Anti-absence activity of mGlu1 and mGlu5 receptor enhancers and their interaction with a GABA reuptake inhibitor: effect of local infusions in the somatosensory cortex and thalamus

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SUMMARY

Objective: Glutamate and GABA are the key neurotransmitter systems in the cortico-thalamo-cortical network, involved in normal and pathological oscillations such as spike-wave discharges (SWDs) characterizing different forms of absence epilepsy. Metabotropic glutamate (mGlu) and GABA receptors are widely expressed within this network. Here, we examined the effects of two selective positive allosteric modulators (PAMs) of mGlu1 and mGlu5 receptors, the GABA reuptake inhibitor, tiagabine, and their interaction in the somatosensory cortex and thalamus on SWDs in WAG/Rij rats.

Methods: Male WAG/Rij rats were equipped with bilateral cannulas in the somatosensory cortex (S1po) or the ventral basal complex of the thalamus (VB), and with cortical EEG electrodes. Rats received a single dose of the mGlu1 receptor PAM, RO0711401, or the mGlu5 receptor PAM, VU0360172, various doses of tiagabine, or VU0360172 combined with tiagabine.

Results: Both PAMs suppressed SWDs regardless of the site of injection. Tiagabine enhanced SWDs when injected in the thalamus, but, unexpectedly, suppressed SWDs in a dose-dependent manner when injected in the cortex. Intracortical co-injection of VU0360172 and tiagabine produced slightly larger effects as compared to either VU0360172 or tiagabine alone. Intrathalamic co-injections of VU0360172 and sub-threshold doses of tiagabine caused an anti-absence effect similar to that exhibited by VU0360172 alone in the first 10 min. At 30 min, however, the anti-absence-effect of VU0360172 was prevented by sub-threshold doses of tiagabine, and the combination produced a paradoxical pro-absence effect at 40 and 50 min.

Significance: These data (i) show that mGlu1 and mGlu5 receptor PAMs reduce absence seizures acting at both thalamic and cortical levels; (ii) demonstrate for the first time that tiagabine, in spite of its established absence-enhancing effect, reduces SWDs when injected in the somatosensory cortex; (iii) indicate that the efficacy of VU0360172 in the thalamus may be critically affected by the availability of (extra)synaptic GABA.

Key words: Glutamate, GABA; Absence Epilepsy; WAG/Rij rats; mGlu PAM

INTRODUCTION

Dysfunction in either glutamatergic or GABAergic neurotrasmission is known to be one of the causes responsible for the initiation and spread of seizures, including absence epilepsy. Known as the electroencephalographic hallmark of absence seizures, the archetypical spike-wave discharges (SWDs) are initiated in the deep layers of the somatosensory cortex and quickly spread to the cortico-thalamo-cortical (C-T-C) network.[1,2] This network consists of glutamatergic projections from the deep layer cortical neurons to ventrobasal (VB) thalamic nuclei including the S1po, and to the reticular thalamic nucleus (nRT); glutamatergic projections from VB thalamic nuclei to the cortex and nRT; and GABAergic projection from the nRT to VB thalami nuclei.[2,3] Several mGlu receptor subtypes appear strategically distributed at the synapses of the C-T-C loop. In the cortex, group-I mGlu receptors (namely mGlu1 and mGlu5 receptors) are expressed post-synaptically on GABAergic interneurons.[4,5,6] At thalamic level, group I mGlu receptors are present post-synaptically on glutamatergic neurons of the VB complex reviewed by Ngomba et al., 2011.[7] These neurons express moderate levels of mGlu5 receptor immunoreactivity,[8] while the mGlu1 receptors mainly reside in the perisynaptic area.[9,10,11,12] Modulatory effects of individual mGlu receptor subtypes on both excitatory and inhibitory synaptic transmissions in the C-T-C circuit have been found, and subtype-selective mGlu receptor ligands were proposed as potential candidates for novel antiabsence drugs.[7]

Studies with mGlu receptor ligands have been conducted in WAG/Rij rats, which develop spontaneous absence seizures after 2-3 months of age.[7] Systemic administration of compounds RO0711401 and VU0360172, which behave as selective positive allosteric modulators (PAMs) of mGlu1 and mGlu5 receptors, respectively, decrease the incidence of SWDs in symptomatic WAG/Rij rats.[13,14] No tolerance develops to the anti-absence activity of VU0360172, whereas the activity of RO0711401 declines after the first three days of repeated administrations.[15] Where precisely within the C-T-C circuit activation of mGlu1 or mGlu5 receptors reduces SWDs is unknown. Here, we have addressed this question by locally injecting either VU0360172 or RO0711401 in the VB part of the thalamus or in the somatosensory cortex.

Next to the role of glutamate, GABA also holds a key position in the control of SWDs. It is well known that systemically administered GABA-mimetics, such as tiagabine, acting by blocking the reuptake of GABA *via* the high affinity GABA transporter, GAT-1,[16] aggravate the

incidence of SWDs.[17] Here, we also investigated whether GABAergic regulation of absence seizures differs in the thalamus and cerebral cortex. WAG/Rij rats represent an appropriate model to examine this issue because in these rats as well as in GAERS (genetic absence epileptic rats of Strasbourg, [18] a high excitability of S1po is a prerequisite for the initiation of SWDs [1,2] that are then sustained by an enhanced tonic GABAergic inhibition in the thalamus.[19,20] Finally, using tiagabine, we examined whether, and in which direction, an increased availability of (extra)synaptic GABA influences responses to the mGlu5 receptor PAM, VU0360172, in the thalamus and somatosensory cortex.

Materials and Methods

Animals

One hundred and thirty one male WAG/Rij rats were used for all experiments. Of these, eight animals were excluded because of a wrong position of the cannulas either in the cortex or in the thalamus. All rats were born and raised at Radboud University Nijmegen, The Netherlands, and had a mean body weight of about 350 g at 9 months of age. Rats of this age have about 16–20 SWDs per hour, adding several hundred SWDs per day.[21] The animals were housed in pairs in Macrolon cages, kept under controlled conditions (20ºC, 60% humidity) in a room with a reversed light–dark cycle (white light on from 9 p.m. to 9 a.m.), with food and drinking water always available. After surgery, rats were kept individually. Animals were handled regularly before starting EEG registrations to reduce handling stress imposed by the local injections. The study was performed in accordance with the guidelines of the European Community for the use of experimental animals and was approved by local ethics committee for animal studies (RU-DEC). All efforts were made to reduce discomfort experienced by the animals and to keep the number of animals as low as possible.

