NPY Sensitivity and Postsynaptic Properties of Heterotopic Neurons in the MAM Model of Malformation-Associated Epilepsy

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Pentney, A. R., S. C. Baraban, and W. F. Colmers. NPY sensitivity and postsynaptic properties of heterotopic neurons in the MAM model of malformation-associated epilepsy. J Neurophysiol 88: 2745–2754, 2002; 10.1152/jn.00500.2002. Neuronal migration disorders (NMDs) can be associated with neurological dysfunction such as mental retardation, and clusters of disorganized cells (heterotopias) often act as seizure foci in medically intractable partial epilepsies. Methyloxazymethanol (MAM) treatment of pregnant rats results in neuronal heterotopias in offspring, especially in hippocampal area CA1. Although the neurons in dysplastic areas in this model are frequently hyperexcitable, the precise mechanisms controlling excitability remain unclear. Here, we used IR-DIC videomicroscopy and whole cell voltage-clamp techniques to test whether the potent anti-excitatory actions of neuropeptide Y (NPY) affected synaptic excitation of heterotopic neurons. We also compared several synaptic and intrinsic properties of heterotopic, layer 2–3 cortical, and CA1 pyramidal neurons, to further characterize heterotopic cells. NPY powerfully inhibited synaptic excitation onto normal and normotopic CA1 cells but was nearly ineffective on responses evoked in heterotopic cells from stimulation sites within the heterotopia. Glutamatergic synaptic responses on heterotopic cells exhibited a comparatively small, D2-amino-5-phosphopentanoic acid-sensitive, N-methyl-D-aspartate component. Heterotopic neurons also differed from normal CA1 cells in postsynaptic membrane currents, possessing a prominent inwardly rectifying K+ current sensitive to Cs+ and Ba2+, similar to neocortical layer 2–3 pyramidal cells. CA1 cells instead had a prominent NAM-amino-5-phosphopentanoic acid-sensitive, H11001, similar to neocortical positions, are often associated with neurological dysfunction. In children, for example, cortical disorganization resulting from a migration disorder can be associated with intractable forms of epilepsy, mental retardation, or autism (Aicardi 1994; Friede 1975; Palmini 2000). Because seizures result from the abnormal electrical discharge of a group of neurons, much effort has been directed toward studying clusters of disorganized neurons (i.e., neuronal heterotopia). Interestingly, clinical studies suggest that disorganized brain regions generate seizure activity (Raymond et al. 1994), and surgical resection of this tissue is often an effective form of seizure control (Palmini et al. 1991a,b).

A strong clinical correlation between migration disorders and epilepsy spurred the development of several animal models in which to study epileptogenesis in the disorganized brain (Chevassus-au-Louis et al. 1999a). One such model utilizes prenatal exposure to the teratogenic, DNA methylating agent, methylazoxymethanol (MAM) (Nagata and Matsumoto 1969). MAM injection on gestational day 15 in rats results in diffuse cortical malformations, including microcephaly, heterotopias in area CA1 of hippocampus, and loss of lamination (Chen and Hillman 1986; Singh 1977). These animals exhibit many of the anatomical/molecular properties of human cortical dysplasia and are significantly seizure-susceptible (Baraban and Schwartzkroin 1996; Chevassus-au-Louis et al. 1999a,b; Colacitti et al. 1999; Germano and Sterpet 1997). Similar to clinical studies, hippocampal heterotopias in an experimental model are of particular interest as a potential site of seizure generation. Recent work suggests that hippocampal heterotopic neurons are capable of independent seizure genesis (Baraban et al. 2000), exhibit hyperexcitable firing activity and a loss of functional A-type potassium channels (Castro et al. 2001), and are most similar, both in molecular and electrophysiological properties, to neocortical neurons (Castro et al. 2002; Chevassus-au-Louis et al. 1998).

Although the hyperexcitability of hippocampal heterotopic neurons in the MAM model is now well established, synaptic physiology within a heterotopia has been little studied and virtually nothing is known about modulation of excitation at heterotopic synapses. Here we examined the actions of neuropeptide Y (NPY), a potent, endogenous modulator of hippocampal excitability (Colmers et al. 1988), on excitatory synaptic inputs to heterotopic cells in hippocampi from MAM-treated rats as well as on normotopic CA1 pyramidal neurons (MAM-treated and control untreated rats). While NPY inhibited stratum radiatum (SR) excitatory synaptic input to heterotopic neurons, it had little effect on intra-heterotopic excitation.

