An Examination of Calcium Current Function on Heterotopic Neurons in Hippocampal Slices from Rats Exposed to Methylazoxymethanol

Maria Elisa Calcagnotto and Scott C. Baraban

Summary: Purpose: To study voltage-dependent calcium currents (VDCCs) on hippocampal heterotopic neurons by using whole-cell patch-clamp techniques in brain slices prepared from methylazoxymethanol (MAM)-exposed rats.

Methods: Whole-cell voltage-clamp recordings were obtained from visually identified neurons in acute brain slices by using an infrared differential interference contrast (IR-DIC) video microscopy system. Heterotopic neurons were compared with normotopic pyramidal cells in hippocampal slices from MAM-exposed rats or CA1 pyramidal neurons in slices from controls.

Results: Heterotopic neurons expressed a prominent VDCC, which exhibited a peak current maximum around –30 mV (holding potential, –60 mV) and an inactivation time constant of 48.2 ± 2.4 ms (n = 91). VDCC peak current and inactivation time constants were similar for normotopic (n = 92) and CA1 pyramidal cells (n = 40). Pharmacologic analysis of VDCC, on heterotopic, normotopic, and CA1 pyramidal cells, revealed an ~70% blockade of peak Ca2+ current with nifedipine and amiloride (L- and T-type channel blockers, respectively). Inhibition of VDCC, for all three cell types, also was similar when more specific Ca2+ channel antagonists were used [e.g., ω-conotoxin GVIA (N-type), ω-agatoxin KT (P/Q-type), and sFTX-3.3 (P-type)]. VDCC modulation by norepinephrine (NE) or adrenergic receptor–specific agonists [clonidine (α₂), isoproterenol (β), and phenylephrine (α₁)] was similar for heterotopic and CA1 pyramidal cells.

Conclusions: Heterotopic neurons do not appear to exhibit Ca2+ channel abnormalities that could contribute to the reported hyperexcitability associated with MAM-exposed rats.

Key Words: Dysplasia—Epilepsy—Heterotopia—Ion channel—Calcium—Patch-clamp.
animal model of early-onset epilepsy associated with a neuronal heterotopia [e.g., rats exposed to methylazoxymethanol (MAM) in utero]. Cells within a neuronal heterotopia, in both humans and the MAM model, receive abundant catecholaminergic innervation (25–27). VDCCs are modulated by catecholamines (28–30) and are believed to be a source of independent seizure generation (31,32). Although potassium channels and γ-aminobutyric acid (GABA) transmission have been studied on heterotopic neurons (33,34), virtually nothing is known regarding calcium channels. To examine VDCC function on heterotopic neurons and its potential modulation by catecholamines, whole-cell patch-clamp recordings and pharmacologic studies were performed on tissue slices obtained from MAM-exposed and control rats.

METHODS

Prenatal methylazoxymethanol injection

Pregnant Sprague–Dawley rats were injected with either 0.9% physiologic saline (Control) or 25 mg/kg MAM. MAM was purchased from NCI Chemical Carcinogen (Kansas City, MO, U.S.A.). Intraperitoneal injections (0.3 ml, 15% DMSO) were made on day 15 of gestation (E15). All animal care and use conformed to the NIH Guide for Care and Use of Laboratory Animals and approved by the UCSF Committee on Animal Research.

Hippocampal slice preparation

Acute tissue slices were prepared from male or female Sprague–Dawley rat pups (P14–P25). In brief, rats were decapitated, and the brain rapidly removed in ice-cold oxygenated slicing medium, an artificial cerebrospinal fluid (sACSF) consisting of (in mM) 220 sucrose, 3 KCl, 1.25 NaH2PO4, 2 MgSO4, 26 NaHCO3, 2 CaCl2, 10 dextrose (295–305 mOsm). A hemisphere of brain containing the hippocampus was blocked and glued (cyanoacrylate adhesive) to the stage of a vibroslicer (Leica VTS1000). Horizontal slices (300 μm thick) were cut in 4°C oxygenated (95% O2/5% CO2) slicing medium. The resulting slices were immediately transferred to a holding chamber where they remained submerged in oxygenated recording medium (ACSF) consisting of (in mM) 124 NaCl, 3 KCl, 1.25 NaH2PO4, 2 MgO4, 26 NaHCO3, 2 CaCl2, 10 dextrose (295–305 mOsm). Slices were held at 37°C for 45 min and then at room temperature (33,34). For each experiment, an individual slice was transferred to a submersion-type recording chamber where it was continuously perfused with oxygenated recording medium at 32°C.