Drugs and experimental protocol

VU0360172 (N-clyclobutyl-6-[2-3(fluorophenyl) ethynyl] pyridine-3-carboxamine), a selective mGlu5 receptor PAM, was obtained from Vanderbilt University Medical Center.[22] RO0711401 (9H-xanthene-9-carboxylic acid (4-trifluoromethyl-oxazol-2-yl) amide, a selective mGlu1 receptor PAM, was kindly provided by Hoffmann-La Roche (Basel, Switzerland). Tiagabine (Hydrochloride, monohydrate), a GABA-reuptake inhibitor, was purchased from Siegfried Chemie AG.

In all 3 experiments (Table 1), bilateral microinfusions in the peri-oral region of the somatosensory cortex (S1po) or in the VB thalamic nuclei were performed using artificial cerebro spinal fluid (ACSF) as vehicle. Drugs were soluble in ACSF at concentrations of 1 mg/ml (VU0360172, RO0711401) or 2 mg/ml (tiagabine). In experiments with single injections, drugs were always microinfused in a volume of 1 μl. Doses were 1 μg for VU0360172 and RO0711401, and 0.5, 1 or 2 μg for tiagabine. In experiments in which VU0360172 and tiagabine were co-administered, the two drugs were infused with a 3 min interval. In the S1po, both VU0360172 and tiagabine were infused at doses of 1 μg in a volume of 1 μl (i.e., 1 μl + 1 μl with 3 min of interval). In the thalamus, VU0360172 was infused at the dose of 1 μg whereas tiagabine was infused at the dose of 0.5 μg (always in a volume of 1 μl). ACSF was infused twice with 3 min interval in control animals. Infusions were performed by means of a Hamilton syringe at a flow rate of 1 μl per min and the injection needle remained in the cannula after each single injection for 2 min to prevent backflow. The needle necessary for doing local injections did not protrude from the guide cannulas in order to avoid a spreading depression induced by the local injection.[23]

In all experiments, cortical EEG was recorded and motor behavior was quantified as detailed in the Supporting Information. Surgical procedures, coordinates used for the implantation of guide cannulas, and the procedure we have used to determine the position of the guide cannulas are described in detail in the Supporting Information.

Statistical analysis

The effects of intracortical or intrathalamic drug injections on the incidence and mean duration of SWDs, and locomotor activity were tested in separate repeated-measures ANOVAs with incidence and mean duration of SWDs or amplitude of the PIR as dependent variables. The hourly incidence (number) and mean of SWDs in the 2 hours before injection were determined and differences between groups were analyses with a simple one-factor ANOVA (groups) for all three experiments. For all post-injection analyses, the time of EEG recording (6x10 minutes blocks post injection) was used as the within subjects factor, group (either site of injection of different drugs) was used as between subjects factor. In Experiment 2 and 3 repeated measures were conducted as well, but now different doses of tiagabine in cortex and thalamus (Experiment 2), or the different groups (Experiment 3 co-injection group, ACSF + ACSF), were used as between subjects factors, all followed by post-hoc tests, if appropriate. One way ANOVAs and Duncan post-hoc tests were used to isolate differences between the locations, doses or different drug treated groups at the different 10 minute episodes. Results are expressed as mean ± SEM and calculations were obtained using SPSS 19 software. The level of statistical significance was set at $p < .05$.

Results

Effects of microinfusion of VU0360172, RO0711401 or ACSF in the S1po cortex and ventrobasal thalamus on absence seizures in WAG/Rij rats

EEG was recorded in symptomatic WAG/Rij rats at baseline and following bilateral microinfusion of VU0360172 (1 μg/μl), RO0711401 (1 μg/μl) or vehicle (ACSF, 1 μl) in the S1po region of the somatosensory cortex or in the VB thalamus. A representative trace of a typical SWDs recorded by EEG is shown in Fig. 1A. No significant differences were found in the two hours preceding drug injections among the 6 groups of animals receiving each of the two drugs or vehicle in either the S1po or the VB thalamus with respect to the incidence of SWDs (F=2.048, df 5,45, p =.086, η^2=.16), mean duration of SWDs (F=0.460, df 5,45, p =.804,

η^2=.04), and locomotor activity (measured as the amplitude of the PIR) (*F*=1.607, df 5,45, *p* =.174, η^2=.13).

EEG was recorded for two more hours post drug or vehicle injections in the S1po or the VB thalamus, and the incidence and mean duration of SWDs was measured in blocks of 10 min. Two-way ANOVA for repeated measures applied to the analysis of the incidence of SWDs in blocks of 10 min after bilateral injections of VU0360172 (n = 8), RO0711401 (n = 8), or vehicle (n = 9) in the S1po showed a time effect (F= 69.25, df 5,155, p< .001, η^2= .691), a drug effect (F=29.56, df 2, 31, p < .001, η^2=.656) and a significant interaction between time and drugs (F=10.28, df 10,155 p <.001, η^2=.399). Post-hoc tests revealed that both VU0360172 and RO0711401 reduced SWD incidence in the first 40 min post-injection with no difference between the two drugs at all time-points (Figure 1B).

The same analysis on SWD incidence after injections of VU0360172 (n = 9), RO0711401 (n = 8) or vehicle (n = 9) in the VB thalamus revealed a time effect (F=39.48, df 5,224, p< .001, η^2= .552), a drug effect (F=44.61, df 2,32, p< .001, η^2=.736), and a significant interaction effect between time and drugs (F=5.48 df 14,224 p< .001, η^2=.255), (Figure 1C). Post-hoc analyses across the six 10-min blocks showed varying differences across time. VU0360172 was more (p<.001) effective than RO0711401 in suppressing the incidence of SWD in the first 10 min post-injections, with both drugs showing significant effects as compared to the ACSF control group. Between 10-20 and 20-30 min post-injections, RO0711401 and VU0360172 were equally effective in suppressing SWDs. Between 30 and 50 min post injection RO0711401 lost its activity whereas VU0360172 still significantly reduced the incidence of SWDs.

Differences in the efficacy of VU0360172 injected in the cortex and thalamus were found only after 50 min post-injection (F=14,205, df 2,32, p < .001, η^2=.470), when the SWD-suppressing effects of VU0360172 were larger in the thalamus. This difference was no longer observed at 60 min post-injection. No statistical difference was found between intrathalamic and intracortical injection of RO0711401 with respect to the incidence of SWD.

