LEADING ROLE OF THE PIRIFORM CORTEX OVER THE NEOCORTEX IN THE GENERATION OF SPONTANEOUS INTERICTAL SPIKES DURING BLOCK OF GABA<sub>A</sub> RECEPTORS

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Abstract—Interictal spikes are generated in the cerebral cortex during certain pathological conditions. In normal tissue, interictal spikes are triggered by blocking GABA<sub>A</sub> receptors. We studied the propensity of different areas of the cerebral cortex (including neocortex and piriform cortex) to generate spontaneous interictal spikes during block of GABA<sub>A</sub> receptors in slices of adult mice. Ten sequential brain slices were studied spanning most of the cerebral hemisphere. During block of GABA<sub>A</sub> receptors spontaneous interictal spikes were observed in all slices. Interestingly, interictal spikes recurred at different frequencies in different slices; posterior slices had a higher rate than the frontal slices (approximately 0.2 vs. 0.1 Hz, posterior vs. frontal). Multi-site recordings allowed to monitor discharges traveling across each slice and to derive cross-correlations. It became apparent that for the slices with higher frequency (i.e. posterior slices) the discharges originated in the piriform cortex and spread to the neocortex. A correlation between the frequency of the spontaneous discharges and the probability of events originating from piriform cortex was positive and significant. A further demonstration of this site of origin was provided by severing the connections between the neocortex and the piriform cortex. After interrupting these connections all the neocortical sites in the posterior slices displayed a lower frequency of discharges; similar to slower frontal slices. Meanwhile, the isolated piriform cortex of the posterior slices continued to produce higher frequency discharges. Thus, the higher frequency activity displayed by the posterior slices is intrinsically generated in the posterior piriform cortex from where it spreads to the neocortex. The results indicate that the neocortex and the piriform cortex have a distinct propensity to generate spontaneous interictal spikes, and that the more prone areas impose their activity on the less prone areas during disinhibition. © 2004 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: epilepsy, paroxysmal depolarizing shift, disinhibition, epileptiform discharge.

Different brain regions have different propensities to generate epileptiform activity after the application of chemosolvents, high-frequency electrical stimulation, or other manipulations (for review: De Curtis and Avanzini, 2001; Prince and Connors, 1986; Traub et al., 1996; Jefferys, 1990; McNamara, 1994). The basic form of epileptiform activity is the interictal spike, which in the depth of the cortex appears as a negative spike followed by a positive wave. Spike-wave discharges or interictal spikes (these terms are used interchangeably here) correspond intracellularly to an overt depolarization termed a paroxysmal depolarizing shift (Matsumoto and Ajmone-Marsan, 1964b; Schwartzkroin and Prince, 1978; Gutnick et al., 1982). Particular interest has been devoted to investigating epileptiform events induced by chemoconvulsants (e.g. GABA<sub>A</sub> receptor antagonists) in different parts of the cerebral cortex, such as the neocortex, the piriform cortex and the hippocampus. In the hippocampus, epileptiform events have been shown to originate in the CA3 region from where they spread to CA1 (Schwartzkroin and Prince, 1978; Traub et al., 1993a,b, 1996; Miles et al., 1984; Hablitz, 1984). The piriform cortex appears to have an unusually strong tendency to generate epileptiform events (Hoffman and Haberly, 1991, 1993, 1996; McIntyre and Wong, 1986; Piredda and Gale, 1985; Piredda et al., 1985; Stevens et al., 1988). In the neocortex, interictal spikes are also generated by blocking GABA<sub>A</sub> receptors (Connors et al., 2001; Chagnac-Amitai and Connors, 1989b; Gutnick et al., 1982; Hablitz, 1987; Ralston, 1958; Matsumoto and Ajmone-Marsan, 1964a,b; Gloor et al., 1977). In a previous study, we found that spontaneous rhythmically recurring spike-wave discharges are induced in slices of neocortex when GABA<sub>A</sub> receptors are blocked (Castro-Alamancos and Rigas, 2002). We also found that after-discharges that are generated by blocking GABA<sub>A</sub> and GABA<sub>B</sub> receptors preferentially occur in specific cytoarchitectonic areas of neocortex, but not in others (Castro-Alamancos and Rigas, 2002). This raised the possibility that spike-wave discharges are preferentially generated in specific cortical areas. In the present study, we examined the propensity of different regions of the cerebral cortex (including neocortex and piriform cortex) to generate spontaneous interictal spikes during block of GABA<sub>A</sub> receptors. Cortical slices were obtained sequentially covering most of the extent of the cerebral hemisphere. Our initial observations revealed that posterior cortical slices produced spontaneous interictal spikes with a higher frequency than frontal slices. Cross-correlation analysis and lesion studies demonstrated that the higher frequency activity of the posterior slices originated from the piriform cortex. Thus, the posterior piriform cortex has a stronger intrinsic propensity to generate spontaneous interictal spikes than the neocortex.