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Furthermore, heterotopic neurons shared few membrane and synaptic properties with CA1 pyramidal cells but were virtually indistinguishable from neocortical layer 2–3 neurons. These data support other evidence (Chevassus-au-Louis et al. 1998; Castro et al. 2002) suggesting that MAM-induced heterotopic neurons in area CA1 of the hippocampus were fated to become layer 2–3 neocortical neurons.

**Methods**

**Preparation of slices**

Pregnant Sprague-Dawley rats were injected intraperitoneally with 25 mg/kg of MAM-Acetate (NCI Chemical Carcinogen, Kansas City, MO) on day 15 of gestation. Male and female offspring (17–35 days old) were decapitated according to a protocol approved by the Health Sciences Laboratory Animal Welfare Committee of the University of Alberta. The brain was rapidly removed and placed in ice-cold (2–4°C) carbogen (95% O₂-5% CO₂)-saturated slicing medium containing (in mM) 118 NaCl, 3 KCl, 1.3 MgSO₄, 1.4 NaH₂PO₄, 5 MgCl₂, 26 NaHCO₃, 1.5 CaCl₂, 10 glucose, and 1 kynurenic acid (to block glutamate-mediated excitotoxicity). The brain was hemisected sagittally, and the cerebellum and frontal lobe removed. Blocked tissue was glued to the base of a Plexiglas slicing chamber. Transverse slices (300 μm) containing hippocampus and neocortex were cut with a vibratome (TPI, St. Louis, MO) and immediately transferred to a holding chamber containing carbogen-saturated artificial cerebrospinal fluid (ACSF) consisting of (in mM) 124 NaCl, 3 KCl, 1.3 MgSO₄, 1.4 NaH₂PO₄, 26 NaHCO₃, 2.5 CaCl₂, and 10 glucose. Slices were held at 32°C for 30–60 min, then stored at room temperature for ≤7 h.

**Electrophysiological recordings**

Individual slices were transferred to a glass-bottomed submersion-type recording chamber, anchored with a platinum “harp,” and continuously perfused with oxygenated ACSF at 34–36°C. Neurons were visually identified using an IR-DIC videomicroscopy system, as described by Ho et al. (2000). Whole cell recordings were performed with patch electrodes (3–6 MΩ) pulled from borosilicate glass capillary tubing filled with an intracellular solution consisting of (in mM) 125 K-glucamate, 2 KCl, 5 HEPES, 5 MgATP, 0.3NaGTP, 5 EGTA, 0.1 BAPTA, 10 creatine phosphate, and 3.0 mg/ml bicytin (pH 7.2; 292–298 mosM). Once a seal (>2 GΩ) was formed, the patch was ruptured to gain access to the cell (15–40 MΩ). Whole cell experiments were performed on pyramidal neurons in hippocampal heterotopias, in st. pyramidale of area CA1 of the hippocampus, and in layer 2–3 of the neocortex overlaying the hippocampus in MAM-treated or control animals, 43 CA1 pyramidal cell, and 43 layer 2–3 neocortical neurons. Data were taken only from neurons whose resting membrane potential was stable and negative to −55 mV. Once a stable membrane potential had been observed, neurons were held in voltage-clamp, near their resting potentials (−65 mV for CA1 neurons, and −75 mV for heterotopic and cortical pyramidal cells, except where noted) for the duration of the experiments. Excitatory postsynaptic currents (EPSCs) were evoked via a bipolar, sharpened tungsten stimulating electrode placed in SR of area CA1, within the heterotopia, or in layer 1 of neocortex. A paired-pulse stimulus protocol (1–20 V, 300 μs, 50-ms interstimulus interval) was delivered to the stimulating electrode from a stimulus isolation unit (IsoFlex, AMPLI, Jerusalem), and comparisons were made between cell types based on the first stimulus of the pair. The intensity of the stimulus was adjusted until a submaximal and stable synaptic current was evoked. In most cases, a voltage step (50 ms, 10–20 mV negative to rest) was applied to the neuron during the protocol, after the synaptic responses had subsided to monitor for changes in access resistance (Ho et al. 2000). Passive postsynaptic membrane properties were also routinely examined in virtually every recording with a slow (2.8 s, 60 mV) positive-going voltage ramp starting 40 mV negative to rest and with a family of 100-ms voltage steps, varying, from −40 to +20 mV relative to the resting membrane potential in 10-mV increments, with a 2-s interval between each step. All whole cell currents were recorded using an Axoclamp 2A amplifier (Axon Instruments, Foster City, CA) used in the continuous single-electrode voltage-clamp mode. Data were acquired and membrane potential controlled using pClamp 8 (Axon Instruments). Drugs were applied via the bath.

