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Selective Increase in T-Type Calcium Conductance of Reticular Thalamic Neurons in a Rat Model of Absence Epilepsy

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The properties of voltage-dependent calcium currents were compared in thalamic neurons acutely dissociated from a rat model of absence epilepsy, designated as Genetic Absence Epilepsy Rat from Strasbourg (GAERS), and from a Nonepileptic Control strain (NEC). Two populations of neurons were isolated: thalamocortical relay neurons of the nucleus ventrobasalis (VB) and neurons of the nucleus reticularis (RT) of the thalamus. Whole-cell patch-clamp analysis demonstrated an increase in the amplitude of the calcium (Ca^{2+}) current with a low threshold of activation (I_T) in RT neurons of GAERS in comparison to that of the seizure-free rat strain (−198 ± 19 pA and −128 ± 14 pA, respectively), whereas the sustained component (I_L) was not significantly different. The kinetic properties, voltage dependence, and basic pharmacological sensitivity of the Ca^{2+} conductances were similar in the two populations of neurons. The amplitude of both I_T and I_L in RT neurons increased after birth, and differences in I_T between GAERS and NEC attained significance after postnatal day 11. At corresponding ages, the Ca^{2+} currents in VB thalamocortical relay neurons were not altered in GAERS in comparison to those in NEC.

We conclude that the selective increase in I_T of RT neurons enhances the probability of recurrent intrathalamic burst activity, thereby strengthening the synchronizing mechanisms in thalamocortical systems, and, as such, represents a possible primary neuronal dysfunction that relates to the pathological increase in synchronization underlying the generation of bilateral and synchronous spike and wave discharges (SWDs) in an established genetic model of generalized epilepsy.

[Key words: thalamus, calcium currents, synchronization, spike and wave discharges, absence epilepsy, genetic model]

Idiopathic generalized epilepsies in humans are characterized by the spontaneous occurrence of convulsive or nonconvulsive seizures that correlate with the abrupt appearance of bilateral synchronous spike and wave discharges (SWDs) on the electroencephalogram (EEG). Rhythmic SWDs that involve the entire cortical mantle from the very onset of the seizure are observed in the prototypical form of idiopathic epilepsy, the absence epilepsy, a presumably genetically determined disease (Metrakos and Metrakos, 1961; Roger et al., 1985; Berkovic et al., 1987; Commission on Classification and Terminology of the International League Against Epilepsy, 1989). The abrupt initiation and cessation of EEG discharges in generalized epilepsy suggested the hypothesis that a centrencephalic pacemaker structure projecting diffusely to the cortex could be responsible for bilateral and synchronous SWDs (Jasper and Kershman, 1941). Since the demonstration that SWDs in humans originate from the thalamus (Spiegel et al., 1951; Williams, 1953), the hypothesis that the mechanisms of thalamocortical synchronization could be implicated in the generation of spontaneous SWDs has been extensively investigated (Jasper and Droogleever-Fortuyn, 1946; Avoli and Gloor, 1982; Gloor and Fariello, 1988; Avoli et al., 1990).

It is widely recognized that the thalamus is intimately involved in cortical rhythmogenesis. During the transition toward quiescent sleep (from a desynchronized to a synchronized EEG state), thalamic nuclei generate rhythmic oscillations that progressively entrain the entire thalamocortical system to produce synchronous rhythmic activity termed spindling (Jahnsen and Llinás, 1984; Steriade and Deschenes, 1984; Steriade and Llinás, 1988; McCormick, 1992; Steriade et al., 1993). Important elements during the transition from desynchronized to synchronized states of the EEG are represented by the neurons of the nucleus reticularis thalami (RT). The RT is a shell-shaped nucleus formed by GABAergic neurons that project to the dorsal thalamus and receive axon collaterals from both corticothalamic and thalamocortical fibers (Jones, 1985). Spindle activity is produced in the RT by the peculiar interplay between the intrinsic membrane properties, in particular, a Ca^{2+} conductance with low threshold of activation and Ca^{2+}-dependent potassium or cationic currents (Avanzini et al., 1989; Huguenard and Prince, 1992; Bal and McCormick, 1993), and by the arrangement of RT synaptic interactions within the thalamocortical network (Steriade et al., 1986, 1987; Spreafico et al., 1991; von Krosigk et al., 1993). The potential role of RT neurons in sustaining the pathological synchronization underlying SWDs has been studied in a genetically based animal model that mimics the characteristics of human absence epilepsy, designated as Genetic Absence

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Epilepsy Rat from Strasbourg (GAERS; Marescaux et al., 1992). Absence-like seizures in GAERS occur spontaneously and are characterized by behavioral arrest, staring, and clonic twitching of the vibrissae, associated with high-amplitude SWDs at 7–11 Hz. As for human generalized primary idiopathic epilepsies, in GAERS (1) the SWD trait is inherited, (2) the onset of seizures is age dependent, (3) seizure occurrence increases during phases of transition between sleep and wakefulness, (4) SWDs are suppressed by drugs effective against human absence epilepsy, (5) intercritical EEG activity is normal, and (6) there are no signs of neurological deficits or intercritical behavioral impairment. In agreement with a possible pathogenic role of the thalamus and, in particular, of the RT in the expression of absence seizures, SWDs in GAERS were demonstrated to originate from the lateral thalamic nuclei (Vergnes et al., 1987), and were abolished by local infusion of Ca^{2+} antagonists into the RT (Avanzini et al., 1993).

