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The circuitry of atypical absence seizures in $GABA_BR1a$ transgenic mice

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ABSTRACT

The objective of the current study was to determine the origin of the slow spike and wave discharges (SSWD) in the transgenic mouse with postnatal over-expression of the $GABA_B$ receptor subunit R1a ($GABA_BR1a^{tg}$), a mutant animal with a characteristic phenotype consisting of atypical absence seizures and cognitive dysfunction. Using simultaneous electrocorticographic (ECoG) recordings from cortical and depth electrodes in freely moving $GABA_BR1a^{tg}$ mice, we showed that the SSWD in this model of atypical absence seizures arise exclusively from midline thalamus (MT), reticular nucleus of the thalamus (nRT), and the CA1 region of the hippocampus. Lesioning of the MT and nRT with ibotenic acid abolished SSWD. Microinjection of the $GABA_B$ receptor agonist, (−) baclofen, into MT and nRT exacerbated, and the $GABA_BR$ antagonist, CGP 35348 abolished, SSWD in the $GABA_BR1a^{tg}$ mice. These data suggest that the nRT and MT are necessary for the generation of SSWD in the $GABA_BR1a^{tg}$ model of atypical absence seizures, and indicate that $GABA_BR$-mediated mechanisms within thalamus are necessary for the genesis of SSWD in atypical absence seizures. A putative cortico-thalamo-hippocampal circuit is proposed to explain the unique electrographic findings, ictal behavior, pharmacology, and impairment of cognition that characterize atypical absence seizures.

There are two types of absence epilepsy observed both clinically in children and experimentally in rodents: typical and atypical (Cortez and Snead, 2006). Atypical absence seizures have a greater burden of illness since they are universally associated with severe cognitive impairment (Markand, 2003; Chan et al., 2004) due to the involvement of thalamo-hippocampal circuitry (Cortez et al., 2001; Chan et al., 2004; Wu et al., 2007), while cognition is preserved in typical absence seizures because the epileptiform activity is confined to thalamocortical neuronal pathways, sparing limbic circuitry (Vergnes et al., 1990; Snead et al., 1999; Perez Velazquez et al., 2007). Other features also may be used to distinguish typical from atypical absence seizures. These include ictal behavior and the frequency of spike-wave discharge (Bare et al., 1998; Snead et al., 1999; Cortez et al., 2001; Nolan et al., 2005; Cortez and Snead, 2006). The pharmacological profiles of the two absence types are the same (Delanty and French, 1998) because anti-absence drugs act upon thalamic circuitry which is common to both typical and atypical absence seizures (Cortez and Snead, 2006). In addition, GABAergic agonists, including some anticonvulsant drugs that act through GABAergic mechanisms, exacerbate typical and atypical absence seizures both clinically and experimentally (Cortez and Snead, 2006).

The metabotropic $GABA_B$ receptor ($GABA_BR$) is a heterodimer that is comprised of R1 and R2 subunits, both of which are required for normal receptor functioning (Bettler et al., 2004; Bettler and Tiao, 2006). The R1 subunit contains the binding site for GABA, and the R2 subunit regulates the trafficking of the receptor complex to the cell surface. Several different splice variations of the R1 subunit have been identified, with the R1a and R1b being the predominantly expressed forms within the brain. The R1a subtype is found primarily on excitatory presynaptic terminals, while the R1b subtype is found predominantly at postsynaptic sites (Guetg et al., 2009; Ulrich and Bettler, 2007).

$GABA_BR$-mediated mechanisms are involved in the pathogenesis of absence seizures (Snead, 1995; Danober et al., 1998; Crunelli and Leresche, 2002). $GABA_BR$ agonists have been shown to exacerbate typical (Snead, 1996) and atypical (Cortez et al., 2001; Cortez and Snead, 2006; Wu et al., 2007) absence seizures. Conversely, $GABA_BR$

Abbreviations: AT, anterior nucleus of the thalamus; BMA, basomedial amygdala; EC, entorhinal cortex; ECoG, electrocorticography; GABA, γ-amino butyric acid; $GABA_BR$, $GABA_B$ receptor; $GABA_BR1a^{tg}$, $GABA_B$ receptor subunit R1a transgenic mice; mPFC, medial prefrontal cortex; MT, medial thalamus; nRe, nucleus reunions of the thalamus; nRT, reticular nucleus of the thalamus; SSWD, slow spike-wave; VBT, ventrobasal thalamus.

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antagonists block both types of seizures (Snead, 1992; 1996; Liu et al., 1992; Cortez et al., 2001; Wu et al., 2007). Based on work showing over-expression of $GABA_BR1a$ and R1b subtypes in a pharmacological model of atypical absence seizures in rodent (Snead et al., 2000), we tested the hypothesis that over-expression of the $GABA_B$ receptor R1a subtype in transgenic mouse forebrain neurons would result in spontaneous atypical absence seizures. The resultant transgenic animal, $GABA_BR1a^{tg}$, indeed showed a phenotype with all the features of atypical absence seizures including impaired hippocampal synaptic plasticity, significantly impaired learning ability, and the involvement of the hippocampus in the slow spike-wave discharges (SSWD) that characterize atypical absence seizures (Wu et al., 2007).

