Membrane and synaptic properties of pyramidal neurons in the anterior olfactory nucleus

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Submitted 16 August 2010; accepted in final form 29 November 2010

McGinley MJ, Westbrook GL. Membrane and synaptic properties of pyramidal neurons in the anterior olfactory nucleus. J Neurophysiol 105: 1444–1453, 2011. First published December 1, 2010; doi:10.1152/jn.00715.2010.—The anterior olfactory nucleus (AON) is positioned to coordinate activity between the piriform cortex and olfactory bulbs, yet the physiology of AON principal neurons has been little explored. Here, we examined the membrane properties and excitatory synapses of AON principal neurons in brain slices of PND22–28 mice and compared their properties to principal cells in other olfactory cortical areas. AON principal neurons had firing rates, spike rate adaptation, spike widths, and I-V relationships that were generally similar to pyramidal neurons in piriform cortex, and typical of cerebral cortex, consistent with a role for AON in cortical processing. Principal neurons in AON had more hyperpolarized action potential thresholds, smaller afterhyperpolarizations, and tended to fire doublets of action potentials on depolarization compared with ventral anterior piriform cortex and the adjacent epileptogenic region preendopiriform nucleus (pEN). Thus, AON pyramidal neurons have enhanced membrane excitability compared with surrounding subregions. Interestingly, principal neurons in pEN were the least excitable, as measured by a larger input conductance, lower firing rates, and more inward rectification. Afferent and recurrent excitatory synapses onto AON pyramidal neurons had small amplitudes, paired pulse facilitation at afferent synapses, and GABA modulation at recurrent synapses, a pattern similar to piriform cortex. The enhanced membrane excitability and recurrent synaptic excitation within the AON, together with its widespread outputs, suggest that the AON can boost and distribute activity in feedforward and feedback circuits throughout the olfactory system.

piriform cortex; preendopiriform nucleus; area tempestas; afterhyperpolarization; inward rectifying potassium channels; cable properties; pyramidal neuron

WITH ITS FEEDBACK CONNECTIONS to the ipsilateral and contralateral olfactory bulbs, and feedforward connections to the piriform cortex, the anterior olfactory nucleus (AON) is poised to coordinate the flow of activity between olfactory areas (Alheid et al. 1984; Reyher et al. 1988; Haberly and Price 1978; Yan et al. 2008). The entire AON receives input from the ipsilateral olfactory bulb, but it is divided into subregions by the topography of output projections and cytoarchitecture (Haberly and Price 1978; Brunjes et al. 2005; Meyer et al. 2006). Three subregions (pars lateralis, dorsalis, and ventroposterioralis) have heavy reciprocal feedforward and feedback connections. Collectively termed pars principalis, or anterior olfactory cortex (AOC) (Davis and Macrides 1981; Luskin and Price 1983a), these three subregions have primitive layering, and their principal neurons have pyramidal shapes, not unlike cortical structures elsewhere (Haberly and Price 1978; Haberly 2001; Meyer et al. 2006; Herrick 1924; Brunjes et al. 2005; Brunjes et al. 2009).

Principal neurons in pars principalis of AON are particularly well positioned to influence activity in piriform cortex for several reasons. Tufted cells in the olfactory bulb project selectively to the AON as well as the neighboring ventrostral anterior piriform cortex (APCvR) (Matsutani et al. 1989). Tufted cells show enhanced excitation relative to mitral cells, which project to the entire olfactory cortex (Schneider and Scott 1983; Orona et al. 1984; Scott et al. 1985; Christie et al. 2001; Nagayama et al. 2004). AON projections to the APC terminate directly adjacent to the cell bodies of pyramidal neurons (Haberly and Price 1978; Luskin and Price 1983a). Furthermore, APCvR is densely and reciprocally connected with the underlying preendopiriform nucleus (pEN), which has been implicated in hyperexcitability and seizure generation (Piredda and Gale 1985; Ekstrand et al. 2001). These organizational features suggest that principal cells in AON, APCvR, and pEN serve distinct, important roles in olfactory processing, yet very little is known about the physiological properties of these cells and circuits. Here we examined the membrane and synaptic properties of principal cells in the pars principalis of AON using whole cell recording in brain slices from juvenile mice. We also recorded from pyramidal neurons in the rostral and caudal subdivisions of ventral anterior piriform cortex (APCvR and APCvC) and principal neurons in pEN and compared their properties.

