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Title: REDUCING GABAERGIC INHIBITION RESTORES COGNITIVE FUNCTIONS IN A MOUSE MODEL OF DOWN SYNDROME.

Running title: GABA antagonism as a therapy for Down syndrome

Authors: Marie-Claude Potier^{1*}, Jérôme Braudeau¹, Luce Dauphinot¹, Benoît Delatour¹

Affiliations: Centre de Recherche de l'Institut du Cerveau et de Moelle Epinière, CNRS UMR7225, INSERM UMRS 975, UPMC, ICM, Hôpital Pitié-Salpêtrière, Paris, France.

***Corresponding author:**

Dr. Marie-Claude POTIER

ICM

Hôpital Pitié-Salpêtrière

CNRS UMR7225, INSERM UMRS975, UPMC

47, Bd de l'Hôpital

75013 PARIS, FRANCE

+33157274519

marie-claude.potier@upmc.fr

Abstract:

Alterations in excitatory-inhibitory balance occur in Down syndrome and could be responsible for cognitive deficits observed through the life of all individuals carrying an extra copy of chromosome 21. Excess of inhibition in the adult could produce synaptic plasticity deficits that may be a primary mechanism contributing to learning and memory impairments. In this study we discuss pharmacological treatments that could potentially alleviate neuronal inhibition and have been tested in a mouse model of Down syndrome. γ -aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mature central nervous system that binds to GABA-benzodiazepine receptors, opens a chloride channel and reduces neuronal excitability. These receptors have been extensively studied as targets for treatment of epilepsy, anxiety, sleep, cognitive disorders and the induction of sedation. Molecules that are either antagonists or inverse agonists of the GABA-benzodiazepine receptors are able to reduce inhibitory GABAergic transmission. However modulating the excitatory-inhibitory balance towards increase of cognition without inducing seizures remains difficult particularly when using GABA antagonists. In this study we review data from the literature obtained using inverse agonists selective for the $\alpha 5$ -subunit containing receptor. Such inverse agonists, initially developed as cognitive enhancers for treatment of memory impairments, proved to be very efficient in reversing learning and memory deficits in a Down syndrome mouse model after acute treatment.

Keywords: chloride, cognitive enhancer, Down syndrome, GABA, immediate early genes, inhibition

Down syndrome (DS) is the most common genetic cause of intellectual disability (1/800 live births), and is the consequence of trisomy 21 [1]. Among all clinical features, DS is characterized by varying degrees of cognitive impairments [2]. The life expectancy of individuals with DS being now 55 years, treatments that would increase their autonomy and their quality of life need to be developed. Additionally since people with DS have Alzheimer's disease (AD) neuropathology in their early forties, several clinical trials have been or are being conducted with anticholinesterase drugs (Donepezil and Rivastigmine) that are largely prescribed in AD. Recently Duke University recruited adolescents with DS to evaluate the pro-cognitive effects of rivastigmine (referenced on <http://clinicaltrials.gov>).

Treating cognitive deficits is complex and multi-faceted and it is now accepted that polytherapies will be necessary to improve cognition of individuals with DS. For instance association of intermittent neuropharmacological intervention to constant teaching and educational methods might prove to be adequate to combat cognitive deficits.

The GABA hypothesis in Down syndrome:

A large number of studies have shown that cognitive impairment displayed by mouse models of DS is related to excessive levels of neuronal inhibition in the medial temporal lobe circuitry, causing a failure of memory-associated long-term synaptic potentiation (LTP) in the hippocampus [3, 4]. The deficit of synaptic plasticity translates into structural morphological changes and increased levels of synaptic proteins at hippocampal and cortical inhibitory synapses [5]. Excessive levels of inhibition thus appear essential in mouse models of DS but are probably not unique components of the physiopathology of this disorder. Particularly, the hypothesis needs conclusive results in DS individuals.

GABA and its receptors: what is known and unknown in Down syndrome:

GABA levels

Inhibition in the adult forebrain is mainly controlled by one major inhibitory neurotransmitter, γ -aminobutyric acid (GABA). GABA is synthesized from the excitatory neurotransmitter glutamate, via the action of glutamate decarboxylase (GAD) enzymes, GAD65 and GAD67. Functionally, GABA-mediated inhibition can be classified as phasic (synaptic) and tonic (extrasynaptic).