**Materials**

NPY was purchased from Dr. S. St.-Pierre (Peptidec Technologies, Montreal, QC, Canada); 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo-(-)quinoxaline (NBQX) was purchased from Research Biochemicals International (RBI, Natick, MA); 4-(N-ethyl-N-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride (ZD 7288) was purchased from Tocris (Ellisville, MO); creatine phosphate was purchased from Boehringer (Mannheim, Germany); cesium chloride (CsCl) and barium chloride (BaCl₂) were purchased from Fisher Scientific (Fair Lawn, NJ). All chemicals used in slicing medium and ACSF were obtained from BDH (Toronto, ON, Canada), and all other chemicals were obtained from Sigma (St. Louis, MO).

**Analysis**

Data were analyzed using pClamp 8 (Axon Instruments) and GraphPad Prism 3.02 software (GraphPad, San Diego, CA). Graphs were made using Axum 5.0 (Mathsoft, Cambridge, MA). Neurons were used as their own controls for statistical purposes. Data on NPY and ion channel blockers are only from experiments in which the effects reversed substantially during washout. Numerical data are presented as means ± SE. Statistical comparisons of the results' significance versus zero were made using a Student’s unpaired t-test, and comparisons between cell types were done using a Student’s paired t-test.

**Histochemistry**

The procedure, with some modifications, was similar to the biocytin visualization procedure described elsewhere (Schiller et al. 1997). Briefly, slices with biocytin-filled neurons were fixed in ice-cold, 4% paraformaldehyde in 100 mM phosphate-buffered saline (PBS). Thereafter, the slices were rinsed in PBS, then in PBS containing 1% H₂O₂ to quench endogenous peroxidases. Sections were rinsed thoroughly in PBS and then 2% Triton X-100 in PBS for 1 h to increase the penetration of reagents. Slices were then incubated for 2 h in avidin-biotinylated horseradish peroxidase according to the manufacturer’s protocol (ABC-Elite, Vector Labs, Peterborough, UK) and then rinsed thoroughly in PBS. Cells were visualized using diaminobenzidine (0.05%) in 0.01% H₂O₂ PBS, and the reaction was quenched by rinsing again in standard PBS. Finally, slices were mounted on slides with an aqueous medium, and photographed with a color digital camera (Dage DC330, Dage-MTI, Michigan City, IN)

**Results**

**Cell morphology**

Results are based on recordings from >100 heterotopic neurons and 35 normotopic CA1 neurons from MAM-treated animals, 43 CA1 pyramidal cell, and 43 layer 2–3 neocortical neurons from untreated rats. We did not include physiological data from the recordings of neocortical neurons from MAM-treated rats in this study, as the MAM treatment used interferes with the formation of neocortical layers 2–4 (Jones et al. 1982), thus making it difficult to unambiguously assign a
neurons from age-matched controls (Fig. 1, A–D) shows typical heterotopic neurons found in area CA1 of the hippocampi of MAM rats. In accordance with previous Golgi staining studies (Singh 1980), nor-motopic CA1 pyramidal neurons from MAM-treated rats appear to be much smaller in size compared with CA1 pyramidal neurons from age-matched controls (Fig. 1, E and G). Biocytin-filled neocortical neurons from MAM rats appear deformed compared with control layer 2–3 neocortical pyramidal cells, and the dendrites of these cells were not aligned in as orderly a fashion (Fig. 1, F and H).

