Giant Spontaneous Depolarizing Potentials in the Developing Thalamic Reticular Nucleus

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1Department of Neurology and Neurological Sciences, Stanford University School of Medicine, Stanford, California; and 2Laboratory of Genetic Neuropharmacology, McLean Hospital, Mailman Research Center, Harvard Medical School, Belmont, Massachusetts

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Pangratz-Fuehrer S, Rudolph U, Huguenard JR. Giant spontaneous depolarizing potentials in the developing thalamic reticular nucleus. J Neurophysiol 97: 2364–2372, 2007. First published January 24, 2007; doi:10.1152/jn.00646.2006. The thalamic reticular nucleus (nRt) provides a major source of inhibition in the thalamocortical circuit and is critically involved in the generation of spindle oscillations. Here we describe the properties of thalamic giant depolarizing potentials (tGDPs) that were observed in nRt during early development. GDPs persisted in presence of ionotropic glutamate receptor agonists and were completely abolished by GABAAR antagonist SR 35591. tGDPs occurred primarily between p3 and p8 in 30–50% of cells and occasionally up until p15. tGDPs lasted 0.4 –3 s with peak conductances of 2–13 nS and occurred at frequencies between 0.02 and 0.06 Hz. We used mice with a benzodiazepine-insensitive α3 subunit [α3(H126R)] to probe for the identity of the GABA receptors responsible for tGDP generation. Benzodiazepine enhancement of tGDP amplitude and duration persisted in nRt neurons in α3(H126R) mice, indicating that the GABAARs containing α3 are not critical for tGDP generation and suggesting that tGDPs are mediated by GABAARs containing the α5 subunit, which is transiently expressed in nRt neurons in early postnatal development. Furthermore, we found that exogenous GABA application depolarized nRt neurons younger than p8, indicating elevated [Cl−] at this developmental stage. Taken together, these data suggest that in immature nRt, long-lasting depolarizing responses mediated by GABA receptors could trigger Ca2+ entry and play a role in functional development of the spindle-generating circuitry.

INTRODUCTION

In the adult mammalian CNS, the GABAergic nucleus reticularis thalami (nRt) is central to rhythm generating circuitry related to sleep and wakefulness within the thalamocortical network. Reciprocal connections between nRt and thalamo-cortical (TC) relay neurons enable intra-thalamic oscillatory activity. The synaptic release of GABA from nRt leads to inhibition of TC neurons and the triggering of rebound bursts, which in turn provide excitatory feedback to nRt, thus completing a reciprocal loop (Huguenard and Prince 1994; Steriade et al. 1985; Warren and Jones 1997). These intrathalamic oscillations, which can occur spontaneously, are thought to be the basis for spindle rhythmicity; however, some evidence suggests that the intra-nRt circuit itself may in some cases act as pacemaker (Destexhe et al. 1994; Steriade et al. 1985). Inhibitory connectivity within nRt regulates inhibitory output and suppresses synchrony by decreasing release of GABA onto TC cells (Huguenard and Prince 1994; Huntsman et al. 1999; Sohal et al. 2000; von Krosigk et al. 1993; Warren et al. 1994). In contrast, the uncontrolled release of GABA could lead to the pathological hypersynchrony of sleep spindles and related rhythms and may be a factor in childhood absence seizures (Steriade and Llinas 1988).

The physiological pattern of sleep spindles is absent in infants up until the second month of age (Lenard 1970; Metcalf 1970), in rodents until postnatal day 10–12 (p10-12) (Jouvet-Mounier et al. 1970) and in ferrets up until p22 (McCormick et al. 1995). This suggests that GABAergic inhibition mediated by nRt undergoes developmental maturation. Therefore, we explored the properties of GABAergic synaptic inhibition in developing mouse nRt prior to the emergence of spindle oscillations. Unexpectedly, we observed the presence of thalamic giant depolarizing potentials (tGDPs) during the first 2 wk of postnatal life. This spontaneous neuronal pattern is mediated by GABAAR receptors (GABAARs). Similar patterns have been reported in neocortex (Yuste et al. 1992), retina (Feller 1999; Wong 1999), spinal cord (Gonzalez-Islas and Wenner 2006; Spitzer 2002), brain stem (Gummer and Mark 1994), and hippocampus (Cherubini et al. 1991; Garaschuk et al. 2000), where they appear to be Ca2+ dependent. The discovery of spontaneous network activity in developing systems (Ben Ari et al. 1990; Goodman and Shatz 1993; Katz and Callaway 1992; Meister et al. 1991; Wong et al. 1993; Yuste et al. 1992) raised the possibility that correlated patterns contribute to functional maturation of neonatal circuitry. In the present study, we focus on the developmental profile and pharmacological properties of tGDPs, a novel pattern of the immature intra-reticular network.