Levels of GABA in the brain of individuals with DS were measured *postmortem* using radioassay, chromatography, or *in vivo* using non invasive magnetic resonance spectroscopy (MRS). *Postmortem* studies have been conducted in adults with DS with AD-like neuropathology. Levels of GABA were found decreased in AD brains but globally unchanged in DS with AD [6]. A significant deficit of GABA has been reported in the hippocampus and in temporal cortex in individuals with DS carrying neocortical neurofibrillary tangles. These results are consistent with losses of cortical neurons containing these neurotransmitters, and resemble the deficits exhibited by the more severely affected cases of AD [7].

In the frontal cortex of fetuses with DS GABA levels were found to be reduced by 60% [8]. This large decrease could be related to the excitatory effects of GABA in immature neurons. The GABA shift from excitatory to inhibitory properties is due to a change in intracellular chloride concentration which is regulated by transporters: the Na-K-Cl cotransporter 1 (NKCC1) that import chloride and the K-Cl cotransporter 2 (KCC2) that export it [9, 10], Figure.

MRS studies in children below 18 (3-17 years old) concluded to a decrease of GABA in the temporal lobes while no change could be identified in the frontal lobes [11, 12]. Gene expression profiles from the hippocampus of trisomic Ts65Dn mice, the best characterized mouse model of DS carrying three copies of chromosome 21 orthologous genes as well as additional three copies of non orthologous genes [13, 14] revealed that GABA synthesis enzymes GAD65 and GAD67 were unchanged as compared to euploids [15]. No change has been reported in the adult brain of DS individuals [16]. GAD67 immunoreactivity was found increased in the brain of Ts65Dn mice and this increase was attributed to the gain of calretinin inhibitory interneurons [17]. Moreover a significant increase in colocalization of GAD65 and the vesicular GABA transporter was reported in the fascia dentata of Ts65Dn mice [5].

GABA receptors

In the central nervous system, GABA is produced and released by inhibitory interneurons and acts on two main classes of membrane-bound receptors: the ionotropic chloride permeable GABA_A receptors (ligand-gated Cl⁻ channels), and the metabotropic (G protein-coupled) GABA_B receptors. Recent research efforts unraveled the positive effects of GABA_B receptors modulation for the treatment of cognitive impairments in Down syndrome [18, 19]. However, the GABA_A receptor family is the predominant type in the brain and has a long history for modulating learning and memory functions. GABA_A receptors are composed of five subunits (α 1–6; β 1–3; γ 1–3; δ ; ϵ ; θ ; π) that form a chloride channel. Each subunit has a transmembrane domain and carries binding sites for GABA and other modulators (benzodiazepines, barbiturates, pentylenetetrazol which are

not endogenous modulators). In the adult brain, the chloride concentration is lower inside neuronal cells as compared to the extracellular compartment and GABA binding to its GABA_A receptor leads to chloride influx. This leads to membrane hyperpolarization and subsequent inhibition of neuronal activity.

The subunit composition and the distribution of GABA_A receptors mediating phasic and tonic inhibitions differ. Indeed phasic inhibition is mediated by low affinity GABA_A receptors localized at the synapse, whereas tonic inhibition arises from activation of high affinity extrasynaptic receptors activated by lower concentrations of ambient GABA [20]. Extrasynaptic GABA receptors are more abundant than those found at synapses and have been suggested to sense GABA spillover.

Each subunit of GABA_A receptors has a distinct regional and cellular distribution in the brain. Using *in situ* hybridization and immunohistochemistry, it was shown that the $\alpha 5$ subunit is mainly localized in the hippocampus, the neocortex and the olfactory bulb in rodents [21, 22]. In human using positron emission tomography (PET) and [¹¹C]Ro15-4513 as a tracer that labels $\alpha 5$ - GABA_A receptors, it was shown that $\alpha 5$ subunit is abundant in the hippocampus, the cortex with an antero-posterior gradient and the nucleus accumbens as well as the olfactory bulb [23].

$\alpha 5$ -containing GABA_A receptors are concentrated in the dendrites of hippocampal cornu ammonis CA1 pyramidal neurons and play a role in regulating tonic inhibition through extrasynaptic receptors [24], Figure. A pool of $\alpha 5$ -containing GABA_A receptors has also been detected at GABAergic synapses on the dendrites of hippocampal pyramidal neurons and could modulate phasic GABAergic inhibition [25]. In the neocortex synapses innervated by bitufted GABAergic interneurons that are parvalbumin and cholecystokinin negative are sensitive to $\alpha 5$ IA [26].