In order to gain insights into the cellular mechanisms that may be involved in the generation of SWDs, we analyzed voltage-dependent Ca^{2+} currents in thalamocortical relay neurons and in RT neurons acutely isolated from GAERS and from a selected 100% seizure-free rat strain (nonepileptic controls, NEC).

Materials and Methods

Acutely isolated neurons were prepared from coronal slices (400 μm), including the RT and the ventrobasal complex of the thalamus (VB) cut by vibratome or tissue chopper from 7–20-d-old GAERS and NEC after halothane anesthesia. Under a stereoscopic microscope, the RT was isolated from the adjacent VB by a cut along the external medullary lamina. The cellular content of the isolated tissue was verified with standard GABA or glutamic acid decarboxylase (GAD) immunostaining (Pape et al., 1994); tissue sections including RT contained a high concentration of immunopositive neurons, whereas GABAergic cells were extremely rare in VB tissue slices. RT cells and relay VB neurons from the same animal were separately dissociated using standard enzymatic/mechanical procedures (Kay and Wong, 1986; Budde et al., 1992). Thalamic slices were incubated in oxygenated medium containing either trypsin (Sigma type XI; 1–4 mg/ml, 60–90 min at room temperature) or papain (Worthington; 14 U/ml, 10–20 min at 36°C), bovine serum albumin (0.5 mg/ml) and (in mM) 120 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 20 PIPES, 25 dextrose (pH 7.35). After washing with enzyme-free medium, the neurons were mechanically dissociated by trituration. Neurons acutely dissociated from the VB were characterized by a large, multipolar, and GABA-negative soma, whereas RT neurons showed a fusiform soma intensely stained by GABA, giving rise to two major dendrites at opposite poles (see also Huguenard and Prince, 1992). Intracellular recordings were performed at room temperature from isolated neurons using the whole-cell variant of the patch-clamp technique (Hamill et al., 1981). Patch electrodes had an access resistance of 4–8 MΩ. Series resistance compensation > 50% was routinely utilized. Records were low-pass filtered at 2.5 kHz (3–8 pole Bessel filter), and a P/4 pulse protocol (pCLAMP; Axon Instruments, USA) was used to remove leak and capacitive current interferences (see Budde et al., 1992). Two different extracellular solution perfused continuously at 0.1–1 ml/min were utilized in the two laboratories to isolate Ca^{2+} currents. The solution used in the Bochum laboratory was (in mM) 120 NaCl, 1 KCl, 10 D-mannitol, 2 $CaCl_2$, 1 $MgCl_2$, 20 TEA-Cl, 6 4-AP, 10 HEPES, and 1.5 μM TTX. The solution in Milano laboratory was (in mM) 80 choline-Cl, 3 KCl, 24 glucose, 5 $CaCl_2$, 1 $MgCl_2$, 50 TEA-Cl, 8 4-AP, 10 HEPES. The solution in the pipette contained (in mM) 128 *N*-methyl-D-glucamine, 10 NaCl, 1 $CaCl_2$, 2 $MgCl_2$, 11 EGTA, 5 Na_2ATP, 0.5 Na_2GTP, and 20 TEA-Cl (pH 7.35); or (in mM) 110 Tris-PO_4 dibasic, 28 Tris-base, 2 $MgCl_2$, 0.5 $CaCl_2$, 11 EGTA, 2 MgATP, 0.2 Tris-GTP, 20 creatine-phosphate, and 50 U/ml creatine-phosphokinase (pH 7.4), in the Bochum and Milano laboratories, respectively.

Results

Whole-cell voltage-clamp experiments were performed in acutely dissociated neurons under conditions where Ca^{2+} currents are isolated. Depolarization of the membrane in the range between −80 and −40 mV from a holding potential of −110 mV evoked a transient Ca^{2+} inward current (I_T), whose amplitude increased with membrane depolarization (Fig. 1*A*). Positive to −40 mV, a more sustained Ca^{2+} current (I_L) was additionally evoked, confirming the existence in RT neurons of two Ca^{2+} conductances with different activation threshold and kinetics (Huguenard and Prince, 1992). By comparing the basic characteristics of Ca^{2+} currents in RT neurons from GAERS and NEC, a striking difference in the amplitude of I_T was observed. As illustrated in Figure 1, depolarizing steps from a holding potential of −110 mV evoked much larger transient I_T currents in RT neurons from GAERS than in NEC, whereas the amplitude of the sustained I_L component was similar in the two groups of neurons. Current–voltage relationships (*I/V* histograms in Fig. 1*A*), constructed from a larger sample of neurons, demonstrated a significant increase in peak current amplitude at potential values negative to −30 mV in GAERS (n = 16) compared with that in NEC (n = 18), with no obvious shift in the peak current–voltage relationship. The sensitivity toward inorganic Ca^{2+} channel antagonists was not significantly different in RT neurons from the two groups of rats (data not shown). Nickel (50 μM) reduced I_T by 54.0 ± 21.4% (n = 9) and by 37.7 ± 8.8% (n = 7), and I_L by 30.0 ± 14.0 (n = 6) and by 33.1 ± 7.6% (n = 7) in RT neurons from GAERS and NEC, respectively. Cadmium (50 μM) reduced I_T by 32.3 ± 23.0% (n = 7) in GAERS and by 57.1 ± 23.2% (n = 6) in NEC, while I_L was completely abolished in RT neurons from both groups of animals (n = 10 and n = 8, respectively). Ethosuccimide, at a saturating concentration (5 mM; Coulter et al., 1989b), reduced I_T by 18.3 ± 6.9% (n = 7) and by 19.5 ± 2.9% (n = 3) in GAERS and in the control group, similar to the reduction observed for the L-type current in the two populations of neurons (18.1 ± 6.3, n = 5; 22.6 ± 6.7, n = 7). Replacement of Ca^{2+} by barium ions (2 mM) resulted in an increase in amplitude of both I_T (23.2 ± 6.3%, n = 7 GAERS; 18.1 ± 3.4 %, n = 7 NEC) and I_L (43.4 ± 13.4%, n = 6 GAERS; 20.8 ± 10.5, n = 3 NEC), as previously reported (Huguenard and Prince, 1992).