None of the treatments caused significant differences in the mean duration of SWDs (Fig. 1D,E) and in locomotor activity (Fig. 1F,G).

Intracortical and intrathalamic injections of tiagabine produced opposite effects on absence seizures in WAG/Rij rats.

Tiagabine was injected at doses of 1 or 2 μg in the S1po (n = 8 in both groups), and 0.5, 1, or 2 μg in the VB thalamus (n = 4, 5, and 5, respectively). Control rats received ACSF (n = 8 in both S1po and thalamus). There were no differences between the groups before drug administration. Intracortical injection of tiagabine reduced the incidence of absence seizures. Two-way ANOVA for repeated measures applied to the analysis of the incidence of SWDs after cortical injections of tiagabine or vehicle showed a time effect ($F=107.93$, df 5,105, $p< .001$, $\eta^2=.837$), with a large reduction of SWDs in the first 40 minutes post injection (Fig. 2A). There was also a dose effect ($F=164.69$, df 2, 21, $p< .001$, $\eta^2=.940$), and an interaction between time and dose ($F=42.77$, df 10,105, $p< .001$, $\eta^2=.803$). Post-hoc analyses showed various differences across the six 10-min blocks: Between 10-40 minutes post-injection, the high dose of tiagabine was more effective in suppressing SWDs compared to the low dose of tiagabine and to ACSF. The low dose also suppressed SWDs during the first 30 min, as compared to ACSF. The low and high doses of tiagabine lost their anti-absence effect at 40 and 50 min post-injections, respectively.

The increase in the incidence of SWDs was obtained after intrathalamic injection of tiagabine. Two-way ANOVA for repeated measures showed a time effect ($F= 73.643$, df 5, 80, $p< .001$, $\eta^2= .822$), with an increase in the incidence of SWDs being present in the first 40 minutes after injection of tiagabine (Fig. 2B). There was also a dose effect ($F= 656.218$, df 3, 16, $p< .001$, $\eta^2=.992$) and an interaction between time and dose ($F=45.84$, df 15, 80 $p< .001$, $\eta^2=.896$). Post-hoc analysis across the six 10-min blocks showed that, between 10 and 30 min post-injection, the highest dose of tiagabine (2 μg) increased the incidence of SWDs to a greater extent than the mid dose (1 μg). The lowest dose of tiagabine (0.5 μg) was inactive. At 40 min post injection, only the highest dose of tiagabine was still effective in reducing the incidence of SWDs.

Treatment with tiagabine did neither change the mean duration of SWDs (Fig. 2C,D) nor locomotor activity scores (Fig. 2E,F).

Effects of combined injections of VU0360172 and tiagabine in the cortex and thalamus

In all experiments, injection of VU0360172 (1 μg in both S1po and VB thalamus) preceded by 3 min the injection of tiagabine (1 μg in the S1po, and 0.5 μg in the VB thalamus; n = 8 and 9, respectively). Control rats received two sequential injections of ACSF in the S1po or in the VB thalamus (n = 8 and 9, respectively). There were no differences between the groups before drug administration. Data obtained after intracortical injections are shown in Fig. 3A. Two-way ANOVA for repeated measures after injections of VU0360172 + tiagabine and ACSF + ACSF showed a time ($F= 14.132$, df 5,70, $p< .001$, $\eta^2= .502$), and a group effect ($F= 102.86$, df 1,14, $p< .001$, $\eta^2= .880$). In the first 10-40 min post injection, the combination of VU0360172 and tiagabine substantially reduced the incidence of SWDs with respect to the control group. In the first 20 min post-injection the reduction in the incidence of SWDs was greater than that previously observed with VU0360172 alone (-67% and -72% with VU0360172 *plus* tiagabine at 10 and 20 min post-injection, respectively, in Fig. 3A vs. -57% and -60% with VU0360172 alone at 10 and 20 min post-injection, respectively, in Fig. 1B). At 50 and 60 min there was a slight reduction in the incidence of SWDs, with no significant difference from the control group.

Data obtained after intrathalamic injections of VU0360172 (1 μg/μl) and tiagabine (0.5 μg/μl) are shown in Fig. 3B. Here, there was also a time effect (blocks) ($F= 5.23$, df 5,80, $p< .001$, $\eta^2= .231$), and an interaction between time and group ($F=2.90$, df 5,80 $p<.001$, $\eta^2=.154$). Post-hoc analysis across the six 10-min blocks showed that in the first 10 min post-injection, the combination between VU0360172 and tiagabine reduced the incidence of SWDs compared to 2 x ACSF. Combination of the two drugs was ineffective at 20 min and caused a significant increase in the incidence of SWDs at 30 and 40 min post injection, again in comparison with 2 x ACSF.

None of these treatments had any effect on the mean duration of SWDs (Fig. 3C,D) and locomotor activity (Fig. 3E,F).

Discussion

Our experiments were a follow-up from the evidence that systemic administration of group-I mGlu receptor PAMs reduces the incidence of SWDs in WAG/Rij rats.[7,13,14] Here, we showed that this effect could be induced by injecting mGlu1 or mGlu5 receptor PAMs in both the S1po and ventrobasal thalamic nuclei. There was a difference in the thalamic and cortical response to RO0711401 and VU0360172. The two drugs were equally effective in reducing SWDs when injected in the cortex; in contrast, the mGlu5 PAM, VU036012, displayed a greater efficacy than the mGlu1 PAM, RO0711401, when injected in the thalamus. The somatosensory cortex is the main site of origin of SWDs in WAG/Rij and GAERS rats,[1] and this region is highly excitable. [2] In the somatosensory cortex, mGlu1α receptors are expressed by somatostatin-positive, calretinin-positive, and calbindin-positive, but not by fast-spiking parvalbumin-positive, GABAergic interneurons.[4,5,6] mGlu5 receptors are found in both somatostatin-positive and parvalbumin-positive GABAergic interneurons,[5,6] as well as in pyramidal neurons.[24] Because the efficacy of intracortically injected VU0360172 and RO0711401 was identical, it is possible that a cell type expressing both mGlu1 and mGlu5 receptors is a common target for the two PAMs. We hypothesize therefore that activation of either mGlu1 or mGlu5 receptors expressed by somatostatin-sensitive and other types of regular spiking GABAergic interneurons suppresses SWDs by enhancing GABAergic inhibition onto pyramidal neurons. This hypothesis is consistent with the finding that intracortical injection of tiagabine, which inhibits the high affinity GABA transporter, GAT-1, reduced SWDs. This leaves us with the suggestion that enhancing mGlu1/5-mediated activation of cortical GABAergic interneurons or inhibiting GABA reuptake would produce the same effect. Hence, it is not surprising that intracortical injection of VU0360172 *plus* tiagabine reduced SWDs to a slightly larger extent than injection of VU0360172 alone.