In the absence of $\alpha 5$ -containing GABA_A receptors tonic inhibition of hippocampal CA1 and CA3 pyramidal cells is about half that found in the control situation, but phasic inhibition is unaltered. This specific reduction of tonic inhibition is sufficient to induce epileptiform activity in the CA3 region that could be reversed by increasing the tonic current [20]. $\alpha 5$ - GABA_A receptors would be recruited only when ambient GABA levels are increased.

Gene expression profiles from the hippocampus of adult Ts65Dn mice revealed that GABA_A and GABA_B receptor subunits were unchanged as compared to euploids [15]. In addition levels of GABA_A $\alpha 1$, $\beta 2$ and $\beta 3$ proteins were found to be unchanged in the hippocampus of adult Ts65Dn mice while the levels of GABA_B receptors R2 were 19% less. This decrease was attributed to compensatory mechanism in response to the increase of G protein coupled inward rectifier potassium channel 2 (GIRK2) levels, an inwardly rectifying potassium channel mapping to the trisomic region [27]. In another recently published study no change in

GABA_A and GABA_B receptors could be identified [19]. However a 20% decrease of GABA_A β 2 and β 3 proteins was found in the dentate gyrus of Ts65Dn adult mice [28]. Finally Martinez-Fernandez *et al.* did not find any change in the binding of RO0154513, a selective GABA_A α 5 inverse agonist, *in vivo* in Ts65Dn mice [29].

GABA receptor ligands

GABA competitive inhibitors antagonize the effects of GABA and thus increase neuronal activity eventually leading to epilepsy. Non competitive GABA antagonists also inhibit the effects of endogenous GABA through a binding site located at the proximity GABA binding site. Other ligands of the binding sites present on GABA_A pentamers have been actively developed with the hope of finding new modulators of GABA transmission.

Administration of non-competitive antagonists of GABA_A receptors (picrotoxin and pentylenetetrazol) were shown to reverse spatial learning disabilities and LTP deficits in DS mouse model suggesting the central role of over-inhibition in learning deficits of the Ts65Dn mice [30, 31].

Other ligands bind to GABA_A receptors and can alternatively modulate GABAergic transmission:

Benzodiazepines synthesized in the 1960's are positive allosteric modulators (PAM) used for their anticonvulsant, anxiolytic, sedative and myorelaxant effects, as well as for the treatment of alcohol withdrawal syndrome. Benzodiazepines bind to GABA_A receptors and increase GABA efficacy by an allosteric modulation thus increasing the opening frequency of channels and chloride flux, leading to stronger inhibition. Benzodiazepine agonists facilitate the action of GABA. They are positive allosteric modulators (PAM). Benzodiazepine antagonists reverse the effects of agonists but have no intrinsic activity on GABA transmission. Finally inverse agonists are competitive ligands of the benzodiazepine site that decrease GABA affinity thus reducing the action of GABA. They are negative allosteric modulators (NAM). They were shown to be convulsant, anxiogenic and promnesiant (increasing learning and memory) [32, 33]. The β -carboline inverse agonist FG7142 turned to have anxiogenic properties that precluded its clinical development [34]. Ro15-4513 a partial inverse agonist developed by Hoffmann-La Roche has been used for blocking the effects of ethanol in the central nervous system [35]. The drug is still being used in positron emission tomography for imaging GABA receptors in humans and animals brains [23].

Since α 1, α 2, α 3 and α 5 subunits drive the sedative/anticonvulsant, anxiolytic and promnesiant actions respectively, an alternative strategy would be to develop molecules that are selective for these subtypes of receptors [36]. Knock-out mice for the α 5 subunit show enhanced learning and memory [37]. In addition in knock-in mice carrying a point mutation invalidating the gene encoding the α 5 subunit associative learning was

