**SLC6A1 Connect Roundtable**
**Developing a Unified Scientific Approach to Treatment Discovery in SLC6A1**
American Epilepsy Society Meeting, New Orleans (New Orleans Marriott)
November 29, 2018

# *Pharmacological Thoughts for SLC6A1*

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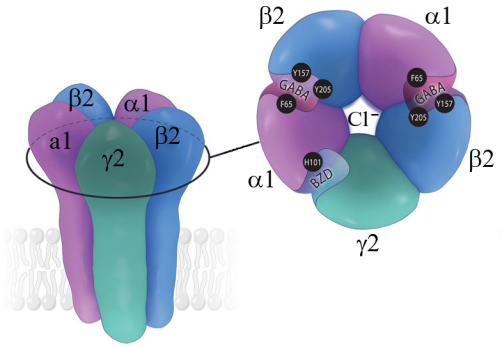


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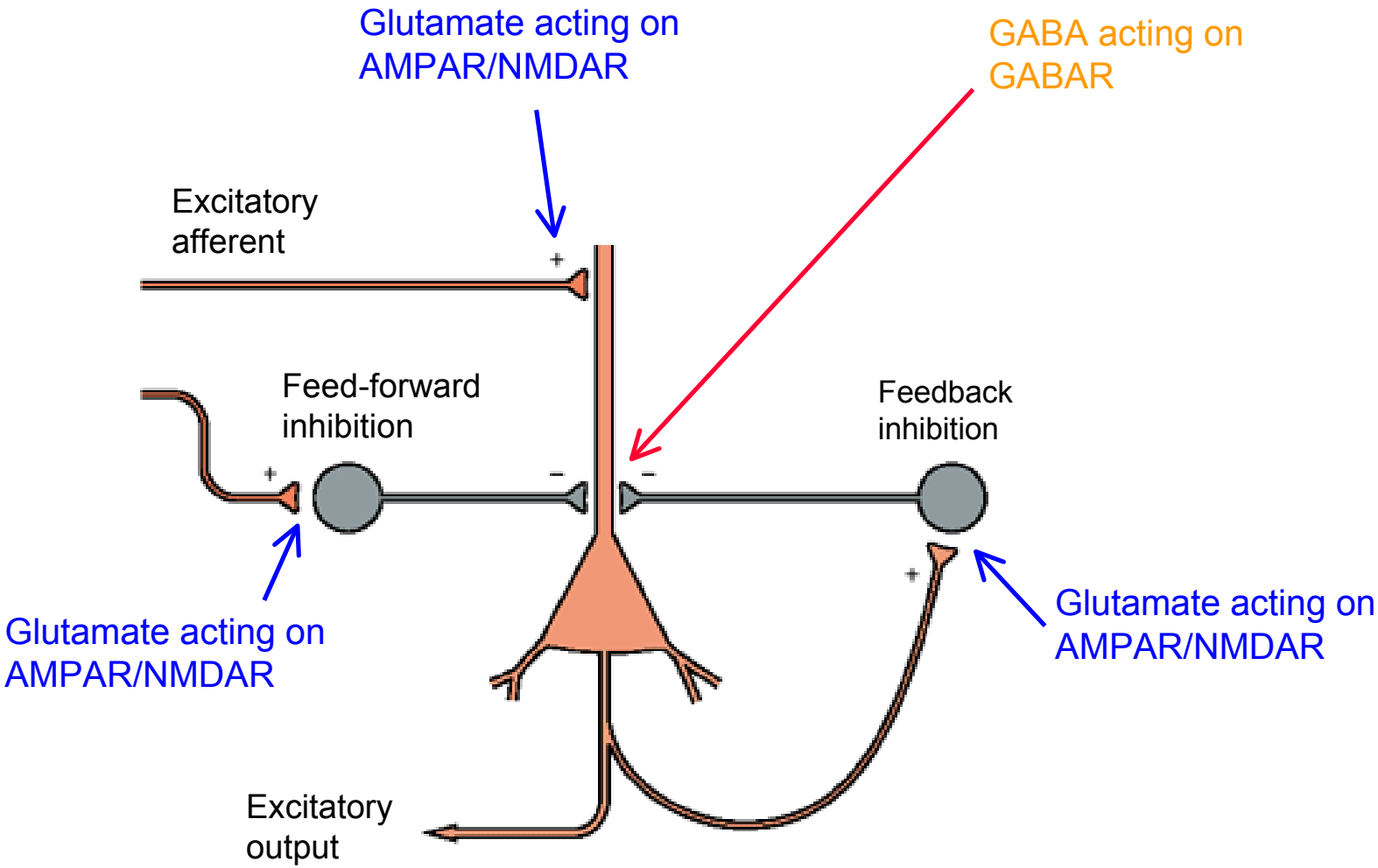
**My Task:** Provide basic pharmacological information to set the stage for thinking about laboratory based research to identify potential treatments for SLC6A1.

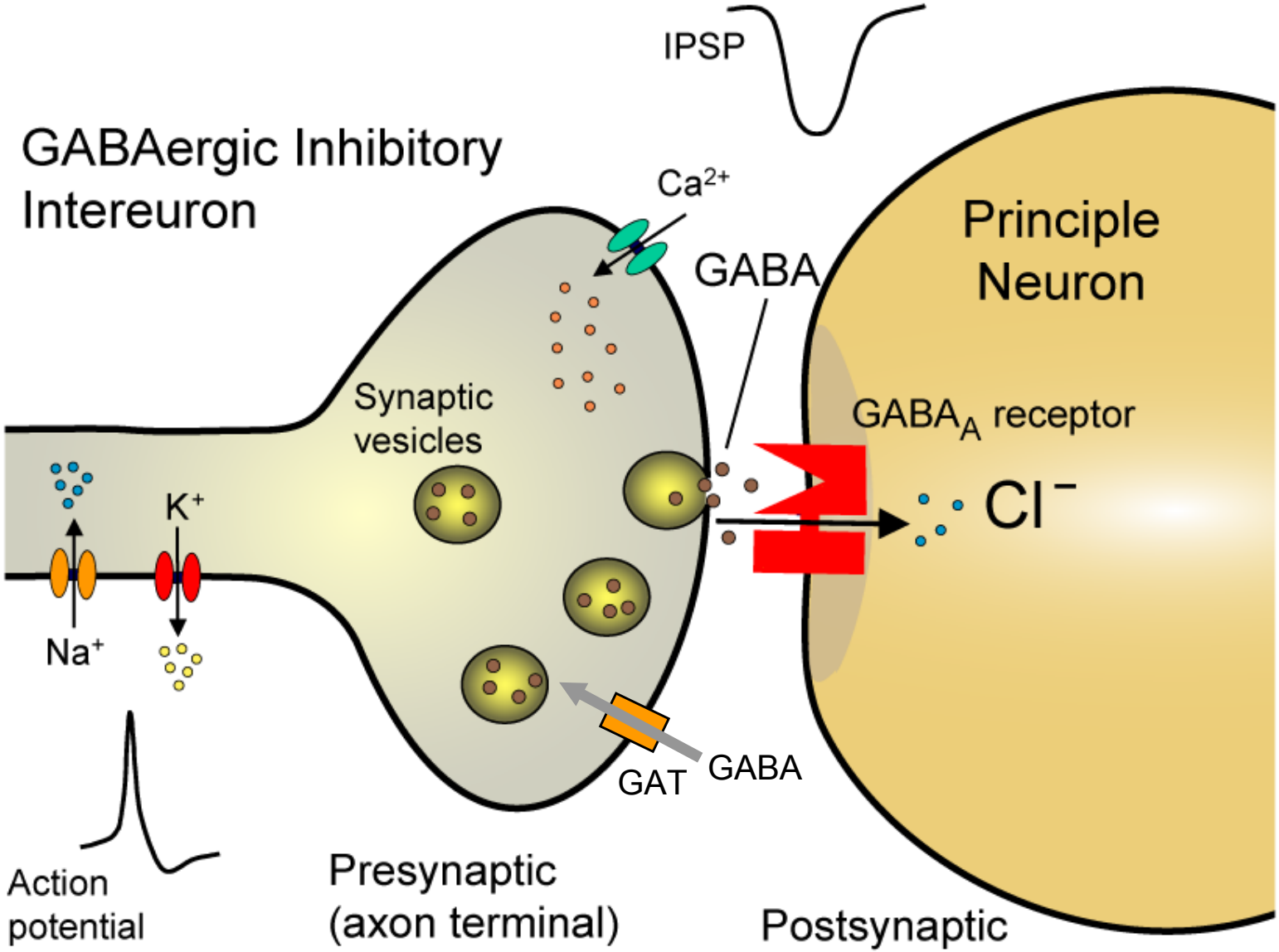
- Pharmacological therapy
- Gene therapy