Both mGlu1 and mGlu5 receptors are present postsynaptically on VB thalamic neurons, and only mGlu5 receptors are found at moderate levels in GABAergic neurons of the reticular thalamic nuclei (reviewed by Ngomba et al., 2011)[7]. Both mGlu1 and Glu5 receptors are coupled to Gq/11, and their activation stimulates phospholipase-Cβ (PLC-β) with ensuing hydrolysis of phosphatidylinositol-4,5-bisphosphate and formation of inositol-1,4,5-trisphosphate and

diacylglycerol (reviewed by Nicoletti et al., 2011). [25] Mutations of PLC-β4 at thalamic level have been shown to enhance bursting of thalamo-cortical projection neurons in a T-type Ca^{2+} channel-dependent fashion, resulting in absence epilepsy.[26] Thus, activation of either mGlu1 or mGlu5 receptors present in VB thalamic neurons might restrain the occurrence of absence seizures by negatively regulating the activity of T-type Ca^{2+} channels on thalamo-cortical cells. However, intrathalamic injection of VU036172 caused a more robust and prolonged suppression of SWDs as compared to intrathalamic injection of RO0711401, suggesting that an additional mechanism that is peculiar to mGlu5 receptors contributes to restrain the occurrence of SWDs. A major difference between mGlu1 and mGlu5 receptors is that only the latter are found on astrocytes, both in the thalamus and cerebral cortex.[27,28] Astrocytes are key players in the regulation of thalamic oscillations because they clear extracellular glutamate and GABA, thereby limiting the activation of extrasynaptic glutamate and GABA receptors.

An increased inhibitory GABAergic transmission at the synapses between reticular thalamic neurons and VB thalamic neurons sustains the occurrence of SWDs (reviewed by Blumenfeld, 2005) [2], and this explains the pro-absence effect seen after systemic administration of drugs that enhance GABAergic transmission, such as tiagabine, vigabatrin, and the neurosteroid allopregnanolone in both GAERS and WAG/Rij rats.[16,29,18,30] Intrathalamic injections of the GABA-transaminase inhibitor, γ-vinyl GABA, the $GABA_A$ receptor agonist, muscimol, or the neurosteroids, allopregnanolone or ganaxolone also increase the incidence of SWDs.[31,32] Tiagabine enhances tonic inhibition of thalamic relay neurons by inhibiting GABA re-uptake, and, therefore, facilitating the endogenous activation of extrasynaptic GABA receptors.[33,19,34] Hence, the enhanced incidence of SWDs found after intrathalamic injection of tiagabine was fully expected and suggests that the pro-absence activity in the thalamus prevails over the anti-absence activity in the cortex after systemic injection of tiagabine.

Experiments in which the mGlu5 receptor PAM, VU036172 was co-injected with sub-threshold doses of tiagabine in the VB thalamus produced interesting results. If combined with tiagabine, VU036172 was still effective in reducing the incidence of SWDs in the early post-injection time (10 min), but then lost this effect at 20 min, and displayed a paradoxical pro-absence effect at 30 and 40 min. This suggests that the regulation of thalamic oscillations by mGlu5 receptors is

shaped by extracellular GABA, or, alternatively, that the mGlu5 receptor controls the activity of the GABA transporter, GAT-1, in astrocytes or in GABAergic fibers afferent to VB thalamic nuclei. Activation of glial mGlu5 receptors is known to regulate the expression of the glial glutamate transporters, GLT-1 and GLAST.[35] If this regulation extends to GAT-1, and we may suggest the following scenario. Pharmacological enhancement of mGlu5 receptors produces pleiotropic effects in the VB thalamus, including an enhanced clearance of extracellular GABA, which might be critical for the suppression of SWDs. In the presence of a GAT-1 inhibitor (e.g., tiagabine) activation of mGlu5 receptors will not stimulate GABA reuptake, and the progressive increase in extracellular GABA will block the anti-absence action mediated by mGlu5 receptor stimulation. Studies on GABA uptake in cultured astrocytes or studies on tonic inhibition in thalamic slices in epileptic and non-epileptic rats are needed to verify this hypothesis.

In conclusion, group I metabotropic receptors are involved in the control of absence seizures through the entire C-T-C network that is responsible for the pathological oscillations associated with SWDs. However, the consequences of stimulation are site dependent: stimulating group I mGlu receptors in the cortex may enhance GABA-ergic inhibition onto pyramidal neurons and reduces SWDs. Stimulation of group I mGlu receptors in thalamic relay neurons might reduce tonic inhibition thereby reducing SWDs. In the presence of tiagabine, activation of thalamic mGlu5 receptors will not stimulate GABA reuptake, and the progressive increase in extracellular GABA will abolish the anti-absence effect mediated by mGlu5 receptor stimulation. A schematic diagram illustrating our hypothesis on the regulation of absence seizures by cortical and thalamic group-I mGlu receptors and the potential interaction with GABAergic transmission is shown in Fig. 4.

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Disclosure statement: Authors declare that there are no conflicts of interest to be disclosed. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Figure legends

Fig. 1. Pharmacological enhancement of mGlu or mGlu5 receptors in the somatosensory cortex or VB thalamus inhibits absence seizures in WAG/Rij rats.

A representative EEG recording of a typical SWD episode is shown in (A). The incidence of SWDs (in blocks of 10 min) in WAG/Rij rats locally infused in the S1po cortex with VU0360172 (1µg; *n* = 8), RO071140 (1µg; *n* = 8), or ACSF (n = 9) is shown in (B). The incidence of SWDs after infusion of VU0360172 (1µg; *n* = 9), RO071140 (1µg; *n* = 8), or ACSF (n = 9) in the ventrobasal thalamus is shown in (C). The injection volume was always 1 µl. Values are means + S.E.M. $p < 0.05$ (Two-way ANOVA + Duncan's t test) compared to the corresponding ACSF values (*), or the corresponding VU0360172 values (#). The corresponding values of mean duration of SWDs and locomotor activity (means + S.E.M.) are shown in (D, E) and (F,G), respectively.

