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# **Deletion of the STOP gene, a microtubule stabilizing factor, leads only to discrete cerebral metabolic changes in mice**

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## **Abstract**

**In mice, the deletion of the STOP protein leads to subtle anatomic changes but induces depleted synaptic vesicle pools, impaired synaptic plasticity, hyperdopaminergic and major behavioral disorders that are alleviated by neuroleptics, hence leading to a “schizophrenic-like” phenotype. In this study, we applied the quantitative autoradiographic [<sup>14</sup>C]-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.**

**MESH Keywords** Animals ; Autoradiography ; Brain ; metabolism ; Glucose ; metabolism ; Male ; Mice ; Mice, Knockout ; Microtubule-Associated Proteins ; genetics ; metabolism

**Author Keywords** cerebral glucose metabolism ; cytoskeleton ; microtubules ; STOP protein ; schizophrenia ; transgenic mice

## **Introduction**

Microtubules play a pivotal role in many vital cell functions, including cell division, 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 microtubule 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 (LCMRgls) in STOP KO mice compared to wild type and heterozygous littermates by means of the quantitative autoradiographic [<sup>14</sup>C]2-deoxyglucose (2DG) technique (Sokoloff et al., 1977) adapted to the mouse (Jay et al., 1985; Boullieret et al., 2000).

## Materials and methods

### Animals and measurement of LCMRgls

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 Directive 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; Boullieret 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.

LCMRgls were measured by the [<sup>14</sup>C]2-deoxyglucose (2DG) technique (Sokoloff et al., 1977) adapted to mice (Jay et al., 1985; Boullieret et al., 2000). The [<sup>14</sup>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 [<sup>14</sup>C]methylmethacrylate standards calibrated for their <sup>14</sup>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. LCMRgls were calculated according to the operational equation of the 2DG method using the integrated specific activity of the ratio between the plasma [<sup>14</sup>C]2DG and glucose concentrations measured for each animal, and the usual rate constants (Sokoloff et al., 1977).

### Statistical analysis

LCMRgls 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 be 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.

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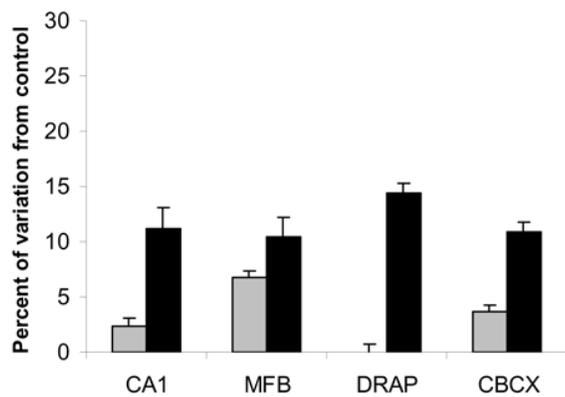
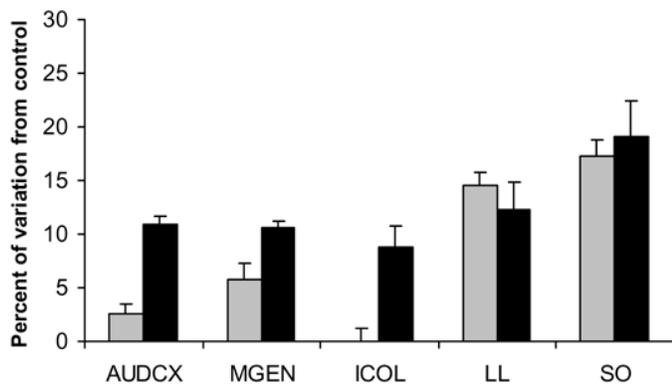
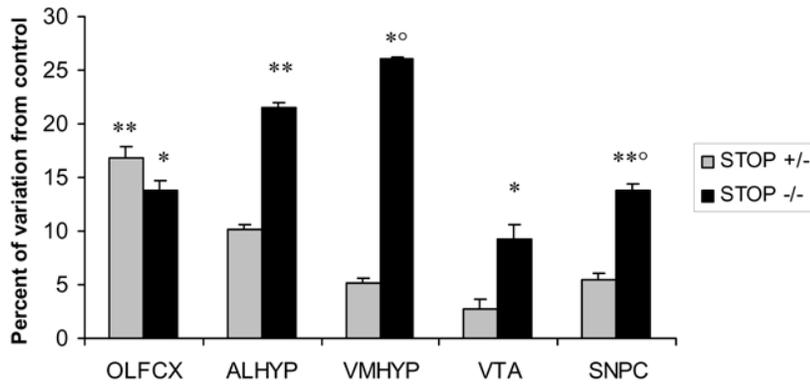
## References:

- Abi-Dargham A , Gil R , Krystal J , Baldwin BM , Seibyl JP , Bowers M , van Dyck CH , Charney DS , Innis RB , Laruelle M 1998; Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am J Psychiatry*. 155: 761- 767
- Aleman A , Bocker KB , Hijman R , de Haan EH , Kahn RS 2003; Cognitive basis of hallucinations in schizophrenia: role of top-down information processing. *Schizophr Res*. 64: 175- 185
- Andreasen NC , Black DW 1991; *Introductory Textbook of Psychiatry*. American Psychiatry Press; Washington, DC
- Andrieux A , Salin PA , Vernet M , Kujala P , Baratier J , Gory-Fauré S , Bosc C , Pointu H , Proietto D , Schweitzer A , Denarier E , Klumperman J , Job D 2002; The suppression of brain cold-stable microtubules in mice induces synaptic defects associated with neuroleptic-sensitive behavioral disorders. *Genes Dev*. 16: 2350- 2364
- Andrieux A , Salin P , Schweitzer A , Begou M , Pachoud B , Brun P , Gory-Faure S , Kujala P , Suaud-Chagny MF , Höfle G , Job D 2006; Microtubule stabilizer ameliorates synaptic function and behavior in a mouse model for schizophrenia. *Biol Psychiatry*. 60: 1224- 1230
- Baas PW , Pienkowski TP , Cimbalnik KA , Toyama K , Bakalis S , Ahmad FJ , Kosik KS 1994; Tau confers drug stability but not cold stability to microtubules in living cells. *J Cell Sci*. 107: 135- 143
- Baratier J , Peris L , Brocard J , Gory-Faure S , Dufour F , Bosc C , Fourest-Lieuvain A , Blanchoin L , Salin P , Job D , Andrieux A 2006; Phosphorylation of microtubule-associated protein STOP by calmodulin kinase II. *J Biol Chem*. 281: 19561- 19569
- Blackwood D , Clair DS , Muir W , Duffy J 1991; Auditory p300 and eye tracking dysfunction in schizophrenic pedigrees. *Arch Gen Psychiatry*. 48: 899- 909
- Bosc C , Andrieux A , Job D 2003; STOP proteins. *Biochemistry*. 42: 12125- 12132
- Bosc C , Cronk JD , Pirolet F , Watterson DM , Haiech J , Job D , Margolis RL 1996; Cloning, expression, and properties of the microtubule-stabilizing protein STOP. *Proc Natl Acad Sci USA*. 93: 2125- 2130
- Boulleret V , Boyet S , Marescaux C , Nehlig A 2000; Mapping of the progressive metabolic changes occurring during the development of hippocampal sclerosis in a model of mesial temporal lobe epilepsy. *Brain Res*. 852: 255- 262
- Breier A , Su TP , Saunders R , Carson RE , Kolachana BS , de Bartolomeis A , Weinberger DR , Weisenfeld N , Malhotra AK , Eckelman WC , Pickar D 1997; Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. *Proc Natl Acad Sci USA*. 94: 2569- 2574
- Brenner E , Schweitzer A , Andrieux A , Sonnewald U , Nehlig A 2007; Confirmation of hypoglutamatergic activity in the STOP KO mouse: a potential model for chronic untreated schizophrenia. *J Neurosci Res*. Feb 15