METHODS

Animals

Wild-type and mutant mice [α3(H126R)] pups of either sex were used at postnatal days 3–20 (p3–p20). Mutants were mice homozygous for a histidine-to-arginine point mutation at position 126 of the GABAAR receptor α3 subunit (5–6 backcrosses to the 129/SvJ background) that were generated as described previously (Low et al. 2000).

Thalamic slice preparation

Experiments were performed in accordance with approved procedures (Protocol 12321/0) established by the Administrative Panel on Laboratory Animal Care at Stanford University. Mice were deeply

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anesthetized by intraperitoneal injection with pentobarbital (50 mg/kg) until unresponsive and then decapitated. Brains were blocked, removed and placed in ice-cold (4°C), oxygen-equilibrated (95% O₂-5% CO₂) “cutting” solution (containing in mM: 234 sucrose, 11 glucose, 24 NaHCO₃, 2.5 KCl, 1.25 NaH₂PO₄, 10 MgSO₄, and 0.5 CaCl₂) for ~1 min. Horizontal slices (200 μm) were cut with a Vibratome (TPI, St. Louis, MO), hemi-sected and incubated in a preheated (32°C) oxygen-equilibrated chamber filled with artificial CSF (ACSF) (containing in mM: 126 NaCl, 26 NaHCO₃, 10 glucose, 2.5 KCl, 2 MgCl₂•6H₂O, 2 CaCl₂•2H₂O, and 1.25 NaH₂PO₄•H₂O) for 1 h before recording.

Electrophysiology

Whole cell patch-clamp recordings were obtained from nRt neurons in a chamber with a constant flow of ACSF perfusion (2 ml/min) equilibrated with 95% O₂-5% CO₂. Experiments were conducted at room temperature. Glass electrodes (tip resistance: 2.5–3.3 MΩ, KG-33, borosilicate glass, Garner Glass, Claremont, CA) were pulled in multiple stages using a Flaming-Brown micropipette puller (model P-87, Sutter Instruments, Novato, CA). For voltage-clamp recordings, pipettes were filled with high (139 mM) chloride solution [containing in mM: 135 CsCl, 5 lidocaine N-ethyl bromide (QX-314), 2 MgCl₂, 10 ethyleneglycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA; Sigma, St.Louis, MO), and 10 HEPES. The solution was adjusted with CsOH to pH 7.3]. The solution was adjusted with KOH to pH 7.3.

Voltage-clamp recordings were made from visually identified neurons within nRt using a fixed-stage upright microscope (Axioskop, Zeiss, Thornwood, NY) equipped with an insulated ×63 objective, Normarski optics and an infrared-sensitive video camera (Cohu, San Diego, CA). Only one experiment per slice was performed to study pharmacology (n of slices), whereas up to two cells per slice were recorded to describe developmental changes (n of cells). Access resistance of all recorded cells was monitored constantly throughout each experiment and only used for analysis if it was <18 MΩ and stable (25% tolerance) for the duration of the experiment.

tGDP identification

The voltage-clamp manifestations of tGDPs were recorded at a holding potential of ~60 mV and appeared under these recording conditions as inward currents. tGDPs were identified based on three criteria including minimum amplitude, duration, and number of individual components within each event (see RESULTS). The following properties of tGDPs (n > 10 events/cell) were determined: time to peak (TTP), the time required to rise from onset to peak amplitude, 90% width (the time required to decay by 90% from peak amplitude), peak current amplitude and interevent interval (IEI). To confirm that tGDPs represent distinct events and not just the occurrence of multiple concurrent spontaneous occurring inhibitory postsynaptic currents (sIPSCs), we integrated baseline-corrected voltage-clamp recordings, using a sweep length of 2 s. Histograms of net charge per 2-s period revealed two distinct populations (e.g., Fig. 3), confirming that tGDPs are not simply summated sIPSCs.

The onset of each tGDP was defined as the time of occurrence of the first component of a multiphasic response. The first component was defined as the last event before the tGDP that did not fully decay to baseline. The IEIs between tGDPs were determined for each recording. Patterns were defined as “regular” when the coefficient of variation for IEI was ≤0.4, whereas “irregular” tGDP patterns were defined as having coefficients of variation >0.4.