Whole-cell recording

Whole-cell voltage-clamp recordings were obtained from visually identified neurons by using an infrared differential interference contrast (IR-DIC) video microscopy system (35). Conventional whole-cell patch recordings were obtained from identified neurons within 75 μm of the slice surface. Patch electrodes (3–7 MΩ) were pulled from 1.5-mm o.d. borosilicate glass capillary tubing (WPI) by using a micropipette puller (Sutter P-87), coated with Sylgard (Dow Chemical), and fire polished. Intracellular patch pipette solution for whole-cell recordings contained (in mM) 30 tetraethylammonium chloride (TEA), 100 CsCl, 10 HEPES, 10 EGTA, 4 NaCl, 1 MgCl2, 0.5 CaCl2, 3 Na2-ATP, 0.3 Na2-GTP (pH 7.25; 285–290 mOsm). To isolate Ca2+ currents, slices were perfused with low sodium solution containing in (mM) 100 NaCl, 3 KCl, 1.25 NaH2PO4, 2 MgSO4, 26 NaHCO3, 2 CaCl2, 10 dextrose 0.001, tetrodotoxin (TTX), 5 4-aminopyridine (4-AP), 20 TEA (295–305 mOsm). VDCCs were recorded at a holding potential of −60 mV. Ca2+ currents were activated by 100-ms depolarizing voltage steps between −70 mV and +20/+40 mV after a 50-ms hyperpolarizing prepulse to −130 mV (see inset in Fig. 2B). Admittedly, space-clamp problems can arise during Ca2+ current activation in acute brain slices, resulting in a partially clamped action current at the outset of the voltage step. However, because the goal of these studies was to record VDCCs on heterotopic neurons, our protocol necessitates the use of acute hippocampal slices with identifiable regions of dysplasia rather than a dissociated cell preparation. As such, all data used for comparison of peak current amplitudes and generation of I-V curves were obtained at time points in the peak of activation step where voltage control problems are less significant. Additionally, VDCC protocols chosen for these studies are identical to those previously published for acute hippocampal slices (20,21,24,30).

Current was recorded with an Axopatch 1D amplifier (Axon Instruments), and monitored on an oscilloscope (Tektronix). Whole-cell voltage-clamp data were low-pass filtered at 1 kHz (−3 dB, eight-pole Bessel), digitally sampled at 10 kHz, and monitored with pCLAMP software (Axon Instruments) running on a PC Pentium computer (Dell Computers). Whole-cell access resistance was carefully monitored throughout the recording, and cells were rejected if values changed by >25% (or exceeded 20 MΩ); only recordings with stable series resistance of <20 MΩ were used for VDCC analysis.