The objectives of the experiments presented in this paper were to define the thalamo-hippocampal circuitry participating in the generation of SSWD in the $GABA_BR1a^{tg}$ genetic model of atypical absence seizures, and to determine whether $GABA_BR$-mediated mechanisms within this circuitry are required for the generation of the SSWD that characterize atypical absence seizures.

1. Experimental procedures

1.1. Animals: Generation of $GABA_BR1a$ transgenic mice

The experimental protocol was approved by the Hospital for Sick Children Animal Committee, which conforms to the rules and regulations of the Canadian Council on Animal Care in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985), the UK Animals Scientific Procedures Act 1986 or the European Communities Council Directive of 24 November 1986 (86/609/EEC). We created a mutant mouse that over-expressed the $GABA_BR1a$ isoform in forebrain (the $GABA_BR^{tg}$) using a hemagglutinin tagged human $GABA_BR1a$ cDNA (provided by Glaxo Smith Wellcome Company, UK) with the CaMKIIα promoter (provided by E.R. Kandel, Columbia University, NY) as previously described (Wu et al., 2007).

1.2. Electrocorticographic (ECoG) mapping studies: Localization of slow spike-wave discharges

$GABA_BR^{tg}$ mice and C57Bl/6 wild type controls 2 to 3 months of age were used for these experiments. All surgical procedures were performed according to protocols reviewed and approved by the animal care committee in the Hospital for Sick Children, and complied with regulations of the Canadian Council on Animal Care. The surgeries and depth electrode implantations were done as previously described (Wu et al., 2007).

The following coordinates were used for depth electrode placement (Paxinos and Franklin, 2001; Fig. 1): midline thalamus (MT): Bregma −1.70 mm, midline left (L) and right (R) 0.45 mm, depth 3.25 mm; ventrobasal thalamus (VBT): Bregma −1.70 mm, midline L and R 1.8 mm, depth 3.6 mm; reticular nucleus of the thalamus (nRT): Bregma −0.58 mm, midline L and R 1.1 mm, depth 3.8 mm. Anterior nucleus of the thalamus (AT): Bregma −0.70 mm, midline left (L) and right (R) 0.75 mm, depth 3.50 mm. CA1 pyramidal layer of the hippocampus (CA1): Bregma −2.3 mm, midline L and R 1.8 mm, depth 1.5 mm; the lateral entorhinal cortex (EC): Bregma −3.16 mm, midline L and R 3.9 mm, depth 4.5 mm and the basomedial amygdala (BMA): Bregma −1.34 mm, midline L and R 2.3 mm. All coordinates were measured in millimeters (mm) with skull surface flat.

After surgery, all animals were monitored daily by veterinary technicians for 5 days of recovery. For all seizure mapping studies, ibotenic acid lesioning studies, and microinjection studies, ECoG and