Pharmacology and method of application

GABAAR mediated tGDPs were recorded in the presence of (±)-2-amino-5-phosphonopentanoic acid (APV, 100 μM, Sigma), 6,7-dimethoxyquinoline-2,3-dione (DNQX, 20 μM, RBI, Natick, MA) to block ionotropic glutamate receptors. Concentrated solutions of clonazepam (CZP, 100 nM) and gabazine (GBZ, 10 μM; all Sigma) were stored at −20°C, diluted with ACSF before experiments and were applied via multi-barrel local perfusion unless otherwise stated.

Data collection and analysis

Data were filtered at 2 kHz, collected, and sorted with locally written software [Metatape, WDetecta and WinScanSelect (J. R. Huguenard)], then analyzed using PClamp 9 (Axon Instruments, Union City, CA) and Origin (MicroCal Software, Inc., Northampton, MA). Data are presented as the means ± SE. Student’s t-test was used to assess statistical significance unless otherwise stated and differences were regarded significant if P < 0.05 (*). Higher levels of significance are indicated as ** (P <0.01), *** (<0.001), or **** (<0.0001).

RESULTS

Whole cell voltage-clamp recordings were obtained from nRt neurons in mouse slices of six different age groups (p3/4, p5/6, p7/8, p9/11, p12/15, and p16/30); each group represents a total number of 50–57 neurons.

Identification of tGDPs in nRt

Examples of tGDPs at p4 and p8 are shown in Fig. 1A, I–2, and B, I–2, respectively. Here, we recorded tGDPs at ~60 mV in voltage-clamp mode at room temperature in the presence APV (100 μM) and DNQX (20 μM) to block ionotropic glutamate receptors. We established three absolute criteria for all age groups to reliably isolate tGDPs from simple sIPSCs. The three basic properties include amplitudes >100 pA (±700 pA), durations of 0.4–3 s (defined as time of decay from peak to 10% of the peak amplitude; i.e., 90% width), and multi-component appearance consisting of a minimum of three transients. Additionally, there was usually “build-up” of synaptic responses during each tGDP, such that several small transients preceded the peak response. This activity pattern occurred spontaneously at frequencies of 0.02–0.06 Hz and was completely abolished by GABAAR antagonist SR 95531 (10 μM; Fig. 5C), thus we termed these events thalamic giant GABAergic spontaneous depolarizing potentials (tGDPs). In the following analysis, when we use the term tGDP, we are referring to the events (synaptic currents) that would cause membrane depolarization if they were recorded in current clamp. We used voltage-clamp experiments in this study as it allowed us to accurately detect and characterize the individual components of each multiphasic synaptic response. In Fig. 1, representative continuous recordings and individual tGDP barrages are shown at two distinct time points during development (Fig. 1A and B). There were fewer individual transients within each tGDP at p3/4 (mean number of transients per tGDP = 4.34 ± 0.23) compared with tGDPs at p7/8 (Fig. 1B2, 5.63 ± 0.05) or at later time points (p9/11: 7.04 ± 0.07; P12/15: 4.93 ± 0.14, not shown). In general, there were fewer and smaller transients during the decay phase (mean ratio between pre- and postpeak transients at p3/4 = 1.3; at p7/8 = 1.48 and at p12/15 = 1.39; ns as in the preceding text). tGDPs generally showed similar kinetic properties (e.g., amplitude, duration, time to peak) throughout the duration of a recording.
either more or less mature mice. (Table 1), and longer intervals were observed with slices from tGDPs were recorded, the briefest IEIs were observed at p7/8 slices with tGDPs at these ages, respectively). Within slices where tGDPs were not observed later than p15. Regular tGDP patterns incidence declined in more mature slices at p9/11 and p12/15 (37 and 55% of 11 of 50; 22%, and 2366 S. PANGRATZ-FUEHRER, U. RUDOLPH, AND J. R. HUGUENARD

FIG. 1. Whole cell patch-clamp recordings of thalamic giant GABAergic spontaneous depolarizing potentials (tGDPs) in thalamic reticular nucleus (nRt) neurons. Sample traces illustrate prominent features of GDPs recorded at p4-8. A1: example nRt cell at p4 shows predominately slow and large events with durations of ~800 ms and amplitudes ~400 pA. A2: representative tGDP from A1 is shown on faster time scale. B1: recordings from a p8 cell. B2: representative tGDP from B1 is shown on an extended time base. The recording in A1 shows an irregular pattern with highly variable interevent intervals (IEIs) between tGDPs. In B1, a regular pattern with constant IEIs is displayed. C: histograms of tGDP IEIs for cells in A and B are shown where the p4 cell with irregular events (top) displays a random distribution, while the p8 cell with regular events has a narrow distribution (bottom). D: population data depict the incidence (% of slices showing regular or irregular tGDP patterns) as a function of age. tGDP incidence peaks at p7/8 and is mainly characterized by irregular pattern up until this point. Incidence decreases until p15 with a progressive increase in the proportion of slices with regularly patterned events. Numbers above each column indicate the number of slices at each developmental stage that displayed tGDPs.

