentiated neurons and could be important for STOP function in synaptic plasticity (Baratier et al., 2006). Furthermore, STOP proteins have the capacity to associate with Golgi material (Gory-Faure et al., 2006) and in light of studies indicating that the presence of Golgi material in neurites and synapses may be important for synaptic plasticity (Horton and Ehlers, 2004), a Golgi interaction with STOP could be important for STOP function in synapses.

The STOP protein was recently deleted in mice. The neuronal deficits induced by the STOP protein deletion do not induce overt anatomical alterations but affect short- and long-term synaptic plasticity in the hippocampus where depleted glutamatergic synaptic vesicle pools were found (Andrieux et al., 2002). The cerebral reduction of glutamate release to the synaptic cleft leads to reduced stimulation of postsynaptic glutamate receptors and increased glutamine metabolism in the vicinity of the postsynapse (Brenner et al., 2007). Correlatively,

synaptophysin, VGlut1, GAP-43 and spinophilin mRNAs are decreased in the hippocampus in STOP null mice (Eastwood et al., 2007). STOP KO mice display also a multiplicity of behavioral disorders, mainly disorganized activity with frequent shifts between hyperlocomotion and prostration, dramatic perturbations of maternal behavior including nurturing defects, anxiety-like behavior, severe social withdrawal, perturbed interactions with the physical environment and decreased time spent in feeding and sleeping (Andrieux et al., 2002). They also exhibit alterations in tests usually used to measure “schizophrenic-like behavior”, mainly in sensory-motor gating mechanisms and locomotor hypersensitivity to stress (Fradley et al., 2005). These mice also exhibit increased dopaminergic neurotransmission and increased efflux of dopamine in the nucleus accumbens upon stimulation (Brun et al., 2005). Most behavioral and dopaminergic disturbances are alleviated by neuroleptic treatment (Andrieux et al., 2002; Brun et al., 2005; Fradley et al., 2005) and by the treatment with a microtubule stabilizing agent, epothilone D (Andrieux et al., 2006) that is a taxol-related compound that interacts with tubulin to stabilize microtubules (Kolman, 2004; Wang et al., 2005).

In the STOP KO mouse, the neurotransmission disturbances are mainly characterized by hypo-glutamatergy associated with hyper-dopaminergy, currently considered to be a landmark of schizophrenia (Frankle et al., 2003). In order to confirm whether or not these neurochemical defects may correlate with functional deficits reminiscent of schizophrenia, as reported in the human literature (for review see Wong and Van Tol, 2003), in the present study, we measured the basal cerebral metabolic rates for glucose (LCMRglcs) in STOP KO mice compared to wild type and heterozygous littermates by means of the quantitative autoradiographic [^{14}C]2-deoxyglucose (2DG) technique (Sokoloff et al., 1977) adapted to the mouse (Jay et al., 1985; Bouilleret et al., 2000).

Materials and methods

Animals and measurement of LCMRglcs

STOP KO male mice, heterozygous and control wild-type (WT) littermates were generated as previously described (Andrieux et al., 2002). Mice were housed eight per cage and maintained in quiet, uncrowded facilities (room temperature of 21-22°C) on 12 hour light-dark schedule (7:00 a.m., lights on) and given unlimited access to lab chow and water. Males only were used for these experiments to eliminate confounding effects of variable estrogen level on neuronal excitability (Murphy et al., 1998) and fluctuations in metabolic rates with the estrus cycle (Nehlig et al., 1985). The autoradiographic experiments were performed on a total number of 23 mice, eight WT, seven heterozygous and eight STOP KO mice. All animal experimentation was performed in accordance with the rules of the European Committee Council Direction of November 24, 1986 (86/69/EEC) and the French Department of Agriculture (Licence N°67-97).