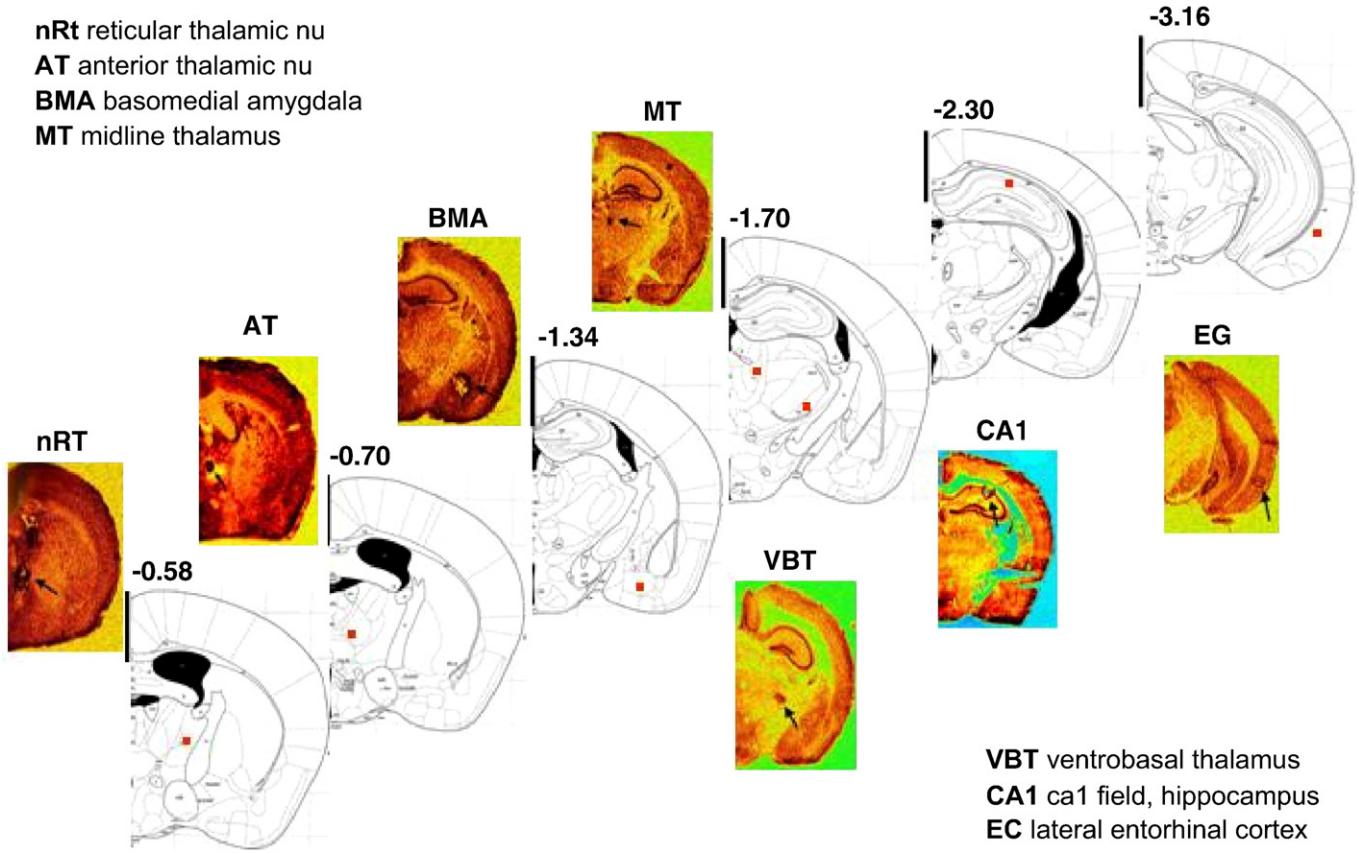

Fig. 1. Superimposed slice drawings, made from the stereotaxic mouse brain atlas from Paxinos and Franklin (2001) at the indicated distances (in millimeters) from the bregma. Red dots represent the recording and microinjection sites. Adjacent histologies indicate the location of the tips of the depth electrodes. nRT: reticular nucleus of thalamus; AT: anterior thalamic nucleus; BMA: basomedial amygdala; MT: midline thalamus, VBT: ventrobasal thalamus; CA1: hippocampus CA1 field; EC: entorhinal cortex.

depth recordings were performed and the data analyzed as previously described (Cortez et al., 2001; Wu et al., 2007) The slow spike-wave discharge (SSWD) duration was measured for consecutive 20-min epochs over a one-hour recording period as described previously (Cortez et al., 2001). SSWD was scored in each animal only if the electrode derivations demonstrated the distinct 4–6 Hz SSWD morphology, with amplitudes four to six times higher than baseline (Cortez et al., 2001; Chan et al., 2004). The cumulative duration was pooled for animals within a group, and the values were compared between groups by a Student's unpaired *t*-test using SigmaStat software. Electrode placement was confirmed histologically in all experiments.

1.3. Lesion studies: Ibotenic acid-induced lesions in MT, RT and CA1

Based on the results from ECoG and depth electrode mapping studies described above, only those brain areas that showed the typical 4–6 Hz SSWD morphology, i.e. nRT, MT, and CA1 (see Results section), were lesioned in this group of experiments in the $GABA_BR^{tg}$ mice. RT, MT and CA1 lesions were induced by ibotenic acid (Sigma-Aldrich, Canada). This drug is an excitotoxin that preserves fibers of passage, but overexcites and then destroy neurons (Schwarcz et al., 1979; Jarrard, 2002). The technique of ibotenic acid lesioning was modified after Chaveau et al. (2005). Briefly, ibotenic acid was dissolved in distilled phosphate buffered saline (PBS, 0.1 μm filtered; final pH: 7.4) to a final concentration of 10 μg/μl. Mice were anaesthetized with a sodium pentobarbital. The skull was surgically exposed and a hole was drilled. A permanent stainless steel guide cannula (o.d. = 0.4 mm, i.d. = 0.3 mm) with a stylet (o.d. = 0.26 mm) of the same length for microinjection was implanted bilaterally for each brain area studied, under stereotaxic guidance using the same coordinates as described above for the ECoG and depth electrode mapping studies for the corresponding regions. Cortical ECoG and depth recordings were utilized to monitor the outcome of the ibotenic acid-induced lesions or phosphate buffered saline control infusions in nRT, MT, or CA1. Following the installation of cannulae and electrodes, mice were allowed to recover for 5 days. We made ECoG and depth electrode recordings for an 80 minute baseline period in lesion and control $GABB_BR^{tg}$ mice prior to microinjection. Ibotenic acid or phosphate buffered saline (100 nl/side) was delivered at a speed of 100 nl/min over 1 min. The injection tubing was left in the cannulae for an additional 30 s following drug administration to prevent leakage of the injected drug. After drug administration, the stylet was reinstalled into the guide cannula. We again recorded the ECoG and depth electrodes for 80 min in both control and ibotenic acid-lesioned animals, 24 h post-microinjection, and analyzed the data using Grasslab Reviewer software. Histologic confirmation of cannula placement and lesions was made in all animals.