METHODS

Slice preparation. Brain slices were prepared from C57BL/6J mice (300 μm, Leica VT 1200S) at PND22–28, which is after the development of basic properties of excitation in piriform cortex (Schwob et al. 1984; Franks and Isaacson 2005). Isoflurane-anesthetized mice were euthanized by decapitation. The brain was cut coronally at the superior colliculus and sagitally at the midline. The right hemisphere was mounted on an acrylic block specially machined so that slices contained the AON, APCvR, and pEN, and layering within these regions was easily discerned. Dissection and slicing were conducted in ice-cold carbogenated saline (in mM): 83 NaCl, 26.2 NaHCO₃, 2.5 KCl, 1 NaH₂PO₄, 3.3 MgSO₄, 0.5 CaCl₂, 22 glucose, 72 sucrose. Slices were incubated in the dissection solution (33°C for 35–45 min) and subsequently placed at room temperature (also in dissection solution, for 30–150 min) until recording. All animal handling and experimental procedures were approved by the Institutional Animal Care and Use Committee at Oregon Health and Science University in accordance with National Institutes of Health guidelines for ethical treatment of animals.
Targeting principal neurons. Recordings of AON neurons were restricted to the broad cell body layer of the lateral part of the AON, deep to the axodendritic layer that borders the lateral olfactory tract (LOT) and posterior from pars externa of AON. This region, known as pars principalis, has three anatomical subdivisions. We largely recorded from pars lateralis with possibly a few recordings in pars ventroposterioralis or pars dorsalis. For simplicity, we refer to the recorded area as AON. Principal neurons in AON were targeted based on morphology, having a teardrop-shaped cell body, and one or two tapering apical dendrites oriented toward the pial surface. Periform pyramidal neurons were targeted in the deep half of layer II (layer IIb) to avoid semilaminar neurons (Suzuki and Bekkers 2006). Principal neurons in pEN were targeted based on multipolar morphology (Tseng and Haberly 1989). A few neurons that had membrane physiology consistent with fast-spiking GABAergic interneurons (McGinley, unpublished observations) were excluded from analysis.

Electrophysiological recording. Voltage and current clamp data were low-pass filtered online at 10 kHz and acquired at 25 kHz using a Multiclamp 700B amplifier (Molecular Devices). Recordings were further low-pass filtered offline (2 kHz) except for calculations of the series resistance and curve fitting to charging transients. Neurons with high series resistance (>20 MΩ) or unstable recordings were excluded from further analysis. Series resistance in the bath was 11.8 ± 0.4 MΩ (n = 230). In most experiments, a potassium-based pipette solution was used. The pipette solution contained (in mM): 135 K-glucuronate, 5 NaCl, 10 HEPES, 12 phosphocreatine, 3 MgATP, 0.3 NaGTP, 0.1 EGTA, 0.025 CaCl2 (pH, 7.3; 285 mOsM). The bath solution (33°C) contained (in mM): 119 NaCl, 26.2 NaHCO3, 2.5 KCl, 1 NaH2PO4, 1.0 MgSO4, 2.0 CaCl2, and 22 glucose (300 mOsM). In some experiments, as indicated, R-CPP (5 μM), picrotoxin (100 μM), or CGP55845 (10 μM) were included in the bath to block NMDA, GABA_A, or GABA_B receptors, respectively. The calculated potassium reversal potential was ~105 mV. Voltage measurements were not corrected for the calculated liquid junction potential (16.3 mV).

Series resistance was corrected in current clamp recordings. For whole cell voltage clamp of synaptic responses, the compensation was not applied. The holding potential for voltage clamp measurements was ~75 mV.