A femoral artery and vein were catheterized with polyethylene catheters (Clay Adams PE 10) under light halothane anesthesia. Because of the erratic and unpredictable locomotor behavior of STOP KO mice, the animals were placed in loose-fitting abdomino-pelvic plaster casts to immobilize the lower part of their body, as originally described and performed in numerous studies (Sokoloff et al., 1977; Nehlig et al., 1984). Both catheters were running outside the left hindpaw to allow easy access for injection and blood sampling. The animals were allowed to recover from surgery for at least 3 h before the onset of the experiment. A minimal duration of 2 h is usually applied to allow the animals to fully recover from anesthesia (Sokoloff et al., 1977; Nehlig et al., 1984, 1985; Bouilleret et al., 2000). Catheters were inserted between 8:30 a.m. and 11:30 a.m. The 2DG measurements were performed between 1:30 and 3:30 p.m. All the animals were sacrificed between 2:30 and 3:30 p.m. Not more than two to three mice were studied on a given day. This procedure was followed to keep experimental and surrounding conditions as constant as possible to avoid disturbances in brain metabolism unrelated to the deletion of the STOP gene.

LCMRglcs were measured by the [^{14}C]2-deoxyglucose (2DG) technique (Sokoloff et al., 1977) adapted to mice (Jay et al., 1985; Bouilleret et al., 2000). The [^{14}C]2DG (4.625 MBq/kg, specific activity 1.65-2.04 GBq/mmol, NEN, France) was injected as an i.v. pulse. Timed arterial blood samples were drawn over the following 45 min for the measurement of plasma

glucose and 2DG concentrations. At approximately 45 min after the injection of the tracer, the animals were sacrificed by decapitation. Brains were rapidly removed, frozen and cut into 20 µm coronal sections. According to the classical procedure described by Sokoloff et al. (1977), within each series of ten consecutive sections, the first four ones were taken for autoradiography, the fifth one was stained with thionine and the next five ones were discarded. Sections were autoradiographed on Amersham Biomax MR film along with [^{14}C]methylmethacrylate standards calibrated for their ^{14}C concentration in brain tissue. The autoradiographs were then digitized and analyzed by densitometry with an image processing system (Biocom 500, Les Ulis, France). The localization of specific nuclei was assessed on the adjacent sections stained with thionine according to the mouse brain atlas of Franklin and Paxinos (1997). The whole surface of the brain regions studied was delineated by hand with the image processing system and the optical density of each brain region was read bilaterally in a minimum of four consecutive brain sections for symmetrical regions and in eight sections for central regions. LCMRglcs were calculated according to the operational equation of the 2DG method using the integrated specific activity of the ratio between the plasma [^{14}C]2DG and glucose concentrations measured for each animal, and the usual rate constants (Sokoloff et al., 1977).

Statistical analysis

LCMRglcs were determined in 64 regions of three groups of 7-8 animals, i.e., WT, heterozygous and STOP KO mice. A global statistical analysis was performed using an analysis of variance for repeated measures (within factor, structures and between factor, group). To locate the differences in LCMRglcs in each structure between the WT control group, the heterozygous and STOP KO mice groups, post-hoc analyses were performed using with Bonferroni's t-test corrected for multiple comparisons. Conservative multiple comparisons were chosen to reduce the likelihood of type II errors in view of the large number of statistical comparisons performed.

Results

In heterozygous mice, LCMRglcs significantly increased over the levels recorded in WT mice in one single region, the olfactory cortex (Table 1, Figure 1). This significant increase over control levels was also found in STOP KO mice. In STOP KO mice, LCMRglcs were also significantly increased over control WT levels in two dopaminergic cell groupings, the ventral tegmental area and the substantia nigra pars compacta, and two hypothalamic nuclei, the anterolateral and ventromedial (Table 1, Figures 1, 2). In the latter nucleus, LCMRglcs were also significantly higher in STOP KO than in heterozygous mice.

In addition, LCMRglcs were increased, though non significantly, by values equal to or larger than 10% in 14 regions of the KO STOP mice (Table 1, Figures 1, 2). Metabolic increases, recorded in STOP KO compared to WT mice occurred in the dorsal raphe nucleus (+14%), cerebellar cortex (+11%), anterior hypothalamic nucleus (+11%), CA1 pyramidal cell layer of the hippocampus (+11%) and median forebrain bundle (+10%). Likewise, rates of LCMRglc were higher in most areas of the auditory system of STOP KO compared to WT mice. This was the case for the auditory cortex, medial geniculate body, inferior colliculus, lateral lemniscus and superior olive in which metabolic rates were increased by 9-19%.

There were no metabolic differences between WT and STOP KO mice in the limbic system, including the other parts of the hippocampus, in the cortex and the thalamus.