1.4. Microinjection of $GABA_BR$ agonist and antagonist in MT, nRT and CA1

Similar to the ibotenic acid lesioning studies, only those brain areas of $GABA_BR^{tg}$ mice that showed the typical 4–6 Hz SSWD morphology in the ECoG and depth electrode mapping studies, i.e. nRT, MT, and CA1, were utilized for microinjection studies. The coordinates for these brain regions were as described above for the ECoG and depth electrode mapping studies. (−) Baclofen (Sigma-Aldrich, Canada), a $GABA_BR$ agonist and CGP 35348 (Sigma-Aldrich, Canada), a $GABA_BR$ antagonist, were dissolved in distilled phosphate buffered saline (PBS, 0.1 μm filtered), concentration of 10 μg/μl for (−) baclofen, 20 μg/μl for CGP 35348. Surgeries for implanting guide cannulae and depth and cortical ECoG recording electrodes were done as described above. Microinjections were performed bilaterally in awake, freely moving animals as described above. (−) Baclofen, CGP 35348, or phosphate buffered saline (300 nl/side) was delivered at a speed of 100 nl/min over 3 min. A baseline ECoG and depth electrode recording was made for 80 min prior to, during, and 80 min following microinjection in control, (−) baclofen-, and CGP 35348-treated $GABA_BR^{tg}$ mice. The SSWD was quantitated using the Grass Lab reviewer software. At the end of the experiments, cannula placement was confirmed histologically.

2. Histology

Histological confirmation was made in all lesioning and microinjection studies. Following sacrifice with pentobarbital, the brains were removed rapidly and chilled in isopentane (−40 °C) for 60 s. Coronal brain sections were cut at 20 μm using a cryostat (Leica CM1900, Leica Instruments, Nussloch, Germany), thaw-mounted on gelatin-coated slides, and stained with Cresyl Violet. The placement of the dialysis probes and of the ibotenic acid-induced lesions was verified under light microscopy with reference to Paxinos and Franklin (2001).

3. Results

3.1. Atypical absence seizures in $GABA_BR1^{tg}$ mice

The phenotype of the $GABA_BR1a^{tg}$ has been described in detail elsewhere (Wu et al., 2007). Briefly, these transgenic mice showed spontaneous, recurrent, bilaterally synchronous, 4–6 Hz SSWD on ECoG recordings. The discharges occurred coincidently, but not precisely time-locked, with absence-like behaviors such as staring, facial myoclonus, and whisker twitching. There was no complete immobility during these ictal events.

3.2. Localization of the spontaneous SSWD in $GABA_BR1a^{tg}$ mice

We performed ECoG from epidural electrodes and seven different brain regions within thalamus and hippocampus in freely moving transgenic mice (Fig. 1). The results of the ECoG mapping studies are summarized in Fig. 2. There were only a few scattered SWD observed in any of the brain regions recorded in wild type control mice. In the $GABA_BR1a^{tg}$ mice 4–6 Hz SSWD were consistently recorded from the cortical electrodes, but there was minimal occurrence of SSWD recorded from AT, VBT, EC and BMA regions and no significant difference in SSWD duration between these regions in transgenic vs. wild type mice. However, SSWD was observed frequently in the $GABA_BR1a^{tg}$ emanating from MT, nRT and CA1 regions and the SSWD duration in these regions was significantly greater than that observed

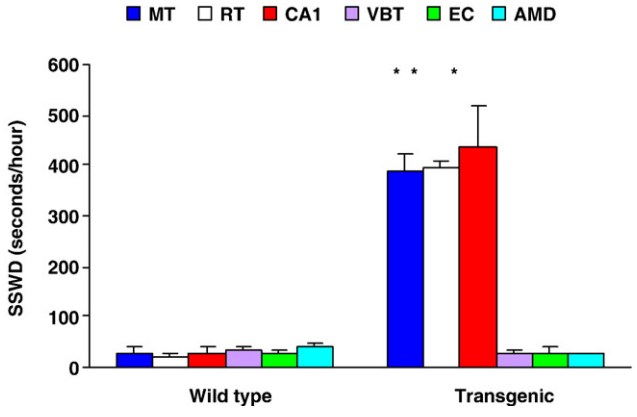

Fig. 2. Quantification of slow spike-wave discharge (SSWD) duration (in seconds/hour) in six different brain regions from wild type and $GABA_BR1A$ transgenic mice. Data are shown as mean ± SEM, $n = 6$, $^*P < 0.001$ (Student's *t*-test). The SSWD could be recorded only from midline thalamus, reticular nucleus of the thalamus, and the CA1 region of the hippocampus. SSWD also were not observed in basolateral amygdala (data not shown). RT: reticular nucleus of thalamus; AT: anterior thalamic nucleus; MT: middle thalamus, VBT: ventrobasal thalamus; CA1: hippocampus CA1 field; EC: entorhinal cortex.