facilitated [38]. For the promnesiant, more generally nootropic activity that translates into higher learning and memory performances, the pharmaceutical industry has searched for new ligands selective of the $\alpha 5$ -containing GABA_A receptors [39, 40]. Several new series of molecules specific for the $\alpha 5$ -containing GABA_A receptors have been discovered that showed enhanced learning and memory properties in animal models: PWZ-029 [41] a triazolophthalazines $\alpha 5$ inverse agonist ($\alpha 5$ IA) from Merck laboratories [42-46] and more recently the triazolobenzodiazepine Ro4938581/RG1662 from Hoffmann-La Roche [47]. The $\alpha 5$ IA has a higher efficacy as a negative allosteric modulator of GABAergic transmission on the $\alpha 5$ subunit as compared to the $\alpha 1$ subunit and is a weak positive allosteric modulator on the $\alpha 2$ and the $\alpha 3$ subunit while affinities are nearly similar for all subunits (0.88, 0.68, 0.61 and 0.66 nM for the $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$ subunits respectively) [44]. Differently Ro4938581 has higher affinity for the $\alpha 5$ -containing GABA_A receptors (4.6nM) than for the $\alpha 1$ (174nM) $\alpha 2$ (185nM) and $\alpha 3$ -containing GABA_A receptors (80nM) and is a negative allosteric modulator of $\alpha 5$ -containing GABA_A receptors only [47]. Table 1 shows the *in vivo* comparative pharmacological profile of $\alpha 5$ IA and Ro4938581. The overall data indicate a lack of effect of the two drugs on anxiety-related behaviors and on convulsant/proconvulsant activity and most importantly promnesiant effects in different learning and memory paradigms. In humans at doses that are not anxiogenic $\alpha 5$ IA raised a receptor occupancy of 50% [48]

GABA_A- $\alpha 5$ selective inverse agonists in DS

After the publication of Fernandez *et al.* (2007) demonstrating that GABA_A antagonists can stably reverse cognitive deficits in DS mouse models, we reasoned that GABA_A- $\alpha 5$ selective inverse agonists would act as negative allosteric modulators of GABAergic transmission and reduce inhibition as GABA_A antagonists do. This would result in improved cognition in DS mice without deleterious convulsant effects of GABA_A antagonists.

The triazolophthalazines from Merck being unavailable when requested, we resynthesized the $\alpha 5$ IA ($\alpha 5$ inverse agonist) following the steps described in Chambers *et al.* (2004) [46] and in their patent (WO 98/50385). We then tested the $\alpha 5$ IA in the Ts65Dn mice in two learning and memory tasks: the Morris water maze (MWM) and the novel object recognition (NOR) that test respectively spatial reference memory (hippocampal-dependent) and short term non spatial memory which relies on parahippocampal cortical areas [49]. We showed that after acute treatment (single 5 mg/kg intra peritoneal i.p. dose before each training session) $\alpha 5$ IA can restore learning and memory deficits of Ts65Dn mice both in the MWM and the NOR tasks [50]. In the NOR task $\alpha 5$ IA also increased the performance of wt mice. Interestingly in the same task Ts65Dn mice treated with $\alpha 5$ IA not only recovered from their deficit but performed as well as treated wild type euploid (wt) mice.

We showed that $\alpha 5$ IA has no convulsant nor proconvulsant activity in wt and Ts65Dn mice. Surprisingly we observed that the convulsant methyl β -carboline-3-carboxylate (β -CCM) inverse agonist had no effect in Ts65Dn mice (while clearly inducing convulsant effects in euploid mice) suggesting that either β -CCM is metabolized differently in Ts65Dn mice or that it is not potent enough to counterpart the increased inhibition in DS mice possibly associated to higher levels of GABA (Table 2).

Locomotor activity is increased in Ts65Dn mice as compared to wt, and treatment did not modify this difference. In the elevated plus maze that evaluates anxiety, Ts65Dn were less anxious, spending more time in the open arms as compared to wt. However after $\alpha 5$ IA treatment (15 mg/kg, three fold higher than the active dose in MWM and NOR), Ts65Dn and wt mice performed equally, indicating that $\alpha 5$ IA does not show anxiogenic activity. Two main questions remain to be answered: i) what would be the effects of chronic injections of $\alpha 5$ IA? ii) are the promnesiant effects long lasting? iii) what would be the effects of $\alpha 5$ IA in mouse models carrying full trisomy 21?

Since the promnesiant effects observed in Ts65Dn mice after $\alpha 5$ IA treatment were dose-dependent, we hypothesized that $\alpha 5$ IA acted through binding to $\alpha 5$ -containing GABA_A receptors and decreased GABAergic transmission. We could not find any difference between the amounts of $\alpha 5$ transcripts in Ts65Dn and in wt brains [15]. Comparing electrophysiological properties of $\alpha 5$ IA in Ts65Dn and wt mice will be necessary in order to test whether structural/functional changes occur in the GABA_A receptor subunits. Recently Martinez-Fernandez *et al.* reported that Ro4938581, the negative $\alpha 5$ -containing GABA_A allosteric modulator from Hoffmann La Roche was also able to rescue LTP and cognitive deficits of Ts65Dn thus validating our initial results with a structurally different ligand [29].