The transient and sustained Ca^{2+} current components were separated using a conditioning pulse protocol. A 100 msec prepulse to −50 mV between the holding potential (−110 mV) and the depolarizing voltage steps inactivated I_T. The I_L current activated at membrane potentials positive to approximately −40 mV, and the amplitude was not significantly different in RT neurons from GAERS and NEC (Fig. 1*B*). The I_T current was isolated by digital subtraction of the records obtained without and with the conditioning prepulse (Fig. 1*C*). In both groups of RT neurons, I_T was rapidly activated through depolarizing voltage steps and peaked at around −50 mV. The average peak amplitude at −50 mV was significantly larger in GAERS [−198 ± 19 pA (mean ± SEM; n = 16)] than in NEC (−128 ± 14 pA; n = 17) ($p < 0.005$). The amplitude of the T-type Ca^{2+} current in thalamic neurons increases significantly during postnatal development (see Fig. 3; see also Pirchio et al., 1990). The I_T amplitude differences observed in the present study are not related to development, since the two populations of RT neurons were isolated at the same postnatal age (postnatal days: 15.6 ± 0.6, n = 16 GAERS; 15.6 ± 0.6, n = 18 NEC). The possibility that the differences in I_T amplitude resulted from differences in cell size could be largely ruled out by using the whole-cell capacitance as an index of membrane surface area, and which was