# Basic Cortical Circuit

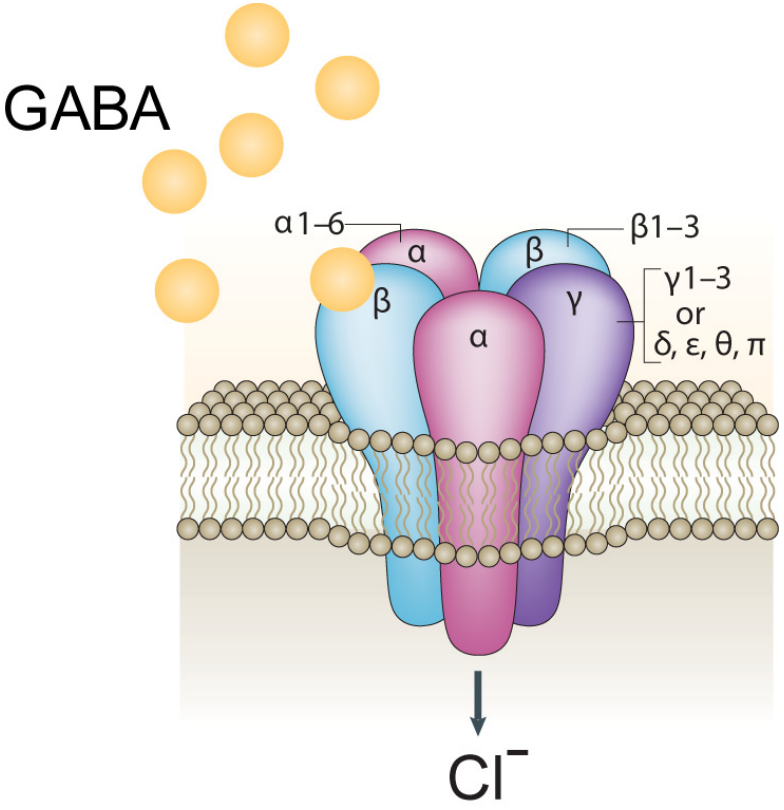


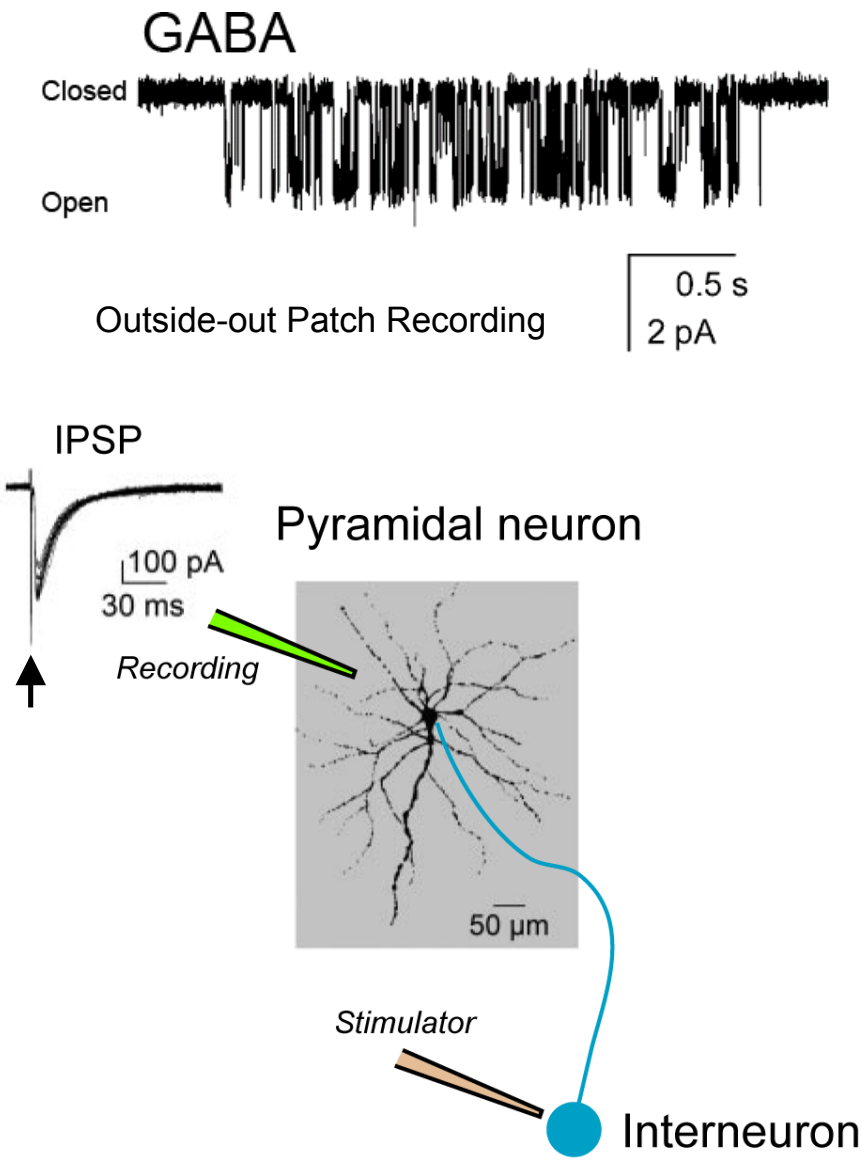

GABAR = GABA receptor
AMPAR = AMPA receptor
NMDAR = NMDA receptor

# GABAergic Synapse

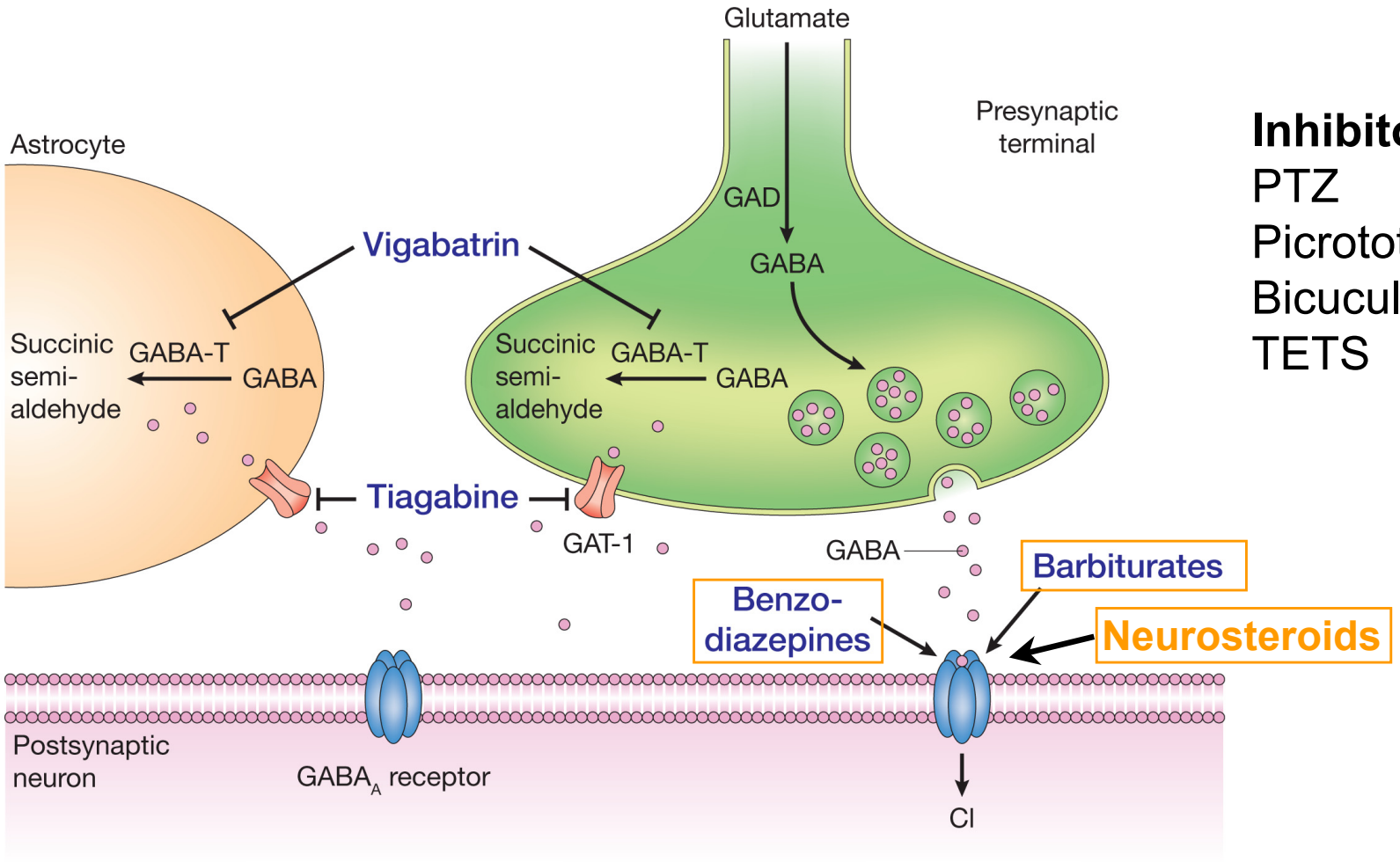


# Subunit Architecture and Physiological Role of $GABA_A$ Receptors



# Inhibitory Synapse




**Inhibitors**
PTZ
Picrotoxin
Bicuculline
TETS

Article

HET

Human and Experimental Toxicology
2016, Vol. 35(2) 109–113

DOI: 10.1177/0960327115579206
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# Review of toxicity and trends in the use of tiagabine as reported to US poison centers from 2000 to 2012

**HA Spiller[1,2], D Wiles[1], JL Russell[1] and MJ Casavant[1,2]**

**Abstract**

**Background:** Tiagabine is a novel antiepileptic that acts by increasing synaptic and extracellular gamma-aminobutyric acid concentrations. Information concerning overdose of tiagabine is limited. After introduction, an increasing number of off-label uses suggested that tiagabine use would increase. However in 2005 and 2008, warnings from the Food and Drug Administration (FDA) were issued on the risk of seizures in non-epileptic and increased suicide ideation. We evaluated the temporal trends associated with these two warnings as well as clinical outcomes from tiagabine overdose.