Discussion

The present data show that, in basal conditions, the levels of functional activity display a general tendency to increase throughout the brain which may reflect the ubiquitous distribution of the protein (Couégnas et al., 2007). However, metabolic rates significantly increased in STOP KO compared to WT mice only in a few scattered regions.

The dopaminergic system

The most striking differences recorded in STOP KO mice compared to WT control mice were significant increases in LCMRglcs in the two dopaminergic cell groupings, the ventral tegmental area and the substantia nigra, pars compacta and the tendency to an increase (+10%) in the median forebrain bundle containing the fibers running from the ventral tegmental area and the substantia nigra, pars compacta to the nucleus accumbens and striatum, respectively. Conversely, in the nucleus accumbens and striatum, LCMRglcs were identical in STOP-KO and wild type mice, which is in accordance with normal basal levels of dopamine measured by microdialysis in the extracellular space of those structures (Brun et al., 2005). This is also in agreement with a normal response to tonic stimulation in the nucleus accumbens and striatum. The tonic stimulation corresponds to the discharge of dopaminergic neurons in a low regular spiking mode allowing to establish a steady state level of dopamine efflux responsible for the basal level of the monoamine (Brun et al., 2005 and references therein). However, the STOP-KO mice were reported to display an enhanced response to phasic stimulation in the nucleus accumbens. The phasic stimulation corresponds to a high frequency bursting discharge eliciting transient increases of dopamine efflux on top of the dopamine basal tone (Brun et al., 2005 and references therein). These larger responses to phasic stimulation in the STOP-KO mice may be facilitated by a higher functional activity in the regions containing dopaminergic cells bodies, mainly the ventral tegmental area, as measured here already at the basal level. Likewise, models of schizophrenia (Grace, 1991) and positron emission tomography (PET) studies in humans have reported a presynaptic dopaminergic abnormality in schizophrenic patients translating into elevated dopamine release in response to stimulation but no increase in basal dopaminergic impregnation (Laruelle et al., 1996; Breier et al., 1997; Abi-Dargham et al., 1998; Ginovart et al., 1999) which is consistent with the data of the present study.

Hypothalamus and sleep

The deletion of the STOP gene led also to metabolic increases in the anterolateral and ventromedial hypothalamic nuclei. STOP KO mice spend less time sleeping and exhibit fragmentation of sleeping times (Andrieux et al., 2002). The anterolateral hypothalamic

nucleus is involved in sleep regulation and the ventromedial nucleus in emotions (Szymusiak et al., 1998). The stimulation of ventromedial hypothalamic regions strongly excites the stimulatory reticular formation, hence causing wakefulness, attention and agitation. Conversely, the stimulation of the anterior part of the hypothalamus leads to somnolence and sometimes sleep (Ticho and Radulovacki, 1991) while the lesion of this region results in persistent insomnia (Szymusiak and Satinoff, 1984; Sallanon et al., 1989). Thus, the increased metabolic activity of these two nuclei may reflect the disturbed regulation of wakefulness and sleep and decreased sleeping time observed in STOP KO mice (Andrieux et al., 2002). In schizophrenic patients, some sleep disturbances were reported, expressed mainly as sleep-onset and maintenance insomnia, and increased latency to various phases of sleep (Monti and Monti, 2004).

The olfactory cortex and hippocampus

There is also a significant change in LCMRglcs in the primary olfactory cortex and this increase appears to be quite robust because it can be recorded to the same extent in both homozygous STOP KO mice and heterozygous mice which means that the lack of one copy of the gene is already disturbing functional activity of this region. This may be in relation with the particularly high density of the STOP protein in the olfactory glomeruli (Andrieux et al., 2002; Couégnas et al., 2007) which leads to a change in function already after a partial gene deletion. Olfactory function has not yet been tested in heterozygous or homozygous STOP KO mice. In schizophrenic patients, phosphorylation-independent MAP2 expression which participates in the modification of synaptic organization is significantly reduced (Rioux et al., 2004) and cell cycle alterations can be found in the olfactory epithelium (McCurdy et al., 2006). Substantial olfactory deficits in schizophrenic patients, such as deficits in odor identification, discrimination, treatment of information and memory are well documented (Moberg et al., 1999; Hudry et al., 2002; Rupp et al., 2005). Furthermore, in humans, olfactory identification is impaired in relatives of patients with familial schizophrenia (Kopala et al., 2001) or in monozygotic twins discordant for schizophrenia (Ugur et al., 2005), which

may relate to the increase in metabolic activity measured here in the olfactory cortex of heterozygous mice.

