Published in final edited form as:

Epilepsia. 2008 May ; 49(5): 921–925. doi:10.1111/j.1528-1167.2008.01536.x.

Increased thalamic inhibition in the absence seizure prone DBA/2J mouse

Heneu O. Tan*, **Christopher A. Reid***, **Cindy Chiu**, **Mathew V. Jones¹**, and **Steven Petrou**
Howard Florey Institute, The University of Melbourne, Parkville VIC 3010, Australia

¹Department of Physiology, University of Wisconsin, Madison, Wisconsin, USA

Summary

Genetic background plays a significant role in producing variable seizure outcomes in patients and animal models. The neurobiological mechanism underlying this heterogeneity is not clear. Here we compare GABAergic synaptic properties within the thalamocortical circuit of two commonly used inbred mice strains, the C57B/6 and spike- and-wave discharge (SWD) prone DBA/2J. Differences between the strains occur in amplitude, kinetics and frequency of miniature inhibitory postsynaptic currents (mIPSC) in a region specific manner. The biggest difference in synaptic inhibition was seen in the thalamus where DBA/2J mice showed a doubling of mIPSC frequency when compared to C57B/6. EEG analysis revealed higher power in the 6-12Hz band during non-rapid eye movement sleep in DBA/2J mice. Increased susceptibility of the DBA/2J strain to develop SWDs and increase in the 6-12Hz EEG power may be due to a hypersynchronous ventrobasal thalamus as a consequence of increased GABAergic input.

Keywords

DBA/2J; C57B/6; absence seizures; thalamocortical; sleep; GABA_A receptor

Introduction

Advances in the study of human genetics have allowed the identification of a number of mutations directly linked to epilepsy (Scheffer and Berkovic, 2003). It is becoming increasingly clear that genetic background is a significant contributor to clinical heterogeneity with incomplete penetrance and phenotypic variability common features of familial epilepsy (Wallace et al., 2001). Phenotypic heterogeneity can be modeled in rodents using mice strains with known seizure susceptibility differences (Yu et al., 2006; Tan et al., 2007).

C57B/6 and DBA/2J have the lowest and highest susceptibility to seizures, respectively, among frequently studied inbred strains of mice (Ferraro et al., 2007). A recently developed mouse model of familial absence epilepsy, based on the R43Q mutation in the $\gamma 2$ subunit of the GABA_A receptor, recapitulates the human condition showing electrographic spike-and-wave discharges (SWD) associated with behavioral arrest (Wallace et al., 2001; Tan et al., 2007). DBA/2J mice harboring this mutation exhibit more severe SWDs than C57B/6 mice,

Corresponding author: Steven Petrou, Howard Florey Institute, The University of Melbourne, Parkville VIC 3010, Australia, Contact: spetrou@unimelb.edu.au, tel; +3 8344 1957 fax: + 3 9347 0446.

*These authors contributed equally to this publication.

None of the authors has any conflict of interest to report in relation to this paper.

mirroring the clinical heterogeneity seen in patients (Wallace et al., 2001; Tan et al., 2007). The neurobiological mechanism underlying such heterogeneity remains unclear.

Abnormal hypersynchrony of the thalamocortical circuit is responsible for the generation of SWDs. GABAergic inhibition plays a complex role in this circuit. Paradoxically, enhancing GABA_A-mediated synaptic transmission, specifically in the ventrobasal thalamus (VB) *increases* SWD frequency in rodent models of absence seizures (Hosford et al., 1997). Interstrain heterogeneity in GABAergic transmission has been observed in other brain nuclei (DuBois et al., 2006). Therefore, differences in thalamocortical GABAergic inhibition between the C57B/6 and DBA/2J mice may underlie susceptibility to SWDs. Here, we investigate basal differences in GABA_A receptor-mediated inhibition in the thalamocortical circuit of C57B/6 and DBA/2J mice. Epidural EEG power analysis was also performed during sleep to determine differences in basal thalamocortical activity between the strains.

Method

Brain slice electrophysiology

Following anaesthesia with 1-3% isoflurane, mice were decapitated, cortical and thalamocortical slices were cut (300 μ m thick) and mIPSC's were recorded as described in Tan et al (2007). Drugs and salts were obtained from Sigma (Australia).

In some cases, biocytin was included in the intracellular solution. After electrophysiological recording, brain slices were fixed with 4% paraformaldehyde. Biocytin was visualized using Vectastain ABC Elite kit (Vector laboratories) according to manufacturer's instructions. Dendritic arbors were traced and documented using the Neurolucida system (MBF Biosciences).