tGDP regularity increases during development

The pattern of tGDP occurrence within individual slices was initially irregular at p3-4 and increased in regularity over the next several days of development (Fig. 1). Irregular patterns (e.g., Fig. 1, A1 and C, top) were characterized by a high coefficient of variation of IEIs, whereas regular patterns (e.g., Fig. 1, B1 and C, bottom) displayed events with a characteristic regularity (see METHODS). The differences between these patterns can be seen in the IEI histograms in Fig. 1C. IEIs were distributed across a broad range (5–200 s) in the p4 slice in Fig. 1A1, whereas they were confined to values very near 5 s in the p8 slice in Fig. 1B1.

Figure 1D summarizes the incidence of tGDPs as percent of the total number of slices per age group with gray and black subdivisions representing the percentage of slices with regular and irregular patterns, respectively. At p3/4, tGDPs appeared in about a third of all slices (n = 17 of 54; 31%, Fig. 1D) and were characteristically irregular in their intervals. tGDP incidence increased significantly at p5/6 [n = 23 of 50 slices; 46% with only 1 of these slices (4%) showing a regular pattern], whereas peak activity was detected at p7/8, characterized by highest tGDP incidence [n = 28 of 50 slices; 56%; 2 slices (7%) with regular pattern] and frequency (lowest IEI, Table 1) of events. tGDP incidence declined in more mature slices at p9/11 and p12/15 (n = 11 of 50; 22%, and n = 9 of 50; 18%, respectively), whereas tGDPs were not observed later than p15. Regular tGDP patterns were observed with highest incidence at p9-p15 (37 and 55% of slices with tGDPs at these ages, respectively). Within slices where tGDPs were recorded, the briefest IEIs were observed at p7/8 (Table 1), and longer intervals were observed with slices from either more or less mature mice.

Developmental changes in tGDP properties

We identified tGDPs in 18–55% of all recordings depending on the age group. From this large sample, we randomly selected 9–20 cells per developmental stage for further analysis. Figure 2 presents a summary of population data illustrating the developmental changes in tGDPs from p3 to p15. At p3/4, tGDPs were characterized by peak current amplitudes between 108 and 666 pA. As shown in Fig. 2A, tGDP amplitudes at this age were significantly larger (mean amplitude = 399 ± 12 pA; n = 15; Fig. 2A) compared with all other age groups. Mean peak amplitude decreased over the following several days (mean amplitude at p5/6 = 219 ± 18 pA; n = 20, and at p12/15 = 108 ± 7; n = 9; P < 0.00001; 1-way ANOVA). We next analyzed the distribution of amplitudes for each age group (Fig. 2B). Amplitudes were calculated from a minimum of five cells per age group (n of events per cell >15), and the histogram was fitted with a single Gaussian distribution. This figure illustrates that although amplitudes were overlapping in their distributions, their peak currents shifted to significantly smaller events in nRt neurons older than p5/6. The

| Table 1. Interevent intervals during development |
|---|---|
| Age | IEI, s |
| P3/4 | 51.7 ± 6.5 |
| P5/6 | 48.6 ± 8.1 |
| P7/8 | 37.3 ± 7.1 |
| P9/11 | 53.3 ± 9.2 |
| P12/15 | 57.6 ± 11.3 |

IEI, interevent interval.
A progressive reduction in duration (mean 90% width at p3/4) was paralleled by a developmental decrease in tGDP amplitude was paralleled by a progressive reduction in duration (mean 90% width at p3/4 = 1692 ± 204 ms; n = 15, p7/8 = 613 ± 50 ms; n = 20 and at p12/15 = 535 ± 125 ms; n = 9, P < 0.00001; 1-way ANOVA, Fig. 2D). Change in TTP showed the opposite trend (Fig. 2C). At p3/4, values were significantly smaller (range between 1.2 and 71.4 ms, mean = 14.05 ± 1.76 ms; n = 15), compared with p7/8 (range between 1.9 and 115.5 ms, mean = 96.3 ± 23.4 ms; n = 20, P < 0.001; 1-way ANOVA). After p9/11, both the number of prepeak transients (p9/11 = 2.4 ± 0.3; n = 11, p12/15 = 1.64 ± 0.19; n = 9) and the TTP declined. In summary, we observed not only the highest incidence of tGDPs at p7/8 but also the highest frequency (shortest IEIs, Table 1) and the longest TTP.