Drugs

In some voltage-clamp experiments, pharmacologic agents were added to the perfusion medium: cadmium (CdCl2, 200 μM), nickel (NiCl2 250 μM), amiloride (1 mM), nifedipine (10 μM), norepinephrine (NE; 10–100 μM), clonidine (5 μM), phenylephrine (5 μM), isoproterenol (5 μM) (from Sigma), and ω-Agatoxin TK (AgTX, 0.1 μM), ω-Conotoxin GVIA (CgTX, 1 μM), synthetic FTX-3.3 (or arginyl polyamine) (sFTX-3.3; 1
In experiments using CdCl₂, phosphate was omitted and MgSO₄ was substituted for MgCl₂ to prevent the precipitation of cadmium. Toxin stock solutions were prepared according to the supplier’s specifications (i.e., using 0.1% BSA, 100 ml NaCl, 10 mM Tris (pH 7.5) and 1 mM EDTA as a diluent) and stored at –20°C. Toxin stock solutions were diluted to final working concentrations on the experimental day. CgTX and AgaTX were added to perfusate containing 0.1% cytochrome C (Sigma) to prevent adherence to connecting tubing and glass walls. Amiloride and nifedipine were prepared fresh and applied in the dark, as they are light-sensitive compounds. NE and adrenergic agonists were prepared fresh and protected from both light and oxidation (40 μM ascorbic acid in ACSF). All other drugs were prepared from stock solutions, storage at –20°C. Drugs were tested by using a 5-min bath-application protocol at a flow rate of ~3 ml/min, unless otherwise indicated. Each cell was exposed to only one drug challenge, and only one cell was recorded per slice when drugs were applied.

**Statistical analysis**

VDCCs were analyzed off-line by using Clampfit software (Axon Instruments). Kinetic analysis of the VDCCs was performed with a single-exponential function. Results are presented as mean ± SEM. To compare results between different cell types, we used a one-way analysis of variance (ANOVA) on the SigmaStat program (Jandel Scientific). Significance level was taken as p < 0.05.

**RESULTS**

Hippocampal slices from MAM-exposed rats contained distinct clusters of displaced neurons (heterotopia) and loss of lamination (Fig. 1A), as described previously (32,36). Neurons were selected for whole-cell voltage-clamp studies based on their location and morphology under direct IR-DIC visualization (Fig. 1B). Experimental data were obtained from hippocampal heterotopic pyramidal-like neurons (n = 91). For comparison, control data were obtained from normotopic pyramidal neurons in hippocampal slices from MAM-exposed rats (e.g., pyramidal cells located within the normal CA1 laminar; n = 40) and CA1 pyramidal neurons in slices from age-matched control rats (n = 92).

** Calcium current on heterotopic neurons**

To study Ca²⁺ channel function on hippocampal heterotopic neurons, we examined whole-cell Ca²⁺ currents by using visualized patch-clamp recording techniques (35). Whole-cell VDCC was recorded in the presence of 1 μM tetrodotoxin (Na⁺ channel blocker), 20 μM tetraethylammonium chloride, and 5 μM 4-aminopyridine (K⁺ channel blockers). VDCC displayed a current peak maximum, for CA1 pyramidal cells, during depolarizing steps to approximately –30 mV (n = 92). By using the same voltage-clamp protocol and identical recording conditions for slices from MAM-exposed rats, we observed a current peak maximum for normotopic (n = 40) and heterotopic pyramidal (n = 91) neurons during depolarizing steps similar to the values observed in CA1 pyramidal cells [i.e., around –30 mV (Fig. 2A–C)]. The inactivation time constant for VDCC evoked at –30 mV (depolarizing step for the maximum peak current value) was 49.9 ± 2.6 ms for control CA1 pyramidal cells (n = 45). In MAM-exposed rats, the inactivation time constant at the same depolarizing step was 43.8 ± 2.3 ms for normotopic cells (n = 21; p > 0.1) and 48.2 ± 2.4 ms for heterotopic pyramidal neurons (n = 38; p > 0.5). Qualitative properties of VDCC (Fig. 2A) and current–voltage plots (Fig. 2B) also failed to reveal significant differences between these three cell types.