In the hippocampus, the deletion of the STOP gene leads to an impairment of long-term potentiation and long-term depression at the level of Schaffer collateral-CA1 pyramidal cell synapses where LCMRglcs increased by 11% over WT mice levels but this change was not statistically significant. Conversely, long-term potentiation and paired-pulse facilitation were identical in STOP KO and WT mice at mossy fiber-CA3 pyramidal cell synapses (Andrieux et al., 2002), where LCMRglcs were identical in STOP KO and WT mice. Thus, the tendency to a metabolic increase only in CA1 but not in CA3 pyramidal cells may be in relation with the specific change of electrophysiological properties at the level of the Schaffer collaterals-CA1 pyramidal cells pathway (Andrieux et al., 2002) and with the specific reduction of synaptophysin mRNA expression in CA1 versus CA3 regions in STOP null mice (Eastwood et al., 2007). These regional differences in function in the hippocampus were observed despite a similar density of the STOP lacZ reporter gene in CA1 and CA3 pyramidal cells (Couégnas et al., 2007). Moreover, glutamatergic vesicle pools are depleted in the hippocampus (Andrieux et al., 2002) and glutamate synthesis is reduced in heterozygous and STOP KO mice leading to reduced stimulation of postsynaptic glutamate receptors (Brenner et al., 2007). These disturbances are in accordance with the hypothesis of an alteration of glutamatergic neurotransmission as a potential underlying cause of schizophrenia (Goff and Coyle, 2001; Frankle et al., 2003).

The auditory system

Functional activity was increased in the whole auditory pathway of STOP KO mice, starting at the level of the first posterior relays, superior olive and lateral lemniscus followed by inferior colliculus, midbrain medial geniculate body and the projection on the auditory cortex. These increases ranged from 9 to 19% in all regions but were not statistically significant. Schizophrenic patients have a characteristic pattern of response to test sounds with reduced amplitude and increased latency of the p300 wave of the auditory evoked potentials (Blackwood et al., 1991; Mathalon et al., 2000). This pattern is a trait marker of the

disease (Mathalon et al., 2000), indicating some impairment of auditory function and processing of sensory information (Siegel et al., 1984). In addition, schizophrenia-related auditory hallucinations (Andreasen and Black, 1991; Spitzer et al., 1994) seem to originate in abnormal coactivation in regions related to the acoustical processing of external stimuli (Aleman et al., 2003; Hubl et al., 2004). In STOP KO mice, the auditory function per se does not seem to be altered (JL Puel and A Andrieux, unpublished observations) but the auditory evoked potentials were not measured and need to be studied in more detail in STOP KO mice. Moreover, the amount of change in functional activity in a given structure necessary to impact on the function of this structure is not known

To which extent might STOP KO mice be considered a model of schizophrenia?

Although number of metabolic changes recorded in STOP KO mice may reflect a schizophrenic phenotype, differences can also been noted. The “hypofrontality” classically reported in naïve and medicated schizophrenic patients, characterized by decreased functional activity in anterior brain regions (Weinberger et al., 1986; Geraud et al., 1987; Liddle et al., 1992; Min et al., 1999), was not found in STOP KO mice. Hypometabolism in frontal (and sometimes parietal) cortex is usually associated with negative symptoms at rest (Wolkin et al., 1992; Kaplan et al., 1993; Yuasa et al., 1995; Sabri et al., 1997; Lahti et al., 2006) which were reported in STOP KO mice (Andrieux et al., 2002; Fradley et al., 2005). Positive symptoms correlate positively with increased functional activity in the hippocampal/parahippocampal region (Lahti et al., 2006) which tended to be slightly activated in STOP KO mice. Conversely to human reports (Clark et al., 2001), no metabolic change could be recorded in the thalamus of STOP KO mice studied in controlled basal conditions, despite the quite strong expression of the STOP lacZ reporter gene in several thalamic nuclei (Couégnas et al., 2007).