Fig. **2 –** Effect of tiagabine locally infused in the somatosensory cortex or ventrobasal (VB) thalamus on absence seizures in WAG/Rij rats.

The effects of two doses of tiagabine (1µg; n=8; or 2µg; *n* = 8) or ACSF (*n* = 8) infused in the S1po cortex, and the effects of three doses of tiagabine (0.5 µg/µl; n=4; 1µg/µl; n=5; 2µg/µl; *n* = 5) or ACSF (1 µl; *n* = 8) insued in the VB thalamus are shown in (A) and (B), respectively. The injection volume was always 1 µl. Values are means + S.E.M. $p < 0.05$ (Two-way ANOVA + Duncan's t test) compared to the corresponding ACSF values (*), to the corresponding values obtained with 1 µg tiagabine (#) in (A), or to the corresponding values obtained with 0.5 µg tiagabine (§) in (B). In (B), the same symbol (either * or §) is referred to the overlapping values obtained in rats treated with 1 or 2 µg of tiagabine. The corresponding values of mean duration of SWDs and locomotor activity (means + S.E.M.) are shown in (C,D) and (E,F), respectively.

Fig. 3 – Combined effect of VU0360172 and tiagabine on the incidence of absence seizures in WAG/Rij rats.

The effects of intracrotical injections of VU0360172 (1 μg) *plus* tiagabine (1 μg) (n = 8) or ACSF + ACSF (n = 8) on the incidence of SWDs are shown in (A); the effects of intrathalamic injections of VU0360172 (1 μg) *plus* tiagabine (0.5 μg) (n = 9) or ACSF| + ACSF (n = 9) are shown in (B). Values are means + S.E.M. Values are means + S.E.M. **p*< 0.05 (Two-way ANOVA + Duncan's t test) vs. the corresponding ACSF values. The corresponding values of mean duration of SWDs and locomotor activity (means + S.E.M.) are shown in (C,D) and (E,F), respectively. VU0360172 and tiagabine were microinfused with 3 min of interval. Control rats received two injections of ACSF with 3 min of interval.

Fig. 4 – Mechanistic hypothesis of the role played by cortical (A) or thalamic (B) mGlu1 and mGlu5 receptors in the modulation of absence seizures. The diffusion of either GABA (rounded dots and arrows in green) or Glutamate (rounded dots and arrows in red) within and out of the synaptic terminals and glial cells are depicted.

In the S1po cortex, pharmacological enhancement of mGlu1 or mGlu5 receptors might reduce absence seizures by activating somatostatin (SST)-positive GABAergic interneurons, and tiagabine might produce the same effect by enhancing GABA levels at the synapses between between SST^+ interneurons and pyramidal neurons.

In the ventrobasal thalamus, tiagabine enhances absence seizures by increasing synaptic and extrasynaptic GABA levels, thereby facilitating the activity of T-type voltage-sensitive Ca^{2+} channels. mGlu1 and mGlu5 receptors might protect against absence seizures by directing modulating T-type Ca^{2+} channels (modulation of T-type channels by phospholipase C has been reported[26]) or through a blockade of the glial glutamate transporter[35]. Tiagabine might act *via* blockade of the presynaptic and glial GABA transporter.

Data on the interaction between VU0360172 and tiagabine raise the possibility that mGlu5 receptors regulate the expression or activity of GAT-1 in GABAergic terminals and/or astrocytes.

Table 1. Overview of the 3 local injection experiments with drugs, doses, and side of drug application.

The injection volume was always 1 µl for each drug or ACSF injection.

	Drug	Dose	Brain region
Experiment 1	VU0360172	1 µg (n=8 & n=9)	S1Po & VB
	RO0711401	1 µg (n=8 & n=8)	S1Po & VB
	ACSF	(n=9 & n = 9)	S1Po & VB
Experiment 2	Tiagabine	1 µg (n=8) 2 µg (n=8)	S1Po
	Tiagabine	0.5 µg (n=4) 1 µg (n=5) 2 µg/µl (n=5)	VB
	ACSF	(n=8 & n=8)	S1Po & VB
Experiment 3	VU0360172 followed by tiagabine (3 min interval)	1 µg + 1 µg (n=8)	S1Po
	VU0360172 + followed by tiagabine (3 min interval)	1 µg + 0.5 µg (n=9)	VB
	ACSF followed by ACSF (3 min interval)	(n=8)	S1Po
	ACSF followed by ACSF (3 min interval)	(n=9)	VB