EEG analysis

Mice were anaesthetised with 1-3% isoflurane and four silver electrodes implanted on each quadrant of the skull. Electrodes were placed 3 mm lateral of the midline and 0.5 and 4.0 mm, caudal from bregma. A ground electrode was placed 2.5 mm rostral from bregma and 0.5 mm lateral from the midline. Mice were allowed to recover for at least 3 days after surgery. EEGs were recorded in freely moving mice for 4-6 hours during daylight hours. Signals were low pass filtered at 200 Hz, AC coupled at 0.1 Hz and sampled at 1 kHz using Powerlab 16/30 (ADInstruments) with synchronised video monitoring using the Quicktime video synchronization module (ADInstruments).

States of vigilance were scored offline using published criteria (Fonck et al., 2005). Wakefulness was characterized by motor activity accompanied by low overall EEG signal power. Non-rapid eye movement (nREM) sleep was characterized by inactivity and high EEG signal power. Power spectra were calculated using a Hann window that produced a final resolution of 0.5 Hz. For quantitative comparison between C57B/6 and DBA/2J mice, three 5 minute epochs were averaged to create a representative power spectrum. Spontaneous SWD's were not observed in either strain during the course of recordings.

All group data were expressed as mean $\pm$ SEM. mIPSC data sets were Winsorized and a Bonferroni correction applied for multiple comparisons within each brain region. A t-test was performed using a corrected alpha value of 0.0125 (Prism, Graphpad Software). A Mann-Whitney test was used for the non-parametric power density EEG data with $p < 0.05$ taken as statistical significance (Prism, Graphpad Software). Animal care and handling conformed to guidelines according to the HFI AEC.

Results

GABAergic inhibition is enhanced in the VB of DBA/2J mice

The thalamocortical circuit comprise three brain regions: the somatosensory cortex, the thalamic reticular nucleus (TRN) and the VB. Whole-cell voltage clamp analysis was performed in each brain region in C57B/6 and DBA/2J strains (Fig. 1A). The close proximity of the TRN and VB provide a technical concern regarding identification of neurons from each region. Three different criteria were used to confirm identity. First, the two regions have distinct anatomical structure; the TRN is translucent and the VB has a darker appearance with the presence of dense striations. Second, the two nuclei have distinctive GABAergic current kinetics (Fig. 1B). And finally, TRN neurons have bipolar dendrites while VB neurons have radially projecting dendrites (Fig. 1C).

Four biophysical parameters of GABA_A receptor-mediated mIPSCs were analyzed; the amplitude, rise-time, decay and frequency. Parameter and region specific changes were noted and are summarized in Table 1. Potentially, the most physiologically relevant difference was in the frequency of mIPSCs, which were 106% greater in the VB and 146% greater in the TRN of DBA/2J mice compared to C57B/6 (Fig. 1D). No difference in cortical mIPSC event frequency was observed (Table 1).

The mIPSC decay in the TRN was faster in the DBA/2J strain (Table 1). No strain differences in mIPSC decay were observed in the VB or the cortex. No differences in average mIPSC amplitude were seen in either the VB or TRN. Cortical mIPSC amplitude was 14% greater in the DBA/2J strain compared to C57B/6 strain (Table 1). In summary, based on mIPSC properties alone, the largest strain difference in inhibition would appear to reside in the two thalamic nuclei, where the DBA/2J mice would be predicted to have enhanced inhibition.

Higher 6-12Hz power during non-REM sleep in DBA/2J mice

Physiologically, the thalamocortical circuit is responsible for generating normal spindle oscillations that occur during sleep (Steriade, 2005). Differences in basal GABAergic function between strains may be reflected in such activity. Power spectrum analysis of epochs of epidural EEGs were performed during awake and sleep states in C57B/6 (n = 7) and DBA/2J (n = 6) mice (Fig. 2). DBA/2J mice showed two fundamental differences in their EEG power spectrum during non-REM sleep when compared to C57B/6. They exhibited lower power in the 2-4Hz band and greater power in the 6-12Hz band (Fig. 2D and E). No significant differences were observed in the power spectrum during awake states (Fig. 2C).

Discussion

Synaptic basis of mIPSC changes

An increase in frequency of mIPSCs in the VB and TRN is likely to reflect either an increase in the number of inhibitory synapses and/or a change in probability of GABA release. Earlier work has shown that an increase in mIPSC frequency in the amygdala was associated with a decrease in paired-pulse facilitation of the evoked IPSCs, implicating a change in probability of transmitter release (DuBois et al., 2006). Post-synaptic differences (amplitude and kinetics) could reflect changes in GABA_A receptor subunit expression (Hood and Buck, 2000) or changes in cellular morphology (DuBois et al., 2006).