tGDPs do not arise from simple summation of sIPSCs

Multiphasic IPSCs could arise from concerted network events or from the nearly simultaneous occurrence of multiple individual spontaneous synaptic currents. To distinguish between these two possibilities, we calculated the total synaptic charge transferred per each 2-s period throughout a recording. Occasional summation of randomly occurring sIPSCs would be reflected by a continuous distribution of charge transferred per unit period. On the other hand, if tGDPs are generated by a mechanism distinct from sIPSCs, then we would expect to see a bimodal distribution with little charge transferred during periods with only sIPSCs and much greater charge transferred during tGDPs. Figure 3 shows example traces with tGDPs for slices from different age groups. In Fig. 3A1, a representative trace at p5 is displayed, whereas the charge transfer per 2-s window is shown in Fig. 3A2. Charge transfer was generally quite low (<15 pC). Occasionally large sIPSCs were seen (Fig. 3A, I and 2*) with charge transfer on the order of 10 pC. However, tGDPs were associated with much greater charge (>50 pC). Thus charge transfer in this cell displayed a bimodal distribution (Fig. 3A4), inconsistent with the idea that tGDPs could arise from simple summation of sIPSCs. The distribution demonstrates a first peak (<30 pC), which represents sIPSC occurrence, and the second peak (>30 and <180 pC), representing tGDPs. An example at p9 is shown in Fig. 3B, which demonstrated the same bimodal distribution of charge transfer per unit time (Fig. 3B, 2 and 4), although there was a developmental decrease in overall charge transfer, consistent with the kinetic changes depicted in Fig. 2.

tGDPs are mediated by depolarizing GABA responses

Early in postnatal life, GABAergic transmission can be depolarizing and thus excitatory instead of inhibitory (Ben Ari et al. 1989; Cherubini et al. 1991). Depolarizing GABA responses contribute to giant depolarizing potentials (GDPs) in developing hippocampal circuits. They are generated by a synergism between excitatory GABAergic and ionotropic glutamatergic receptors (Ben-Ari et al. 1997; Cherubini et al. 1991; Owens et al. 1996). In immature hippocampal neurons, the reversal potential for chloride ($E_{Cl^-}$) is more depolarized than the resting membrane potential due to accumulation of intracellular chloride [Cl$^-$] (Cherubini et al. 1991). Therefore we tested whether the [Cl$^-$] concentration was higher in immature than in adult nRt neurons (Fig. 4). We used a noninvasive technique to estimate $E_{GABA}$ by recording in cell-attached mode with high K$^+$-filled pipettes (Verheugen et al. 1999). $V_m$ was estimated based on the expected reversal potential.
potential (−0 mV) for voltage-gated K⁺ currents. Thus with isonic K⁺ in the pipette, K⁺ currents reverse direction when $V_m$ equals $V_h$. To determine whether $E_{GABA}$ was hyperpolarized or depolarized compared with resting membrane potential in p14-20 nRt neurons, we measured the change in membrane potential produced by GABA application. Depolarizing voltage ramps (from $V_h = −100$ to +200 mV) were applied to elicit K⁺ currents before (Fig. 4A, top), during (A2, middle), and after (A3, bottom) bath application of 50 μM GABA. Figure 4A–3 shows averaged current responses ($n = 10$) to voltage ramps at p3. In control conditions, ramp stimuli activated voltage-gated K⁺ currents, which were inward from onset up to −55 mV and reversed direction at higher $V_m$ values. For our calculations, we corrected for the linear leak current at values below activation threshold and then determined the $E_K$ as the voltage at which the activated K⁺ current response intersected the linear leak (“correction”). At p3, we calculated mean $V_m$ in control as $−77 ± 1$ mV ($n = 3$). In the presence of 50 μM GABA, we observed a shift to more depolarized values for $V_m$ (mean $V_m$ for p3 in presence of GABA: $−65 ± 2$ mV; $n = 3$). This effect was reversible during washout (mean $V_m$ after washout: $−83 ± 5$ mV, $P < 0.01$, $n = 3$, 1-way ANOVA). By contrast, GABA induced a hyperpolarization in more mature neurons (p20, mean $V_m$ in control: $−87 ± 2$ mV, in GABA: $−96 ± 3$ mV and during washout: $−82 ± 4$ mV, $P < 0.05$, $n = 4$, 1-way ANOVA). Figure 4B compares how the average $V_m$ changed during GABA application for two representative neurons at p3 and p20. For recordings at p3 (Fig. 4C) GABA depolarized nRt $V_m$, and a similar, but not significant trend was seen at p7. By contrast, at p14-20, GABA hyperpolarized $V_m$. This is in accordance with findings that at more mature ages GABA acts mainly as “stabilizer” of $V_m$ (Verheugen et al. 1999). In summary (Fig. 4D), we observed a significant depolarization of $V_m$ in the presence of GABA at p3, compared with a smaller but significant hyperpolarization at p14-20. Presumably, active chloride extrusion mechanisms which maintain a lower [Cl⁻] concentration in adult neurons (Misgeld et al. 1986) are not yet fully developed at this age (Fukuda et al. 1998; Ganguly et al. 2001; Toyoda et al. 2003).