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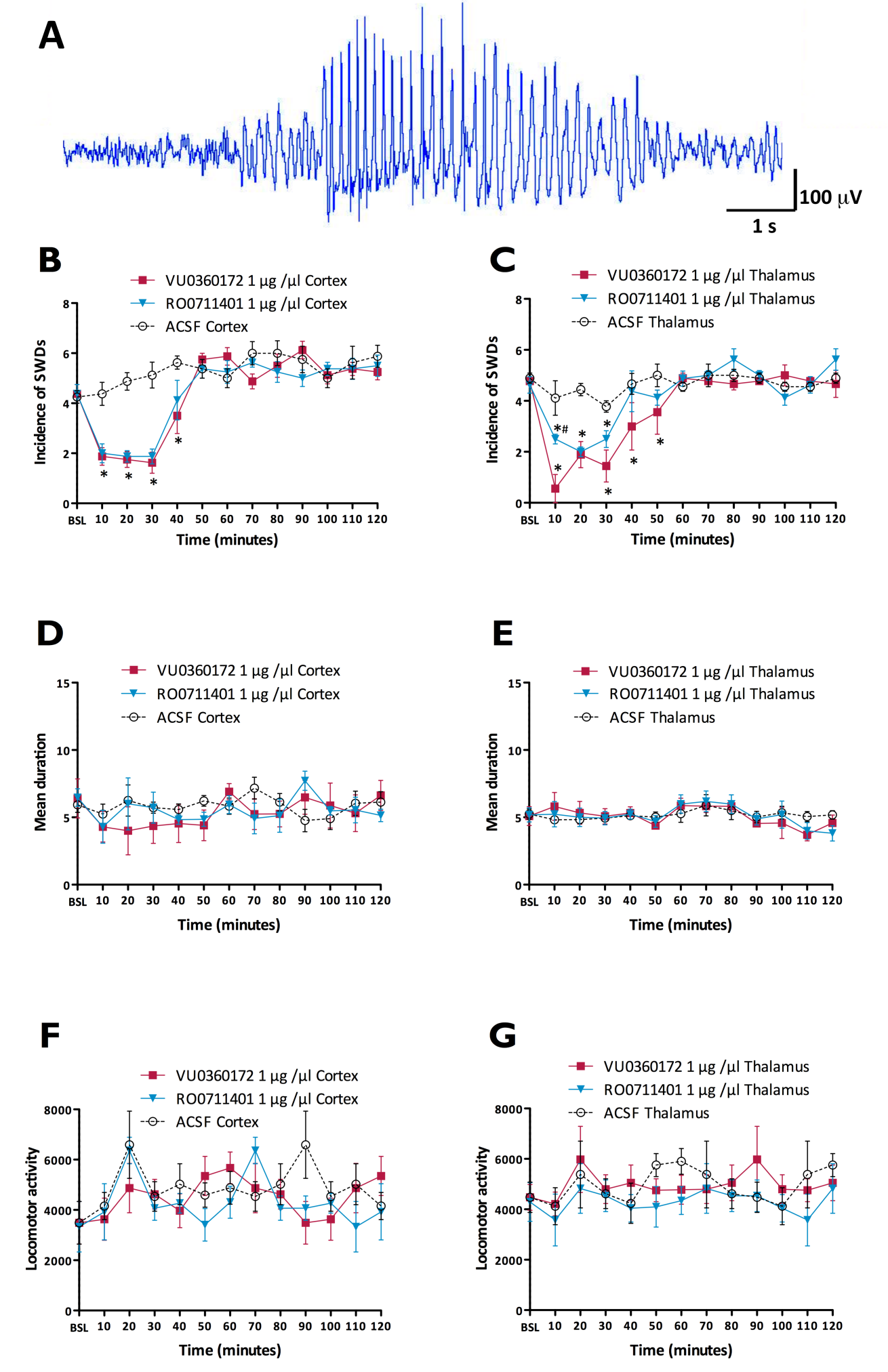
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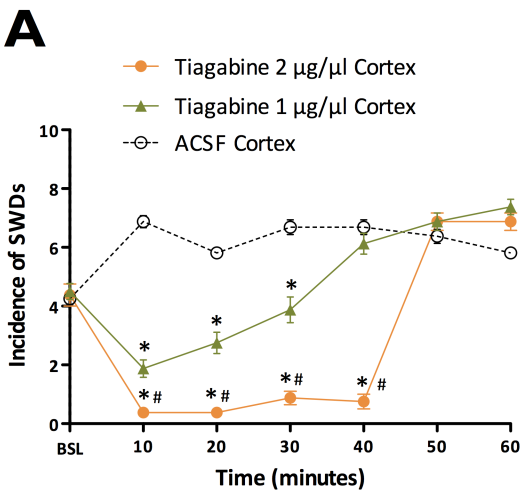
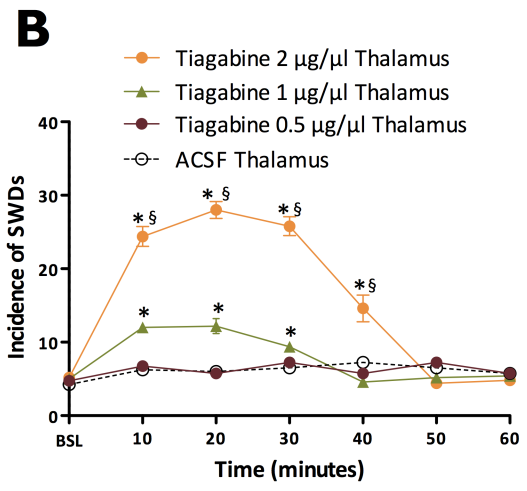
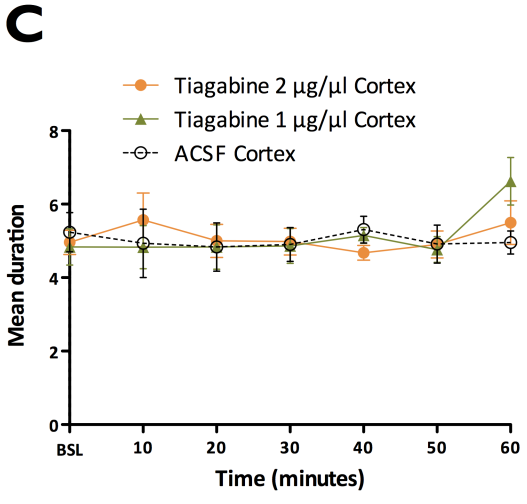
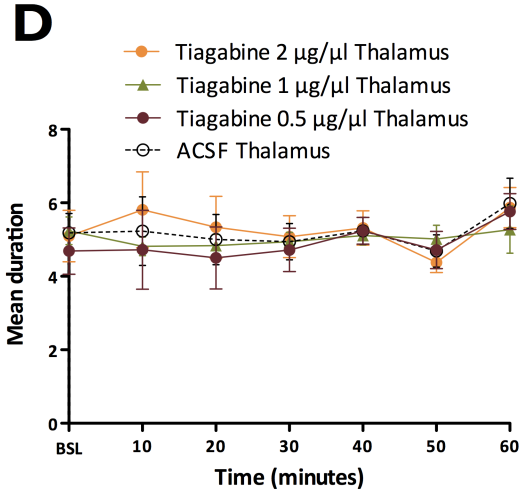
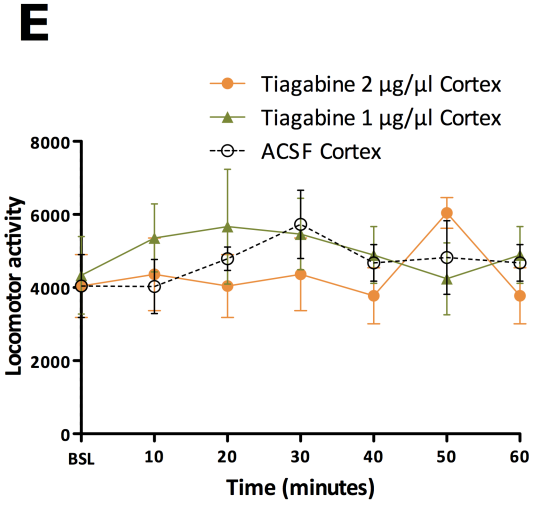
100 µV
1 s
B
VU0360172 1 µg /µl Cortex
RO0711401 1 µg /µl Cortex
ACSF Cortex
Incidence of SWDs
Time (minutes)
C
VU0360172 1 µg /µl Thalamus
RO0711401 1 µg /µl Thalamus
ACSF Thalamus
Incidence of SWDs
Time (minutes)
D
VU0360172 1 µg /µl Cortex
RO0711401 1 µg /µl Cortex
ACSF Cortex
Mean duration
Time (minutes)
E
VU0360172 1 µg /µl Thalamus
RO0711401 1 µg /µl Thalamus
ACSF Thalamus
Mean duration
Time (minutes)
F
VU0360172 1 µg /µl Cortex
RO0711401 1 µg /µl Cortex
ACSF Cortex
Locomotor activity
Time (minutes)
G
VU0360172 1 µg /µl Thalamus
RO0711401 1 µg /µl Thalamus
ACSF Thalamus
Locomotor activity
Time (minutes)