Could differences in GABA_A synaptic function explain absence seizure susceptibility?

A complete description of inhibition would have to take into consideration a number of neuronal and synaptic properties. However, the largest effect predicted by our results is a significant enhancement of GABA_A receptor-mediated inhibition onto the thalamus of DBA/2J compared to C57B/6 mice. Systemic administration of GABA_A receptor agonists exacerbate experimental seizures, with microinjections into the VB sufficient to increase SWD frequency (Hosford et al., 1997; Snead et al., 1999). Microinjection of the antiepileptic drug carbamazepine also exacerbates SWDs by enhancing GABA_A receptor-mediated inhibition in the VB (Liu et al., 2006).

Hyperpolarization-dependent activity is a hallmark of pacemaker currents. Thalamocortical neurons express T-type calcium channels and hyperpolarization-activated cyclic nucleotide-gated channels (HCN) that rely on hyperpolarization to function (Steriade, 2005). During SWDs and sleep spindles (7-14Hz) low threshold spiking, which relies on these channels, occurs in thalamocortical neurons following strong hyperpolarization (Steriade, 2005). The larger GABA_A receptor-mediated inhibition in the VB is likely to lead to more synchronous thalamic firing in DBA/2J mice compared to C57B/6 mice. This may be responsible for the higher 6-12Hz power noted in DBA/2J mice during sleep. It could also be responsible for the increased severity of SWDs in DBA/2J mice that harbor the R43Q mutation (Tan et al., 2007). Although there is no precedence, differences noted in other nuclei could also alter seizure susceptibility.

Modelling the influence of genetic background on seizure susceptibility

Inbred mice strains can model complex monogenic inheritance in which a mutation is necessary but not sufficient to manifest epilepsy (Yu et al., 2006; Tan et al., 2007). Investigating basal differences in neuronal function of different strains, as done in this study, may well lead to a better understanding of predisposing and protective neurobiological factors as determined by genetic background and impact clinical epileptology.

Acknowledgments

The authors would like to thank Prof Sam Berkovic and A/Prof. Terry O'Brien for critical reading of this manuscript. This work was funded by NHMRC (SB, CAR, SP), Molly McDonnell Foundation Grant (CAR) and NIH (NS046378 to MVJ). 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.

The authors would like to thank Prof Sam Berkovic and A/Prof. Terry O'Brien for critical reading of this manuscript. This work was funded by NHMRC (CAR, SP), Molly McDonnell Foundation Grant (CAR) and NIH (NS046378 to MVJ).

References

- DuBois DW, Perlegas A, Floyd DW, Weiner JL, McCool BA. Distinct functional characteristics of the lateral/basolateral amygdala GABAergic system in C57BL/6J and DBA/2J mice. *J Pharmacol Exp Ther.* 2006; 318:629–40. [PubMed: 16651400]
- Ferraro TN, Smith GG, Schwebel CL, Lohoff FW, Furlong P, Berrettini WH, Buono RJ. Quantitative Trait Locus for Seizure Susceptibility on Mouse Chromosome 5 Confirmed with Reciprocal Congenic Strains. *Physiol Genomics.* 2007
- Fonck C, Cohen BN, Nashmi R, Whiteaker P, Wagenaar DA, Rodrigues-Pinguet N, Deshpande P, McKinney S, Kwoh S, Munoz J, Labarca C, Collins AC, Marks MJ, Lester HA. Novel seizure phenotype and sleep disruptions in knock-in mice with hypersensitive alpha 4* nicotinic receptors. *J Neurosci.* 2005; 25:11396–411. [PubMed: 16339034]
- Hood HM, Buck KJ. Allelic variation in the GABA A receptor gamma2 subunit is associated with genetic susceptibility to ethanol-induced motor incoordination and hypothermia, conditioned taste