- Brun P , Bégou M , Andrieux A , Mouly-Badina L , Clerget M , Schweitzer A , Scarna H , Renaud B , Job D , Suaud-Chagny MF 2005; Dopaminergic transmission in STOP null mice. *J Neurochem*. 94: 63- 73
- Clark C , Kopala L , Li DK , Hurwitz T 2001; Regional cerebral glucose metabolism in never-medicated patients with schizophrenia. *Can J Psychiatry*. 46: 340- 345
- Clementz BA , Geyer MA , Braff DL 1998; Poor P50 suppression among schizophrenia patients and their first-degree biological relatives. *Am J Psychiatry*. 155: 1691- 1694
- Couégnas A , Schweitzer A , Andrieux A , Ghandour MS , Boehm N 2007; Expression pattern of STOP lacZ reporter gene in adult and developing mouse brain. *J Neurosci Res*. 85: 1515- 1527
- Denarier E , Fourest-Lieuvain A , Bosc C , Pirolet F , Chapel A , Margolis RL , Job D 1998; Nonneuronal isoforms of STOP protein are responsible for microtubule cold stability in mammalian fibroblasts. *Proc Natl Acad Sci USA*. 95: 6055- 6060
- Dustin P 1984; *Microtubules*. 2 Springer Verlag; Berlin
- Eastwood SL , Lyon L , George L , Andrieux A , Job D , Harrison PJ 2007; Altered expression of synaptic protein mRNAs in STOP (MAP6) mutant mice. *J Psychopharmacol*. Oct 18
- Fradley RA , O'Meara GF , Newman RJ , Andrieux A , Job D , Reynolds DS 2005; STOP knockout and NMDA NR1 hypomorphic mice exhibit deficits in sensorimotor gating. *Behav Brain Res*. 163: 257- 264
- Frankle WG , Lerma J , Laruelle M 2003; The synaptic hypothesis of schizophrenia. *Neuron*. 39: 205- 216
- Franklin KBJ , Paxinos G 1997; *The Mouse Brain in Stereotaxic Coordinates*. Academic Press; San Diego
- Galiano MR , Bosc C , Schweitzer A , Andrieux A , Job D , Hallak ME 2004; Astrocytes and oligodendrocytes express different STOP protein isoforms. *J Neurosci Res*. 78: 329- 337
- Geraud G , Arne-Bes MC , Guell A , Bes A 1987; Reversibility of hemodynamic hypofrontality in schizophrenia. *J Cereb Blood Flow Metab*. 7: 9- 12
- Ginovart N , Farde L , Halldin C , Swahn CG 1999; Changes in striatal D2-receptor density following chronic treatment with amphetamine as assessed with PET in nonhuman primates. *Synapse*. 31: 154- 162
- Goff DC , Coyle JT 2001; The emerging role of glutamate in the pathophysiology and treatment of schizophrenia. *Am J Psychiatry*. 158: 1367- 1377
- Gory-Faure S , Windscheid V , Bosc C , Peris L , Proietto D , Franck R , Denarier E , Job D , Andrieux A 2006; STOP-like protein 21 is a novel member of the STOP family, revealing a Golgi localization of STOP proteins. *J Biol Chem*. 281: 28387- 28396
- Grace AA 1991; Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience*. 41: 1- 24