The STOP protein is a candidate to participate in the role that microtubules may play in the pathophysiology of schizophrenia (Kerwin, 1993). The STOP deletion-induced changes in the expression of synaptic protein mRNAs are reminiscent of those reported in schizophrenia (Eastwood et al., 2007). A recent case-control genetic study on 35

schizophrenic patients reported an association between the STOP gene and schizophrenia. In patients, the mRNAs of two isoforms of the STOP mRNA, and mainly isoform 2 were up-regulated in the prefrontal cortex compared to control cases (Shimizu et al., 2006). Thus, variable regional changes in the cellular content of the STOP protein may underlie the expression of the disease. However, the gene KO technology, as applied here, leads to generalized suppression of the protein which most likely explains the discrepancies between the disorder and the present model. Further work in both humans and animals applying more subtle and regional gene deletions may be necessary to obtain a model closer to the pathophysiology of the disorder.

Acknowledgments

The present study was supported by grants from the Institut National de la Santé et de la Recherche Médicale (INSERM U 366, 405 and 666) and from the Fondation pour la Recherche Médicale. The authors wish to thank D. Job and M.F. Suaud-Chagny for advice and careful reading of the manuscript.

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Legend to Figure 1

Figure 1: Effects of the deletion of the STOP gene on LCMRglcs in heterozygous and homozygous STOP KO mice. Values represent means ± S.D. of 7-8 animals and are expressed as percent of the corresponding level in wild type animals.

* $p < 0.05$, ** $p < 0.01$, statistically significant differences from levels in wild type mice

° $p < 0.05$, statistically significant difference from levels in heterozygous mice.

Abbreviations: OLFCX: olfactory cortex, ALHYP: anterolateral hypothalamus, VMHYP: ventromedian hypothalamus, VTA: ventral tegmental area, SNPC: substantia nigra, pars compacta, AUDCX: auditory cortex, MGEN: medial geniculate body, ICOL: inferior colliculus, LL: lateral lemniscus, SO: superior olive, CA1: hippocampal CA1 pyramidal cell layer, MFB: median forebrain bundle, DRAP: dorsal raphe, CBCX: cerebellar cortex.

Figure 2: 2DG autoradiograms of brain sections of a wild type control (WT) and a STOP KO mouse taken at the level of the midbrain. Compared to the wild type mouse, brain metabolism is increased in the STOP KO mouse at the level of the substantia nigra pars compacta (SNPC), the ventral tegmental area (VTA), the auditory cortex (AUDCX), the CA1 pyramidal cell layer of hippocampus (HIP) and the thalamus (THAL).