- aversion, and withdrawal in BXD/Ty recombinant inbred mice. *Alcohol Clin Exp Res*. 2000; 24:1327–34. [PubMed: 11003197]
- Hosford DA, Wang Y, Cao Z. Differential effects mediated by GABAA receptors in thalamic nuclei in lh/lh model of absence seizures. *Epilepsy Res*. 1997; 27:55–65. [PubMed: 9169291]
- Liu L, Zheng T, Morris MJ, Wallengren C, Clarke AL, Reid CA, Petrou S, O'Brien TJ. The mechanism of carbamazepine aggravation of absence seizures. *J Pharmacol Exp Ther*. 2006; 319:790–8. [PubMed: 16895979]
- Scheffer IE, Berkovic SF. The genetics of human epilepsy. *Trends Pharmacol Sci*. 2003; 24:428–33. [PubMed: 12915053]
- Snead OC 3rd, Depaulis A, Vergnes M, Marescaux C. Absence epilepsy: advances in experimental animal models. *Adv Neurol*. 1999; 79:253–78. [PubMed: 10514819]
- Steriade M. Sleep, epilepsy and thalamic reticular inhibitory neurons. *Trends Neurosci*. 2005; 28:317–24. [PubMed: 15927688]
- Tan HO, Reid CA, Single FN, Davies P, Chiu C, Murphy S, Clarke AL, Dibbens L, Krestal H, Mulley J, Jones MV, Seeburg P, Sakmann B, Berkovic SF, R S, Petrou S. Reduced cortical inhibition in a mouse model of familial Childhood Absence Epilepsy. *PNAS*. 2007; 104:17536–41. [PubMed: 17947380]
- Wallace RH, Marini C, Petrou S, Harkin LA, Bowser DN, Panchal RG, Williams DA, Sutherland GR, Mulley JC, Scheffer IE, Berkovic SF. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nat Genet*. 2001; 28:49–52. [PubMed: 11326275]
- Yu FH, Mantegazza M, Westenbroek RE, Robbins CA, Kalume F, Burton KA, Spain WJ, McKnight GS, Scheuer T, Catterall WA. Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat Neurosci*. 2006; 9:1142–9. [PubMed: 16921370]

FIG. 1. Enhanced thalamic inhibition in the DBA/2J mice. **(A)** Representative GABA_A receptor-mediated mIPSC traces from the somatosensory cortex (CTX), Thalamus (TRN) and Ventrobasal nucleus (VB) in DBA/2J and C57BL/6 mice. **(B)** Normalized mIPSCs from TRN and VB in a C57BL/6 mouse illustrating distinct differences in kinetics **(C)** Neurolucida tracings of biocytin labeled neurons highlighting distinct dendritic morphology in the TRN and VB. **(D)** Average pooled data of mIPSC frequency in different brain regions of the two mice strains. See Table 1 for numbers. * p < 0.0125.

FIG. 2.

Differences between strains in EEG power during non-REM sleep (**A**) *Upper*, raw epidural EEG signal recorded from a C57B/6 mouse. *Lower*, power spectrum derived from epochs during awake and nREM sleep states taken from periods marked by black bars (**B**) *Upper*, raw epidural EEG signal recorded from a DBA/2J mouse. *Lower*, power spectrum derived from epochs during awake and nREM sleep states taken from periods marked by black bars. (**C**) Average of absolute EEG power in each strain during the awake state. (**D**) Average of absolute EEG power in each strain during nREM sleep. (**E**) Relative EEG power (normalized to overall power) for the two strains in two frequency bands during non-REM sleep. * $p < 0.05$

TABLE 1

Regional differences in mIPSC characteristics between DBA/2J and C57B/6 strains.

		Amplitude (pA)	Decay (ms)	Rise time (ms)	Frequency (Hz)
Cortex	C57 (10)	58.1 ± 1.8	7.6 ± 0.4	0.29 ± 0.01	4.1 ± 0.5
	DBA (24)	66.0 ± 1.7	8.0 ± 0.3	0.28 ± 0.01	4.4 ± 0.4
	p=	0.01	0.41	0.25	0.73
TRN	C57 (6)	48.6 ± 2.2	54.6 ± 2.5	0.43 ± 0.02	1.3 ± 0.2
	DBA (5)	44.4 ± 6.5	42.8 ± 1.2	0.39 ± 0.01	3.2 ± 0.5
	p=	0.50	0.003	0.15	0.006
VB	C57 (9)	79.4 ± 6.0	7.9 ± 0.6	0.65 ± 0.03	1.6 ± 0.3
	DBA (14)	79.2 ± 3.0	7.2 ± 0.4	0.50 ± 0.01	3.3 ± 0.4
	p=	0.98	0.30	0.001	0.007

Numbers in parentheses represents cell counts recorded in each strain. These cells were derived from 3-5 animals in each group. Significance set at $p < 0.0125$ following Bonferroni correction.