NPY actions in the MAM brain

Hippocampal heterotopic neurons are hyperexcitable and a potential source of seizure genesis in MAM-treated rats (Baraban et al. 2000; Castro et al. 2001). NPY inhibits excitatory synaptic transmission in the hippocampus (Colmers et al. 1988) and thereby exerts a powerful antiepileptic role in rodents (Baraban et al. 1997; Bindokas et al. 1998; Colmers et al. 1988; Klapstein and Colmers 1997; Marsh et al. 1999; Vezzani et al. 1999). In the present study, we examined the modulatory effect of NPY in acute hippocampal slices from MAM- and untreated control rats. NPY (1 μM) potently inhibited EPSCs elicited by stimulation in SR both in normal CA1 pyramidal (control: 85.7 ± 1.78%, n = 11, P < 0.0001) and normotopic CA1 pyramidal neurons (MAM: 72.7 ± 4.15%, n = 8, P < 0.0001; Fig. 2, A, B, and E). The same concentration of NPY had a significantly smaller, yet still significant, effect on EPSCs evoked in heterotopic cells by SR stimulation (MAM: 54.1 ± 4.10%, n = 16, P < 0.0001; Fig. 2, C and E). When the stimulating electrode was placed within the heterotopia, 1 μM NPY had no effect on EPSCs evoked in 8 of 19 heterotopic neurons and only weakly inhibited EPSCs in the remaining 11 cells (10.96 ± 1.38%, n = 11, P < 0.0001; Fig. 2, D and E). Thus intra-heterotopic excitatory connections are far less responsive to the actions of NPY than are extra-heterotopic, presumably Schaffer collateral, inputs. It is important to note that eliciting EPSCs in heterotopic neurons from the stratum radiatum was only successful ~40% of the time, presumably because the fibers of the SR tend to avoid the heterotopia as has been shown previously using carbocyanine tracing (Chevassus-au-Louis et al. 1999b). However, EPSCs that were evoked in heterotopic neurons from the SR were done so using a similar stimulus intensity as was needed when the stimulating electrode was placed within the heterotopias.

Characterization of EPSC responses in the MAM brain

To further characterize the synaptic properties of hippocampal heterotopic neurons, we examined the pharmacological and biophysical properties of evoked glutamatergic EPSCs. To examine functional NMDA-mediated synaptic responses on heterotopic neurons, we perfused slices with an NMDA receptor antagonist, D-2-amino-5-phosphopentanoic acid (APV). At a holding potential of −45 mV, virtually all NMDA receptors are free from the blockage by magnesium that occurs at more negative membrane potentials (Nowak et al. 1984). In neurons held at −45 mV, we found that 50 μM APV inhibited the EPSCs on layer 2–3 cortical neurons evoked from layer 1 of the neocortex (52.6 ± 6.23%, n = 7, P > 0.001) and CA1 pyramidal cells (59.1 ± 8.56%, n = 7, P > 0.007) from normal animals significantly more than its inhibition of EPSCs on hippocampal heterotopic neurons when the stimulating electrode was placed in the SR (22.3 ± 1.78%, n = 7; Fig. 3B). Addition of 3 μM NBQX, an α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor-selective antagonist (Sheardown et al. 1990) following APV caused a further reduction of EPSC amplitude to approximately 7% of predrug

![Figure 1](jneurosci.org/content/88/11/2747/F1.large.jpg)
levels for all three cell types (data not shown). Thus the glutamatergic EPSCs recorded here in these experiments were composed mainly of NMDA and AMPA components.

Qualitative observations of synaptic responses, evoked from the SR in heterotopic and from layer 1 of the neocortex in layer 2–3 neurons, suggested they were quite fast compared with EPSCs in normotopic CA1 pyramidal neurons from both MAM and control animals (Fig. 4, A–D). To compare this quantitatively, we determined decay time constants of the EPSC (τEPSC) for each cell type using a standard exponential fitting model. At a holding potential of −75 mV, there was no significant difference between the τEPSC of heterotopic (4.8 ± 0.20 ms, n = 26) and layer 2–3 neocortical neurons from control animals (5.1 ± 0.26 ms, n = 23, P > 0.39; Fig. 4E). However, the τEPSC in control CA1 pyramidal cells held at −75 mV was much greater than in the other cell types (7.9 ± 0.95 ms, n = 16, P = 0.0001) and was not significantly different from those recorded in normotopic CA1 pyramidal neurons (7.4 ± 0.71 ms, n = 5, P > 0.8; Fig. 4E). When neurons were held at −65 mV, there was still no significant difference between the τEPSC in heterotopic and layer 2–3 pyramidal neurons (4.3 ± 0.30 ms, n = 21 vs. 4.9 ± 0.30 ms, n = 17, P > 0.15), and they remained significantly smaller than either the τEPSC of normal CA1 cells (7.864 ± 0.4091 ms, n = 22, P < 0.0001) or normotopic CA1 pyramidal neurons (MAM: 8.1 ± 0.68 ms, n = 12), which did not significantly differ from one another. (P > 0.95).