in the wild type controls and from AT, VPL, EC, and BMA in the transgenic animals; (Fig. 2; $P<0.001$; $n=6$, Student's t-test). Therefore, the subsequent lesioning and microinjection experiments were focused upon MT, nRT, and CA1.

3.3. Effect of ibotenic acid-induced lesions on spontaneous SSWD duration in $GABA_BR1a^{tg}$ mice

As described above, we determined from the ECoG and depth electrode mapping studies that the MT, nRT and CA1 brain regions are involved in the generation of SSWD in the $GABA_BR1a^{tg}$ mice. However, the requirement for participation of each of these brain regions in the genesis of the SSWD in $GABA_BR1a^{tg}$ is not known. To address this question, we created an ibotenic acid lesion bilaterally in each of the three regions and monitored the outcome with cortical ECoG recordings. The data show (Fig. 3) that ibotenic acid-induced lesions of MT and nRT resulted in a significant decrease in SSWD duration in the transgenic mice (Fig. 2, $P<0.001$, $n=6$, Student's t-test). The SSWD duration per hour value (seconds) was 287 ± 53 vs. 68 ± 22 before and after ibotenic acid lesioning respectively of the MT in the transgenic mice, and 245 ± 48 vs. 56 ± 9 before and after ibotenic acid lesioning respectively of the nRT region in the transgenic mice, respectively. Ibotenic acid lesioning of CA1 of the transgenic mice failed to induce a statistically significant change in SSWD duration as recorded from the ECoG and depth electrodes in MT and nRT. In the control experiments, there was no statistically significant effect either before or after effect of phosphate buffered saline infusion on SSWD duration in MT, nRT, or CA1 of $GABA_BR^{tg}$ mice (data not shown).

3.4. Effect of (−) Baclofen and CGP 35348 on spontaneous SSWD duration GABABR1atg transgenic mice

In order to test further the hypothesis that the SSWD seen in the $GABA_BR1a^{tg}$ mice are the direct result of over-expression of $GABA_BR1a$ in those regions in the brain from whence the SSWD emanate, we investigated the effect of the microinjection of the $GABA_BR$ receptor agonist (−) baclofen or the $GABA_BR$ antagonist CGP 35348, into MT, nRT, or CA1 on SSWD duration in $GABA_BR1a^{tg}$ mice. (−) Baclofen significantly increased the SSWD duration when given into either MT [271 ± 38 before vs. 695 ± 119 after (−) baclofen] or nRT [260 ± 61 before vs. 734 ± 79 after (−) baclofen treatment] ($P<0.001$, $n=6$, Student's t-test; Fig. 4). (−) Baclofen administration into the CA1 region failed to produce a significant change in SSWD duration. Conversely, microinjection of the $GABA_BR$ antagonist, CGP 35348, significantly decreased, i.e. almost abolished, the SSWD duration in $GABA_BR1a^{tg}$ mice when the drug was administered into MT (285 ± 33 before, vs. 65 ± 6 after CGP 35348 treatment) or nRT (251 ± 32 before, vs. 61 ± 14 after CGP 35348 treatment; $P<0.001$, $n=6$, Student's t-test; Fig. 5). CGP 35348 administration into CA1 had no statistically significant effect on SSWD duration. In the control experiments, there was no statistically significant before and after effect of phosphate buffered saline infusion on SSWD duration in MT, nRT, or CA1 of $GABA_BR^{tg}$ mice (data not shown).

4. Discussion

We have demonstrated in the $GABA_BR1a^{tg}$ mouse model of atypical absence seizures that the epileptiform discharges emanate solely from nRT and MT and project to the CA1 as well as the cerebral cortex. In addition, we have shown that $GABA_BR$-mediated mechanisms within the thalamic arm of this cortico-thalamo-hippocampal circuitry are critical for the genesis of SSWD in this genetic model of atypical absence seizures. These data shed light on the circuitry that underpins atypical absence seizures and give credence to the hypothesis (Chan et al., 2004) that the differences between the typical and atypical absence seizures in terms of the ictal behavior, morphology of epileptiform discharges, and neurocognitive outcome, are circuitry-dependent.