**Method:** A retrospective review of all single substance tiagabine exposures in National Poison Data System (NPDS) from 2000 to 2012.

**Results:** A total of 2147 patients had ingested tiagabine, with a mean of 165 year$^{-1}$. This was disproportionally distributed, with a steep rise leading up to 2004 (max 559 year$^{-1}$) and then a significant decline ($p < 0.05$) between 2005 and 2006. The number of cases reported to NPDS mirrored the sales of tiagabine. Clinical effects were predominantly neurological, with the most commonly reported effects being drowsiness (27%), agitation (19%), confusion (12%), seizures (11%), and tachycardia (10%). In all, 758 patients (35%) showed a major or moderate medical outcome, with no deaths reported. A disproportionate share of the major outcomes was in the suicide attempt group (73%). The majority of patients (75%) were treated in a health-care facility (HCF).

**Conclusions:** The HCF usage is likely due to high rate of symptomatic patients (59%) and the large proportion of suicide attempt cases. The frequency of tiagabine cases in NPDS mirrored pharmaceutical sales, with steep declines temporally related to the 2005 FDA warning.

**Keywords**

Tiagabine, FDA warning, toxicity

## Introduction

Tiagabine is a novel antiepileptic that acts by binding to the gamma-aminobutyric acid (GABA) uptake transporter in presynaptic neurons and glial cells resulting in increased synaptic and extracellular GABA concentrations. The increased concentration of the inhibitory neurotransmitter GABA is believed to be responsible for the antiepileptic effects. Information concerning overdose of tiagabine is limited to case reports and a single case series.[1–9] These reports suggest the effects in supratherapeutic doses and overdoses that follow the GABA neurological mechanism, with lethargy, confusion, and coma. Seizures, convulsive, and nonconvulsive status epilepticus have also been reported. The epileptogenic mechanism for tiagabine is not known but suggestions have included possible mediation via GABA receptors in the thalamus and stimulation of dopamine, serotonin, or glycine receptors.[9,10] An atypical

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**Table 1.** Reported clinical effects with single substance ingestion of tiagabine.

| Clinical effect | *N* | Percent of total (%) |
|---|---|---|
| Drowsiness/lethargy | 570 | 26.5 |
| Agitated | 405 | 18.9 |
| Confusion | 260 | 12.1 |
| Total with SZ | 226 | 10.5 |
| SZ—single | 89 | 4.1 |
| SZ—multiple | 81 | 3.8 |
| SZ—status epilepticus | 56 | 2.6 |
| Tachycardia | 213 | 9.9 |
| Dizziness | 106 | 4.9 |
| Coma | 99 | 4.6 |
| Hypertension | 84 | 3.9 |
| Vomiting | 55 | 2.6 |
| Mydriasis | 45 | 2.1 |
| Slurred speech | 39 | 1.8 |
| Dystonia | 35 | 1.6 |
| Hypotension | 23 | 1.1 |
| Hallucinations/delusions | 23 | 1.1 |
| Bradycardia | 16 | 0.7 |
| Muscle rigidity | 15 | 0.7 |
| Respiratory depression | 26 | 1.2 |
| Muscle weakness | 11 | 0.5 |
| Headache | 10 | 0.5 |
| Fasciculation | 8 | 0.4 |
| Fever | 8 | 0.4 |
| Acidosis | 7 | 0.3 |
| Tachypnea | 6 | 0.3 |
| Chest pain | 3 | 0.1 |

SZ: seizures.

*Seizure* 1999; **8**: 314–317
Article No. seiz.1999.0303, available online at http://www.idealibrary.com on IDEAL

CASE REPORT

# Tiagabine-induced absence status in idiopathic generalized epilepsy

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Several medications such as baclofen, amitriptyline and even antiepileptic drugs such as carbamazepine or vigabatrin are known to induce absence status epilepticus in patients with generalized epilepsies. Tiagabine (TGB) is effective in patients with focal epilepsies. However, TGB has also been reported to induce non-convulsive status epilepticus in several patients with focal epilepsies and in one patient with juvenile myoclonic epilepsy. In animal models of generalized epilepsy, TGB induces absence status with 3–5 Hz spike-wave complexes.

We describe a 32-year-old patient with absence epilepsy and primary generalized tonic–clonic seizures since 11 years of age, who developed her first absence status epilepticus while treated with 45 mg of TGB daily. Administration of lorazepam and immediate reduction in TGB dosage was followed by complete clinical and electroencephalographic remission. This case demonstrates that TGB can induce typical absence status epilepticus in a patient with primary generalized epilepsy.

*Key words:* absence status; status epilepticus; generalized epilepsy; seizure induction; tiagabine.

## INTRODUCTION

Absence status epilepticus is defined as a generalized absence seizure lasting for more than half an hour in the context of a primary generalized epilepsy[1]. During the time of mental clouding of differing degree, bilateral synchronous spike-wave discharges can be recorded by EEG. Of all the forms of non-convulsive status epilepticus, generalized absence status is the most commonly encountered[2]. The incidence of absence status in patients with absence epilepsy is estimated to be between 2 and 10%[3].

Several antiepileptic drugs have been reported to induce absence status in patients and animal models. Tiagabine (TGB) inhibits the reuptake of $\gamma$-aminobutyric acid (GABA) into the presynaptic terminals and glia cells, thereby making more GABA available at the synapses[4]. It increases the extracellular concentration of GABA after oral administration[5,6]. In clinical trials TGB had a dose-related anticonvulsant effect in patients with focal epilepsies[7–11]. However, in animal models the frequency of interictal spike-wave discharges increased in rats when treated with TGB[12–14].

In addition, TGB was also reported to induce non-convulsive status epilepticus in several patients with partial epilepsy[15,16] and in one patient with juvenile myoclonic epilepsy[6]. There have been no detailed reports of this effect of TGB in patients with absence epilepsy.

We describe a 32-year-old patient with absence epilepsy and generalized tonic–clonic seizures since the age of 11 years, in whom TGB induced her first absence status.

## CASE REPORT

A 32-year-old woman was admitted with absence status epilepticus lasting for more than 6 hours.

At the age of 11 years, she began having absences and generalized tonic–clonic seizures. The diagnosis of absence epilepsy was supported by generalized spike-wave complexes during hyperventilation and a positive family history for epilepsy. Seizures were