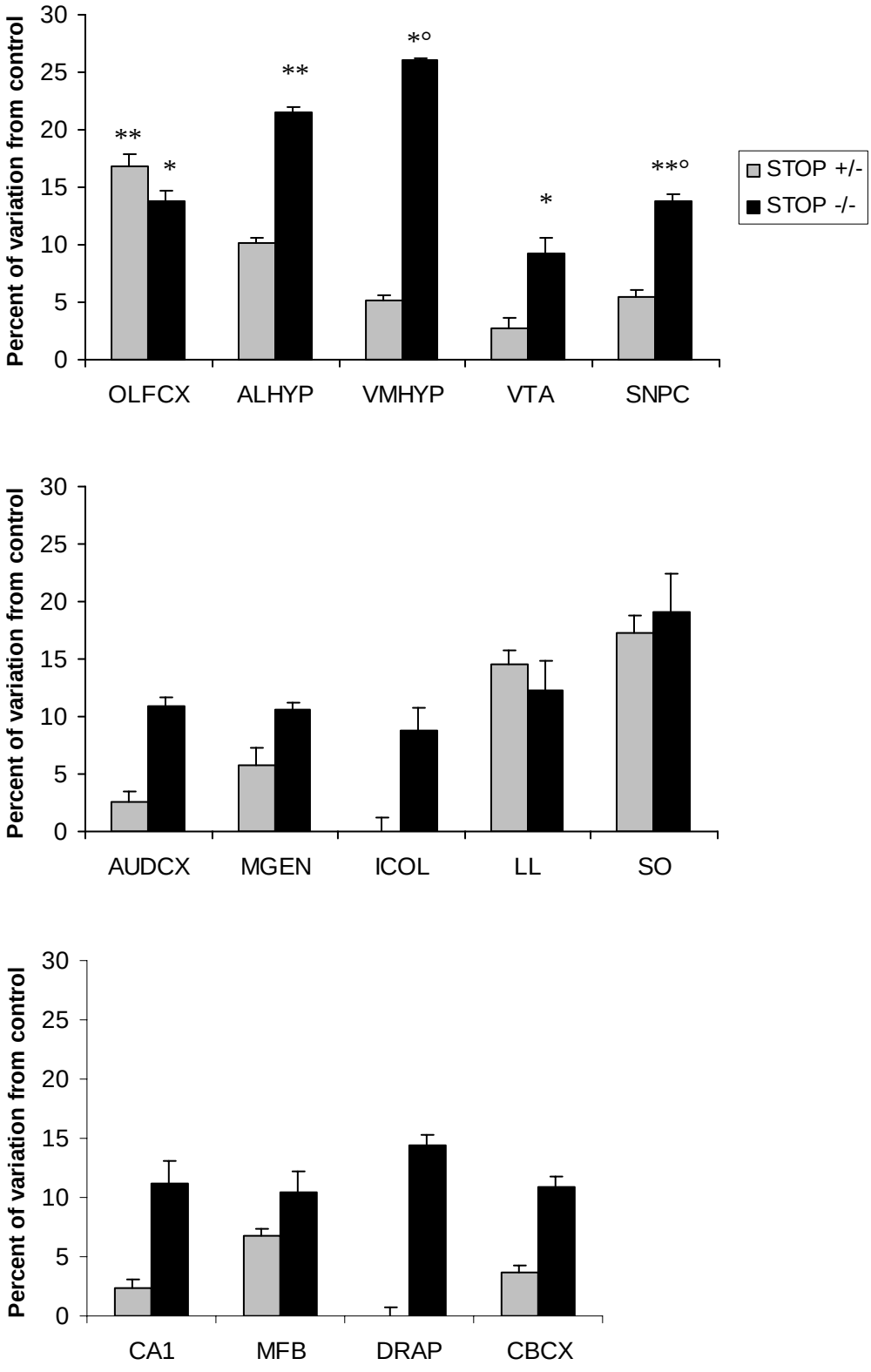

Percent of variation from control
30
25
20
15
10
5
0
**
*
**
*°
*
**°
OLFCX
ALHYP
VMHYP
VTA
SNPC
STOP +/-
STOP -/-
AUDCX
MGEN
ICOL
LL
SO
CA1
MFB
DRAP
CBCX

Figure 1

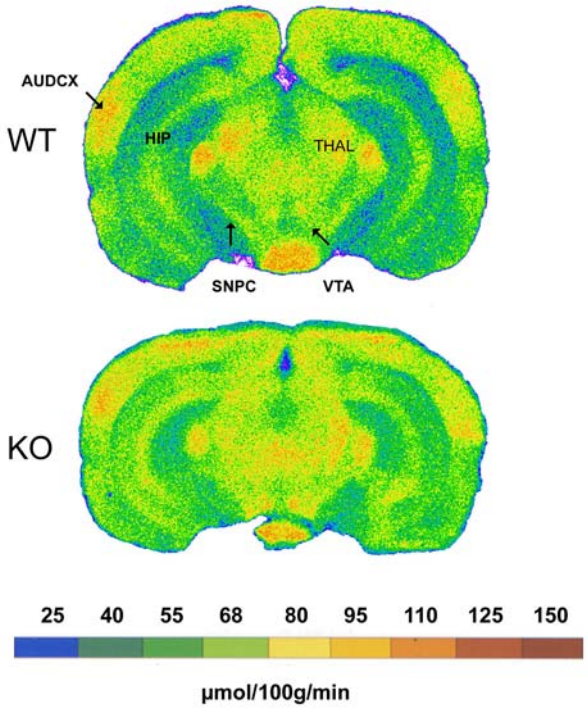
Table 1. Local cerebral metabolic rates for glucose in wild type (WT), heterozygous and STOP KO mice.