**Pharmacology of calcium current on heterotopic cells**

Further to characterize VDCC on heterotopic neurons, we used a variety of pharmacologic manipulations designed to block Ca²⁺ channels. First, slices were bathed in a low-Na⁺ ACSF supplemented with the dihydropyri-
dine L-type Ca\(^{2+}\) channel blocker, nifedipine (10 \(\mu\)M) (37,38). An example of the effect of 10 \(\mu\)M nifedipine on VDCC in a heterotopic cell is illustrated in Fig. 3A. Second, slices were perfused in low Na\(^{+}\) ACSF supplemented with a T-type Ca\(^{2+}\) channel blocker, amiloride (1 mM) (39). Third, slices were bathed in a low-Na\(^{+}\) ACSF containing inorganic Ca\(^{2+}\) channel blockers (200 \(\mu\)M CdCl\(_2\) or 250 \(\mu\)M NiCl\(_2\)). The level of inhibition observed with normotopic, heterotopic, and CA1 pyramidal cells was similar for all of these manipulations (Fig. 3B; \(p > 0.2\)). In an additional set of pharmacologic studies, slices were perfused in low-Na\(^{+}\) ACSF containing Ca\(^{2+}\) channel subunit–specific toxins: 0.1 \(\mu\)M AgaTX (P/Q-type), 1 \(\mu\)M CgTX (N-type), or 1 \(\mu\)M sFTX-3.3 (P-type). An example of the effect of sFTX-3.3 on VDCC in a heterotopic cell is illustrated in Fig. 4A. In agreement with data using less-specific VDCC blockers (e.g., nifedipine, amiloride, nickel, and cadmium), the level of inhibition observed with channel subunit–specific toxins was similar for all three cell types (Fig. 4C; \(p > 0.5\)).

**Adrenergic modulation of calcium current on heterotopic cells**

Because hippocampal heterotopic neurons in the MAM model receive excessive innervation by catecholaminergic fibers (25–27), and it is well established that catecholamines modulate Ca\(^{2+}\) channel activity (40) or hippocampal neuronal excitability (28,40,41,42), we investigated adrenergic modulation of VDCC on heterotopic neurons. Bath application of norepinephrine (NE, 10–100 \(\mu\)M), a potent agonist at \(\alpha_1\), \(\alpha_2\), and \(\beta\)-adrenergic receptors, significantly reduced VDCC peak current amplitude in a dose-dependent manner on both CA1 pyramidal control neurons and heterotopic cells (Fig. 5). An example of the effect of 10 \(\mu\)M NE on VDCC in a heterotopic cell is illustrated in Fig. 5A. NE produced a similar level of VDCC inhibition for both cell types (Fig. 5C and D). In additional experiments, we tested noradrenergic receptor-specific agonists dissolved in low-Na\(^{+}\) ACSF: clonidine, 5 \(\mu\)M (\(\alpha_2\)); phenylephrine, 5 \(\mu\)M (\(\alpha_1\)); and isoproterenol, 5 \(\mu\)M (\(\beta\)). Among these agonists, the \(\alpha_2\)-adrenergic agonist clonidine caused the most significant inhibition of VDCC, whereas the \(\beta\)-agonist, isoproterenol, produced very little effect. Again, NE-induced inhibition of VDCC by using receptor-specific agonists was similar for all cell types and all drugs tested. Despite the massive catecholaminergic innervation of hippocampal heterotopia (25,43), inhibition of VDCC in heterotopic cells by adrenergic agonists was comparable to control cells (Fig. 5D; \(p > 0.4\)).