- Guillaud L , Bosc C , Fourest-Lieuvain A , Denarier E , Pirollet F , Lafanechere L , Job D 1998; STOP proteins are responsible for the high degree of microtubule stabilization observed in neuronal cells. *J Cell Biol.* 142: 167- 179
- Guyton AC 1989; Anatomie et Physiologie du Système Nerveux Central. Décarie/Vigot; Montréal/Paris
- Horton AC , Ehlers MD 2004; Secretory trafficking in neuronal dendrites. *Nat Cell Biol.* 6: 585- 591
- Hubl D , Koenig T , Strik W , Federspiel A , Kreis R , Boesch C , Maier SE , Schroth G , Lovblad K , Dierks T 2004; Pathways that make voices: white matter changes in auditory hallucinations. *Arch Gen Psychiatry.* 61: 658- 668
- Hudry J , Saoud M , D'Amato T , Daléry J , Royet JP 2002; Ratings of different olfactory judgements in schizophrenia. *Chem Senses.* 27: 407- 416
- Jay TM , Jouvét M , des Rosiers MH 1985; Local cerebral glucose utilization in the free moving mouse: a comparison during two stages of the activity-rest cycle. *Brain Res.* 342: 297- 306
- Kaplan RD , Szechtman H , Franco S , Szechtman B , Nahmias C , Garnett ES , List S , Cleghorn JM 1993; Three clinical syndromes of schizophrenia in untreated subjects: relation to brain glucose activity measured by positron emission tomography (PET). *Schizophr Res.* 11: 47- 54
- Kapur S , Remington G 1996; Serotonin-dopamine interaction and its relevance to schizophrenia. *Am J Psychiatry.* 153: 466- 476
- Kerwin RW 1993; Glutamate receptors, microtubule associated proteins and developmental anomaly in schizophrenia: an hypothesis. *Psychol Med.* 23: 547- 551
- Kolman A 2004; BMS-310705 Bristol-Myers Squibb/GBF. *Curr Opin Investig Drugs.* 5: 1292- 1297
- Kopala LC , Good KP , Morrison K , Bassett AS , Alda M , Honer WG 2001; Impaired olfactory identification in relatives of patients with familial schizophrenia. *Am J Psychiatry.* 158: 1286- 1290
- Lahti AC , Holcomb HH , Weiler MA , Medoff DR , Frey KN , Hardin M , Tamminga CA 2004; Clozapine but not haloperidol Re-establishes normal task-activated rCBF patterns in schizophrenia within the anterior cingulate cortex. *Neuropsychopharmacology.* 29: 171- 178
- Lahti AC , Holcomb HH , Weiler MA , Medoff DR , Tamminga CA 2003; Functional effects of antipsychotic drugs: comparing clozapine with haloperidol. *Biol Psychiatry.* 53: 601 - 608
- Lahti AC , Weiler MA , Holcomb HH , Tamminga CA , Carpenter WT , McMahon R 2006; Correlations between rCBF and symptoms in two independent cohorts of drug-free patients with schizophrenia. *Neuropsychopharmacology.* 31: 221- 230
- Laruelle M , Abi-Dargham A , van Dyck CH , Gil R , D'Souza CD , Erdos J , McCance E , Rosenblatt W , Fingado C , Zoghbi SS , Baldwin RM , Seibyl JP , Krystal JH , Charney DS , Innis RB 1996; Single photon emission computerized tomography imaging in amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc Natl Acad Sci USA.* 93: 9235- 9240
- Liddle PF , Friston KJ , Frith CD , Hirsch SR , Jones T , Frackowiak RS 1992; Patterns of cerebral blood flow in schizophrenia. *Br J Psychiatry.* 160: 179- 186
- Margolis RL , Wilson L 1978; Opposite end assembly and disassembly of microtubules at steady state in vitro. *Cell.* 13: 1- 8
- Mathalon DH , Ford JM , Pfefferbaum A 2000; Trait and state aspects of P300 amplitude reduction in schizophrenia: a retrospective longitudinal study. *Biol Psychiatry.* 47: 434- 449
- McCurdy RD , Feron F , Perry C , Chant DC , McLean D , Matigian N , Hayward NK , McGrath JJ , Mackay-Sim A 2006; Cell cycle alterations in biopsied olfactory neuroepithelium in schizophrenia and bipolar I disorder using cell culture and gene expression analyses. *Schizophr Res.* 82: 163- 173
- Min SK , An SK , Jon DI , Lee JD 1999; Positive and negative symptoms and regional cerebral perfusion in antipsychotic-naive schizophrenic patients: a high-resolution SPECT study. *Psychiatry Res.* 90: 159- 68
- Mitchison T , Kirschner M 1984; Dynamic instability of microtubule growth. *Nature.* 312: 237- 242
- Moberg PJ , Agrin R , Gur RE , Gur RC , Turetsky BI , Doty RL 1999; Olfactory dysfunction in schizophrenia: a qualitative and quantitative review. *Neuropsychopharmacology.* 21: 325- 340
- Monti JM , Monti D 2004; Sleep in schizophrenia patients and the effects of antipsychotic drugs. *Sleep Med Rev.* 8: 133- 148