Potential contributions of ionotropic glutamate receptors to tGDPs

In the experiments described so far, we had applied APV+DNQX to isolate GABA$_A$R-mediated events. The presence of tGDPs under these conditions indicates that the activation of ionotropic glutamate receptors is not required for tGDP generation. To determine whether AMPA or NMDA receptors might nevertheless contribute to tGDP generation, we tested the effect of ionotropic glutamate receptor blockade on tGDPs in a separate series of experiments in p5-9 mice. The effects of bath-applied APV+DNQX are shown in Fig. 5, A and B, where we observed only minor changes, including a modest but significant reduction in tGDP amplitude (257 ± 34 to 170 ± 29 pA, $n = 8$, $P < 0.05$, paired Student’s $t$-test) without obvious changes in IET or 90% width (not shown). Thus we conclude that ionotropic glutamate receptor activation is not essential for tGDP generation, indicating significant differences in the mechanism underlying the genera-
tion of giant potentials in nRt compared with the CA3 region in hippocampus (Ben Ari et al. 1989). Finally, the GABAR antagonist SR95531 (gabazine, GBZ) effectively blocked tGDP activity at all ages (Fig. 5C). This result provides further support that GABAergic transmission plays the central role in tGDP generation.

**tGDPs are mediated by α5 subunit containing GABARs**

In mature neurons of the thalamic reticular nucleus, α3 subunits of the GABAR are highly expressed, whereas other alpha subunits are almost undetectable (Pirker et al. 2000; Wisden et al. 1992). We recently observed (Pangratz-Fuehrer et al. 2004) that the α5 subunit is transiently expressed in synaptic GABARs in nRt during early development. Given that GABARs in nRt do not express the α1 or α2 subunit, the final series of experiments was designed to determine whether tGDP properties are mediated by GABAR containing α3 or α5. To distinguish between these two possibilities, we compared the effects of clonazepam (CZP, 100 nM) on tGDPs recorded from thalamic slices obtained from α3(H126R) and their wild-type controls (WT; Fig. 6). The α3(H126R) point-mutation (Low et al. 2000) affects selectively the GABAR α3 subunit by rendering it insensitive to benzodiazepines (BZs) with otherwise normal function. We speculated that if tGDPs were mediated by α3 subunit-containing GABARs, we would observe no BZ-induced effect in the mutant. Alternatively, if they were mediated by GABAR containing α5, tGDPs would be enhanced. As shown in Fig. 6A, bath perfusion with CZP increased tGDP amplitude and decay kinetics to a similar extent in both genotypes. Example tGDPs in Fig. 6B and population data in Fig. 6C demonstrate that CZP strongly increased 90% width from 902 ± 70 ms to 1,319 ± 95 ms (46%) in WT and from 962 ± 106 to 1,544 ± 146 ms (60%) in α3 mutant mice (n = 3 for each genotype; P < 0.01, paired Student’s t-test). Furthermore, tGDP amplitude was significantly increased by ~25% in both genotypes, in the WT from 198 ± 22 to 244 ± 28 pA and in the α3 mutant from 173 ± 22 to 213 ± 14 pA. The increase in decay time was not correlated with any alterations in IEIs (not shown). In conclusion, because we did not observe any differences in the extent of CZP sensitivity between genotypes, the most likely explanation is that the α5 subunit primarily contributes to tGDP modulation by BZs during the first postnatal week.
DISCUSSION

In the present study, voltage-clamp recordings in immature thalamic mouse slices have clearly shown the existence of patterned spontaneous synaptic activity in the nRt. In particular, we have observed GABA-mediated tGDPs within the first 2 wk of postnatal life. This suggests that in thalamus, similar to other developing networks, spontaneous intrinsic activity within nRt may regulate development and refinement of neuronal circuits. Further work will be required to determine the specific role tGDPs play in the maturation of nRt circuitry.