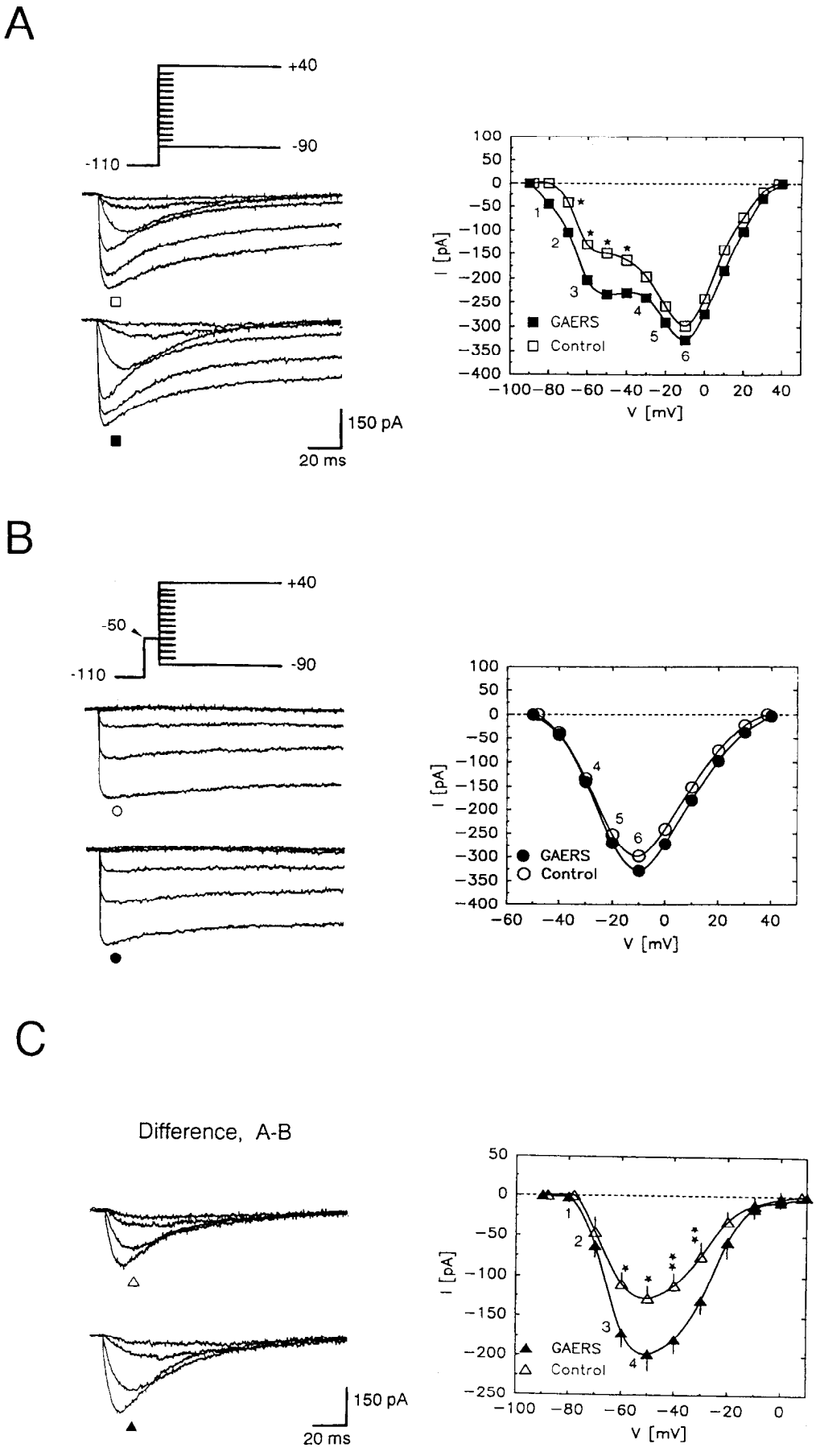


Figure 1. Whole-cell Ca^{2+} currents in RT neurons isolated from GAERS (*closed symbols*) and NEC (control; *open symbols*). *A*, Depolarizing voltage steps from −110 mV evoke a transient Ca^{2+} current, I_T, followed by a sustained component, I_L, at more positive potentials. Note the increase in the transient component in GAERS, which is also indicated by the increase in the typical shoulder separating the two current components with different activation threshold in the *I/V* relationship. *B*, A conditioning prepulse to −50 mV (duration 100 msec) inactivates I_T, and the remaining I_L is very similar in RT neurons from GAERS and NEC. *C*, Digital subtraction of I_L (from experiments in *B*) from the total Ca^{2+} current (from *A*) isolates I_T, whose amplitude is significantly increased in GAERS. *I/V* relationships of peak currents are averaged from recordings in $n = 16$ and $n = 18$ RT neurons from GAERS and from NEC, respectively; *numbers* indicate examples shown as original traces; bars in *C* represent SEM, *asterisks* mark significant differences (**, $p < 0.001$; *, $p < 0.005$; two-tailed t test) between GAERS and NEC. Neurons from both groups of animals were dissociated at postnatal day 16.