Abbreviations: ACSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; BMI, bicuculline.

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EXPERIMENTAL PROCEDURES

Slices were prepared from adult (≥7 weeks) BALB/C mice as previously described (Castro-Alamancos and Rigas, 2002). Mice were deeply anesthetized with sodium pentobarbitone (60 mg/kg) and upon losing all responsiveness to a strong tail pinch the brain was rapidly extracted and placed in ice cold buffer solution. Slices (400 μm thick) were cut in the thalamocortical plane (Agmon and Connors, 1991) using a vibratome. Six sequential slices of the right hemisphere were taken from each animal and were kept in an interface chamber at 32.5 °C. The slices were bathed constantly (1–1.5 ml/min) with artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl (126), KCl (3.5), NaH2PO4 (1.25), NaHCO3 (26), MgSO4·7H2O (1), dextrose (10), CaCl2·2H2O (1). The ACSF was bubbled with 95% O2 and 5% CO2. This ACSF composition has been shown to produce spontaneous slow activity in neocortical slices (Sanchez-Vives and McCormick, 2000; Castro-Alamancos and Rigas, 2002). All procedures were reviewed and approved by the Animal Care Committee of McGill University and Drexel University College of Medicine. The number of animals used in this study was kept to a minimum and every effort was made to minimize their suffering.

Field recordings were made using low-impedance pipettes (approximately 0.5 MΩ) filled with ACSF. Data were stored and analyzed using Experimenters’ Workbench (Data Wave Technologies, Longmont, CO, USA) and Origin (Microcal Software, Northampton, MA, USA) software. Every slice studied was photographed in the interface chamber using a CCD camera in order to allow subsequent identification (see Fig. 1).

Statistical analyses were performed using Origin (Microcal Software) and Datasm (Bates College, Lewiston, ME, USA) and consisted of t-tests and repeated measures analysis of variance (ANOVA) with three factors (site/slice/cut) and a Fischer’s test for post hoc analysis.

RESULTS

Experimental setup and initial observations

Ten sequential slices of the mouse brain spanning most of the fronto-posterior axis of the right cerebral hemisphere were studied. Slices were named 1 through 10 from posterior to frontal (Fig. 1). Slice 5 was defined as the first slice that included an intact thalamocortical connection (i.e. intact thalamocortical axons course from thalamus to neocortex), and the other slices were sequentially labeled based on that criterion. Moreover, since all slices were photographed, subsequent identification was possible after the experiments. Note that since slices are not cut in the coronal plane they cannot be compared directly with existing mouse atlases throughout the extent of the slice (Paxinos and Franklin, 2001).

A comparison of only the ventrolateral quadrant of the slices will be at different distances from bregma. A comparison of only the ventrolateral quadrant of the slices (i.e. area of the piriform cortex) with the atlas of Paxinos and Franklin (2001) revealed that slice 1 corresponds roughly to 2.8 mm posterior to bregma and slice 10 corresponds to 0.8 mm anterior to bregma. Due to space and time constraints six slices were studied per experiment (animal) and several series of experiments were performed. One series of experiments included the first six slices (i.e. 1–6), and another series of experiments included the last six slices (i.e. 5–10). Since these two series of experiments had two common slices (i.e. slices 5 and 6), they served as a means of controlling for variability between experiments (see below). Moreover, another series of experiments included slices 1–3 and 8–10, which allowed comparing the most frontal and posterior slices in the same experiment (see below). Recordings were performed from multiple sites (sites 1–5; see Fig. 1) of each slice (slices 1–10) under control conditions and application of bicuculline (BMI) or both. The recording sites extended from the neocortex (sites 1–4) to the piriform cortex (site 5). Site 4 was generally located around the transition area between insular cortex and neocortex for the frontal slices.
and between perirhinal cortex and neocortex for the posterior slices. The experimental procedure involved using two recording electrodes. One electrode was always located in site 1, the steady site, while the other electrode was moved from sites 2–5 recording for 4–5 min at each site. A necessary condition for a successful experiment was that the activity in site 1 was not significantly different throughout the mapping procedure for each slice (i.e. the frequency of events in site 1 varied less than 10%).