Comparisons of membrane properties in pyramidal cells from MAM-treated and normal animals

During the previous experiments on synaptic actions, we routinely observed postsynaptic properties in some detail to determine whether any changes in synaptic responses with NPY application might be explained by alterations in postsynaptic properties. The first noticeable difference between heterotopic and normotopic CA1 neurons from MAM-treated animals was that heterotopic neurons rested at a significantly more negative potential than did either normotopic CA1 neurons (−74.6 ± 0.42 mV, n = 102 vs. −64.9 ± 0.42 mV, n = 35, P < 0.0001) or CA1 neurons from control animals (−64.4 ± 0.54 mV, n = 43, P < 0.0001). However, the resting potentials of layer 2–3 neocortical neurons were not significantly different (−74.19 ± 0.57 mV, n = 43) from those of heterotopic neurons (P > 0.5).

No evidence was found for an effect of NPY on postsynaptic properties in any neurons tested, consistent with earlier reports (Colmers et al. 1987, 1988; McQuiston and Colmers 1996). However, we did observe differences in membrane steady-state current-voltage relationships between the different types of neurons studied here. These differences prompted a systematic comparison of the postsynaptic properties of neurons from MAM-treated and control animals.

First, we examined steady-state, current-voltage relationships using a slow voltage ramp protocol. As the membrane...
inwardly rectifying potassium current, voltage ramp (Fig. 6A) is consistent with the presence of an inwardly rectifying potassium current in heterotopic and layer 2–3 cells. Cs⁺ reduced the membrane conductance in all three types of cells, having an effect at every membrane potential in CA1 pyramidal neurons (−110 mV: 34.1 ± 9.72% inhibition, n = 9; −60 mV: 19.5 ± 7.42% inhibition, n = 9) and only affecting the conductance of heterotopic and layer 2–3 neocortical cells at potentials more negative than −90 and −80 mV, respectively (HET at −110 mV: 83.9 ± 8.27% inhibition, n = 6; COR at −110 mV: 98.8 ± 5.36% inhibition, n = 11). Conversely, in 6 mM K⁺, Ba²⁺ does not significantly alter membrane conductance in CA1 pyramidal neurons but has a strong inhibitory effect on the steady-state conductance of both heterotopic and cortical neurons at −80 mV and below (HET at −110 mV: 75.2 ± 8.73% inhibition, n = 6; COR at −110 mV: 60.4 ± 6.28% inhibition, n = 11).