Changes in gene expression after chronic treatment with $\alpha 5$ IA:

In order to get insights into the mode of action of $\alpha 5$ IA *in vivo* we first investigated the expression of the murine osteosarcoma viral oncogene homolog fos protein, an immediate early gene (IEG) activated following behavioral stimulation. The fos protein (as well as other immediate early genes products) is hence classically used to map evoked neuronal activity [51, 52]. We showed that fos translation triggered by a behavioral episode (exploration of a new environment) is strongly increased after treatment with $\alpha 5$ IA both in Ts65Dn and wt mice in the cortex, the CA1 region of the hippocampus but not in the dentate gyrus that lacks high density of $\alpha 5$ -containing GABA_A receptors. We then established gene expression profiles of Ts65Dn and wt hippocampi collected after chronic

treatment (during 12 days) with $\alpha 5$ IA and compared expression levels to vehicle-treated mice. We analyzed separately the three-copy genes and the immediate early genes. Three triplicated genes were found to be differentially expressed between Ts65Dn and wt mice and among them the superoxide dismutase 1 (*Sod1*) gene which has been shown to participate in several clinical features of DS [15]. Interestingly $\alpha 5$ IA treatment corrected the elevated levels of *Sod1* present in Ts65Dn mice. It will be important to test whether Sod1 could be a marker of $\alpha 5$ IA efficiency *in vivo*.

In euploid mice, chronic treatment with $\alpha 5$ IA increased IEGs expression, particularly of c-Fos and the activity-regulated cytoskeleton-associated protein (Arc) genes. In Ts65Dn mice, deficits of IEGs activation particularly of c-Fos and early growth response 2 (*Egr2*) were rescued after treatment with $\alpha 5$ IA but did not reach the levels obtained after $\alpha 5$ IA treatment of wt mice. At the protein level we could not find any deficit of fos basal levels between Ts65Dn and wt mice. Differences between the two analysis at the protein and at the transcript levels could be explained in various ways: i) transcripts were analyzed after training the mice in the MWM task during two weeks while Fos immunoreactivity was measured after the short-lasting exploration–memorization of a new environment ii) transcripts were quantified after 12 days of treatment while for Fos immunoreactivity, acute treatment was applied. Analysis at the transcript and at the protein levels will need to be performed after similar behavioral stimulation and equivalent treatment regimen to reach conclusion.

Increased activation of IEGs could allow a more efficient storage of information during memory process and correct deficits in learning and memory functions that have been described in DS.

Use of allosteric modulators of $\alpha 5$ -containing GABA_A receptors in other disease conditions:

Negative allosteric modulators (NAM) of $\alpha 5$ -containing GABA_A receptors

As a general cognitive enhancer and GABAergic inhibitor, $\alpha 5$ IA could be a target for increasing cognition not only in the normal brain but also in memory-challenging conditions and in neuropsychiatric disorders [53]. Indeed we found that $\alpha 5$ IA can reverse cognitive deficits in a mouse model of DS without showing any adverse effects. Another study by Nutt *et al.* has shown the benefits of GABA $\alpha 5$ -inverse agonists for treating alcohol-induced amnesia in human [54].

After stroke in mice, tonic inhibition is increased in the peri-infarct zone; it is mediated by extra synaptic GABA_A receptors and caused by an impairment for GABA transporter function. This over inhibition could be alleviated using $\alpha 5$ IA as well as by lowering the number of $\alpha 5$ -containing GABA_A receptors [55].

Inhalation of anesthetics promotes anterograde amnesia. It was recently shown that $\alpha 5$ -containing GABA_A receptors are necessary for the development of post-anesthetic deficits in recognition [56]. These receptors could thus be targeted to restore memory even after elimination of the anesthetic.