In control buffer slices displayed spontaneous slow oscillations, as previously described (Sanchez-Vives and McCormick, 2000; Castro-Alamancos and Rigas, 2002). For example, in site 1 these events occurred at a frequency of 0.08 ± 0.06 Hz (mean ± S.D.; n=45 slices) and their amplitude was 0.30 ± 0.15 mV. The probability of observing spontaneous slow oscillations in the neocortex varied widely between different slices and cortical sites (see Table 1). On average approximately 51% of the slices displayed slow oscillations in site 1. There were some notable exceptions. Site 1 of slice 10 displayed no activity in control buffer. Also, spontaneous slow oscillations were found to be routinely absent from site 5 (piniform cortex) and also quite rare in site 4 of all slices, while they are usually present in sites 1–3 of all slices. For instance, the probability of observing spontaneous oscillations in control buffer was 5% in site 5 of all slices, while the probability in sites 1–3 of all slices was on average 55%.

Application of a GABA<sub>A</sub> receptor antagonist (BMI; 10 μM) transformed the slow-wave oscillations into large amplitude 1.7 ± 0.4 mV (n=93 slices) interictal spikes that recurred spontaneously, as previously described for frontal slices (Castro-Alamancos and Rigas, 2002) and in vivo (Castro-Alamancos, 2000). During BMI, interictal spikes were observed in all slices and cortical sites irrespective of the presence of spontaneous slow oscillations during control buffer.

Table 1. Frequency (Hz; mean ± S.D.) and probability of occurrence (% of slices that displayed spontaneous activity) of spontaneous activity in the five sites of the 10 slices during control buffer (n = number of experiments).

<table>
<thead>
<tr>
<th>Slice</th>
<th>n</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Site 4</th>
<th>Site 5</th>
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<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>0.04±0.03</td>
<td>0.06±0.03</td>
<td>0.14±0.13</td>
<td>0</td>
<td>0.02±0.00</td>
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<tr>
<td>2</td>
<td>9</td>
<td>0.05±0.02</td>
<td>0.18±0.11</td>
<td>0.17±0.12</td>
<td>0.05±0.03</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>0.08±0.03</td>
<td>0.07±0.06</td>
<td>0.06±0.03</td>
<td>0.04±0.02</td>
<td>0</td>
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<tr>
<td>4</td>
<td>7</td>
<td>0.02±0.02</td>
<td>0.06±0.08</td>
<td>0.03±0.02</td>
<td>0.02±0.01</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>23</td>
<td>0.06±0.03</td>
<td>0.11±0.08</td>
<td>0.08±0.04</td>
<td>0</td>
<td>0.01±0.00</td>
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<tr>
<td>6</td>
<td>20</td>
<td>0.12±0.07</td>
<td>0.18±0.11</td>
<td>0.12±0.09</td>
<td>0.01±0.00</td>
<td>0.02±0.01</td>
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<tr>
<td>7</td>
<td>10</td>
<td>0.10±0.08</td>
<td>0.12±0.14</td>
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<td>8</td>
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<td>9</td>
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<tr>
<td>10</td>
<td>9</td>
<td>0</td>
<td>0.05±0.05</td>
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<td>0.01±0.00</td>
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Fig. 2 illustrates an example of this activity. A comparison of the activity recorded from site one of the different slices revealed that interictal spikes recurred at different frequencies (Fig. 2; see also Table 2). For instance while the frontal slices 7–10 displayed interictal spikes at a frequency of 0.10±0.02 Hz, the posterior slices 1–6 displayed a significantly higher frequency of 0.2±0.07 Hz (t-test; P<0.0001). This difference cannot be attributed to the fact that in some cases the frontal and posterior slices were taken from different experiments. Thus, slices 5–6 were common for two series of experiments that included either frontal or posterior slices and the interictal spike frequency was not significantly different between these two groups of experiments (n=20 experiments per group; P=0.36). Moreover, in other cases (n=8 animals) we recorded from three frontal slices (8–10) and three posterior slices (1–3) within the same experiment and found that the posterior slices had a significantly higher frequency than the frontal slices (0.19±0.05 vs. 0.11±0.2 Hz; P<0.0001). Thus, the posterior slices displayed about double the interictal spike frequency than the frontal slices (approximately 0.2 vs. 0.1 Hz, posterior vs. frontal). In conclusion, during block of GABA<sub>A</sub> receptors posterior slices (slices 1–6) produce a significantly higher frequency spontaneous interictal spike activity than frontal slices (slices 7–10).