**DISCUSSION**

We described the properties of voltage-dependent Ca\(^{2+}\) currents on heterotopic neurons in the MAM model of brain malformation–associated epilepsy. HVA- and LVA-type voltage-dependent Ca\(^{2+}\) currents were ob-
served on all heterotopic neurons. VDCC current-activation thresholds and inactivation time constant values were not different between hippocampal heterotopic and control CA1 pyramidal neurons. No differences in VDCC peak current amplitude, voltage dependence, or pharmacology were observed. The response to exogenously applied catecholamines also was similar for VDCCs recorded on heterotopic and control cells. Nifedipine blocked ~70% of the peak Ca\(^{2+}\) current on heterotopic cells. Similar blocking effects (~70%) were obtained with amiloride. The response to these agents was comparable for all three cell types, suggesting that the expression of functional L- and T-type channels on these cells is similar. Several “N-like” HVA neuronal Ca\(^{2+}\) channels also can be distinguished by using peptide toxins obtained from the venom of predatory invertebrates (snails and spiders). These toxins have high-affinity for Ca\(^{2+}\) channels, and it is well established that N-type Ca\(^{2+}\) channels are sensitive to micromolar levels of ω-conotoxin GVIA (6,46); P/Q-type channels are sensitive to ω-agatoxin IVA (47); P-type are sensitive to FTX-3.3 (48), and R-type channels resistant to these toxins (49). Except for their pharmacology, N-P/Q-, and R-types of Ca\(^{2+}\) channels appear functionally similar. The cell body of vertebrate neurons also can express a specific mixture of various HVA Ca\(^{2+}\) channels, including L type (50). We found that ω-conotoxin GVIA, ω-agatoxin KT, and sFTX-3.3 (the synthetic analogue of FTX-3.3) inhibited VDCC on heterotopic cells (~30%, ~30%, and ~50%, respectively). Again, we observed nearly identical responses to these toxins in normotopic and control CA1 pyramidal cells, suggesting that the specific distribution of Ca\(^{2+}\) channel subtypes is similar for all three hippocampal cell types. Because significant differences were not observed with any of the calcium

![Diagram](image)

**FIG. 5.** Adrenergic modulation of voltage-dependent calcium currents (VDCCs). A: Superimposed traces of a maximum peak current amplitude (evoked at ~20 mV) from a heterotopic neuron before (black trace) and ~5 min after application of norepinephrine (NE), 10 µM (gray trace) and its respective I/V curve before (●) and after (○) NE. B: Plot illustrating the percentage of peak current amplitude after application of AgaTX, 0.1 µM; CgTX, 1 µM; or sFTX-3.3, 1 µM; for CA1 control (black), MAM CA1 normotopic (gray), and heterotopic (white) cells. N = 3 cells for each experimental manipulation. Note the similarity of effect of Ca\(^{2+}\) channel blockers for all cell types here and in Fig. 3.
channel blockers tested, further isolation and analysis of specific calcium channel components was not pursued.

**Catecholaminergic modulation of heterotopic neurons**

Neurotransmitters that affect intracellular second-messenger systems modulate Ca\(^{2+}\) channel function and thus influence neuronal output. Norepinephrine, for example, decreases N-, P/Q-, and R-type VDCCs and therefore the amount of neurotransmitter released at a given synapse (51). In neurons, these modulatory actions can significantly reduce neurotransmitter release and depress fast synaptic transmission. Because release of neurotransmitters is a steep function of presynaptic Ca\(^{2+}\) entry, a depression of Ca\(^{2+}\) current would significantly reduce synaptic output. Such presynaptic inhibition could explain how some neurotransmitters block excitatory synaptic transmission in the hippocampus (e.g., direct inhibition of presynaptic voltage-dependent Ca\(^{2+}\) channels (52)). Because of the importance of NE-mediated modulation of excitability and anatomic studies in human tissue from patients with cortical dysplasia (53,54) or tissue sections from MAM-exposed rats (25, 26,55) demonstrating that dysplastic or heterotopic cell regions were heavily innervated by catecholaminergic fibers, we were interested in determining whether VDCC modulation by exogenous NE was altered in the MAM model.

We observed that VDCC on heterotopic neurons is depressed by adrenergic receptor activation in a dose-dependent manner. Although immunohistochemistry revealed an abundance of catecholaminergic fibers innervating cortical/hippocampal regions in the MAM brain (26,55), an altered inhibitory catecholaminergic effect to exogenously applied agonists, which could potentially contribute to abnormal modulation of excitatory synaptic transmission in the malformed hippocampus, was not observed. Responsiveness of VDCC on heterotopic neurons to NE application was similar to that measured for normal CA1 pyramidal cells. Similar findings were observed by using adrenergic receptor–specific agonists. Previously NE was reported to modulate hippocampal excitability via activation of \(\alpha_\text{1}\) (28) and \(\alpha_\text{2}\) adrenoreceptors (56) but not \(\beta\)-adrenoreceptors. Our results in both dysplastic and normal hippocampus are consistent with NE-mediated modulation of VDCCs via \(\alpha\)-adrenergic receptors, more specifically \(\alpha_\text{2}\)-adrenoreceptors.