Developmental profile of tGDPs in nRt

tGDPs share similar properties with neonatal patterns observed in other maturing networks. However, our data indicate that tGDP activity is unique in some ways among endogenous network activities. Although the time window for incidence of spontaneous network activity was similar in all published studies (in the 1st week of postnatal life), the incidence of tGDPs in thalamic slices was considerably lower than that of giant depolarizing potentials (GDPs) (Ben Ari et al. 1989) or early neonatal oscillations (ENOs) (Garaschuk et al. 1998) in hippocampus CA3 and CA1 slices, respectively. tGDPs were present in only 31–56% of all thalamic slices between p3 and p8, whereas GDPs and ENOs were observed in 85% of hippocampal recordings between p0/1 and p8 (Ben Ari et al. 1989; Garaschuk et al. 1998; Wong et al. 1993; Yuste et al. 2005). It is noteworthy that our studies were carried out in mice in contrast to studies in hippocampus that recorded from slices obtained from rats. In addition, we cannot exclude that tGDPs are already present in nRt neurons younger than p3/4.

Patterned activity in nRt

With the discovery of spontaneous activity in immature networks (Ben Ari et al. 1989; Garaschuk et al. 1998; Wong et al. 1993; Yuste et al. 1992), several different neuronal patterns have been described so far, such as synchronous and asynchronous patterns in the CA1 region of rat hippocampus (Garaschuk et al. 1998). Furthermore, previous studies have correlated early network activity with the appearance of Ca2+ oscillations (Ben Ari et al. 1989; Flint et al. 1999; Garaschuk et al. 1998; Wong et al. 1993; Yuste et al. 2005). Although patterns of GDPs and ENOs are generally rhythmic, giant GABAergic events in nRt display a developmentally regulated time course that gradually transforms early “irregular” activity at p3/4 into “regular” activity in neurons ≥p8.

Potential mechanisms underlying tGDPs

The exact mechanisms for generation of immature network activities are incompletely understood. In hippocampus (Ben Ari et al. 1989; Garaschuk et al. 1998) and the retina (Wong et al. 1993), a key role has been attributed to the excitatory influence of the neurotransmitter GABA. For example, the generation of GDPs in hippocampus is based on joint operation of glutamate and GABAA receptors. However, in nRt, blocking ionotropic glutamate receptors merely reduced tGDP amplitude, whereas blocking GABAAR abolished them completely. The fact that the reversal potential of GABA (\(E_{\text{GABA}}\)) for immature nRt neurons at p5 is depolarized compared with adult cells suggests that GABA is functionally excitatory and tGDPs are primarily GABA mediated. The reticular nucleus is characterized by strong interconnections between individual GABAergic cells that are mediated by both chemical and electrical synapses (Deleuze and Huguenard 2006). The depolarizing influence of the former in early development (Fig. 4) together with the excitatory coupling afforded by gap junctions would create a recurrent excitatory network within nRt. This would explain the persistence of the tGDPs even in the absence of ionotropic glutamatergic excitation. On the other hand, the fact that tGDPs are detectable even after p8 indicates that GABA-mediated depolarization of nRt neurons might not be
the only mechanism that can trigger tGDP generation. We therefore suggest that other mechanisms could contribute to a transient increase in intracellular calcium, such as metabotropic glutamate receptor (mGluR) activation. Studies in juvenile rats have shown that the activation of mGluRs in nRt can coordinate synchronous rhythms among electrically coupled neurons (Long et al. 2004).

Furthermore, the reciprocal recurrent connectivity between relay and nRt neurons may create an accessory network that could amplify the internal network response. Hence, the mildly suppressive effects of CNQX and APV on tGDPs (Fig. 5) may result from disruption of the accessory network.

Taken together, these data suggest that several mechanisms may be responsible for generating and propagating patterned activity in the immature nRt, including spontaneous GABA release, perhaps triggered by voltage-gated Ca\(^{2+}\) channels; gliamate release from thalamocortical or corticothalamic axon terminals with resulting activation of mGluRs; astroglia associated Ca\(^{2+}\) oscillations as observed for the thalamic ventrobasal complex (Crunelli et al. 2002; Parri and Crunelli 2002; Parri et al. 2001); and/or via gap-junction-mediated synchronization.

