Neurobiology of Disease 48 (2012) 317–328

Contents lists available at SciVerse ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

The loss of interneuron functional diversity in the piriform cortex after induction of experimental epilepsy

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ARTICLE INFO

Article history:
Received 25 May 2012
Revised 28 June 2012
Accepted 5 July 2012
Available online 16 July 2012

Keywords:
Experimental epilepsy
Interneuron
Kv 1.6
Piriform cortex
Firing pattern

ABSTRACT

Interneuronal functional diversity is thought to be an important factor in the control of neural network oscillations in many brain regions. Specifically, interneuron action potential firing patterns are thought to modulate brain rhythms. In neurological disorders such as epilepsy where brain rhythms are significantly disturbed interneuron function is largely unexplored. Thus the purpose of this study was to examine the functional diversity of piriform cortex interneurons (PC; an area of the brain that easily supports seizures) before and after kindling-induced epilepsy. Using cluster analysis, we found five control firing behaviors. These groups were termed: non-adapting very high frequency (NAvHF), adapting high frequency (AHF), adapting low frequency (ALF), strongly adapting low frequency (sALF), and weakly adapting low frequency (wALF). A morphological analysis showed these spiking patterns were not associated with any specific interneuronal morphology although we found that most of the cells displaying NAvHF firing pattern were multipolar. After kindling about 40% of interneuronal firing pattern changed, and neither the NAvHF nor the wALF phenotypes were found. We also found that in multipolar interneurons a long-lasting potassium current was increased. A qPCR analysis indicated Kv1.6 subtype was up-regulated after kindling. An immunocytochemical analysis showed that Kv1.6 protein expression on parvalbumin (multipolar) interneurons increased by greater than 400%. We also examined whether these changes could be due to the selective death of a subset of interneurons but found that there was no change in cell number. These data show an important loss of the functional diversity of interneurons in the PC. Our data suggest that under pathophysiological condition interneurons are plastic resulting in the attenuation of high frequency network oscillations in favor of low frequency network activity. This may be an important new mechanism by which network synchrony is disturbed in epileptic seizures.

Introduction

The piriform cortex (PC) is a three layered phylogenetically old cortical structure (paleocortex) that is part of the limbic system. In addition to olfactory sensation and memory processing (Haberly, 2001), the PC has also been implicated in the development of seizures (Loscher and Ebert, 1996; Racine et al., 1988). As in all cortical networks, the GABAergic interneuron system within the PC is important for the regulation of neuronal excitability and rhythmicity of the neural network, participating in both feed-forward and feedback inhibitory loops (Haberly, 1983; Kelly et al., 2002; Neville and Haberly, 2004). These circuits have been shown to modulate associative long-term potentiation (Kanter and Haberly, 1993) and generate oscillatory activities in the PC (Neville and Haberly, 2003). It has also been shown that the PC is a heterogeneous structure with anatomical differences along the rostro-caudal axis, including differences in layer thickness (Haberly and Price, 1978), the number of interneurons per layers (Haberly et al., 1987; Loscher et al., 1998) as well differences in the laminar distribution/orientation of associational fibers (Haberly and Price, 1978; Luskin and Price, 1983). These morphological traits are thought to reflect different functional roles along the anterior–posterior axis of the PC (Neville and Haberly, 2004). In light of this diverse functionality, the focus on morphological and electrophysiological characteristics of interneuronal subpopulations in the PC has increased. A recent study by Suzuki and Bekkers (2010) has demonstrated in mouse PC a variety of interneuron firing patterns that are similar in many ways to those found in other limbic regions. These included those that fired at high (>50 Hz) and low (<50 Hz) frequencies and those that fired at a constant (non-adapting) or decreasing (adapting) rate. The role of these interneuron firing patterns in the PC has not been examined in the detail that various firing patterns have been studied in other brain regions such as the hippocampus and neocortex.

Additionally, whether these firing patterns are amenable to change in response to either physiological or pathophysiological stimuli has yet to be explored. Given the intimate roles or "subdivision of labor" (Klausberger and Somogyi, 2008) that these neurons

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Available online on ScienceDirect (www.sciencedirect.com).

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doi:10.1016/j.nbd.2012.07.002

seem to control with regard to defining differing oscillatory patterns in neural circuits, disturbances in their firing patterns may have wide ranging effects on behavior. Here we have investigated the hypothesis that in the kindling model of epilepsy interneuron firing patterns may be disturbed. We found that in contrast to control tissue, kindled neurons lose the non-adapting phenotype and the ability to fire at very high frequencies (>100 Hz). This change was not accounted for by cell death but rather an increase in the expression of the Kv1.6 subtype of potassium channels on multipolar interneurons which normally are capable of firing at very high frequency non-adapting rates. Our observations indicate that there is a loss of interneuron firing diversity in the PC after the induction of kindled induced epileptic seizures.

Materials and methods

Adult male Sprague Dawley rats (Charles River), weighing 200–250 g at the time of the study (n = 45), were used. All experiments were conducted in accordance with the guidelines of the Canadian Council on Animal Care and protocols approved by The University of Western Ontario Animal Care Committee.

Electrode implantation

Animals (n = 20) were anesthetized with Ketamine–Dormitor mixture (0.1 ml/100 g; i.p.) and implanted with two bipolar stimulating/recording electrodes bilaterally in the basolateral amygdala (BLA) with the following coordinates: 2.6 mm posterior to Bregma, 4.5 mm lateral to midline and 8.0 mm ventral (Paxinos and Watson, 1986). Post-mortem histological confirmation of intracranial location of electrodes in BLA was performed for all kindled rats.