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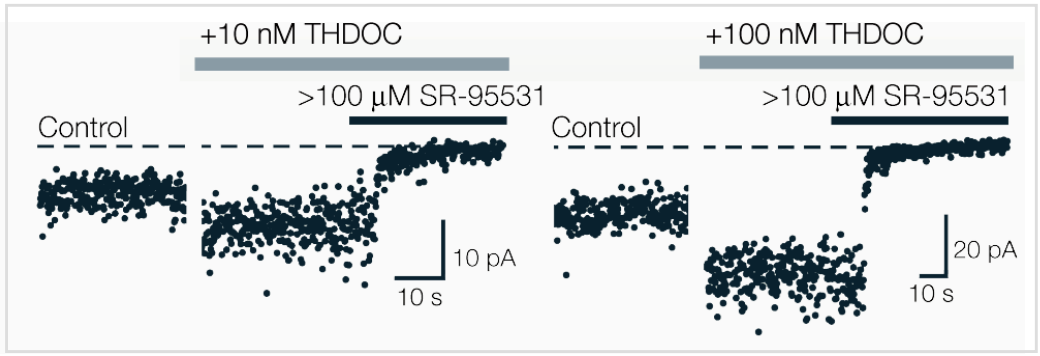
+10 nM THDOC
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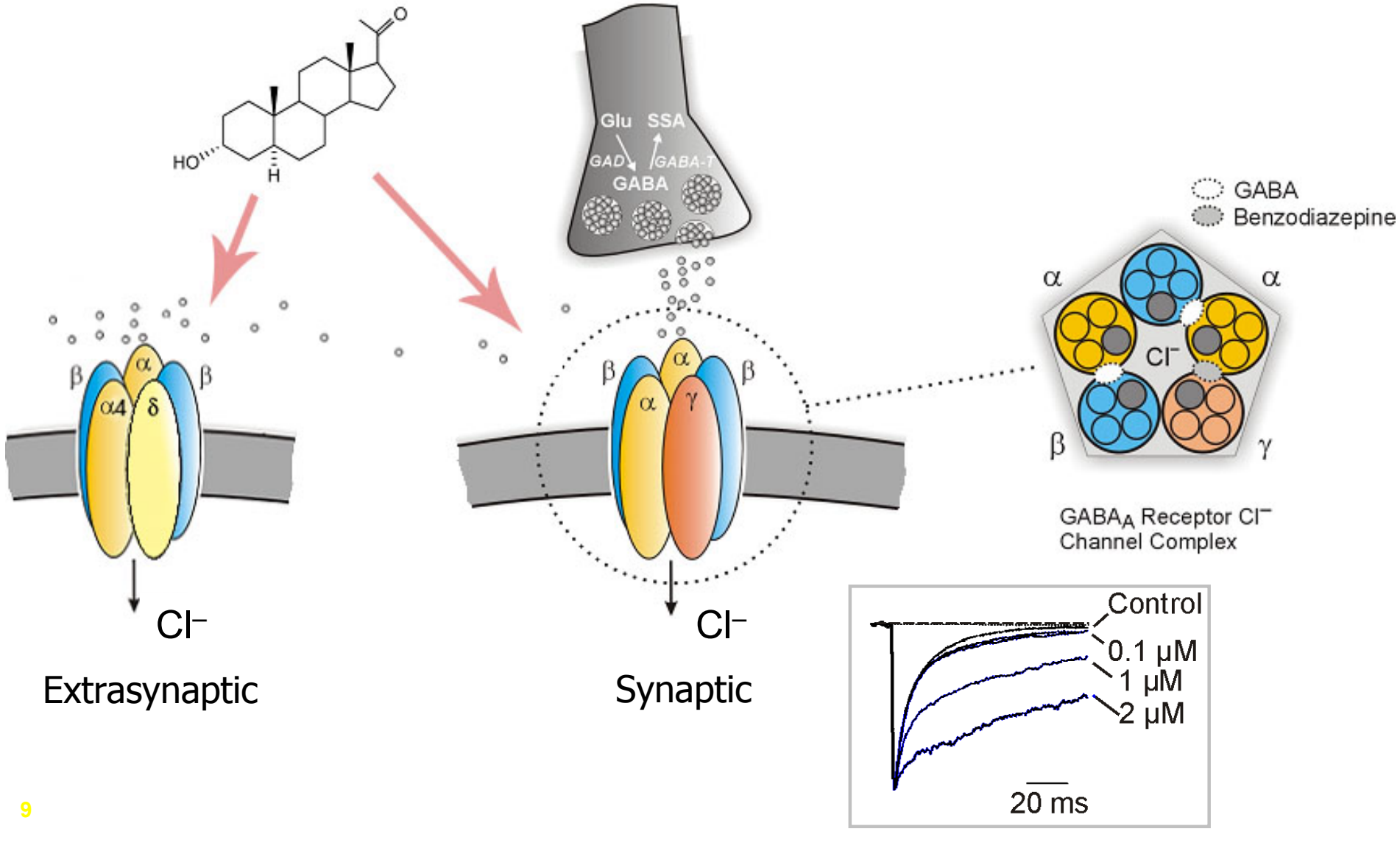
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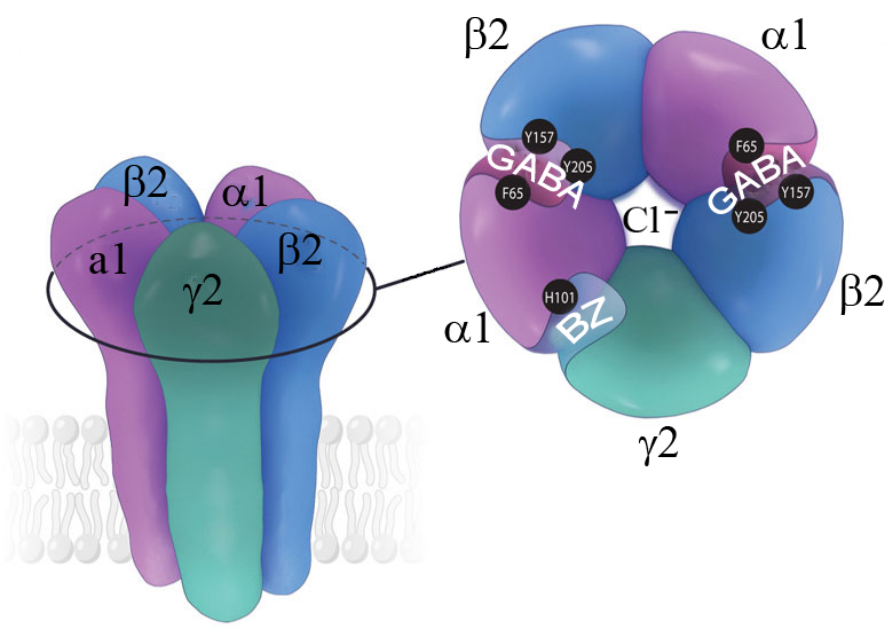
Cl⁻
Synaptic
GABA
Benzodiazepine
α
α
Cl⁻
β
γ
GABA$_A$ Receptor Cl⁻
Channel Complex
Control
0.1 µM
1 µM
2 µM
20 ms

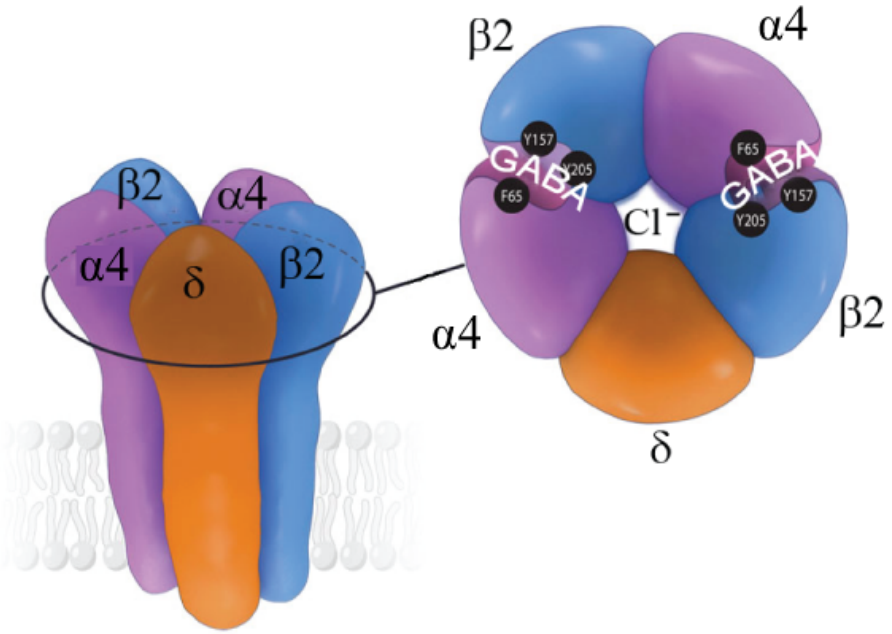
# Pharmacology of Synaptic and Extrasynaptic $GABA_A$ Receptors

## Synaptic




**Benzodiazepines, zolpidem active**
**Neurosteroids active**

## Extrasynaptic




Benzodiazepines, zolpidem inactive
**Neurosteroids active**

[Meldrum BS and Rogawski MA, Neurotherapeutics 2007;4:18-61]

# Anticonvulsant Activity of Intravenous Muscimol in Rats

*Neuropharmacology*, Vol. 18, pp. 885 to 889
Pergamon Press Ltd 1979. Printed in Great Britain

## ANTICONVULSANT ACTIVITY OF MUSCIMOL AGAINST SEIZURES INDUCED BY IMPAIRMENT OF GABA-MEDIATED NEUROTRANSMISSION

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(*Accepted* 28 *May* 1979)

**Summary**—Muscimol is a potent *in vivo* agonist of gamma-aminobutyric acid (GABA) when tested iontophoretically and binds to GABA receptors *in vitro*. A study of muscimol effects on seizures induced by agents which impair GABA-mediated neurotransmission was performed in the rat. Muscimol delayed the onset of isoniazid-and picrotoxin-induced convulsions. The tonic forelimb extension component of bicuculline and metrazole seizures was abolished by muscimol. Inhibition of forelimb extension was chosen as an endpoint for comparison of clinically effective antiepileptics with muscimol. The order of potency was diazepam > muscimol ≫ phenobarbital > phenytoin. Muscimol had no effect on strychnine-induced convulsions. It is concluded that muscimol penetrates rat brain and specifically antagonizes seizures caused by GABA receptor blockade or depletion of brain GABA.

Gamma-aminobutyric acid (GABA) is an amino acid which satisfies many of the criteria for a naturally-occurring inhibitory transmitter in the vertebrate central nervous system (Roberts, Chase and Tower, 1976; DeFeudis, 1977). Experimental evidence implicates a hypofunctional central GABA system in certain neurologic disorders (Hornykiewicz, Lloyd and Davidson, 1976) including epilepsy (Meldrum, 1975). Muscimol (3-hydroxy-5-aminomethylisoxazol), a structural analog of GABA, is a potent GABA agonist at the crustacean neuromuscular junction (Wheal and Kerkut, 1976) and at bicuculline-sensitive postsynaptic receptors in the mammal (Krogsgaard-Larsen, Johnston, Curtis, Game and McCulloch, 1975; Enna, Collins and Snyder, 1977). A delay in the onset of isoniazid-induced seizures after muscimol treatment has been reported (Naik, Guidotti and Costa, 1976). However, abolition of convulsive behavior elicited by impairment of GABAergic neurotransmission has not been demonstrated after muscimol treatment. The present authors studied the anticonvulsant activity of muscimol against seizures induced by a variety of drugs known to interfere with GABA-mediated neurotransmission and other convulsant chemicals. The anticonvulsant profile of muscimol was compared to that of several clinically effective antiepileptics.