	Wild type mice (n = 8)	Heterozygous mice (n = 7)	Variation from WT mice (%)	STOP KO mice (n = 8)	Variation from WT mice (%)
Cerebral cortex					
Olfactory	100.4 ± 4.6	117.3 ± 9.7**	+ 17	114.2 ± 9.1*	+ 14
Prefrontal	74.8 ± 6.2	77.0 ± 6.6	+ 3	80.4 ± 9.9	+ 7
Frontal	84.0 ± 13.3	84.6 ± 6.0	+ 1	87.5 ± 8.8	+ 4
Anterior cingulate	98.3 ± 16.9	98.9 ± 9.4	+ 1	98.0 ± 8.1	0
Piriform	64.8 ± 8.4	62.0 ± 5.2	- 4	63.6 ± 3.0	- 2
Parietal	85.5 ± 11.9	84.6 ± 6.2	- 1	86.6 ± 0.9	+ 1
Motor	82.8 ± 13.1	84.1 ± 5.5	+ 2	87.2 ± 3.3	+ 5
Auditory	113.7 ± 7.2	116.6 ± 9.9	+ 3	126.1 ± 8.9	+ 11
Entorhinal	61.1 ± 5.2	62.8 ± 5.3	+ 3	60.8 ± 1.7	0
Visual	89.1 ± 12.5	90.3 ± 11.9	+ 1	85.5 ± 8.7	+ 4
Sensory systems					
Medial geniculate	119.3 ± 15.7	126.1 ± 19.4	+ 6	131.9 ± 6.8	+ 11
Lateral geniculate	101.1 ± 11.6	106.2 ± 7.4	+ 5	109.2 ± 10.9	+ 8
Superior colliculus	84.6 ± 8.3	87.0 ± 9.8	+ 3	86.8 ± 7.4	+ 3
Inferior colliculus	158.6 ± 19.3	158.0 ± 18.7	0	172.7 ± 32.2	+ 9
Lateral lemniscus	110.9 ± 17.7	127.1 ± 13.5	+ 15	124.5 ± 28.6	+ 12
Superior olive	125.4 ± 13.8	147.0 ± 19.7	+ 17	149.3 ± 41.3	+ 19
Cochlear nucleus	119.0 ± 7.6	120.6 ±15.0	+ 1	118.2 ± 9.9	- 1
Vestibular nucleus	145.9 ± 15.0	145.6 ±7.7	0	155.3 ± 11.7	+ 6
Limbic system					
Medial septum	67.7 ± 6.4	71.7 ± 3.6	+ 6	72.4 ± 9.7	+ 7
Lateral septum	65.0 ± 5.6	66.8 ± 3.3	+ 3	72.0 ± 14.2	+ 7
Nucleus accumbens	62.9 ± 6.0	63.1 ± 1.8	0	62.4 ± 2.9	- 1
Hippocampal CA1	48.0 ± 7.6	49.1 ± 4.0	+ 2	53.4 ± 8.8	+ 11
Hippocampal CA2	49.5 ± 7.8	46.1 ± 3.0	- 7	49.1 ± 5.9	- 1
Hippocampal CA3	65.6 ± 10.8	62.6 ± 2.0	- 5	66.4 ± 7.1	+ 1
Hippocampal CA4	51.2 ± 7.8	50.6 ± 1.7	- 1	50.2 ± 5.5	- 2
Dentate gyrus	60.4 ± 3.9	57.8 ± 2.8	- 4	62.6 ± 2.4	+ 4
Medial amygdala	39.7 ± 5.0	40.5 ± 3.3	+ 2	39.6 ± 4.4	0
Central amygdala	37.4 ± 3.4	38.4 ± 2.3	+ 3	33.4 ± 4.6	- 11
Basolateral amygdala	71.8 ± 8.8	71.4 ± 5.6	0	74.2 ± 5.5	+ 3
Ventral tegmental area	77.1 ± 7.6	79.2 ± 4.6	+ 3	84.2 ± 3.8*	+ 9
Mesencephalic reticular formation	66.1 ± 8.6	61.5 ± 7.9	- 7	62.1 ± 8.6	- 6
Mammillary body	135.9 ± 16.5	154.4 ± 17.7	+ 14	144.8 ± 22.7	+ 7
Pontine grey	49.1 ± 7.0	52.6 ± 4.9	+ 7	52.1 ± 6.6	+ 6
Dorsal raphe	72.0 ± 8.7	72.0 ± 5.0	0	82.4 ± 6.4	+ 14
Median raphe	96.9 ± 6.4	101.0 ± 3.0	+ 4	96.9 ± 1.1	0
Locus coeruleus	72.4 ± 3.4	71.7 ± 3.4	- 1	75.9 ± 3.2	+ 5
Medullary reticular formation	61.1 ± 6.5	63.5 ± 2.1	+ 4	68.5 ± 4.5	+ 12
Hypothalamus					
Anterior	48.5 ± 6.7	52.0 ± 3.6	+ 7	53.8 ± 3.9	+ 11
Anterolateral	45.1 ± 7.9	49.7 ± 2.0	+ 10	54.8 ± 0.5**	+ 22
Paraventricular	47.3 ± 6.4	48.2 ± 3.6	+ 2	49.6 ± 2.0	+ 5
Median forebrain bundle	60.3 ± 4.7	64.4 ± 3.0	+ 6	66.6 ± 11.1	+ 10
Ventromedial	40.7 ± 5.7	42.8 ± 3.7	+ 5	51.3 ± 5.7*°	+ 26
Dorsomedial	48.1 ± 4.3	47.5 ± 4.4	- 1	52.0 ± 5.8	+ 8
Thalamus					