To further compare the neuronal properties of these cell types, a series of 100-ms voltage-clamp steps was applied from a holding potential of −75 mV. At a step to −115 mV, an inwardly rectifying potassium currents are potentiated by increases in extracellular K⁺ (Pennefather and DeCoursey 1994). Therefore elevating the extracellular potassium to 6 mM increased $K_{IR}$ on heterotopic and neocortical neurons. In the presence of 6 mM K⁺, the membrane conductance increased relative to control K⁺ in all three cell types but increased the conductance most at −110 in heterotopic and layer 2–3 cells. Ca²⁺ reduced the membrane conductance in all three types of cells, having an effect at every membrane potential in CA1 pyramidal neurons (−110 mV: 34.1 ± 9.72% inhibition, n = 9; −60 mV: 19.5 ± 7.42% inhibition, n = 9) and only affecting the conductance of heterotopic and layer 2–3 neocortical cells at potentials more negative than −90 and −80 mV, respectively (HET at −110 mV: 83.9 ± 8.27% inhibition, n = 6; COR at −110 mV: 98.8 ± 5.36% inhibition, n = 11). Conversely, in 6 mM K⁺, Ba²⁺ does not significantly alter membrane conductance in CA1 pyramidal neurons but has a strong inhibitory effect on the steady-state conductance of both heterotopic and cortical neurons at −80 mV and below (HET at −110 mV: 75.2 ± 8.73% inhibition, n = 6; COR at −110 mV: 60.4 ± 6.28% inhibition, n = 11).
inward relaxation was observed, possibly consistent with the presence of a hyperpolarization-activated cation current, \( I_h \) (Halliwell and Adams 1982; Mayer and Westbrook 1983; Pape 1996). However, comparison of the time constant of this current relaxation at \(-115 \text{ mV} \) \( (\tau_{111}) \) showed that heterotopic and layer 2–3 neocortical neurons exhibited fast inward currents (MAM: 13.8 ± 0.26 ms, \( n = 26 \) vs. control: 15.2 ± 0.71 ms, \( n = 21 \)) that did not differ significantly \( (P > 0.1; \text{Fig. 7}) \). Conversely, CA1 pyramidal neurons exhibit a much slower, more prominent inward relaxation (36.6 ± 1.19 ms, \( n = 29 \), \( P < 0.0001; \text{Fig. 7} \)). Further detailed analysis of neuronal firing properties (e.g., action potential morphology, afterhyperpolarizations, firing frequency, input resistance) were not made as this information has been presented elsewhere (Baraban and Schwartzkroin 1995, Castro et al. 2002).

The strong inhibition of steady-state conductance by \( \text{Ba}^+ \) in heterotopic and layer 2–3 neurons suggests a very small \( I_h \) in these cells (Pape 1996). We thus examined the effect of a selective \( I_h \) blocker, ZD 7288 (Harris and Constantini 1995; Gasparini and DiFrancesco 1997), on membrane conductance.
using the voltage step protocol described in the preceding text. Comparison of currents elicited by a voltage step to \(-115\) mV, in the absence or presence of ZD 7288 (50 \(\mu\)M), demonstrated a strong inhibition of \(I_h\) in CA1 pyramidal neurons by ZD 7288 but only a very minor effect on heterotopic and neocortical neurons (Fig. 8). By subtracting current traces taken before and after application of ZD 7288, we were able to isolate the net \(I_h\) (Fig. 8, A–C), which we compared at \(-115\) mV in each cell type. As expected from the results with Ba\(^{2+}\), in the preceding text, the amplitude of \(I_h\) in CA1 pyramidal cells was substantial (103.6 ± 9.81 pA, \(n = 6\)) and significantly larger than the amplitude of \(I_h\) in heterotopic (12.0 ± 4.74 pA, \(n = 8\), \(P > 0.0005\)) and layer 2–3 neocortical neurons (14.7 ± 4.07 pA, \(n = 5\), \(P > 0.004\); Fig. 8D). Consistent with the hypothesis that heterotopic and neocortical neurons share similar intrinsic properties, the amplitudes of \(I_h\) measured for heterotopic and layer 2–3 pyramidal neurons were not significantly different (\(P > 0.7\)). The lesser effect of ZD 7288 in the heterotopia and cortex indicates that a much weaker \(I_h\) is present in heterotopic and layer 2–3 pyramidal cells than in CA1 pyramidal cells, consistent with the effects of Ba\(^{2+}\) in the voltage ramp experiments.

**Discussion**

Neuronal heterotopias are frequently seizure foci in patients suffering from epilepsies associated with a cortical malformation (Palmini et al. 1991a). Here we show that heterotopic neurons in area CA1 of the hippocampus of MAM-treated rats differ from neighboring normotopic CA1 pyramidal neurons (or control CA1 pyramidal neurons) in their connectivity, synaptic function and ion channel and presynaptic receptor complement. Hippocampal heterotopic neurons in these animals most closely resemble layer 2–3 neocortical neurons, as suggested previously (Castro et al. 2002; Chevassus-au-Louis et al. 1998), consistent with the hypothesis that these animals model a neuronal migration disorder. These results add to our growing knowledge of how neurons function in a disorganized brain, suggest that a variety of physiological functions are altered when neurons migrate incorrectly, and implicate alterations in presynaptic NPY receptors as one source of hyperexcitability in experimental heterotopias.