**Conclusion**

We described, for the first time, the physiologic and pharmacologic properties of VDCC in an animal model featuring nodular heterotopia. We detected no differences in VDCCs between CA1 pyramidal cells, normotropic, and hippocampal heterotopic cells. Heterotopic neurons, therefore, do not appear to exhibit Ca\(^{2+}\) channel abnormalities that could contribute to the observed hyperexcitability in the MAM model. Our results suggest that some other property of heterotopic neurons accounts for the differences in intrinsic firing properties reported (32,57)—a lack of functional A-type Kv4.2 potassium channels is one possibility (33)—and that VDCC responsiveness to exogenous adrenergic agonists is unchanged in these animals. Nonetheless, these findings add to our growing understanding of how heterotopic neurons function in the MAM model of malformation-associated epilepsy and could yield insights into the human condition.

**Acknowledgment:** This work was supported by funds from the National Institutes of Health (R01 NS40272) and Parents Against Childhood Epilepsy (P.A.C.E.). As always, we thank Peter A. Castro for expert technical assistance.

**REFERENCES**

1. Kennedy MB. Regulation of neuronal function by calcium. Trends Neurosci 1989;12:417–20.
2. Llinas RR, Sugimori M, Cherkesky B. Voltage-dependent calcium conductances in mammalian neurons: the P channel. Ann N Y Acad Sci 1989;560:103–11.
3. Nowycky MC, Fox AP, Tsien RW. Three types of neuronal calcium channel with different calcium agonist sensitivity. Nature 1985;316:440–3.
4. Eliot LS, Johnston D. Multiple components of calcium current in acutely dissociated dentate gyrus granule neurons. J Neurophysiol 1994;72:762–7.
5. Beck H, Steffens R, Heinemann U, et al. Properties of voltage-activated Ca\(^{2+}\) currents in acutely isolated human hippocampal granule cells. J Neurophysiol 1997;77:1526–37.
6. Bean BP. Classes of calcium channels in vertebrate cells. Annu Rev Physiol 1989;51:367–84.
7. Miller RJ. Multiple calcium channels and neuronal function. Science 1987;235:46–52.
8. Carbone E, Swandulla D. Neuronal calcium channels: kinetics, blockade and modulation. Prog Biophys Mol Biol 1989;54:31–58.
9. Huguenard JR. Low-threshold calcium currents in central nervous system neurons. Annu Rev Physiol 1996;58:329–48.
10. Ozawa S, Tsuzuki K, Ino M, et al. Three types of voltage-dependent calcium current in cultured rat hippocampal neurons. Brain Res 1989;495:329–36.
11. Dunlap K, Luebke JJ, Turner TJ. Excitatory Ca\(^{2+}\) channels in mammalian central neurons. Trends Neurosci 1995;18:89–98.
12. Takahashi T, Momiyama A. Different types of calcium channels mediate central synaptic transmission. Nature 1993;366:156–8.
13. Bading H, Ginty DD, Greenberg ME. Regulation of gene expression in hippocampal neurons by distinct calcium signaling pathways. Science 1993;260:181–6.
14. Llinas RR. The intrinsic electrophysiological properties of mammalian neurons: insights into central nervous system function. Science 1988;242:1654–64.
15. Furukawa K, Fu W, Li Y, et al. The actin-severing protein gelsolin modulates calcium channel and NMDA receptor activities and vulnerability to excitotoxicity in hippocampal neurons. J Neurosci 1997;17:8178–86.
16. Fletcher CF, Lutz CM, O’Sullivan TN, et al. Absence epilepsy in trotting mutant mice is associated with calcium channel defects. Cell 1996;87:607–17.
17. Burgess DL, Noebels JL. Single gene defects in mice: the role of voltage-dependent calcium channels in absence models. Epilepsy Res 1999;36:111–22.
18. Kim D, Song I, Keum S, et al. Lack of the burst firing of thalamic cortico relay neurons and resistance to absence seizures in mice lacking alpha (1G) T-type Ca(2+) channels. Neuron 2001;31:35–45.
Kohr G, Lambert CE, Mody I. Calbindin-D28K (CaBP) levels and calcium currents in acutely dissociated epileptic neurons. *Exp Brain Res* 1991;85:543–51.