The classically described circuitry for typical absence seizures is shown in Fig. 6A. The typical absence seizure in rodent is initiated in layer 5/6 of the perioral region of the somatosensory cortex and then projected to the ventrobasal thalamus, primarily the ventromedial nucleus, and the caudal nRT. It should be noted however, that MT in these experiments indicate a group of midline thalamic nuclei, such as central medial thalamic, reuniens, etc., since we did not target individual midline nuclei stereotactically (Meeren et al., 2002; Aker et al., 2006; Polack et al., 2007; Çavdar et al., 2008). The circuitry within the thalamus serves as an intrinsic oscillatory unit whose function depends critically on reciprocal synaptic connectivity between excitatory thalamocortical relay neurons and inhibitory thalamic reticular neurons along with a robust post-inhibitory rebound mechanism in relay neurons (Steriade, 2005; Beenhakker and Huguenard, 2009). The end result is that the cortical structures provide the excitatory drive and that the thalamus organizes, amplifies, and synchronizes the seizure activity. This reverberating thalamocortical circuitry is responsible for the electrographic, behavioral, and pharmacological characteristics of typical

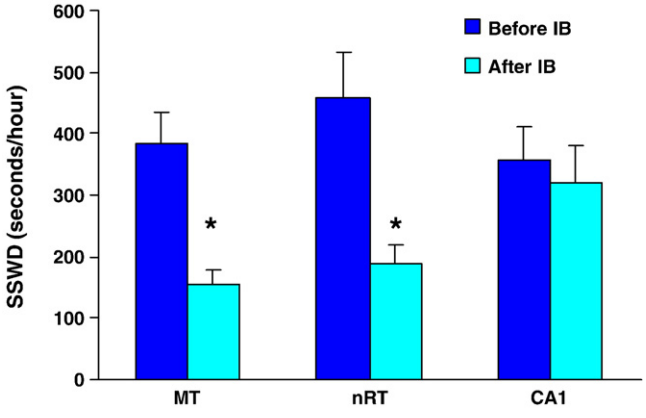

Fig. 3. Quantification of slow spike-wave discharge (SSWD) duration (in s/h) as recorded from monopolar cortical electrodes before and after brain microinjection of ibotenic acid (IB) in midline thalamus (MT), reticular nucleus of the thalamus (nRT) and CA1 regions in the $GABA_BR1a$ transgenic mouse model. Data are shown as mean ± SEM, $n=6$ for each region. The SSWD duration was significantly [$^*P<0.01$ (Student's t-test)] attenuated after lesioning of the nRT and MT, but not CA1.

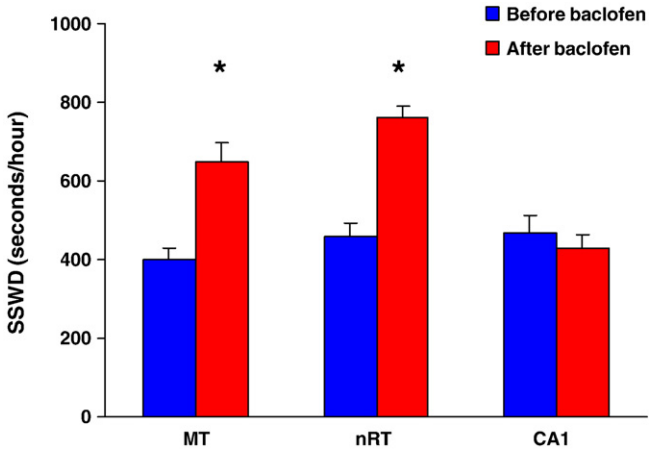

Fig. 4. Quantification of slow spike-wave discharge (SSWD) duration (in s/h) as recorded from monopolar cortical electrodes before and after brain microinjection of the $GABA_BR$ agonist, (−) baclofen, in midline thalamus (MT), reticular nucleus of the thalamus (nRT) and CA1 regions in the $GABA_BR1a$ transgenic mouse model. Data are shown as mean ± SEM, $n=6$ for each region, The SSWD duration was significantly [$^*P<0.01$ (Student's t-test)] increased following microinjection of baclofen into the nRT and MT, but not CA1.

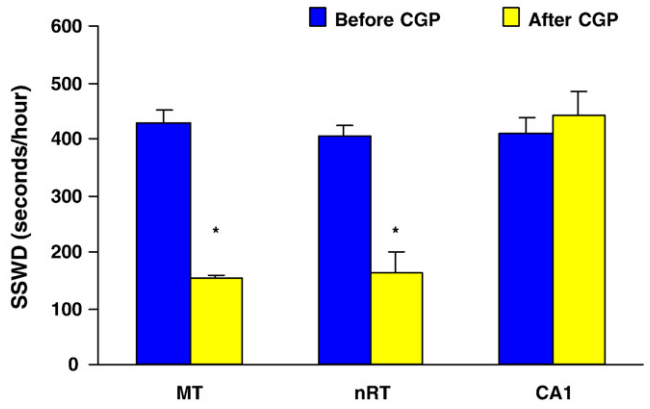

Fig. 5. Quantification of slow spike-wave discharge (SSWD) duration (in s/h) as recorded from monopolar cortical electrodes before and after brain microinjection of the $GABA_BR$ antagonist, CGP 35348, into midline thalamus (MT), reticular nucleus of the thalamus (nRT) and CA1 regions in the $GABA_BR1a$ transgenic mouse model. Data are shown as mean ± SEM, $n=6$ for each region, The SSWD duration was significantly [$^*P<0.01$ (Student's t-test)] attenuated following microinjection of CGP 35348 into the nRT and MT, but not CA1.

absence seizures. There are no projections to or from the hippocampus in this circuitry, hence there are no limbic characteristics to the ictal behavior of typical absence seizures, nor are there learning and memory deficits (Getova et al., 1997) in typical absence seizures.