For analysis of membrane properties, neurons were recorded across regions in the same slices, often in pairs, to avoid variability or artificial differences resulting from slice condition. We applied a family of 21 evenly spaced current steps to each neuron (starting from rest, 800-ms duration, 5-s pause between steps, 2–3 repetitions per cell) with amplitudes ranging from −2.5 to +2.5 times the rheobase in each neuron (15- to 30-pA increments). Rheobase was defined as the amplitude of the smallest depolarizing current step that elicited one or more action potentials on most trials. Single action potentials at rheobase were scored as 1.25 Hz in step that elicited one or more action potentials on most trials. Action potentials in principal neurons across regions, measured at threshold (Fig. 2, A and B, gray traces), had similar amplitudes (peak - threshold; F = 3.7; P = 0.29; AON, 86.9 ± 2.0 mV; APCV-R, 81.6 ± 1.4 mV; APCV-C, 82.9 ± 1.4 mV; pEN, 82.3 ± 1.4 mV) and half widths (F = 4.3; P = 0.23; AON, 1.31 ± 0.07 ms; APCV-R, 1.21 ± 0.07 ms; APCV-C, 1.29 ± 0.06 ms; pEN, 1.35 ± 0.04 ms). These values are typical of pyramidal neurons in cerebral cortex (Larkman and Mason 1990; Krahe and Gabbiani 2004). The current necessary to trigger an action potential (rheobase) was similar in AON and periformal pyramidal neurons, but larger in pEN (Fig. 2C; F = 7.9; P < 0.05), consistent with the larger slope conductance in

Passive cable analysis. For cable analysis of dendritic structure, we used the ball-and-stick model of Rall (1969) with procedures outlined by Jackson (1992). An average of 20–50 charging transients, from depolarizing and hyperpolarizing voltage pulses, were fit with sums of three exponentials (see Supplementary Methods; Supplemental Material for this article is available online at the Journal website.).

Statistics. Data are presented as means ± SE. Statistical comparisons were made with the two-tailed Student’s t-test or ANOVA (F statistic and P value reported) followed by Tukey’s post hoc (P value reported) for multiple comparisons, or Friedman ANOVA was used, and the χ2 value is reported. Statistics were calculated using OriginPro 8 (OriginLab, Northampton, MA).

RESULTS

Active properties of principal neurons in olfactory cortical subregions. Despite its large size and extensive interconnectivity across the mammalian olfactory system, the physiological properties of AON neurons have not been reported. Thus, we performed whole cell recordings from 122 neurons in AON to assess their membrane properties and compared them with pyramidal neurons in layer IIb in rostral and caudal ventral anterior periform cortex (APCV_R and APCV_C, respectively, n = 52) and principal neurons in the pEN (n = 21; Fig. 1A). An example family of voltage responses to current steps, for a typical neuron from each region, is shown in Fig. 1B. Principal cells in all regions responded to depolarizing currents with a pattern of accommodating action potentials. Hyperpolarizing currents produced modest voltage sags, suggesting activation of a small I_h component.

To quantitatively compare membrane properties, we randomly chose nine neurons from AON, six neurons from layer IIb of APCV_R, seven neurons from layer IIb of APCV_C, and nine neurons in pEN for more detailed analysis. I-V relationships are shown in Fig. 1C. I-V curves had similar slopes near rest, as reflected in the slope conductance at −85 mV (Fig. 1D), although there was a small but significant difference between pEN and APCV_C (F = 4.7; P < 0.01). I-V curves differed more substantially at −105 mV (F = 7.1, P < 0.001), where pEN neurons showed more inward rectification (Fig. 1D). The larger conductance in pEN neurons at hyperpolarized voltages was reflected in the twofold larger “difference” conductance than all other regions (subtraction of slope conductance).

Because the voltage sag was small in all regions (Fig. 1B), the inward rectification in pEN probably did not result from I_h, because the voltage sag was small in all regions (Fig. 1E; see Fig. 2B). Furthermore, the time constant of the sag was not different between regions (F = 0.56, P = 0.91; AON, 132 ± 6 ms; APCV_R, 137 ± 19 ms; APCV_C, 141 ± 26 ms; pEN, 125 ± 27 ms).