**Limited NPY susceptibility within heterotopias**

The present results represent, to our knowledge, the first report of an altered pre-synaptic response to NPY by heterotopic neurons. NPY has a substantial inhibitory effect on the amplitudes of EPSCs elicited by SR stimulation in all neurons tested, including CA1 pyramidal cells of both MAM-treated and untreated animals and heterotopic neurons. By contrast, NPY had only a very weak effect on inputs to heterotopic neurons when the stimulating electrode was placed within the heterotopia. As NPY acts presynaptically (Colmers et al. 1988, McQuiston and Colmers 1996; Qian et al. 1997), this suggests that the excitatory inputs to heterotopic neurons have few, if any, NPY receptors on their terminals. The fibers of SR have a powerful presynaptic response to NPY (Qian et al. 1997). While the origin of excitatory inputs within the heterotopia is unclear, evidence here and elsewhere suggests that the en passant fibers of the SR are not the exclusive source. Earlier studies using carbocyanine tracing indicate that SR fibers avoid the neurons of a heterotopia (Chevassus-au-Louis et al. 1999b), consistent with such ectopic neurons expressing a complement of cell surface markers suppressing their integration within the hippocampus. Because the SR fibers contribute a large majority of excitatory synaptic inputs to CA1 neurons (Shepherd and Harris 1998; Shepherd et al. 2002), this raises the possibility that a considerable number of excitatory inputs originate within the heterotopia. As the intra-heterotopic inputs appear to lack NPY receptors, poorly regulated heterotopic-heterotopic excitation may contribute to epileptiform activity found in these disorganized cell regions (Baraban et al. 2000) and may also be a factor in human neuronal migration disorders where dysplastic areas frequently form epileptic foci (Palmini et al. 1991b).

**Reduced NMDA component in heterotopic EPSPs**

In the present study, we have shown that the NMDA receptor antagonist APV does not have as strong an inhibitory effect on the EPSCs of heterotopic neurons as it does on those of layer 2–3 cortical and CA1 pyramidal cells. The reduced effect of d-APV in hippocampal heterotopic neurons seen here agrees with studies showing a relatively minor NMDA component in EPSCs for dysplastic neurons in the freeze-lesion model of cortical dysplasia (Luhmann and Raabe 1996; Luhmann et al. 1998). However, evidence from the same model suggests that NMDA receptors play an important role in the initiation and propagation of epileptiform discharges (Defazio and Hablitz 2000). Although there is recent evidence that the expression of NMDA receptors in disorganized areas of MAM-treated brain tissue is qualitatively similar to that in controls (Rafiki et al. 1998), the proportion of NMDA receptor type 2B subunits is increased in cortical dysplasias of humans (Najm et al. 2000) and experimental animals (Defazio and Hablitz 2000; Rafiki et al. 1998), suggesting a role for this subunit in epileptogenicity. While the present study shows that NMDA has a reduced role in the generation of EPSCs in heterotopic neurons of the MAM model, this increased expression of NR2B may play a part in the propagation of epileptiform activity in disorganized tissue.

![Image](http://jn.physiology.org/lookup/suppl/doi:10.1152/jn.00395.2001/-/DC1/fig7.png)
Also, while this reduced NMDA effect may seem inconsistent with the intrinsically hyperexcitable nature of these neurons (Baraban et al. 2000), a synaptic hyperexcitability mediated by AMPA-receptors has been reported in a chronic model of temporal lobe epilepsy (Lothman et al. 1995). The changes in NMDA (and AMPA) receptor complement in heterotopias of the MAM model must be more thoroughly examined.

Hippocampal heterotopic neurons have similar properties to layer 2–3 cortical neurons