**Spatiotemporal properties of interictal spikes**

During application of BMI interictal spikes occurred in all cortical sites and slices examined including sites 4 and 5 that were generally silent during control buffer, and also in site 1 of slice 10, which was also silent (see Table 2). Thus, BMI produced synchronous activity over all five sites recorded, and it seemed that interictal spikes spread from one site to another. This became apparent using cross-correlation analysis, which was computed by using thresh-
revealed that the interictal spikes could originate in different sites depending on the slice. For instance, in some slices activity would consistently start in site 5 and spread up to site 1 (Fig. 3, upper panel); in other cases activity would originate in any of the other sites and spread from there. Fig. 3 shows several examples from different slices where the activity originated in different sites. It became apparent that the slices in which site 5 steadily preceded all the other sites (i.e. more than 80% of the events originating at site 5) had a higher frequency of spontaneous interictal spike activity. This is illustrated in Fig. 4. A linear correlation analysis between the frequency of the spontaneous interictal spike activity and the probability of events originating from site five was positive and significant ($r=0.73$, $P<0.0001$; $n=180$; Fig. 4, upper panel). Fig. 4 (middle and lower panels) also shows that this relationship was only present for the posterior slices (slices 1–6; $r=0.63$; $P<0.0001$) and not for the frontal slices, which had a lower frequency of spontaneous interictal spike activity. Thus, during BMI the higher frequency activity of posterior slices may originate in the piriform cortex (site 5) from where it spreads to the neocortex (sites 1–4).

Effect of interrupting the connection between piriform cortex and neocortex

Since the higher frequency activity observed in the posterior slices seems to originate in the piriform cortex (site 5) we reasoned that interrupting the connection between the piriform cortex and the neocortex would affect the frequency of activity observed in the neocortex. The next experiment was performed to test this hypothesis by comparing the spontaneous activity during BMI in all sites and slices before and after a cut was performed to interrupt the connections between site 5 and the other sites 1–4 (see Fig. 1). The cut extended from the pia through the white matter interrupting all connections between both cortical regions. A three-way ANOVA of the spontaneous interictal spike frequency was performed. The three factors used were: the “slice,” the “site” and the effect of the “cut.” Fig. 5 shows that in intact slices the frequency of spontaneous interictal spike activity for all sites within a slice was fairly regular because the activity would spread between sites. Thus, “site” was not a significant factor in the ANOVA ($P=0.91$). The frontal slices (7–10) had a significantly lower frequency of spontaneous interictal spike activity