Action potentials in principal neurons across regions, measured at threshold (Fig. 2, A and B, gray traces), had similar amplitudes (peak - threshold; F = 3.7; P = 0.29; AON, 86.9 ± 2.0 mV; APCV_R, 81.6 ± 1.4 mV; APCV_C, 82.9 ± 1.4 mV; pEN, 82.3 ± 1.4 mV) and half widths (F = 4.3; P = 0.23; AON, 1.31 ± 0.07 ms; APCV_R, 1.21 ± 0.07 ms; APCV_C, 1.29 ± 0.06 ms; pEN, 1.35 ± 0.04 ms). These values are typical of pyramidal neurons in cerebral cortex (Larkman and Mason 1990; Krahe and Gabbiani 2004). The current necessary to trigger an action potential (rheobase) was similar in AON and periformal pyramidal neurons, but larger in pEN (Fig. 2C; F = 7.9; P < 0.05), consistent with the larger slope conductance in
pEN. AOC pyramidal neurons had a lower threshold for action potential initiation (F = 9.2; P < 0.05; Fig. 2D) and a smaller afterhyperpolarization (F = 11.2; P < 0.01; Fig. 2E) than pyramidal neurons in piriform cortex, which would be expected to enhance firing.

To analyze intrinsic firing patterns, we calculated f-I curves for principal neurons across regions. F-I curves were shallower in pEN (Fig. 3A), mirroring the differences in rheobase shown in Fig. 2. Furthermore, average firing rates at threshold, and at 2.5× rheobase, were lower in pEN than in all other regions (F = 5.7; P < 0.005; F = 10.3; P < 0.02; Fig. 3, C and D). The percent of neurons that fired bursts (2 or more APs) at threshold was also lowest in pEN (0%) and highest in AON (78%) compared with piriform cortex (APCvR, 50%; APCvC, 38%). Firing rates in all regions adapted to long current pulses, as shown for AON (Fig. 3B). However, there was a rostral-to-caudal decrease in adaptation across AON and APC (Fig. 3E; F = 8.4, P < 0.05), whereas pEN neurons had comparable adaptation to AON (Fig. 3E). These results suggest enhanced excitability in the rostral corner of piriform cortex.

Passive properties of principal neurons. To understand how differences in passive properties contribute to the shaping of activity, we analyzed passive charging transients in re-
responses to voltage steps from rest, in neurons from each region (Fig. 4A, inset). Three time constants were necessary to accurately fit charging transients (Fig. 4A), suggesting substantial dendritic charging (Rall 1969). Differences between regions in time constants and their associated amplitudes suggested a more extended electrotonic structure in AON (Supplementary Fig. 1). Therefore, we applied a uniform cable and lumped soma model and extracted passive parameters (Rall...
The dendritic length (in units of length constants) was longest in AON and decreased in the rostro-caudal direction (Fig. 4B; F = 6.0, P < 0.005). The membrane time constant (τ_{mem}) also showed a rostro-caudal gradient (F = 6.1, P < 0.002; AON, 4.8 ± 0.5 ms; APCV-R, 5.3 ± 0.7 ms; APCV-C, 8.9 ± 1.4 ms; pEN, 8.8 ± 0.9 ms), such that the membrane time constant was slower in APCV-C than in AON (P < 0.02). pEN neurons had a membrane time constant comparable to APCV-C neurons (P = 0.99998) and slower than AON neurons (P < 0.01). The somatic-to-dendritic resistance ratio (ρ) was larger in AON and intermediate in pEN (Fig. 4C; F = 17.2, P < 2E-7). These results suggest a greater filtering of dendritic inputs in AON pyramidal neurons.

Weak single fiber LOT inputs to AON and APCV. Afferent and associational synapses in piriform cortex show distinct features thought to be important for cortical function, in terms of plasticity, modulation, and synaptic strength (Bower and Haberly 1986; Hasselmo and Bower 1990, 1992; Tang and Hasselmo 1994; Linster and Hasselmo 2001; McNama et al. 2004; Franks and Isaacson 2006). To examine the synaptic properties of AON pyramidal neurons, we measured the strength of single afferent inputs from the LOT using minimal stimulation (Raastad et al. 1992). Single shocks (0.1 Hz) of low amplitude (3–10 V) evoked failures on some trials and successes on other trials. In 14 of 18 neurons, we isolated a single shock strength at which similar amplitude successes occurred on some trials, and failures occurred on other trials, indicating that the successes resulted most likely from a single fiber input (Fig. 5A). The amplitude of the single fiber input with the potassium-based pipette solution was 16.1 ± 2.4 pA (Fig. 5B). For the remaining four neurons, a small increase in shock strength (≤1 V) resulted in an abrupt transition from all failures to all successes, thus providing a least-upper-bound (LUB) estimate of the single fiber input strength. The LUB estimate of 28.4 ± 3.5 pA was larger than the well-isolated single fiber input estimates (P < 0.02), suggesting that LUB estimates represented the recruitment of multiple fibers. To address the possibility that our single fiber measurements were underestimates because of reduced voltage clamp control with a potassium-based pipette solution, we used a cesium-based internal solution in six additional neurons. In five of six neurons, threshold stimulation indicated a single fiber amplitude of 28.5 ± 7.0 pA. The remaining neuron had a LUB estimate of 57.3 pA. These values were larger than with a potassium-based solution (P < 0.02) but not as large as the single fiber input strengths previously reported in piriform cortex (Franks and Isaacson 2006).