A
Tiagabine 2 µg/µl Cortex
Tiagabine 1 µg/µl Cortex
ACSF Cortex
Incidence of SWDs
Time (minutes)
BSL
10
20
30
40
50
60


B
Tiagabine 2 µg/µl Thalamus
Tiagabine 1 µg/µl Thalamus
Tiagabine 0.5 µg/µl Thalamus
ACSF Thalamus
Incidence of SWDs
Time (minutes)


C
Tiagabine 2 µg/µl Cortex
Tiagabine 1 µg/µl Cortex
ACSF Cortex
Mean duration
Time (minutes)


D
Tiagabine 2 µg/µl Thalamus
Tiagabine 1 µg/µl Thalamus
Tiagabine 0.5 µg/µl Thalamus
ACSF Thalamus
Mean duration
Time (minutes)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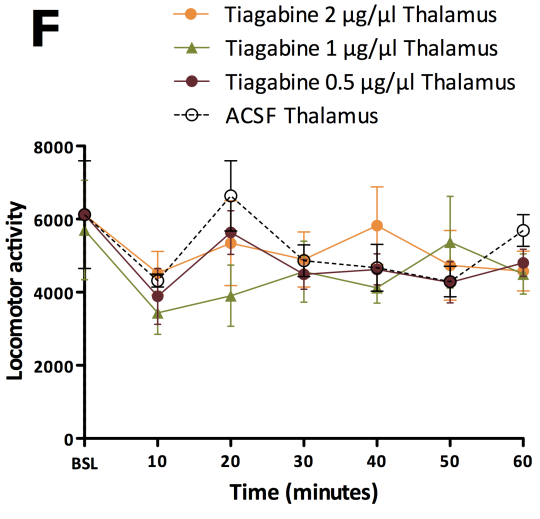
ACSF Cortex
Locomotor activity
Time (minutes)


F
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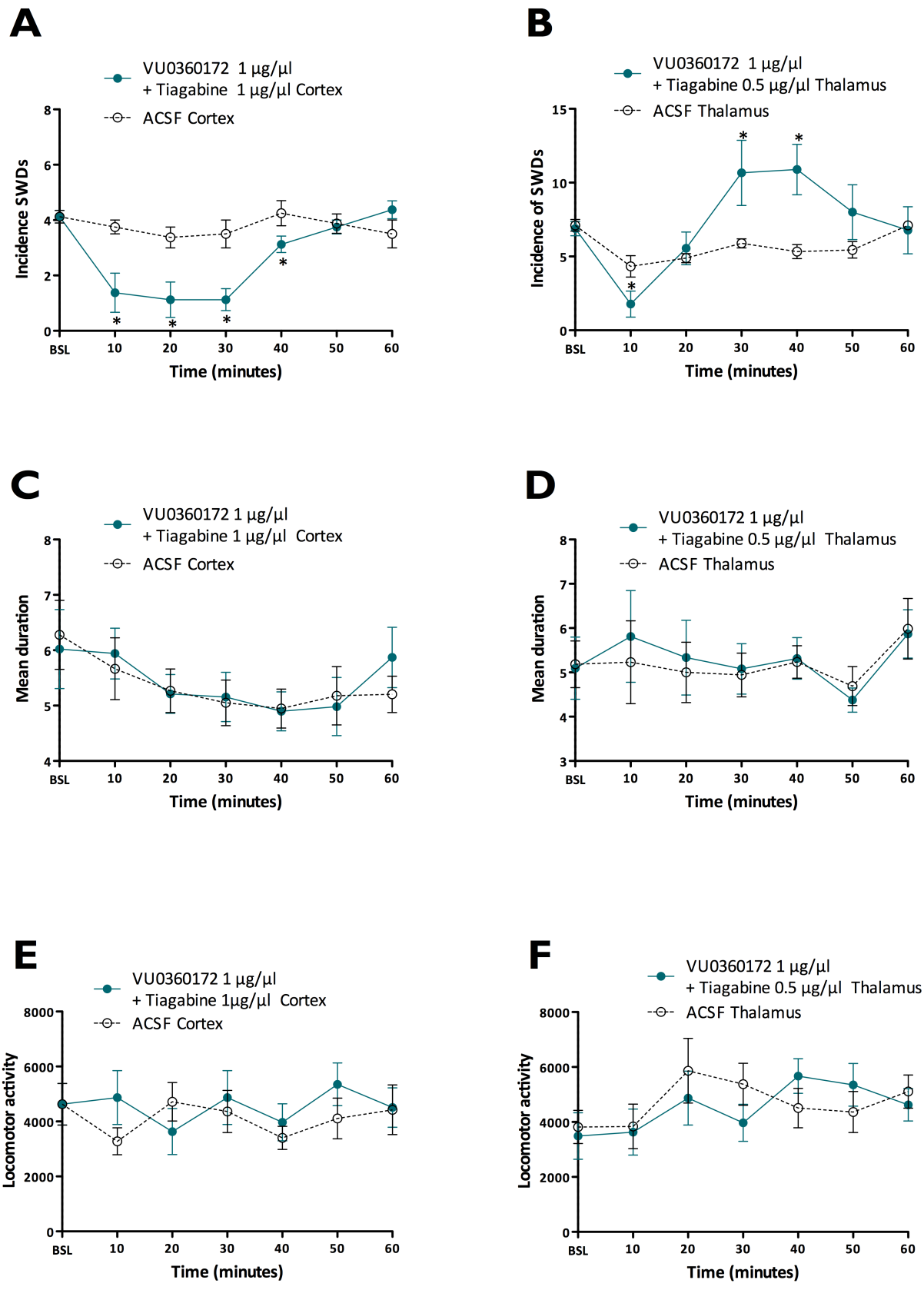
Tiagabine 1 µg/µl Thalamus
Tiagabine 0.5 µg/µl Thalamus
ACSF Thalamus
Locomotor activity
Time (minutes)