### METHODS

Male Sprague–Dawley rats (200–275 g) were used in all experiments. Animals were housed in constant temperature, humidity and light cycles. Food and water were available *ad libitum*.

Key words: tonic forelimb extension, picrotoxin, isoniazid, bicuculline, pentylenetetrazole, strychnine.

*Drugs*

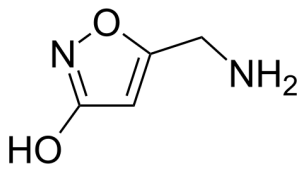
Seizures were produced by systemic administration of the following convulsants: picrotoxin (Smith Kline & French); isonicotinic acid hydrazide (Isoniazid, Sigma); bicuculline (Sigma); strychnine sulfate (Smith Kline & French); pentylenetetrazole (Metrazole, Kroll Pharmaceuticals). These compounds were tested for anticonvulsant activity: muscimol (Smith Kline & French); diazepam (Smith Kline & French); phenobarbital sodium (Smith Kline & French); dipheylhydantoin sodium (Phenytoin, Sigma). Muscimol, phenobarbital, isoniazid, strychnine and picrotoxin were dissolved in water or saline. Diazepam and bicuculline were administered in dilute HCl (pH 3). Phenytoin was dissolved in water with the addition of NaOH to a final pH of 11.

*Chemically-induced convulsions*

Picrotoxin was injected subcutaneously into rats and the latency to onset of convulsions was measured. A mean onset time was determined in control (non-anticonvulsant treated) animals for each experimental day. A rat treated with an anticonvulsant agent was considered "protected" if it failed to convulse within two standard deviations of the mean onset time for the control group. Rats were also injected subcutaneously with isoniazid and monitored for the onset of convulsive activity. Time to onset of clonic and tonic seizure components was noted. An animal was considered "protected" if it failed to have a seizure episode within two standard deviations of the mean onset time for a control group treated only with isoniazid. Bicuculline, strychnine and pentylenetetrazole were injected intravenously. The time to onset of the components of seizure activity was recorded and the pattern of seizure development observed. Animals



Muscimol




GABA

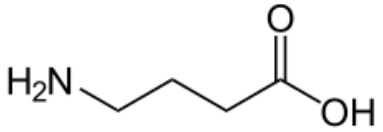



Table 4. Summary of anticonvulsant testing. Comparison of muscimol with standard antiepileptic agents

| Compound | Convulsant | | |
|---|---|---|---|
| | Bicuculline | Strychnine | Pentylenetetrazole |
| Muscimol | 1.0 (0.7–2.0) | No effect at 8 mg/kg | 0.49 (0.32–1.5) |
| Diazepam | 0.32 (0.07–0.49) | 0.82 (0.4–1.4) | 0.09 (0.04–0.14) |
| Phenobarbital | 7.9 (5.1–10.7) | 35.9 (22.2–63.8) | 4.1 (2.2–7.4) |
| Phenytoin | 9.3 (5.0–13.3) | 136.2 (80.0–354.4) | Not tested |

Muscimol, diazepam and phenobarbital were given intravenously. Phenytoin was administered intraperitoneally. All convulsants were injected intravenously 30 min after anticonvulsant treatment. Table values are $ED_{50}$ (mg/kg) with 95% Fieller limits; $n$ = 5–10/dose group.

*Epilepsia*, 36(10):960–965
Lippincott–Raven Publishers, Philadelphia


# Age-Related Differences in the Effects of $GABA_A$ Agonists Microinjected Into Rat Substantia Nigra: Pro- and Anticonvulsant Actions

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*Laboratory of Developmental Epilepsy, Departments of *Neurology, †Neuroscience, and ‡Pediatrics, Albert Einstein College of Medicine, Bronx, New York, U.S.A.*

**Summary:** GABAergic transmission in the substantia nigra pars reticulata (SNR) has an important role in the control of experimental seizures. In the flurothyl seizure model, SNR microinjection of the selective $GABA_A$ receptor agonist muscimol results in a biphasic dose–response curve in adults: Intermediate doses are anticonvulsant, but high doses have proconvulsant effects. Another $GABA_A$ agonist, THIP (4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridin-3-ol), also produces anticonvulsant effects at lower doses, whereas higher doses tend to produce a proconvulsant effect. In 16-day-old rat pups, no anticonvulsant but only proconvulsant effects of muscimol occur, and at lower doses than in adults. These data suggest that the immature SNR is significantly more sensitive to the proconvulsant effects of $GABA_A$ receptor agonists than is the SNR of adults. We hypothesize that the age-related differences in nigral GABAergic response may be due to ontogenic changes in $GABA_A$-sensitive neuronal circuits in the SNR. **Key Words:** Muscimol—THIP—Flurothyl—Seizures—Epilepsy—γ-Aminobutyric acid.

Both clinically and experimentally, it is recognized that the immature brain is at increased risk for sustaining epileptic seizures, particularly generalized seizures (1). An important goal of neuroscience research is to identify the neuronal circuitry and synaptic pharmacology underlying this ontogenic phenomenon. Such understanding will help direct the development of more efficacious and selective age-specific therapies than those currently available.

The substantia nigra pars reticulata (SNR) has been identified as a critical site in rat brain for the anticonvulsant action of GABAergic drugs (2). Since it was shown that GABAergic transmission in SNR can attenuate seizures (3,4), a series of investigations (5,6) led to the hypothesis that experimental manipulations that inhibit firing in SNR neurons bilaterally can limit convulsive and subconvulsive seizure activity produced in several experimental models of epilepsy (2). However, studies suggest that the ability of SNR inhibition to control seizures may be age-dependent: drugs that are anticonvulsant when microinjected into the SNR of adult rats may be ineffective (7) or even proconvulsant (8) when injected into the SNR of rat pups.

Our laboratory has studied developmental differences in the anticonvulsant pharmacology of GABA transmission in rat SNR. We measured the susceptibility of rats to seizures induced by flurothyl (hexafluorodiethyl ether), a volatile inhaled convulsant well suited to developmental studies (9). We commonly compare adult seizure responses with those of 16-day-old rat pups, an age at which such pups are most susceptible to seizures (1).

We previously showed the effects on flurothyl seizures of some GABAergic agents to be similar regardless of the age of the rats examined. Doses of the presynaptic GABA agonist γ-vinyl-GABA (GVG) that increase GABA in SNR result in the suppression of fluorothyl seizures in both adult (10) and 16-day-old rats (11). Conversely, microinjections into SNR of bicuculline, a competitive $GABA_A$ receptor antagonist (12) significantly facilitate flurothyl seizures in both adults (13) and pups

Received August 27, 1994; revision accepted April 13, 1995.
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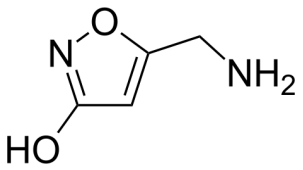
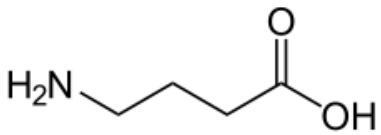
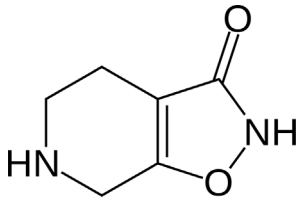
Muscimol

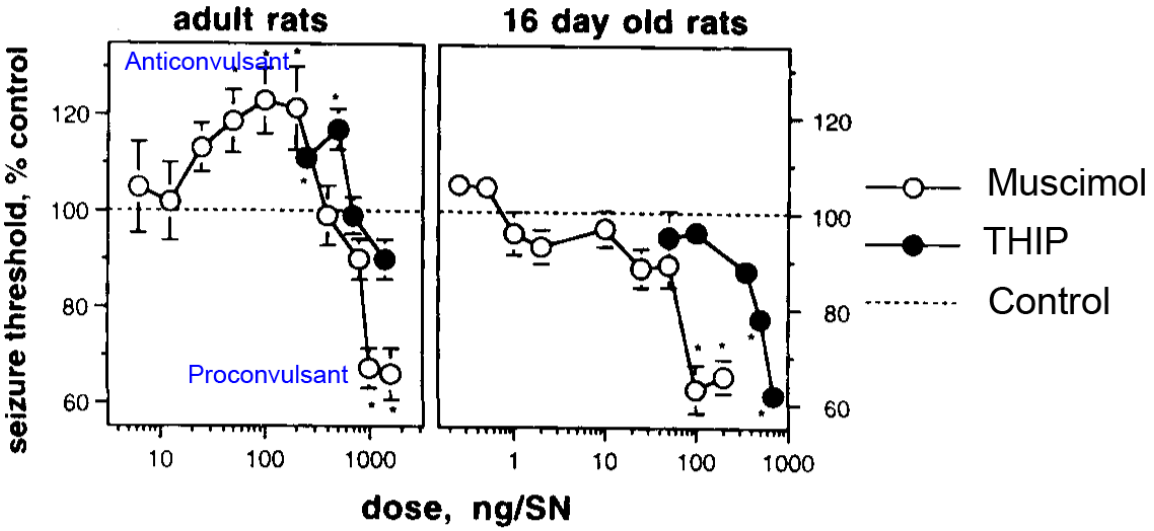


GABA



Gaboxadol (THIP)