- Murphy DD , Cole NB , Greenberger V , Segal M 1998; Estradiol increases dendritic spine density by reducing GABA neurotransmission in hippocampal neurons. *J Neurosci.* 18: 2550- 2559
- Nehlig A , Lucignani G , Kadekarom M , Porrino LJ , Sokoloff L 1984; Effects of acute administration of caffeine on local cerebral glucose utilization in the rat. *Eur J Pharmacol.* 101 : 91- 100
- Nehlig A , Porrino LJ , Crane AM , Sokoloff L 1985; Local cerebral glucose utilization in normal female rats: Variations during the estrous cycle and comparison with males. *J Cereb Blood Flow Metab.* 5: 393- 400
- Pazdur R , Kudelka AP , Kavanagh JJ , Cohen PR , Raber MN 1993; The taxoids: paclitaxel (Taxol) and docetaxel (Taxotere). *Cancer Treat Rev.* 19: 351- 86
- Rioux L , Ruscheinsky D , Arnold SE 2004; Microtubule-associated protein MAP2 expression in olfactory bulb in schizophrenia. *Psychiatry Res.* 128: 1- 7
- Rupp CI , Fleischhacker WW , Kemmler G , Kremser C , Bilder RM , Mechtcheriakov S , Szeszko PR , Walch T , Scholtz AW , Klimbacher M , Maier C , Albrecht G , Lechner-Schoner T , Felber S , Hinterhuber H 2005; Olfactory functions and volumetric measures of orbitofrontal and limbic regions in schizophrenia. *Schizophr Res.* 74: 149- 161
- Sabri O , Erkwoh R , Schreckenberger M , Cremerius U , Schulz G , Dickmann C , Kaiser HJ , Steinmeyer EM , Sass H , Buell U 1997; Regional cerebral blood flow and negative/positive symptoms in 24 drug-naive schizophrenics. *J Nucl Med.* 38: 181- 188
- Sallanon M , Sakai K , Denoyer M , Jouvét M 1989; Long lasting insomnia induced by preoptic neuron lesions and its transient reversal by muscimol injection into the posterior hypothalamus. *Neuroscience.* 32: 669- 683
- Shimizu H , Iwayama Y , Yamada K , Toyota T , Minabe Y , Nakamura K , Nakajima M , Hattori E , Mori N , Osumi N , Yoshikawa T 2006; Genetic and expression analyses of the STOP (MAP6) gene in schizophrenia. *Schizophr Res.* 84: 244- 252
- Siegel C , Waldo M , Mizner G , Adler LE , Freeman R 1984; Deficits in sensory gating in schizophrenic patients and their relatives. Evidence obtained with auditory evoked responses. *Arch Gen Psychiatry.* 41: 607- 612
- Spitzer RL , Gibbon M , Skodol AE , Williams JBW , First MB 1994; DSM-IV Casebook. American Psychiatry Press; Washington, DC
- Sokoloff L , Reivich M , Kennedy C , Des Rosiers MH , Patlak CS , Pettigrew KD , Sakurada O , Shinohara M 1977; The [<sup>14</sup>C]deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure and normal values in the conscious and anesthetized albino rat. *J Neurochem.* 28: 897- 916
- Szymusiak R , Alam N , Steininger TL , McGinty D 1998; Sleep-waking discharge patterns of ventrolateral preoptic/hypothalamic neurons in rats. *Brain Res.* 803: 178- 188
- Szymusiak R , Satinoff E 1984; Ambient temperature-dependence of sleep disturbances produced by basal forebrain damage in rats. *Brain Res Bull.* 12: 295- 305
- Ticho SR , Radulovacki M 1991; Role of adenosine in sleep and temperature regulation in the preoptic area of rats. *Pharmacol Biochem Behav.* 40: 33- 40
- Ugr T , Weisbrod M , Franzek E , Fuller U , Sauer H 2005; Olfactory impairment in monozygotic twins discordant for schizophrenia. *Eur Arch Psychiatry Clin Neurosci.* 255: 94- 98
- Wang H , Wang Z , Wang S , Li M , Nan L , Rhie JK , Covey JM , Zhang R , Hill DL 2005; Preclinical pharmacology of epothilone D, a novel tubulin-stabilizing antitumor agent. *Cancer Chemother Pharmacol.* 56: 255- 260
- Weinberger DR , Berman KF , Zec RF 1986; Physiologic dysfunction of dorsolateral prefrontal cortex in schizophrenia. I. Regional cerebral blood flow evidence. *Arch Gen Psychiatry.* 43: 114- 124
- Wolkin A , Sanfilippo M , Wolf AP , Angrist B , Brodie JD , Rotrosen J 1992; Negative symptoms and hypofrontality in chronic schizophrenia. *Arch Gen Psychiatry.* 49: 959- 965
- Wong AHC , Van Tol HHM 2003; Schizophrenia: from phenomenology to neurobiology. *Neurosci Biobehav Rev.* 27: 269- 306
- Yuasa S , Kurachi M , Suzuki M , Kadono Y , Matsui M , Saitoh O , Seto H 1995; Clinical symptoms and regional cerebral blood flow in schizophrenia. *Eur Arch Psychiatry Clin Neurosci.* 246: 7- 12