Anteromedial	115.2 ± 10.7	114.4 ± 13.9	- 1	113.5 ± 6.9	- 1
Anteroventral	115.5 ± 12.7	116.0 ± 9.1	0	122.8 ± 7.2	+ 6
Ventromedial	114.1 ± 9.3	114.2 ± 11.2	0	114.6 ± 9.8	0
Mediodorsal	112.8 ± 12.6	120.1 ± 15.4	+ 6	117.3 ± 11.3	+ 4
Lateral	105.7 ± 14.4	113.2 ± 11.1	+ 7	112.3 ± 10.2	+ 6
Ventroposteromedial	85.3 ± 8.5	88.7 ± 7.6	+ 4	87.0 ± 10.6	+ 2
Posteromedial	78.0 ± 7.0	80.0 ± 8.4	+3	81.8 ± 8.2	+ 5
Posterior	95.6 ± 10.0	101.8 ± 11.0	+ 6	102.8 ± 11.9	+ 8
Paraventricular	62.8 ± 5.3	60.3 ± 2.8	- 4	62.6 ± 9.0	0
Motor system					
Caudate nucleus	98.9 ± 5.8	99.6 ± 3.7	+ 1	98.1 ± 8.0	- 1
Globus pallidus	55.0 ± 4.8	55.3 ± 3.8	0	54.8 ± 6.0	0
Subthalamic nucleus	86.7 ± 10.2	92.1 ± 5.5	+ 6	88.2 ± 5.0	+ 2
Substantia nigra, pars reticulata	52.7 ± 7.0	51.2 ± 6.0	- 3	53.6 ± 4.9	+ 2
Substantia nigra, pars compacta	79.3 ± 7.8	83.6 ± 5.6	+ 5	90.2 ± 5.8**°	+ 14
Red nucleus	86.9 ± 8.6	93.7 ± 7.0	+ 8	94.2 ± 8.7	+ 8
Cerebellar cortex	56.7 ± 3.9	58.8 ± 3.6	+ 3	62.9 ± 4.4	+ 11
Dentate nucleus	99.4 ± 6.3	103.2 ± 9.0	+ 4	99.9 ± 6.9	+ 1
Fastigial nucleus	110.6 ± 8.2	115.8 ± 8.7	+ 5	108.9 ± 11.0	- 2
Interpositus nucleus	113.1 ± 8.8	112.9 ± 8.8	0	107.3 ± 5.8	- 5
White matter					
Genu of the corpus callosum	27.2 ± 2.5	29.7 ± 4.1	+ 9	27.0 ± 9.8	- 1
Cerebellar white matter	35.5 ± 3.1	35.9 ± 4.8	+ 1	33.8 ± 3.1	- 5

Values expressed as µmol/100g/min represent means ± S.D. of the number of animals in parentheses.
* $p < 0.05$, ** $p < 0.01$, statistically significant differences from wild type mice
° $p < 0.05$, statistically significant differences between heterozygous and STOP KO mice

Figure 2


AUDCX
WT
HIP
THAL
SNPC
VTA
KO
25 40 55 68 80 95 110 125 150
μmol/100g/min