Neurobiology of Disease

# GABA Transporter Deficiency Causes Tremor, Ataxia, Nervousness, and Increased GABA-Induced Tonic Conductance in Cerebellum

**Chi-Sung Chiu,[1,6] Stephen Brickley,[2] Kimmo Jensen,[3,4] Amber Southwell,[1] Sheri Mckinney,[1] Stuart Cull-Candy,[5] Istvan Mody,[3] and Henry A. Lester[1]**

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GABA transporter subtype 1 (GAT1) knock-out (KO) mice display normal reproduction and life span but have reduced body weight (female, −10%; male, −20%) and higher body temperature fluctuations in the 0.2–1.5/h frequency range. Mouse GAT1 (mGAT1) KO mice exhibit motor disorders, including gait abnormality, constant 25–32 Hz tremor, which is aggravated by flunitrazepam, reduced rotarod performance, and reduced locomotor activity in the home cage. Open-field tests show delayed exploratory activity, reduced rearing, and reduced visits to the central area, with no change in the total distance traveled. The mGAT1 KO mice display no difference in acoustic startle response but exhibit a deficiency in prepulse inhibition. These open-field and prepulse inhibition results suggest that the mGAT1 KO mice display mild anxiety or nervousness. The compromised GABA uptake in mGAT1 KO mice results in an increased $GABA_A$ receptor-mediated tonic conductance in both cerebellar granule and Purkinje cells. The reduced rate of GABA clearance from the synaptic cleft is probably responsible for the slower decay of spontaneous IPSCs in cerebellar granule cells. There is little or no compensatory change in other proteins or structures related to GABA transmission in the mGAT1 KO mice, including GAT1-independent GABA uptake, number of GABAergic interneurons, and $GABA_A$-, vesicular GABA transporter-, GAD65-, and GAT3-immunoreactive structures in cerebellum or hippocampus. Therefore, the excessive extracellular GABA present in mGAT1 KO mice results in behaviors that partially phenocopy the clinical side effects of tiagabine, suggesting that these side effects are inherent to a therapeutic strategy that targets the widely expressed GAT1 transporter system.

*Key words:* tiagabine; epilepsy; flunitrazepam; cerebellum; inhibition; tremor

## Introduction

GABA is the principal inhibitory neurotransmitter in the mammalian brain, where it activates $GABA_A$, $GABA_B$, and $GABA_C$ receptors. GABA released from presynaptic terminals is removed from the vicinity of the synaptic cleft by GABA transporters, and this action is believed to be a key event in terminating synaptic currents. GABA transporters are also involved in maintaining a low extracellular GABA concentration throughout the brain, preventing excessive tonic activation of synaptic and extrasynaptic receptors. GABA transporters may also play a role in replenishing the supply of presynaptic transmitter. Furthermore, GABA transporters may reverse, under both normal and pathological circumstances, to release GABA (Richerson and Wu, 2003, 2004).

Of the three GABA transporters identified in the CNS, GABA transporter subtype 1 (GAT1) is highly expressed in the olfactory bulb, neocortex, cerebellum, superior colliculus, and substantia nigra, where it is predominantly found in axons, presynaptic terminals, and glial cells. GAT2 is weakly expressed throughout the brain, primarily in arachnoid and ependymal cells. GAT3 expression is densest in the olfactory bulb, midbrain regions, and deep cerebellar nuclei, where it is found predominantly on glial cells (Radian et al., 1990; Ikegaki et al., 1994; Itouji et al., 1996; Yan et al., 1997; Engel and Wu, 1998; Barakat and Bordey, 2002; Chiu et al., 2002).

The GAT1 inhibitor tiagabine is a clinically useful antiepileptic drug with few cognitive side effects (Aldenkamp et al., 2003), but it also causes tremor (its major side effect), ataxia, dizziness, asthenia, somnolence (sedation), and nonspecific nervousness (Adkins and Noble, 1998; Pellock, 2001; Schachter, 2001). It is important to know whether these side effects arise directly from increased extracellular concentration of GABA in the CNS or, instead, from actions on unintended targets. For instance, GAT1

Received Aug. 16, 2004; revised Jan. 25, 2005; accepted Jan. 25, 2005.

This research was supported by National Institutes of Health Grants DA-01921, NS-11756, MH-49176, NS-030549, and DA-010509, National Science Foundation Grant 0119493, the Wellcome Trust, a Royal Society-Wolfson Award (S.C.-C.), and a Della Martin Fellowship (C.-S.C.). We are indebted to members of Caltech and University of California Los Angeles groups for advice, Limin Shi and Paul Patterson for use and help with the startle system, and J. Crawley for comments on this manuscript.

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DOI:10.1523/JNEUROSCI.3364-04.2005

 0270-6474/05/253234-12$15.00/0



**mGAT1 KO mouse is slightly more sensitive than the WT mouse to PTZ-induced seizures**

PTZ (40 mg/kg, i.p.) decreased observable activity in WT and heterozygotes while causing preconvulsive states and mild seizures in mGAT1 KO mice

PTZ (70 mg/kg), all WT and heterozygotes survived with severe seizures, whereas mGAT1 KO mice showed severe seizures, and one of three died

nature medicine

# Enhanced tonic $GABA_A$ inhibition in typical absence epilepsy

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**The cellular mechanisms underlying typical absence seizures, which characterize various idiopathic generalized epilepsies, are not fully understood, but impaired γ-aminobutyric acid (GABA)-ergic inhibition remains an attractive hypothesis. In contrast, we show here that extrasynaptic $GABA_A$ receptor–dependent 'tonic' inhibition is increased in thalamocortical neurons from diverse genetic and pharmacological models of absence seizures. Increased tonic inhibition is due to compromised GABA uptake by the GABA transporter GAT-1 in the genetic models tested, and GAT-1 is crucial in governing seizure genesis. Extrasynaptic $GABA_A$ receptors are a requirement for seizures in two of the best characterized models of absence epilepsy, and the selective activation of thalamic extrasynaptic $GABA_A$ receptors is sufficient to elicit both electrographic and behavioral correlates of seizures in normal rats. These results identify an apparently common cellular pathology in typical absence seizures that may have epileptogenic importance and highlight potential therapeutic targets for the treatment of absence epilepsy.**



Typical absence seizures characterize numerous idiopathic generalized epilepsies and appear in the electroencephalogram (EEG) as bilaterally synchronous spike-and-wave discharges (SWDs) accompanied by behavioral arrest[1,2]. Whereas absence seizures are known to arise in thalamo–cortical networks[2–4], the underlying cellular mechanisms are not fully understood. Impaired GABAergic inhibition remains an attractive hypothesis[5,6], and $GABA_A$ receptor ($GABA_AR$) subunit mutations have been identified in human cohorts with typical absence seizures, albeit as part of a complex phenotype[7–9]. However, although some of these mutations compromise $GABA_AR$ function in heterologous expression systems[10], only modest changes in $GABA_AR$ inhibition have so far been identified in the thalamo-cortical network of rodents with spontaneous SWDs[11–13]. Furthermore, systemic or intrathalamic administration of agents that promote GABAergic inhibition, including the antiepileptic drugs vigabatrin and tiagabine, initiate or exacerbate seizures in humans and rodents[14–18]. Thus, augmented rather than impaired $GABA_AR$ inhibition may be a feature of absence seizures.

Activation of $GABA_AR$s generates two types of inhibition: the transient activation of synaptic $GABA_AR$s ($sGABA_AR$s) elicits inhibitory postsynaptic currents (IPSCs), or 'phasic' inhibition; and the activation of peri- or extrasynaptic $GABA_AR$s ($eGABA_AR$s) by ambient GABA causes a persistently active, or tonic, current[19,20]. Because in thalamocortical neurons, major players in thalamo–cortical networks during SWDs, >90% of $GABA_AR$ inhibition is tonic[21–24], we have examined the prospect of aberrant tonic inhibition in experimental absence seizures. Our data indicate that enhanced tonic $GABA_A$ inhibition is a common feature of diverse genetic and pharmacological models of typical absence epilepsy and may be a requirement for the appearance of absence seizures.