<table>
<thead>
<tr>
<th>Slice</th>
<th>$n$</th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Site 4</th>
<th>Site 5</th>
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<tr>
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<td>30</td>
<td>0.17±0.07</td>
<td>0.17±0.06</td>
<td>0.17±0.07</td>
<td>0.17±0.07</td>
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<td>2</td>
<td>26</td>
<td>0.17±0.06</td>
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<td>3</td>
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<td>0.19±0.07</td>
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<td>23</td>
<td>0.18±0.08</td>
<td>0.17±0.07</td>
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<td>0.20±0.13</td>
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<tr>
<td>5</td>
<td>36</td>
<td>0.21±0.05</td>
<td>0.19±0.05</td>
<td>0.21±0.06</td>
<td>0.24±0.08</td>
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<td>6</td>
<td>33</td>
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<td>0.13±0.04</td>
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<td>29</td>
<td>0.11±0.02</td>
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<td>27</td>
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than the posterior slices (2–5), and this was reflected in the significant effect of the factor “slice” \( (P<0.0001) \). Moreover, this was significant for all five sites examined \( (P<0.005) \). It was also apparent that in some of the posterior slices (i.e. slices 4–5) the frequency was higher in the piriform cortex area (sites 4–5), suggesting that these sites might be the origin of the higher frequency activity in those slices; this was reflected in a significant two-way interaction between “slice” and “site” \( (P<0.0001) \). After interrupting the cortical connection between site 5 and the other neocortical sites by performing a cut between sites 4 and 5, all the posterior slices (1–6) displayed a lower

Fig. 3. Examples of cross-correlations from different slices in which the spontaneous interictal spikes originated from different sites. The timing of events at each site are plotted with respect to site 1 (time zero) and each site is color coded. Upper, In this slice the events would consistently (100% of events) start around site 5 (red) and spread from there to site 1 (black at 0) by passing through sites 4, 3 and 2. This example corresponds to a slice 5. Middle, In this slice the events would consistently start around site 1 (100% of events) and spread to site 5. This example corresponds to a slice 9. Lower, In this slice the events would either start around site 5 (35% of events) and spread to site 1 or events would start around site 1 (65% of events) and spread from there to site 5. This example corresponds to a slice 4.
frequency of spontaneous interictal spike activity in sites 1–4, while the higher frequency activity in site 5 was unaffected (Fig. 5). In fact, after the cut sites 1–4 of the posterior slices displayed a frequency of spontaneous interictal spike activity that is quite similar to the activity of frontal slices. Statistically, this was reflected in a significant main effect of the factor “cut” (P < 0.0001) and a significant two-way interaction between “slice” and “cut” (P < 0.0001).

These results indicate that the higher frequency activity displayed by the posterior slices is intrinsically generated in the piriform cortex from where it spreads to the neocortex.

The ANOVA also showed a three-way interaction (slices×sites×cut). The cut differently affected the activity of sites 1–5 in posterior and anterior slices. While the cut significantly decreased the activity of sites 1–4 in posterior slices 1–5 (P < 0.05), it had no effect on the activity of the same sites in slices 7–10. Thus, after the cut the neocortical activity of the posterior slices resembles the low activity of the frontal slices (approximately 0.1 Hz). Moreover, the degree of significance at which the activity of sites 1–4 in the posterior slices was reduced differed; slices 3–5 were more strongly affected (P < 0.001) than slice 2 (P = 0.01) and slice 1 which was only marginally affected (P = 0.04). On the contrary, in the frontal slices (7–10) the activity in sites 1–4 did not change after the cut, while the activity in site 5 was abolished after the cut, indicating that the spontaneous interictal spikes observed in that site were propagated from sites 1–4.

Cross-correlation analysis during BMI revealed that before the cut activity in most of the posterior slices tended to start in site 5 (i.e. piriform cortex) as described above.
Fig. 6. Origin of spontaneous interictal spike activity in intact slices (A) and after a cut interrupting the connections between sites 4 and 5 (B). Plotted is the number of slices, as a percentage of the total (%), in which the majority (>80%) of all the events began at the site indicated in the x axis. If none of the sites met this criterion then the slice would be included in the column X, indicating that none of the sites were the preferred origin. Each site is represented by a distinct column. After the cut (B) site 5 was eliminated from the plot because events could not spread from there to other sites.

(Fig. 6). Slices were classified according to the site of origin by counting the number of events that started at each site. For example, a slice was classified as a site 1 slice if more than 80% of the events started at site 1. If none of the sites met this criterion then the slice was classified as X. Fig. 6 shows that in more than 75% of slices 2–5 spontaneous events during BMI originated in site 5 (Fig. 6A). Thus, the events in the posterior slices tended to originate in site 5. In contrast, the frontal slices tended not to have a particular site of origin (i.e. most were classified as X), or site 2 was generally the site of origin of the events. After the cut severing the connections between sites 4 and 5 activity in the posterior slices (2–6) did not generally have a preferred site of origin and most were classified in group X. These results demonstrate that the higher frequency spontaneous interictal spike activity observed throughout all sites of the posterior slices during BMI originates in the piriform cortex (site 5).