Our data in the $GABA_BR1a^{tg}$ model of atypical absence showing SSWD emanating from MT, but not from VBT, the classical thalamic source of SWD in typical absence (Vergnes et al., 1990; Meeren et al. 2002; Polack et al. 2007), plus the non-involvement of the MT in typical absence (Liu et al., 1992), suggest a very different circuitry for atypical absence seizures (Fig. 6B). The nRT is common to the neuronal circuitry that subserves both typical and atypical absence seizures because of its synchronizing properties (Beenhakker and Huguenard, 2009; Steriade, 2005) and because both typical and atypical absence seizures respond to drugs that act at the level of the nRT (Coulter et al., 1989; Huguenard and Prince, 1994). However, the remainder of the thalamocortical circuitry that underpins atypical absence seizures is quite different from that for typical absence.

The data showing the projection of the SSWD to the CA1 in the $GABA_BR^{tg}$ model of atypical absence seizures suggest that the midline thalamic nucleus most likely involved in this model of atypical absence seizures is the nucleus reunions (nRE), since this midline thalamic nucleus has direct projections to the CA1 (Bertram and Zhang, 1999; Vertes et al., 2006; 2007; Çavdar et al., 2008). While circuitry involving the nRT, nRE, and CA1 would explain the ictal semiology and the cognitive deficits observed with atypical absence (Chan et al., 2004, 2006; Wu et al., 2007), it does not allow for the focal cortical initiation seen in typical absence, seemingly a requirement for the bilaterally synchronous epileptiform activity that characterizes both typical and atypical absence seizures (Meeren et al., 2002; Polack et al., 2007). Our data do not support the CA1 being the driver of seizures in the $GABA_BR^{tg}$ model of atypical absence seizures since lesioning of the CA1 failed to abort the seizure. Therefore, given the reciprocal projections of nRE to the medial prefrontal cortex (mPFC) (Vertes et al., 2007; Hoover and Vertes, 2007) and nRT (Çavdar et al., 2008), the cortical arm of the circuitry in atypical absence seizures for an atypical absence seizure may reside in layer 5/6 of the medial prefrontal cortex (mPFC) (Fig. 6).

In addition to the current ECoG experimental results in the $GABA_BR1a^{tg}$, there are ample neuroanatomical data that support the hypothesis that the mPFC represents the cortical arm of the cortico-thalamo-hippocampal circuitry that subserves atypical absence seizures. There are direct monosynaptic projections from CA1/subiculum to the mPFC which are quite pronounced, but there are no direct return projections from the mPFC to the CA1 (Hoover and Vertes, 2007).