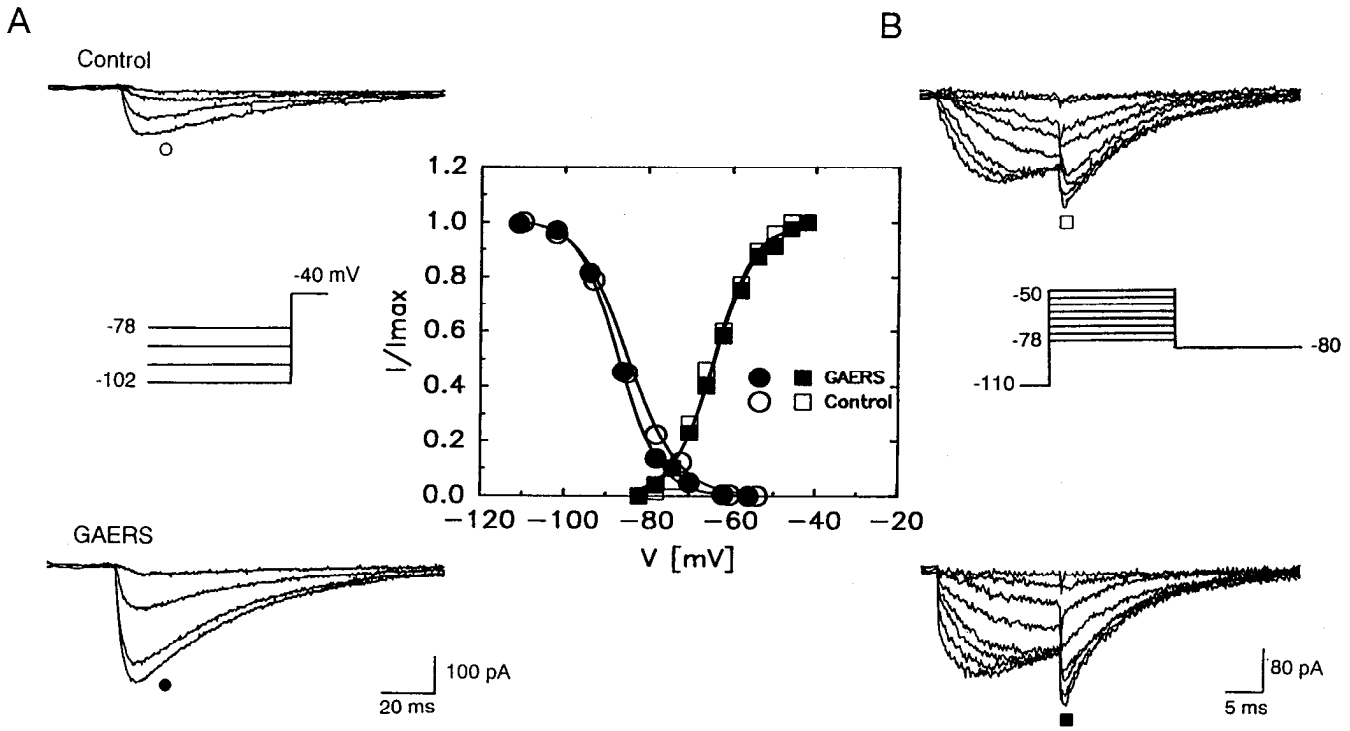


Figure 2. Similar voltage-dependent properties of I_T in RT neurons from GAERS (*closed symbols*) and NEC (control; *open symbols*). *A*, Steady-state inactivation of I_T, as determined by varying a prepulse of 2 sec duration between −110 and −54 mV before stepping to −40 mV. The amplitude of I_T decreases with more positive values of the prepotential, indicating inactivation of the underlying conductance. *B*, Analysis of tail currents. I_T was activated with 15 msec depolarizations to various potentials, and tail currents were evoked upon return to −80 mV. *C*, The inactivation (*circles*) and activation curve (*squares*) of I_T, as obtained from normalized peak currents (experiments in *A*) and tail current amplitudes (*B*), are not significantly different for the two groups of cells. Histogram represents averaged data (n = 10–15); continuous curves are Boltzmann fits.

not significantly different in the two groups of neurons (16.3 ± 0.8 pF, n = 13 GAERS; 15.7 ± 1.2 pF, n = 11 NEC).

A possible explanation for the altered expression of I_T in RT neurons may relate to changes in the voltage-dependent properties of the underlying conductance, for example, a shift in the inactivation and activation curves, or an alteration in the kinetics of the current. Since the time constant of activation of I_T could not be reliably determined, due to the relatively small amplitude of the current, the time-to-peak values were used as an approximation of the activation kinetics. The time-to-peak of I_T decreased with more positive membrane potentials: averaged values in the voltage range between −70 and −40 mV were between 21.2 ± 1.6 msec and 5.1 ± 0.5 msec in GAERS (n = 11) and between 17.8 ± 1.6 msec and 6.6 ± 1.7 msec in NEC (n = 10). The difference did not attain statistical significance (data not shown). The I_T currents inactivated completely with sustained depolarization, and they could not be elicited from holding potentials positive to −60 mV. The time course of current decay was well fitted by a single exponential function of the form $I = A_0 + A_1\exp(-t/\tau)$, where I is the amplitude of the membrane current at the time t, A_0 and A_1 are the amplitude coefficients, and τ is the time constant. The time constant displayed very little voltage dependence, averaging 37.1 ± 2.9 and 25.3 ± 0.9 msec in GAERS (n = 15), 37.4 ± 3.0 and 26.5 ± 2.6 msec in NEC (n = 14) for depolarizations to −70 and −30 mV, respectively (data not shown).

Figure 2*A* illustrates samples of current records used to determine the voltage dependence of steady-state inactivation of the conductance underlying I_T. A 2 sec prepulse was incremented between −110 and −54 mV with 8 mV steps, before delivering a constant test pulse at −40 mV, subthreshold for I_L and near maximal for I_T activation. The peak current progressively reduced as the prepulse potential became less negative, indicating an increased level of inactivation. The normalized peak amplitudes of the currents were averaged and plotted against the prepotential (circles in histogram of Fig. 2). Approximation of a Boltzmann function[1] to the data points indicated a range of steady-state inactivation of I_T that was largely overlapping in the two groups of RT neurons. The I_T current was completely inactivated positive to −60 mV; the values of half inactivation (at −87.0 ± 0.8 and −84.8 ± 1.3 mV) and the slope of the curves (4.9 ± 0.4 and 6.0 ± 0.6 mV^{-1}) were not significantly different in GAERS (n = 15) and NEC (n = 12). The time course of recovery from inactivation of I_T was approximated best by a single exponential function (data not illustrated). The average time constant in RT neurons from GAERS (278.1 ± 20.4 msec; n = 13) was unaltered compared with that in NEC (274.9 ± 13.7 msec; n = 14). To study the activation range of I_T, tail currents were analyzed, obtained by repolarizing the membrane to −80 mV following 15 msec depolarizing commands (4 mV increments) from a holding potential of −110 mV. The most depolarized command steps evoked nearly overlapping tail currents, indicating near-maximal activation of I_T. With fur-

[1] The equation for the Boltzmann function was $I/I_{max} = [1 + \exp((V-V_h)/k)]^{-1}$, where V_h is the potential of the half-(in)activation and k is the slope factor indicating the steepness of the calculated curve. The equation applies to inactivation and activation curves, k assuming a positive and negative value, and V representing the prepotential and the command potential, respectively.

ther depolarization, tail currents were contaminated by I_L. The duration of the depolarizing commands was set to values near peak current activation, and the tail currents evoked by repolarization to potentials outside the range of I_T activation can be assumed to reflect the T-channels activated during the depolarizing command. Since the repolarizing potential is fixed, tail currents can be normalized and plotted against the command potential to produce an activation curve.