A
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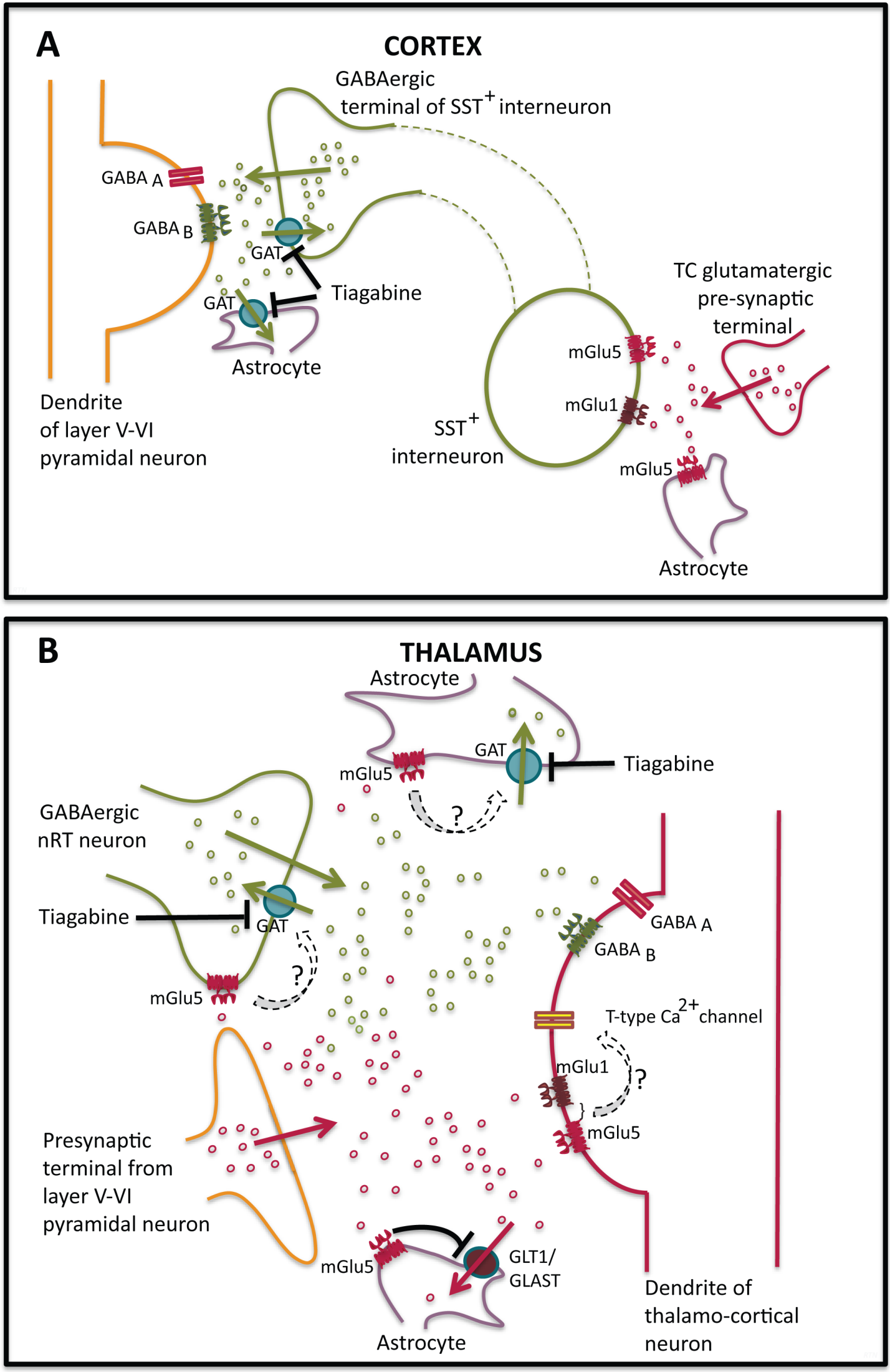
+ Tiagabine 1 µg/µl Cortex
ACSF Cortex
Incidence SWDs
Time (minutes)
B
VU0360172 1 µg/µl
+ Tiagabine 0.5 µg/µl Thalamus
ACSF Thalamus
Incidence of SWDs
Time (minutes)
C
VU0360172 1 µg/µl
+ Tiagabine 1 µg/µl Cortex
ACSF Cortex
Mean duration
Time (minutes)
D
VU0360172 1 µg/µl
+ Tiagabine 0.5 µg/µl Thalamus
ACSF Thalamus
Mean duration
Time (minutes)
E
VU0360172 1 µg/µl
+ Tiagabine 1µg/µl Cortex
ACSF Cortex
Locomotor activity
Time (minutes)
F
VU0360172 1 µg/µl
+ Tiagabine 0.5 µg/µl Thalamus
ACSF Thalamus
Locomotor activity
Time (minutes)
BSL
10
20
30
40
50
60


A
CORTEX
GABAergic
terminal of SST+ interneuron
GABA A
GABA B
GAT
Tiagabine
GAT
Astrocyte
Dendrite
of layer V-VI
pyramidal neuron
SST+
interneuron
TC glutamatergic
pre-synaptic
terminal
mGlu5
mGlu1
mGlu5
Astrocyte
B
THALAMUS
Astrocyte
GAT
Tiagabine
mGlu5
?
GABAergic
nRT neuron
Tiagabine
GAT
?
mGlu5
GABA A
GABA B
T-type Ca2+ channel
mGlu1
?
mGlu5
Presynaptic
terminal from
layer V-VI
pyramidal neuron
mGlu5
GLT1/
GLAST
Astrocyte
Dendrite of
thalamo-cortical
neuron