Positive allosteric modulators (PAM) of $\alpha 5$ -containing GABA_A receptors

Excessive neuronal activity in the hippocampus has been shown to contribute to cognitive impairment in aging. In aged rats with cognitive impairment, pyramidal neurons of the dentate gyrus have elevated excitability [57]. Moreover, aberrant excitability is found in the hippocampus of transgenic mice overexpressing amyloid precursor protein [58] and in mouse model expressing the $\epsilon 4$ allele of the apolipoprotein E (ApoE4) that confers elevated risk for sporadic Alzheimer's disease [59]. In aged rats, the positive allosteric modulators of $\alpha 5$ -containing GABA_A receptors, the thiophene derivatives developed initially by Merck [45, 60], produced memory improvements thus suggesting a novel approach to improve neural function in clinical conditions of abnormal (increased) hippocampal activity [61]. Also the use of levetiracetam, an antiepileptic drug, has been shown to mitigate neuronal hyperactivity in subjects with mild cognitive impairments (a condition at risk for conversion to AD) and to improve memory performance [62].

Modulating chloride ions flux in disease conditions

The symptoms of autism spectrum disorders (ASDs) are the result of deficient GABA neurotransmission and higher intracellular chloride concentrations leading to brain hyper excitability early during brain development [63]. Initial evidence of a deficit in $\alpha 5$ -containing GABA_A receptors in ASD has been shown and support further investigations of the GABA system in this disorder [64]. Interestingly it was recently found that Bumetanide, an inhibitor of the chloride co-transporter NKCC1 that reduces intracellular chloride concentration, produced a significant improvement in infantile autistic syndrome without any side effects [65].

Conclusions:

In DS GABA over inhibition has not been associated to the over expression of specific genes mapping to human chromosome 21. Over inhibition could be a secondary event leading to neural plasticity defects. Yet the amounts of GABA synthesis enzymes (GAD) and chloride co-transporters (NKCC1 and KCC2) need to be measured in this disease. In addition levels of GABA can now be assessed *in vivo* before and after treatments using MRS [66-68]. These new protocols could be applied in DS individuals as well as in mouse models.

Many GABA mimetics are widely used in clinic: they either increase GABA transaminase, glutamate decarboxylase or inhibit GABA reuptake. Unfortunately drugs that would have opposite effects may induce

hyper excitability. Still the effects of drugs acting on the cation-chloride co-transporters (activator of NKCC1 or inhibitor of KCC2) remain to be demonstrated in DS mouse models (Figure).

At the present time, diminishing GABA_A inhibition in DS with negative allosteric modulators of the α 5-containing GABA_A receptors appears as a sensible strategy since two molecules of this class have already proven to be very efficient for correcting cognitive deficits in DS mouse models and one currently being tested in DS individuals [69].

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List of abbreviations:

AD Alzheimer's disease

$\alpha 5$ IA $\alpha 5$ inverse agonist

ApoE apolipoprotein E

Arc activity-regulated cytoskeleton-associated protein

ASD autism spectrum disorder

β -CCM methyl β -carboline -3-carboxylate

CA1 cornu ammonis 1

DS Down syndrome

Egr2 early growth response 2

Fos murine osteosarcoma viral oncogene homolog

GABA γ aminobutyric acid

GAD glutamate decarboxylase

GIRK2 G protein coupled inward rectifier potassium channel 2

IEG an immediate early gene

i.p. intra peritoneal

KCC2 K-Cl cotransporter 2

MRS magnetic resonance spectroscopy

MWM Morris water maze

NAM Negative allosteric modulators

NKCC1 Na-K-Cl cotransporter 1

NOR the novel object recognition

PAM Positive allosteric modulators

PET positron emission tomography

Sod1 superoxide dismutase 1

Conflict of Interest

The authors declare no conflict of interest

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Pharmacological test	Animal wild type	Dose of α 5IA	p value	Dose of Ro4938581	p value
Anxiogenic activity					
Elevated Plus Maze	Rat	1-10 mg/kg p.o. ¹	NS	0.3-3 mg/kg p.o. ³	NS
	Mouse	5 mg/kg i.p. ²	<0.05*		
	Mouse	15 mg/kg 2 weeks ²	NS		
Social Approach Avoidance	Rat			0.3-3 mg/kg p.o. ³	NS
Proconvulsant effects					
Threshold of PTZ seizures	Mouse	10-50 mg/kg i.p. ^{1,2}	NS		
Kindling	Mouse	10 mg/kg i.p. 19days ²	NS		
Audiogenic seizures	Mouse			up to 100 mg/kg ³	NS
Promnesiant activity					
DMTP water maze	Rat	0.03-0.3 mg/kg p.o. 5days ¹	<0.005	0.1-10 mg/kg ³	NS
	Rat	0.3-3 mg/kg p.o. 8 days ¹	<0.005		
	Mouse	1, 5 mg/kg ²	<0.05		
DMTP after scopolamine	Rat			0.3-1 mg/kg ³	<0.01
MWM	Mouse	1-5 mg/kg i.p. ²	0.025		<0.01
MWM on diazepam effect	Rat			1-10 mg/kg ³	<0.001
NOR	Mouse	5 mg/kg i.p. ²	0.005		
Object Retrieval Tasks	Monkey			3-10 mg/kg ³	<0.001
Locomotor activity					
Rotarod	Mouse	0.3-10 mg/kg p.o. ¹	NS		
Open Field	Mouse	5 mg/kg i.p. ²	NS		

¹[43] ²[50] ³[47]*Due to a normalization of anxiety levels (low to normal) in Ts65Dn mice.

Table 1: Pharmacological profile of the two α 5-containing GABA_A receptors inverse agonists: α 5IA and Ro4938581

Injected drug	Genotype	Nb of mice with convulsions	Latency to convulsions
Vehicle	Euploid	0/6	-
β -CCM 3 mg/kg i.p.	Euploid	3/6	213 sec
Vehicle	Ts65Dn	0/6	-
β -CCM 3 mg/kg i.p.	Ts65Dn	0/6	-

Table 2: Effects of the non-selective convulsivant inverse agonist β -CCM in Ts65Dn and euploid wt mice.

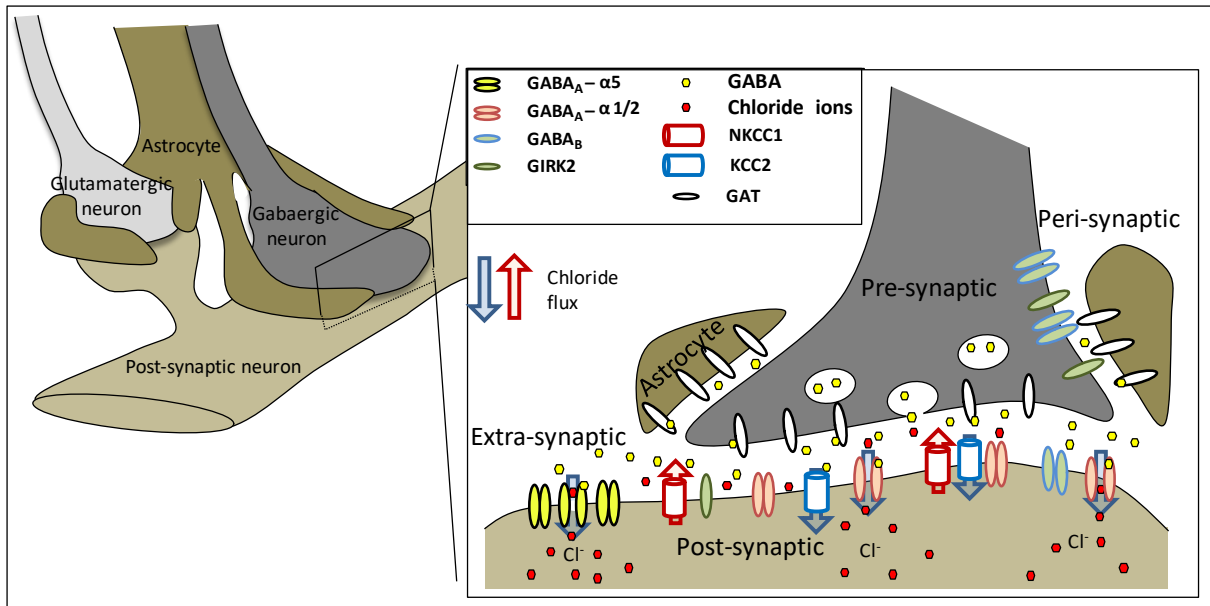


Figure: Mechanisms of GABA inhibition in Down syndrome: increase of the size of inhibitory synapses [5, 17, 70] could produce more GABA that is not able to be transported through GAT thus producing GABA spillover and the activation of extra-synaptic receptors such as $\alpha 5$ -containing GABA_A receptors. Right part is a magnification of the rectangle on the left part of the figure.