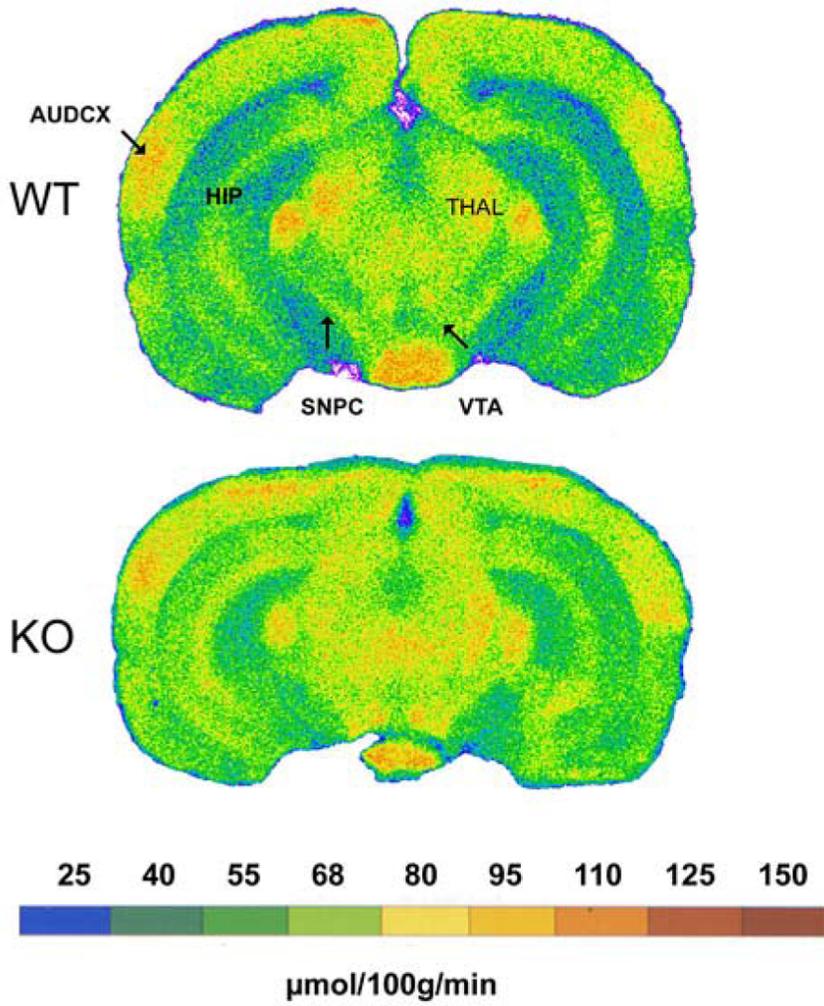
**Figure 1**

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).



**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 (%)
<b>Cerebral cortex</b>					
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
<b>Sensory systems</b>					
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
<b>Limbic system</b>					
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
<b>Hypothalamus</b>					
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
<b>Thalamus</b>					
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
<b>Motor system</b>					
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
<b>White matter</b>					
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  $\mu\text{mol}/100\text{g}/\text{min}$  represent means  $\pm$  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