Vreugdenhil M, Wadman WJ. Enhancement of calcium currents in rat hippocampal CA1 neurons induced by kindling epileptogenesis. *Neuroscience* 1992;49:373–81.

Yarni Y, Hamon B, Lux HD. Development of two types of calcium channels in cultured mammalian hippocampal neurons. *Science* 1987;235:680–2.

Aicardi G, Schwartzkroin PA. Suppression of epileptiform burst discharges in CA3 neurons of rat hippocampal slices by the organic calcium channel blocker, verapamil. *Exp Brain Res* 1990;81:288–96.

Niespodziany I, Kliggaard H, Margineanu DG. Levetiracetam inhibits the high-voltage-activated Ca(2+) current in pyramidal neurons of rat hippocampal slices. *Neurosci Lett* 2001;306:5–8.

Johnston MV, Coyle JT. Histological and neurochemical effects of fetal treatment with methylazoxymethanol on rat neocortex in adulthood. *Brain Res* 1979;170:135–55.

Johnston MV, Grzanna R, Coyle JT. Methylazoxymethanol treatment of fetal rats results in abnormally dense noradrenergic innervation of neocortex. *Science* 1979;203:369–71.

Bardosi A, Ambach G, Hann P. The angiogenesis of the internal angioarchitecture of cortex. *Acta Neuropathol* 1987;75:85–91.

Boehm S. Presynaptic alpha2-adrenoceptors control excitatory, but not inhibitory, transmission at rat hippocampal synapses. *J Physiol* 1999;519:439–49.

Czesnik D, Nezlin L, Rabba J, et al. Noradrenergic modulation of Ca2+ channel currents and synaptic transmission in the olfactory bulb of Xenopus laevis tadpoles. *Eur J Neurosci* 2001;13:1093–100.

Liy YW, Guyenet PG, Bayliss DA. Voltage-dependent calcium currents in bulbarospinal neurons of neonatal rat rostral ventrolateral medulla: modulation by alpha2-adrenergic receptors. *J Neurophysiol* 1998;79:583–94.

Dubeau F, Palmini A., Fish D, et al. The significance of electroclinical, electrophysiological and neurochemical characteristics. *Electrocorticography Clin Neurophysiol* 1998;48:77–96.

Baraban SC, Wenzel HJ, Hochman DW, et al. Characterization of heterotopic cell clusters in the hippocampus of rats exposed to methylazoxymethanol in utero. *Epilepsy Res* 2000;39:8804.

Castro PA, Cooper EC, Lowenstein DH, et al. Hippocampal heterotopia lack functional Kv4.2 potassium channels in the methylazoxymethanol model of cortical malformations and epilepsy. *J Neurosci* 2001;21:6626–34.

Calcagnotto ME, Paredes MF, Baraban SC, et al. Heterotopic neurons with altered inhibitory synaptic function in an animal model of malformation-associated epilepsy. *J Neurosci* 2002;22:7596–605.

Stuart GJ, Dod HU, Sakmann B. Patch-clamp recordings from the soma and dendrites of neurons in brain slices using infrared video microscopy. *Pflugers Arch* 1993;423:511–8.

Chevassus-Au-Louis N, Rafiki A, Jorgueras I, et al. Neocortex in the hippocampus: an anatomical and functional study of CA1 heterotopias after prenatal treatment with methylazoxymethanol in rats. *J Comp Neurol* 1998;394:520–36.

Fox AP, Nowycky MC, Tsien RW. Single-channel recordings of three types of calcium channels in chick sensory neurones. *J Physiol* 1987;394:173–200.