## RESULTS

### Enhanced tonic $GABA_A$ current in genetic models of absence

Genetic absence epilepsy rats from Strasbourg (GAERS) are a well established polygenic model of absence epilepsy that show bilateral spontaneous SWDs and accompanying behavioral arrest from approximately postnatal day 30 (P30)[16]. In thalamocortical neurons, tonic $GABA_A$ currents are generated by extrasynaptic receptors containing the δ subunit[21–23], and, in rats, δ subunit expression is apparent only from approximately P12 (ref. 25). Therefore, we measured tonic $GABA_A$ current amplitude from thalamocortical neurons in slices of the somatosensory ventrobasal thalamus of GAERS from P14 onward and compared it to non–epileptic control (NEC) rats of the same age. We observed no significant difference in tonic current amplitude at P14–P16 ($P > 0.05$ for each day) (**Fig. 1a,b**). At P17, however, there was an approximately twofold increase in tonic current amplitude in GAERS compared to NEC rats ($P < 0.05$) that was sustained in subsequent days (**Fig. 1a,b**) and was independent of whole-cell capacitance (**Supplementary Results** and **Supplementary Fig. 1a**). Comparison of spontaneous IPSC (sIPSC) parameters in GAERS and NEC rats at the same ages revealed no consistent differences (**Supplementary Table 1**), in agreement with previous data obtained from younger GAERS[12]. Notably, there was a significantly ($P < 0.05$) smaller sIPSC peak amplitude, frequency, charge transfer and total current in GAERS at P18, but these changes were not maintained at later ages (**Supplementary Table 1**). These results

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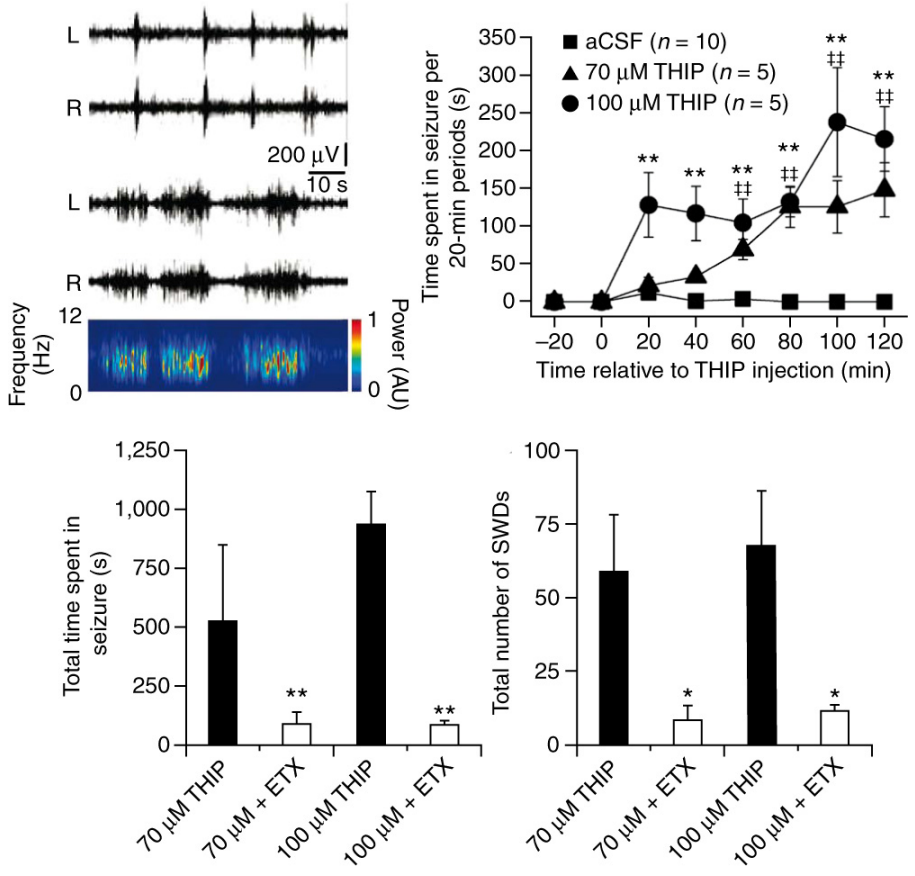
Received 21 October 2008; accepted 15 October 2009; published online 22 November 2009; doi:10.1038/nm.2058



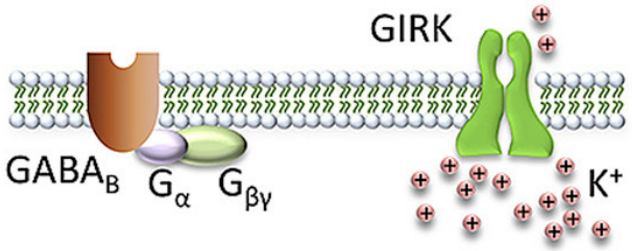
## THIP Induces SWDs in normal Wistar rate

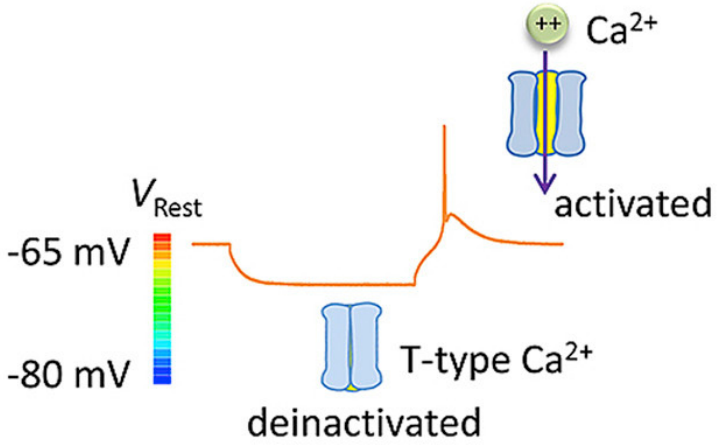


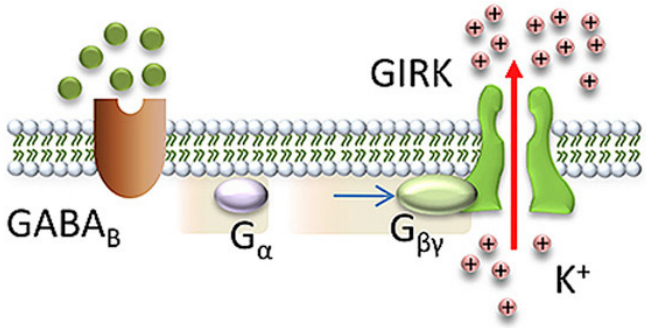

Elevated GABA levels in **GAERS rats** in ventral thalamus because of reduced uptake; also in **stargazer** and **lethargic** mice

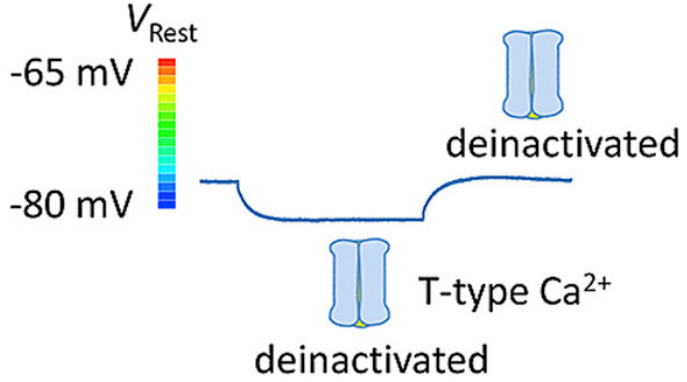
Enhanced tonic GABA current through activation of extrasynaptic GABA-A receptors

# $GABA_B$ Receptors



# Proepileptic Activity of Baclofen is Due to Disinhibition

## $GABA_B$ autoreceptor-mediated cell type-specific reduction of inhibition in epileptic mice

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Contributed by Nancy J. Kopell, July 20, 2013 (sent for review February 2, 2013)

**$GABA_B$ receptors ($GABA_BRs$) mediate slow inhibitory effects on neuronal excitability and synaptic transmission in the brain. However, the $GABA_BR$ agonist baclofen can also promote excitability and seizure generation in human patients and animals models. Here we show that baclofen has concentration-dependent effects on the hippocampal network in a mouse model of mesial temporal lobe epilepsy. Application of baclofen at a high dose (10 mg/kg i.p.) reduced the power of γ oscillations and the frequency of pathological discharges in the Cornu Ammonis area 3 (CA3) area of freely moving epileptic mice. Unexpectedly, at a lower dose (1 mg/kg), baclofen markedly increased γ activity accompanied by a higher incidence of pathological discharges. Intracellular recordings from CA3 pyramidal cells in vitro further revealed that, although at a high concentration (10 μM), baclofen invariably resulted in hyperpolarization, at low concentrations (0.5 μM), the drug had divergent effects, producing depolarization and an increase in firing frequency in epileptic but not control mice. These excitatory effects were mediated by the selective muting of inhibitory cholecystokinin-positive basket cells ($CCK^+$ BCs), through enhanced inhibition of GABA release via presynaptic $GABA_BRs$. We conclude that cell type–specific up-regulation of $GABA_BR$-mediated autoinhibition in $CCK^+$ BCs promotes aberrant high frequency oscillations and hyperexcitability in hippocampal networks of chronic epileptic mice.**

presynaptic inhibition | mTLE model | patch clamp

Neuronal activity in the hippocampus shows oscillations in behavior-relevant frequency ranges including γ frequencies (30–80 Hz) (1). γ activity is prominent in the aroused brain and has been implicated in higher-level brain functions, such as sensory binding, perception (2), and storage and recall of information (3, 4). At the same time, γ frequency oscillations are also prevalent in epileptic patients and are most often observed at seizure onset during in depth EEG recordings (5). The GABAergic system plays a pivotal role in the generation of γ oscillations (6–8). However, it remains to be resolved how distinct GABAergic receptor subtypes, in particular $GABA_B$ receptors ($GABA_BRs$), contribute to the generation and modulation of pathological network oscillatory activity.