The normalized amplitudes of the tail currents were averaged, plotted against the command step, and a Boltzmann function was approximated to the data points (squares in histogram of Fig. 2). The activation curves of I_T were nearly identical in RT neurons from GAERS (n = 13) and NEC (n = 10). Activation started at around −80 mV and was almost complete at −40 mV; the values of half-activation (−64.0 ± 1.2 and −64.4 ± 1.8 mV) and slope (−4.9 ± 0.3 and −4.7 ± 0.4 mV^{-1}) were not significantly different in GAERS and NEC neurons.

As shown in Figure 3, the amplitude of I_T and I_L increased substantially after birth in RT neurons, and differences in I_T amplitude between GAERS and NEC attained significance after postnatal day 11. At the same postnatal age, the I_T current in thalamocortical relay neurons isolated from VB was not significantly altered in GAERS in comparison to NEC (see also Guyon et al., 1993).

Discussion

The choice of the experimental model

The study of the mechanisms underlying epileptic discharges in primary generalized epilepsies requires the utilization of appropriate experimental models, which have to fulfill the criteria described by the terms idiopathic and generalized (Commission on Classification and Terminology of the International League Against Epilepsy, 1989). The strain of Wistar rats with genetically determined seizures designated as GAERS present spontaneous absences correlated with high-amplitude SWDs occurring abruptly on a normal intercritical EEG activity. Neurophysiological, behavioral, genetic, and pharmacological studies carried out in the past 10 years demonstrated that SWDs in GAERS fulfill the requirements for an experimental model of absence epilepsy (Marescaux et al., 1992). The SWD genetic trait in GAERS is inherited, and the onset of seizures is age dependent, starting after 1 month of postnatal life (Vergnes et al., 1986; Marescaux et al., 1992). As for spindles and human generalized SWDs (Kellaway, 1985), seizure occurrence in GAERS increases during phases of transition between arousal and sleep, although the frequency of SWDs in GAERS at 7–11 Hz (Marescaux et al., 1992) is higher than that (2–4 Hz) seen in the EEG of petit mal patients (Kellaway, 1985) and in animal experiments using the penicillin model of generalized epilepsy (Gloor and Fariello, 1988) or the cortically or thalamically induced seizure activity in acute and chronic preparations (cf. Avoli et al., 1990). This heterogeneity of SWDs may indicate that multiple mechanisms may be involved in the genesis of different forms of SWDs (cf. Steriade et al., 1993; von Krosigk et al., 1993). In any case, *in vivo* recordings from the thalamus and cortex in GAERS demonstrated that SWDs were abolished ipsilaterally by a large lesion of the lateral thalamus after a complete callosotomy (Vergnes et al., 1987; Vergnes and Marescaux, 1992). Moreover, Avanzini et al. (1993) demonstrated that SWDs in GAERS are disrupted ipsilaterally to selective lesions of RT induced by stereotaxic injections of the excitotoxine ibotenic acid in previously callosotomized animals. Similarly, inorganic Ca^{2+} antagonists suppressed SWDs when locally infused in RT and only reduced their expression when injected in the lateral thalamic relay nuclei (Vergnes et al., 1987; Avanzini et al., 1993). These data strongly suggested that Ca^{2+}-dependent processes in RT are determinant in the regulation of SWDs in GAERS, and they achieve particular relevance in light of the demonstration in isolated thalamic neurons that the transient calcium current I_T, critical for the generation of oscillatory behavior, is reduced by antiepileptic drugs effective on absence seizures and on GAERS SWDs, like ethosuccimide (Marescaux et al., 1984; Coulter et al., 1989b, 1990; Huguenard and Prince, 1992, 1994a).