**Are tGDPs mediated by a different GABA\(_A\)R subtype?**

In the mammalian brain, GABA\(_A\)Rs mediate the majority of GABAergic signaling and provide fast inhibition. GABA\(_A\)Rs are constructed from pentameric ion channels with several binding sites for allosteric modulators, such as BZs. The kinetic properties and allocation of these receptors is determined by the subunit assembly, particularly by the \(\alpha\) subunit isoform (Sieghart and Sperk 2002). Although considerable research had been devoted to unraveling biophysical properties of these individual GABA\(_A\)R subtypes (Barnard et al. 1998; Mody and Pearce 2004; Okada et al. 2000; Ortinski et al. 2004; Sieghart and Sperk 2002; Vicini et al. 1986), less attention has been paid to whether a distinct \(\alpha\) subtype is associated with GABA\(_A\)R-mediated spontaneous activity in immature networks. We have observed that the expression of GABA\(_A\)Rs containing \(\alpha5\) subunits precedes GABA\(_A\)Rs containing the \(\alpha3\) subunit in mouse nRt neurons during very early development (Pangratz-Fuehrer et al. 2004). When we compared the time course of tGDP incidence with the expression of the GABA\(_A\)R \(\alpha5\) subunit in nRt, we found that both peaked at the end of the first postnatal week. These results are further supported by recent findings of high GABA\(_A\)R \(\alpha5\) subunit expression in nRt at p5, whereas only low expression was detected at p0 and p10 (Studer et al. 2006). Essentially no expression of \(\alpha1\) (Pangratz-Fuehrer et al. 2004) or \(\alpha2\) (Studer et al. 2006) subunits could be detected at the end of the first postnatal week. Taken together with our results that clonazepam strongly enhanced tGDPs in immature nRt neurons of the BZ-insensitive \(\alpha3\) mutant, we suggest that tGDPs are primarily mediated by the GABA\(_A\)R \(\alpha5\) subtype. \(\alpha3\) and \(\alpha5\) GABA\(_A\)R subtypes may be co-expressed for a short period of time prior to synapse maturation around p9. Further support for a transient co-expression of \(\alpha3\) and \(\alpha5\) is suggested by the heightened GABA potency in p5/9 rat nRt neurons compared with p18/25, and p58/74 rats (Gibbs et al. 1996). The turnover and re-arrangement of GABA\(_A\)R subtypes is responsible for mature inhibitory signal transduction in a variety of systems (Dunning et al. 1999; Hollrigel and Soltesz 1997; Okada et al. 2000; Tia et al. 1996; Vicini et al. 2001). Disruptions of the normal developmental sequence may produce long-lasting changes in network excitability. Thus a mouse mutant lacking the adult GABA\(_A\)R subtype (\(\alpha1\)), which is normally highly expressed in mature neocortical neurons, showed impaired GABAergic functions. This may have resulted from a failure of the synaptic receptors to attain a mature phenotype (fast kinetics) required to support high-frequency oscillations (Bosman et al. 2005). By analogy, disruptions in the development of nRt circuitry may then prevent normal development of the spindle generating circuitry potentially leading to disorders of sleep and AE.

**Conclusion**

In summary, we report the existence of spontaneous, large-amplitude depolarizing synaptic events (tGDPs) in developing nRt neurons. These events differ in terms of generation and propagation from neonatal spontaneous activity previously described for other brain regions. The tGDPs are mainly mediated by the GABA\(_A\)R \(\alpha5\)-subtype and reflect a time and location specific network pattern in nRt. The spontaneous tGDPs are intrinsic (generated within nRt), and we speculate that they play a role in the development of intra-thalamic connectivity and furthermore that the switch to \(\alpha3\)-containing GABA\(_A\)Rs is associated with functional maturation of the circuit. Because well-regulated inhibitory output from nRt is central to normal rhythm generation in the thalamocortical system, perturbation of tGDPs might alter activity-dependent nRt network development and disrupt formation of the normal rhythm generating circuitry.

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**References**


Bosman LW, Heinen K, Spijker S, Brussaard AB. Mice lacking the major adult GABA\(_A\) receptor subtype have normal number of synapses, but retain juvenile IPSC kinetics until adulthood. *J Neurophysiol* 94: 338–346, 2005.