While heterotopic neurons shared relatively few physiological properties with normotopic or normal CA1 neurons, most properties studied were indistinguishable from those of layer 2–3 pyramidal cells. For example, heterotopic and cortical neurons both rest at a membrane potential of approximately −75 mV, while CA1 pyramidal cells in MAM-treated and untreated rats rest at approximately −65 mV. Furthermore, the \( \tau_{EPSC} \) of heterotopic and cortical neurons are similar and far faster than that in normal or normotopic CA1 pyramidal cells, consistent with their sharing similar postsynaptic responses to excitatory inputs. Likewise, heterotopic and layer 2–3 cortical neurons exhibit an increased conductance at membrane potentials negative to −80 mV. This conductance was sensitive to both barium and cesium and was enhanced in elevated extracellular potassium, consistent with an inwardly rectifying potassium current (\( I_{IR} \)) (e.g., Williams et al. 1988). In contrast, the steady-state current-voltage relationship of CA1 pyramidal neurons is linear across all membrane potentials examined but is affected by cesium, and not by barium, consistent with an inwardly rectifying cation current (\( I_{h} \)) that has previously been described in hippocampal (Halliwell and Adams 1982) and other neurons (Pape 1996). Consistent with this, the \( I_{h} \)-specific
blocker, ZD 7288, suppressed a prominent current in CA1 neurons but had little effect in heterotopic and layer 2–3 pyramidal cells.

Interestingly, the differences in intrinsic membrane conductances in these neurons may in part contribute to the elevated excitability observed in the heterotopic neurons of area CA1 (Baraban et al. 2000). For example, the primary effect of $I_h$ is to resist changes in membrane potential (Hutcheon et al. 1996; Pape 1996). Blockade of $I_h$ channels in pyramidal neurons has been shown to increase the rate of spike generation (Gasparini and DiFrancesco 1997) and to lead to the enhanced summation of EPSPs (Berger et al. 2001; Magee 1999). Thus the absence of $I_h$ from the heterotopic neurons would tend to heighten their responsiveness to excitation. Furthermore, although the presence of a prominent inwardly rectifying potassium current in the heterotopic cells would tend to reduce their excitability in relatively negative membrane voltage regions, the prominent decrease in this conductance with depolarization would make these cells more excitable if depolarized slightly. The combination of an increased EPSP summation in the absence of $I_h$ coupled with the decreased baseline conductance in the depolarizing subthreshold voltage region with the deactivation of the inward rectifier would be expected to result in a heightened response to excitatory input in these neurons. Heterotopic cells would also be expected to fire more rapidly in the absence of $I_h$. These properties, then, may be involved in the hyperexcitability observed in the rat MAM model (Baraban et al. 2000). Similarly, such alterations would tend to elevate excitability in human cortical dysplasias.

The similarities observed in intrinsic membrane properties of heterotopic and cortical pyramidal cells, as well as the differences seen here between heterotopic and CA1 neurons, indicate that hippocampal heterotopic neurons in MAM-treated rats may actually have been fated to become layer 2–3 cortical neurons. Previous studies have demonstrated that heterotopic neurons express genes specific to neurons of neocortical layer 2–3 (Castro et al. 2002) and that heterotopic and neocortical supragranular neurons have similar developmental features and neuronal firing properties (Castro et al. 2002; Chevassus-Au-Louis et al. 1998). These findings, in combination with the additional electrophysiological similarities described here, provide strong evidence that hippocampal heterotopic neurons in MAM-treated rats are displaced layer 2–3 cortical cells rather than the product of a second wave of CA1 neuron migration during development as previously hypothesized (Zhang et al. 1995).

Although it is rare for heterotopias to appear in the hippocampi of human NMD patients (Mischel et al. 1995), the hippocampal heterotopias of MAM rats are morphologically similar to heterotopic nodules found in human periventricular or subcortical nodular heterotopia (PNH) (Colacitti et al. 1998). While MAM-treated rats have not yet been shown to exhibit spontaneous seizures (Baraban and Schwartzkroin 1995; Chevassus-au-Louis et al. 1999; Germano and Sperber 1997), the abnormal migration of neurons in the MAM-model may provide a clue as to the etiology of neuronal migration disorders such as PNH. The small $I_h$ current observed here, in combination with recent work demonstrating a lack of A-type potassium current on heterotopic neurons (Castro et al. 2001), suggest that altered ion channel function contributes to the ability of heterotopic neurons to fire in a hyperexcitable manner. Furthermore, on-going electrophysiological characterization of the intrinsic and synaptic properties of heterotopic neurons, some of which is presented here, will ultimately lead to a greater understanding of how dysplastic neurons function. In conclusion, it is tempting to speculate that in the larger assemblages of dysplastic neurons that form human cortical dysplasias, the combination of enhanced intrinsic excitability, collateral excitation, and insensitivity to NPY action may increase the propensity of a malformed brain to express focal seizure activity.

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