$GABA_BRs$ mediate slow inhibitory effects and control synaptic transmission and the excitability of neurons in cortical networks. $GABA_BRs$ are expressed both postsynaptically in somatodendritic compartments and presynaptically in axon terminals, in excitatory principal cell and inhibitory interneurons (9–11). The effects of $GABA_BR$ activation on the network are dominated by inhibition leading to an overall dampened population activity. However, if GABAergic interneurons are effected dominantly, as observed for example, during high-frequency stimulation, $GABA_BR$ activation can produce disinhibition in principal cells (12, 13). Accordingly, the role of $GABA_BRs$ in epilepsy and seizure generation remains ambiguous. $GABA_BRs$ are expected to have an overall antiepileptic effect, and indeed, the receptor KO animals show an epileptic phenotype (14). However, there is also evidence that the receptor agonist baclofen can induce seizures in patients after intrathecal application (15, 16). The picture is further complicated by the fact that $GABA_BR$ expression can be altered in both epileptic patients, e.g., in mesial temporal lobe epilepsy (mTLE) (17), and animal models (18). Thus, cell type–specific alterations in $GABA_BR$ expression may change network excitability during the progression of mTLE.

Using a chronic kainate (KA) model of mTLE, which reproduces major electrophysiological and histopathological characteristics of human mTLE (19, 20), we studied the role of $GABA_BRs$ in altered hippocampal network activity. Our results suggest that enhanced and persistent $GABA_BR$ activation in epileptic mice suppresses the inhibitory output from hippocampal interneurons, in particular cholecystokinin (CCK)-expressing basket cells (BCs) onto pyramidal cells (PCs). This reduction in the inhibitory output of interneurons, in turn, leads to disinhibition in hippocampal networks, enhances γ activity, and promotes the transition to pathological hyperexcitability.

### Results

**Dose-Dependent Effects of $GABA_BR$ Activation on Hippocampal Network Activity in a Mouse Model of mTLE in Vivo.** We first analyzed the effects of $GABA_BR$ activation on network activity in vivo in chronic epileptic mice. EEG recordings were carried out

### Significance

Metabotropic $GABA_B$ receptors control synaptic transmission and excitability in neuronal circuits of the brain. Although effects of these receptors are predominantly inhibitory at both cellular and network levels, application of the agonist baclofen can promote excitability and induce seizures in patients and animal models of epilepsy. Here we demonstrate that proepileptic effects of baclofen are concentration dependent and result from disinhibition. Although at high doses, baclofen reduces network excitability due to its combined pre- and postsynaptic inhibitory effects in pyramidal cells, at low doses, it leads to an enhanced presynaptic suppression of the synaptic output of a specific set of inhibitory neurons. This disinhibitory effect promotes high-frequency oscillations and the emergence of pathological discharges in the epileptic hippocampal network.

Author contributions: T.G. designed research; T.D., N.M., C.B., S.G., U.H., A.W., and T.G. performed research; U.H. and C.A.H. contributed new reagents/analytic tools; T.D., N.M., C.B., S.G., J.C.M., I.V., N.J.K., and T.G. analyzed data; and T.D., C.B., J.C.M., I.V., N.J.K., and T.G. wrote the paper.

The authors declare no conflict of interest.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1313505110/-/DCSupplemental.



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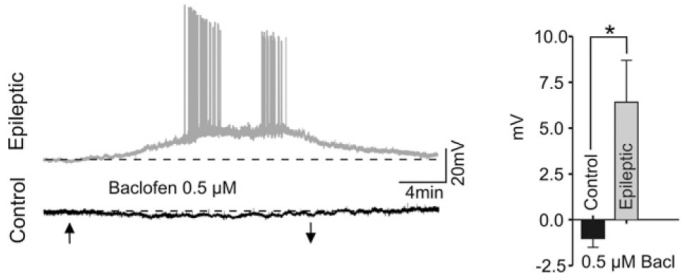
## Chronic kainate model mTLE




- Baclofen augments hippocampal γ oscillations and promotes pathological discharge in the CA3 network of chronic epileptic mice in a dose-dependent manner
- Reduced inhibition of CA3 PCs and enhanced presynaptic $GABA_B$ receptor activation
- Enhanced activation of presynaptic $GABA_B$ receptors leads decreased GABA release and consequent disinhibition and hyperexcitability of pyramidal cells.

*Neuropharmacology* Vol. 29, No. 7, pp. 649–655, 1990
Printed in Great Britain. 

0028-3908/90 $3.00 + 0.00


# CHARACTERIZATION OF GABAERGIC SEIZURE REGULATION IN THE MIDLINE THALAMUS

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(*Accepted* 29 *November* 1989)

**Summary**—This study characterized the role of GABA in the central medial intralaminar nucleus on seizures induced by pentylenetetrazol given systemically. Injections of the direct selective $GABA_A$ agonist, piperidine-4-sulfonic acid or the indirect $GABA_A$ agonists, flurazepam and pentobarbital, in this region depressed arousal and facilitated myoclonic and clonic seizures induced by pentylenetetrazol but only caused slight inhibition of tonic seizures. In contrast the $GABA_B$ agonist (−)baclofen facilitated all three types of seizures. Recording after injection of piperidine-4-sulfonic acid and (−)baclofen revealed marked suppression and slowing of thalamic and cortical electrical activity. Thalamic injections of the $GABA_A$ antagonist, bicuculline methiodide, had opposite behavioral effects, causing hyperactivity and episodes of violent running, not accompanied by EEG discharges. When pentylenetetrazol was infused concommitantly there was marked facilitation of the tonic seizures, which occurred without preceding myoclonic of clonic seizures, or EEG spikes.

These results demonstrate that GABA-mediated neurotransmission in the central medial intralaminar nucleus can control the threshold of seizures and that GABA agonists and antagonists have opposite effects. It is suggested that the central medial intralaminar nucleus is not a site of origination or spread of seizures, but controls seizures indirectly by regulating the excitability of other structures and that different synaptic mechanisms and anatomical connections mediate effects on different types of seizures.

*Key words*—pentylenetetrazol, central medial nucleus, GABA, seizure, epilepsy, thalamus, arousal.

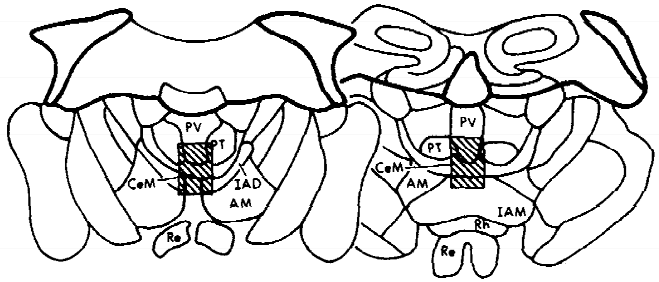
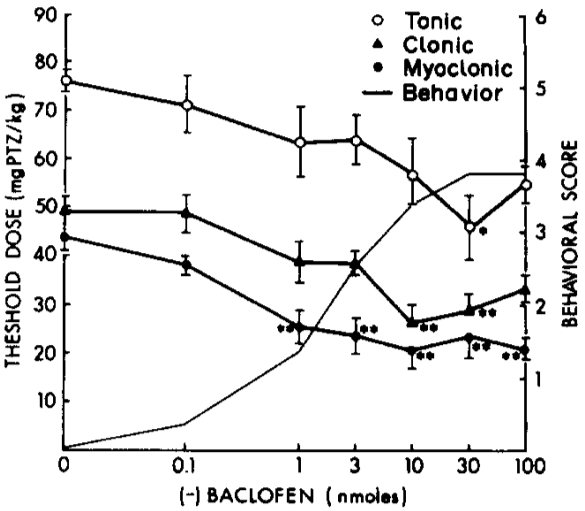
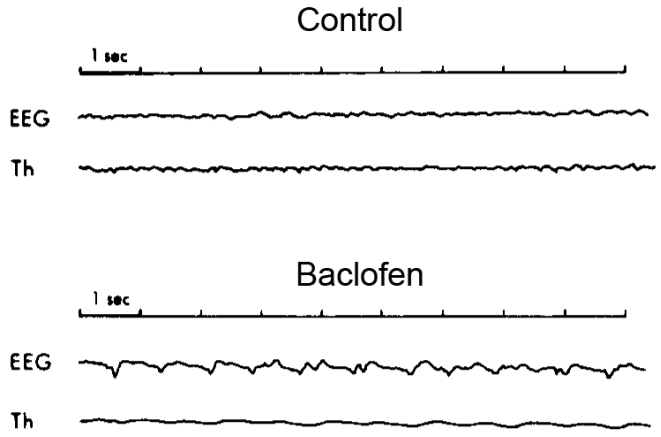



Fig. 1. Diagram showing the target zone in the central medial nucleus in the thalamus. For inclusion in the study, the entire blue injection site had to be confined to this zone. Abbreviations are as follows: AM—anteromedial nucleus; CeM—central medial nucleus; IAD—interanterodorsal nucleus; IAM—interanteromedial nucleus; PT—paratenial nucleus; PV—paraventricular nucleus; Re—reuniens; Rh—rhomboid nucleus.

## Injection of Baclofen into Midline Thalamus



# The End