The electrodes were constructed of two twisted strands of 0.127-mm diameter Diamel-insulated Nichrome wire and were attached to male Amphenol pins. The electrodes were implanted and secured to the skull with jeweler's screws. The electrode assembly was fixed to the skull by dental acrylic cement (McIntyre and Molino, 1972).

Kindling protocol

The kindling protocol began 1 week after the surgery. The afterdischarge threshold (ADT) was determined in each amygdala by delivering a 2-s 60-Hz sine wave stimulus of progressively increasing intensity (delivered once every minute at 15, 25, 35, 50, 75, 100,150, 200, 250, 300 and 350 μA) until an ADT was triggered (Gavrilovici et al., 2006; McIntyre and Plant, 1993). The rats were stimulated daily until six generalized stage 5 convulsions on the Racine's scale were elicited. Seizure severity and duration were recorded daily during the kindling acquisition. Fully kindled rats were allowed to recover for a minimum of 2 weeks (range 2–3 weeks) after the last seizure (Gavrilovici et al., 2006; McIntyre and Plant, 1993).

Tissue preservation, slicing procedures and maintenance

Coronal rat brain slices (350 μm; 1.5 to −0.3 mm relative to Bregma) were performed according to published methodology (Gavrilovici et al., 2006; McIntyre et al., 2002a). The anesthetized rats (Ketamine–Dormitor mixture; 0.1 ml/100 g; i.p.) were perfused through the heart with an ice-cold Ringer solution in which sodium was replaced by choline [containing (in mM): Choline Cl 110, KCl 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 0.5, $MgCl_2$ 7, Na pyruvate 2.4, ascorbate 1.3, dextrose 20] as previously described (Gavrilovici et al., 2006; McIntyre et al., 2002a). After heart perfusion, the brain was removed and temporal lobe was coronally sliced using a Vibratome. The slices were incubated at 37 °C for 30 min and subsequently moved to a room-temperature (22 °C) bath for at least 45 min. Slicing, incubation, and storage were all performed in the choline solution (see above). The Ringer's solution used during electrical recordings was similar to the choline solution except pyruvate and ascorbate were removed, equimolar NaCl replaced the choline Cl, and $CaCl_2$ and $MgCl_2$ were both used at a 2 mM concentration (McIntyre et al., 2002a). All solutions were maintained at pH 7.4 and bubbled with 5%CO2/95%O_2 (carbogen).

Electrophysiology

Patch electrodes were pulled from borosilicate glass capillaries and filled with K^+-gluconate solution having a composition (in mM) of: potassium gluconate 147, KCl 1, $CaCl_2$ 2, HEPES 10, EGTA 10, Glucose 10, MgATP 2, and GTP 0.3 (300 mOsm, pH 7.3–7.4). As this electrode solution has been shown to potentially block some potassium currents in hippocampus we also used an internal solution containing K^+ methanesulfonate (n = 34) to check that the identified spiking patterns were not influenced by the K^+ gluconate based internal solution (Zhang et al., 1994). These recordings were indistinguishable from control recordings. The K^+ methanesulfonate solution was not used throughout this study as we found that the cells did not support this internal solution as effectively as when the K^+ gluconate solution was employed. Whole-cell patch clamp recordings from neurons in layers 1–3 of anterior PC were made with an EPC 9/2 amplifier (HEKA, Lambrecht, Germany). Series resistance compensations were performed in all recordings. The initial access was <20 MΩ and compensated by 50–70%. All experiments were performed at 32 °C. Voltage-gated currents and excitability of the cell were monitored by means of voltage-clamp and current-clamp protocols (PulseFit v 8.0; Heka, Germany). Cell responses were obtained in less than 5 min after forming whole-cell configuration by injecting hyperpolarizing and depolarizing current steps (500 ms pulse; 50 pA increments). Input resistance (R_I) was calculated by linear regression of the current–voltage relationship in response to hyperpolarizing steps (as described in Dietrich et al., 2005) using Origin software (Microcal, Northampton, MA). Firing pattern analysis was performed at the current level that produced reliable repetitive firing (twice the firing threshold), in the presence of NMDA, AMPA and kainate channel blockers (20 μm 2-amino phosphonovaleric acid, APV and 10 μm dinitroquinoxaline-2,3-dione, DNQX; Research Biochemicals, Natick, MA, USA). Interspike interval ratio (II_R) was obtained by dividing the last interspike interval (measured in milliseconds) by the duration of the first interval, as described in Kroner et al. (2007). In a small number of recordings (n = 25) blocking $GABA_A$ receptors with gabazine (10 μM; added to the perfusion bath) did not affect the interneuron firing pattern in kindled animals. The effect of gabazine (10 μM) on GABAergic current (n = 9) was tested in the presence of sodium, NMDA, AMPA and kainate channel blockers, using a KCl electrode solution (as described in Gavrilovici et al., 2006).

Also, in a number of recordings (n = 72) to evaluate the K^+ current, before and after kindling, sodium and calcium channel blockers (250 nM TTX, and 1 mM $NiCl_2$) were added into the bath perfusion. Potassium outward currents were evoked by depolarizing steps between −50 and +10 mV, leak-subtracted and measured at the end of each pulse (PulseFit v 8.0; Heka, Germany).

Finally, a small number of current clamp recordings (n = 15) were performed on sham rats (electrode implanted, but never kindled) of the same age as kindled and control group rats, using identical protocols. The five firing patterns were observed in the sham rats (NAvHF, AHF, ALF, sALF and wALF), and corresponded to the firing patterns seen in control rats.

Cluster analysis

Unsupervised cluster analysis was performed on interneuronal populations of PC using Ward's method with *z*-score normalization and intervals calculated by Euclidian squared distances (SPSS 13, Chicago, Illinois, USA) according to described methodology (Cauli et al., 2000). The analysis was based on their main electrophysiological