Fox AP, Nowycky MC, Tsien RW. Kinetic and pharmacological properties distinguishing three types of calcium currents in chick sensory neurones. *J Physiol* 1987;394:149–72.

Tang CM, Presser F, Morad M. Amlodilie selectively blocks the low threshold (T) calcium channel. *Science* 1988;240:213–5.

Hille B. Modulation of ion-channel function by G-protein-coupled receptors. *Trends Neurosci* 1994;17:531–6.

Madison DV, Nicoll RA. Noradrenaline blocks accommodation of pyramidal cell discharge in the hippocampus. *Nature* 1982;299:636–8.

Madison DV, Nicoll RA. Actions of noradrenaline recorded intra-cellularly in rat hippocampal CA1 pyramidal neurones, in vitro. *J Physiol* 1986;372:221–44.

Beaulieu M, Coyle JT. Fetaly indued noradrenergic hyperinnervation of cerebral cortex results in persistent down-regulation of beta-receptors. *Brain Res* 1982;256:491–4.

Mintz IM, Venema VJ, Swider KM, et al. P-type calcium channels blocked by the spiroxin omega-aga-IVA. *Nature* 1992;358:279–79.

Mintz IM, Adams ME, Bean BP. P-type calcium channels in rat central and peripheral neurons. *Neuron* 1992;9:85–95.

Reynolds II, Wagner JA, Snyder SH, et al. Brain voltage-sensitive calcium channel subtypes differentiated by omega-conotoxin fraction GVIa. *Proc Natl Acad Sci U S A* 1986;83:8804–7.

Brown AM, Sayer RJ, Schwindt PC, et al. P-type calcium channels in rat neocortical neurons. *J Physiol* 1994;475:197–205.

Dupere JR, Moya E, Blagbrough IS, et al. Differential inhibition of Ca2+ channels in mature rat cerebellar Purkinje cells by sFTX-3.3 and FTX-3.3. *Neuropharmacology* 1996;35:1–11.

Randall A, Tsien RW. Pharmacological dissection of multiple types of Ca2+ channel currents in rat cerebellar granule neurons. *J Neurosci* 1995;15:2995–3012.

Westenbroek RE, Hoskins L, Catterall WA. Localization of Ca2+ channel subtypes on rat spinal motor neurons, interneurons, and nerve terminals. *J Neurosci* 1998;18:6319–30.

Hile B. Voltage-gated calcium channels. In: Hile B, ed. *Ion channels of excitable membranes*. 3rd ed. Sunderland, MA: Sinauer Associates, 2001:95–130.

Wu LG, Sagapu P. Presynaptic inhibition of elicited neurotransmitter release. *Trends Neurosci* 1997;20:204–12.

Trottier S, Evrard B, Vignal JP, et al. The serotoninergic innervation of the cerebral cortex in man and its changes in focal cortical dysplasia. *Epilepsy Res* 1996;25:79–106.

Trottier S, Evrard B, Bizerek A, et al. Altered patterns of catecholaminergic fibers in focal cortical dysplasia in two patients with partial seizures. *Epilepsy Res* 1994;19:161–79.

Zoli M, Pich EM, Ciminio M, et al. Morphometrical and microdensitometrical studies on peptide- and tyrosine hydroxylase-like immunoreactivities in the forebrain of rats prenatally exposed to methylazoxymethanol acetate. *Dev Brain Res* 1990;51:65–61.

Scanziani M, Gahwiler BH, Thompson SM. Presynaptic inhibition of excitatory synaptic transmission mediated by alpha adrenergic receptors in area CA3 of the rat hippocampus in vitro. *J Neurosci* 1993;13:5393–401.

Sancini G, Franceschetti S, Battaglia G, et al. Dysplastic neocortex and subcortical heterotopias in methylazoxymethanol-treated rats: an intracellular study of identified pyramidal neurones. *Neurosci Lett* 1998;246:181–5.