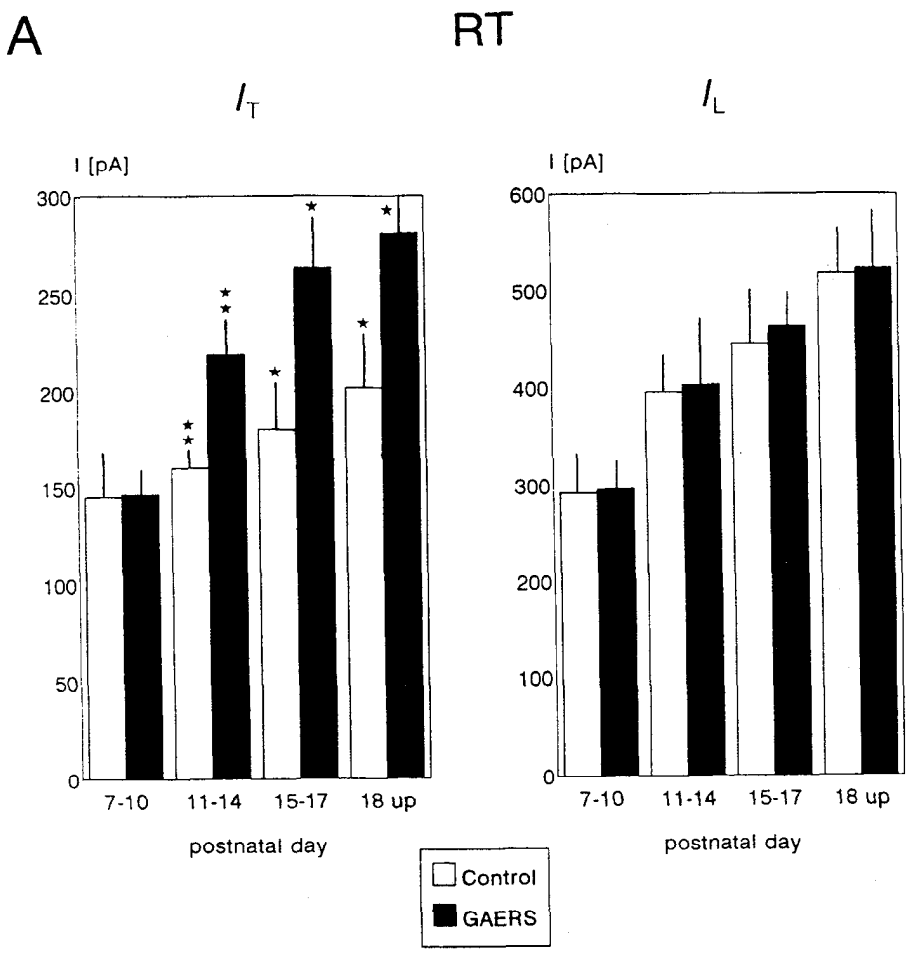
Selective I_T increase in RT

The present study demonstrates that in GAERS the transient Ca^{2+} conductance I_T is selectively augmented in neurons of the RT after the second postnatal week. This alteration seems to be selective in terms of the type of Ca^{2+} conductance and the type of thalamic neuron that are affected, since the L-type current component was not affected in the same sample of RT neurons, and Ca^{2+} currents were not significantly different in thalamocortical relay neurons isolated from the VB complex of the same animals. The latter result confirms the observation by Guyon et al. (1993) of unaltered Ca^{2+} currents in VB slices obtained from adult GAERS. The observed enhancement of I_T amplitude is not due to differences in membrane surface area or postnatal age of the two populations of RT neurons that were studied, and it is not likely to result from an alteration in the gating properties of the underlying conductance, since activation and inactivation curves and the kinetics of the current were similar in neurons dissociated from NEC and GAERS. Following from this, the augmented I_T current in RT neurons from GAERS seems to reflect an increase in the number of available T-type Ca^{2+} channels or an increase in single channel conductance, thereby resembling the transient enhancement of I_T demonstrated in relay neurons after cortical lesions (Chung et al., 1993).

A transient Ca^{2+} current with a nearly voltage-independent, slow rate of inactivation and a rather positive range of activation (V_h = −49 mV), designed as I_T, has been demonstrated by Huguenard and Prince (1992) in neurons isolated predominantly from the most anterior section of the thalamus. The neurons from the dorsal, VB-related portion of the RT studied in the two laboratories participating in the present study (see Materials and Methods) possessed a more rapidly inactivating I_T with negative range of activation (V_h = −64 mV). This heterogeneity suggests that the I_T may be selectively segregated in neurons of the most anterior portion of RT, where neurons also present morphological features different from those of the more caudal RT (Scheibel and Scheibel, 1972; Spreafico et al., 1991; Lübke, 1993), or that two classes of neurons possessing distinctive electrophysiological properties may exist in the RT nucleus (Contreras et al., 1992).

Increased synchronization and SWDs

Several lines of evidence summarized in the introduction indicate that common circuitry and intrinsic mechanisms in the thalamus, of which reciprocal intrathalamic connections and burst firing dependent on the T-type Ca^{2+} conductance represent two important variables, underlie spindle rhythm and SWDs of absence epilepsy. We suggest that the selective, presumably genetically determined, increase in the conductance underlying I_T in RT neurons enhance the propensity for burst firing, thereby