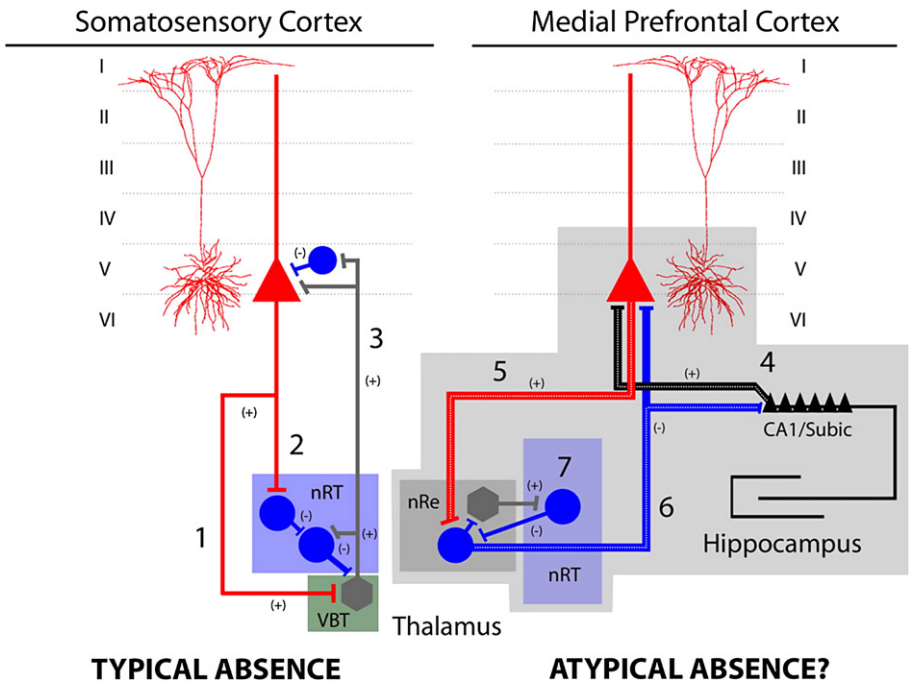

Fig. 6. A. The well established (Meeren et al., 2002; Polack et al., 2007) neuronal circuitry of typical absence seizures involves reciprocal connections between layer 5/6 of the perioral region of somatosensory cortex where the seizures are initiated, the ventrobasal thalamus (VBT) (1 and 3), and the caudal reticular nucleus of the thalamus (nRT) (2) B. Hypothesized circuitry of atypical absence seizures. The initiating epileptiform event for an atypical absence seizure is postulated to occur in layer 5/6 of the medial prefrontal cortex (mPFC) and then project to the nucleus reunions of the thalamus (nRE) (5) which projects back to the mPFC and monosynaptically to the CA1 (6) which in turn projects to the mPFC (4). This reverberating circuit is modulated and driven by reciprocal intrathalamic connections between the nRE and rostral nRT (7). Blue = GABAergic neurons; red = glutamatergic neurons. (−) indicates inhibition; (+) indicates excitation.

Therefore, in the absence of direct mPFC to CA1 projections, it appears that the nRE serves a critical relay from mPFC to CA1 creating a reverberating loop that is modulated by reciprocal intrathalamic connections between nRE and rostral nRT. (Dolleman-Van der Weel et al., 1997; McKenna and Vertes, 2004; Di Prisco and Vertes, 2006; Vertes et al., 2006, 2007; Aker et al., 2006) (Fig. 6). The cognitive outcome and ictal behaviors that characterize atypical absence seizures could well be explained by the involvement of CA1 and mPFC within this circuitry. The hypothesized circuitry also would explain how there can be a cortical trigger for both typical and atypical absence seizures, yet a profound difference in the clinical and electrographic manifestation of the two absence seizure types, the cortical trigger for atypical absence seizures being the mPFC and that for typical absence seizures being the perioral region of the somatosensory cortex.

The excitability of the thalamocortical relay cells in the thalamus is regulated by $GABA_BR$ (Crunelli and Leresche, 2002; Gervasi et al., 2003; Ulrich and Bettler, 2007). Recent data (Ulrich and Bettler, 2007) suggest that the $GABA_BR1a$ subtype may play a regulatory role in this regard since it appears to comprise the $GABA_BR1$ isoform of the thalamic presynaptic $GABA_B$ heteroreceptor on glutamatergic neurons in thalamus. Our microinjection data showing that intrathalamic $GABA_BR$ agonists enhanced and $GABA_BR$ antagonists abolished the atypical absence seizures in the $GABA_BR1^{tg}$ mouse comport with the experimental results of Ulrich and Bettler (2007). Although we have not examined the specific spatial distribution of our expressed R1a transgene with high ultrastructural resolution, the localization of the R1a transgene to presynaptic thalamic sites would be expected in the $GABA_BR1a^{tg}$ mouse since the R1a subtype is normally trafficked to excitatory terminals (Vigot et al., 2006).

There are a number of limitations of our study. First, we did not map out the cortical arm of the circuitry because we used only epidural electrodes for cortical recordings. Therefore, additional experiments are required to provide support for our hypothesis that the mPFC is the cortical trigger for atypical absence seizures. Second, we did not map out the nRT in terms of precisely which anatomic regions of this structure (Aker et al., 2006) might be involved in the hypothesized circuitry. Third, although the published anatomical and electrophysiological data reviewed above strongly implicate the nucleus reuniens in this circuitry, we did not specifically target this nucleus. Rather, we sampled a number of medial thalamic nuclei by virtue of how we did the electrode implantation. Finally, it should be noted that there is a significant nigral input to the control and maintenance of typical absence seizures via the ventral medial thalamic nuclei (Paz et al. 2007). Similarly, there are connections between the pars reticula and the rostral nRT (Çavdar et al., 2008) which could impact the SSWD activity in atypical absence seizures since the rostral nRT is proposed to be involved in the putative circuitry that underpins atypical absence seizures (see above); however, to date we have not addressed the role of nigrothalamic pathways in the $GABA_BR^{tg}$ model of atypical absence seizures.

In summary, our data support the hypothesis that the behavioral, electrographic, and pharmacological manifestations of atypical absence seizures are circuitry-dependent. Further, the data indicate that the circuitry that underpins atypical absence seizures differs markedly from that which subserves typical absence seizures. Finally, our experiments indicate that intrathalamic $GABA_BR$-mediated mechanisms are required for the manifestation of atypical absence seizures in the $GABA_BR^{tg}$ mouse model of this disorder.

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