DISCUSSION

The present study found that application of a GABAₐ receptor antagonist produces spontaneous interictal spikes that recur at different frequencies in different slices. Thus,
frontal slices (termed here slices 7–10) produced significantly lower frequency discharges than posterior slices (slices 1–6). Cross-correlation analysis of the propagating discharges within each slice suggested that the higher frequency activity of posterior slices originated from site 5 in those slices, which corresponds to the piriform cortex. A lesion that interrupted the connections between the piriform cortex and the neocortex (i.e. between sites 4 and 5) demonstrated that the higher frequency activity is intrinsically generated in the piriform cortex from where it drives activity in the neocortex. In conclusion, both the neocortex and the piriform cortex have the intrinsic ability to generate interictal spikes during block of GABA<sub>A</sub> receptors, but because of the intrinsic capability of piriform cortex to generate higher frequency discharges it drives the activity in the neocortex, which has a propensity to generate discharges at a lower frequency. Thus, during certain pathological conditions the piriform cortex may be a principal site for the origination of interictal spikes that can drive such activity in the neocortex.

The present study shows that both neocortex and piriform cortex have the intrinsic capability to generate spontaneous interictal spikes during block of GABA<sub>A</sub> receptors. However, the piriform cortex has a propensity to generate such activity at a higher frequency, about double the activity of the fastest neocortical areas. Epileptiform activity in the piriform cortex has been shown to arise from the endopiriform nucleus (Hoffman and Haberly, 1991, 1993, 1996; Piredda and Gale, 1985; Piredda et al., 1985; Stevens et al., 1988). Thus, it is reasonable to assume that in the present study the faster activity of the posterior slices originated from the endopiriform nucleus. The present findings also revealed that there is a difference between different parts of the piriform cortex. For instance, the frontal part of the piriform cortex did not generate faster interictal spikes than the neocortex, and when it was disconnected from the neocortex it did not generate spontaneous interictal spikes at all. It was only the posterior slices that produced a faster interictal spike generation and this activity persisted after disconnection from the neocortex. In fact, the piriform cortex has been divided into anterior and posterior parts based on cytoarchitectonic criteria (Haberly and Price, 1978a,b). The borderline between anterior and posterior piriform cortex is defined by the disappearance of the lateral olfactory tract, a concomitant increase in layer III and the appearance of the ventral endopiriform nucleus. This division roughly matches the separation between anterior and posterior slices in the present study. Interestingly, kindling studies in vivo have suggested that the posterior piriform cortex, and in particular the deep cell layer of the rostral portion of the posterior piriform cortex, plays a critical role in kindling induced seizures (Honack et al., 1991; Wahnschaffe et al., 1993). However, administration of BMI in different parts of the piriform cortex revealed no differences in seizure susceptibility, or a tendency of the anterior piriform cortex to show higher susceptibility in some species (Piredda and Gale, 1985; Piredda et al., 1985; Ebert et al., 2000). The extensive associative fiber system in the piriform cortex may explain the discrepancy between electrically and chemically induced seizures. It is also interesting that the transition area between anterior and posterior piriform cortex is the piriform area with both higher density and total number of GABAergic interneurons (Loscher et al., 1998). These characteristics of the posterior piriform cortex may provide an explanation for the higher susceptibility of this region to generate interictal spikes after BMI.

Another interesting finding in the present study was that while the piriform cortex presented the highest frequency of spontaneous activity during blockade of GABA<sub>A</sub> receptors, it generally did not generate spontaneous activity in control buffer, while the neocortical sites did. We speculate that the endopiriform nucleus and the overlying upper layers (I–III) of the piriform cortex are incapable of generating slow oscillations, which may be an exclusive capability of the neocortex because of the presence of the infragranular layers (layers V and VI).

In conclusion, the present study shows the stronger propensity of the posterior piriform cortex to generate spontaneous interictal spikes during block of GABA<sub>A</sub> receptors, as compared with any neocortical area.

REFERENCES


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