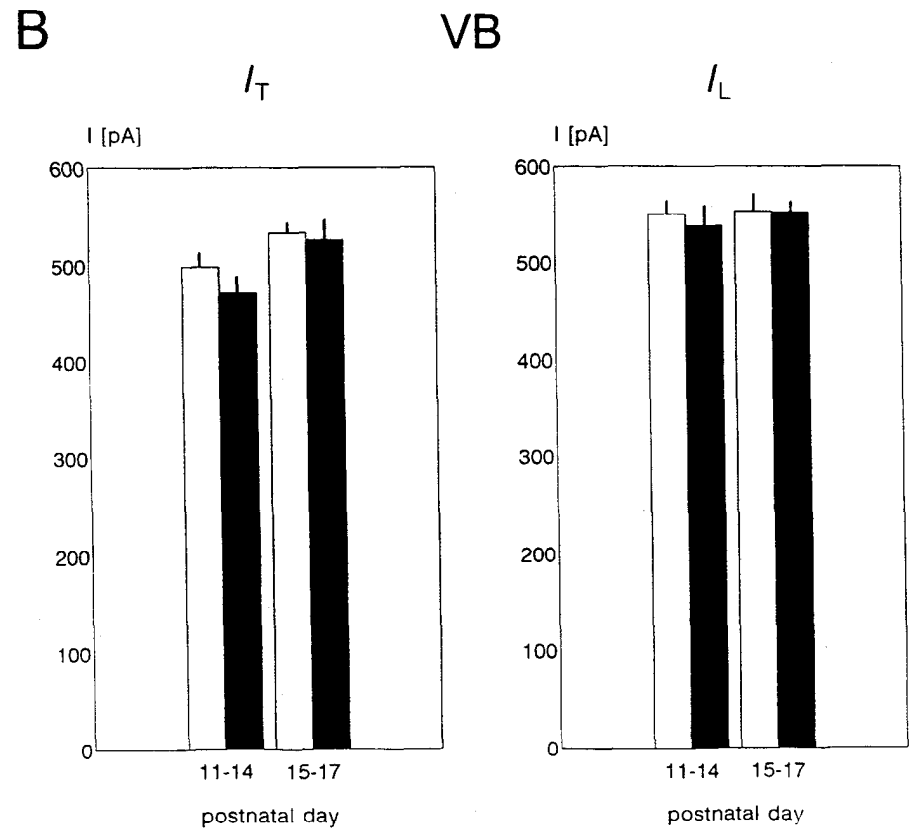


Figure 3. Age-dependent and selective increase of I_T in RT neurons from GAERS. Mean maximal amplitudes of I_T and I_L in RT neurons (*A*) and relay neurons from VB (*B*) acutely dissociated from GAERS (*closed bars*) and NEC (control; *open bars*) at different postnatal ages. Note the selective increase in I_T in GAERS starting at postnatal days 11–14. Bars represent averaged data (± SEM); significant differences between GAERS and NEC are indicated by *asterisks* (**, $p < 0.01$; * *, $p < 0.05$; two-tailed t test). To account for possible differences in cell size at different postnatal days, current amplitudes were normalized to a membrane surface area corresponding to an input capacitance of 20 pF.

entraining recurrent burst activity and contributing to the pathological increase in synchronization underlying SWDs in GAERS. In line with this hypothesis is the recent observation in thalamic slice preparations that the decrease (30–40%) in I_T conductance by succinimides resulted in a dramatic reduction in intrathalamic oscillations through a slight decrease in burst probability of thalamic neurons (Huguenard and Prince, 1994b). The Ca^{2+} currents in thalamocortical relay neurons in VB and possibly in other thalamic nuclei are not affected in GAERS, but relay cells can be assumed to be secondarily recruited by the rhythmic discharges generated in RT. In addition, an increase in $GABA_B$ receptor-mediated responsiveness (Snead, 1992; Hosford et al., 1992) and resulting increase in deinactivation of I_T in relay cells may act to reinforce recurrent rhythmic discharges into pathological forms of synchronization on the level of the relay nuclei (Von Krosigk et al., 1993).

The fact that seizures and SWDs in human absence epilepsy and in genetic animal models are age dependent and are usually absent at birth suggests that the pathological expressivity of the original defect (or defects) depends on the complete maturation of neuronal elements and synaptic circuitry in the thalamocortical system. In GAERS, both the increase in I_T amplitude and the appearance of SWDs are absent at early postnatal stages. Their later developmental course diverges considerably, since I_T augmented with respect to controls 11 d after birth, whereas SWDs are undetectable before the end of the first month of postnatal life. It is conceivable that, as for the physiological thalamocortical synchronizing patterns (Jouvet-Mournier et al., 1970), SWD expression in GAERS requires the complete maturation of the thalamocorticothalamic synaptic network to become fully expressed. It is known that maturation of thalamic and cortical connections in rats is completed within 2 weeks of postnatal age (Jones, 1985). By comparison, other processes such as synaptogenesis and spine formation, which control postsynaptic potential generation in the thalamus and cortex and which are probably needed to express functionally mature rhythmic and synchronous thalamocortical oscillations, are modified during later phases of development and do not reach near-adult stages before the fourth postnatal week (Cragg, 1975; Daniels et al., 1975; Wise et al., 1978; Jones, 1985; for a review see Shatz, 1990).

Changes in the quantitative expression of a single current or membrane receptor during the thalamocortical developmental time table paced by a programmed sequence of gene expression (Desarmenian et al., 1992; Spitzer et al., 1994) may significantly modify the synaptic arrangement and the weight of synapses in a developing network. The possibility that such a process is active in the ontogenesis of SWD generation is strengthened by the notion that synaptic activity and rhythmic oscillations play an important role in organizing synaptic connectivity (Changeaux and Danchin, 1976; Provine and Rogers, 1977; Llinás, 1984; Llinás, 1988; Shatz, 1990; Kalb and Hockfield, 1992; Sillar, 1994). It is known from these studies that during development, neurons produce electrical autorhythmicity, which can entrain homogeneous populations of neurons to oscillate. Network oscillations lead to a dynamic linkage between different brain areas and may reinforce synaptic interactions. Oscillatory rhythmic activity at various frequencies, whose relevance in normal physiological conditions can be easily appreciated by looking at the EEG of an infant, may be an important factor in determining the strength and the ontogenic organization of synaptic connectivity that are necessary to regulate a complex function. The abnormal thalamic oscillatory activity generated in juvenile GAERS by the augmented I_T in RT neurons could, in principle, consolidate excitatory synapses and influence the plastic properties of developing thalamic and cortical neurons toward a persistent state of hyperexcitation, which is expressed by SWDs.

The selective increase in I_T conductance in RT neurons is the first demonstration of a primary neuronal dysfunction suspected to relate to seizure generation in an established animal model of genetic epilepsy and represents a potentially important step in the attempt to identify markers for genetic linkage studies in human epilepsies.

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