To compare LOT input strengths between AON and piriform pyramidal neurons, we recorded pyramidal neurons in APCV in response to minimal stimulation of the LOT. Successful threshold stimulation was achieved in four of five piriform pyramidal neurons. The single fiber input strength was 17.4 ± 4.8 pA, not different from the value obtained in AON (P = 0.82; Fig. 5B). The remaining piriform pyramidal neuron had a LUB estimate of 40.9 pA. Similarly, the single fiber amplitude, with a cesium-based solution, was 33.4 ± 8.7 pA (n = 4), larger than with intracellular potassium (P < 0.05), and not different from cesium-loaded AON neurons (P = 0.6). To further evaluate the strength of single fiber inputs, we performed graded stimulation in five AON principal neurons and six piriform pyramidal neurons. The EPSC amplitude increased gradually with shock strength (Fig. 5, C and D), consistent with the results of minimal stimulation. Our results indicate that LOT inputs to AON and APCV are weak under the conditions of our experiments, similar to afferent synapses in neocortex (Bruno and Sakmann 2006) and the analogous synapses in locusts (Jortner et al. 2007).

In addition to LOT inputs, we examined local excitatory interactions between AON neurons. Extensive recurrent
excitatory connectivity is a hallmark of cortical regions including the piriform cortex (Tsodyks et al. 2000; Holmgren et al. 2003; Haberly and Price 1978; Luskin and Price 1983b; Johnson et al. 2000). In paired recordings of AON principal neurons using potassium electrodes, 3 of 18 pairs exhibited an excitatory monosynaptic connection in one direction (see Fig. 6; 3 of 36 possible connections, or 8.3%), which is similar to layer 2/3 of neocortex (Holmgren et al. 2003). These recurrent connections had small EPSC amplitudes (26 ± 12 pA; see Fig. 6A), also similar to neocortex (Tsodyks and Markram 1997; Holmgren et al. 2003).

Short-term plasticity and pathway-specific GABA<sub>B</sub> modulation in AON. LOT synaptic inputs to piriform pyramidal neurons show paired pulse facilitation (Bower and Haberly 1986; Hasselmo and Bower 1992). Furthermore, and unlike recurrent excitatory connections, LOT inputs to piriform pyramidal neurons lack presynaptic GABA<sub>B</sub> receptors (Tang and Hasselmo 1994). We looked for these patterns in AON. For LOT inputs to AON principal neurons, paired pulse stimulation (interstimulus interval, ISI, 50–1,000 ms) evoked paired pulse facilitation for short ISIs (Fig. 7A) that was unaffected by GABA<sub>B</sub> receptors (P = 0.31; Fig. 7B). With a potassium-based internal solution, the EPSC amplitude was reduced by a small amount in baclofen (Fig. 7C), suggesting postsynaptic shunting or filtering due to GABA<sub>B</sub> receptors. Consistent with this interpretation, baclofen shifted the holding current with a potassium-based (Fig. 7D) but not cesium-based (Fig. 7D) solution and reduced the input resistance with a potassium-based (n = 3; χ² = 6; P < 0.05; control, 69.7 ± 14.6 MΩ; baclofen, 48.1 ± 6.0 MΩ; CGP55845, 78.4 ± 9.7 MΩ) but not with a cesium-based pipette solution (n = 3; χ² = 4.7; P = 0.10; control, 232 ± 53 MΩ; baclofen, 195 ± 42 MΩ; CGP55845, 261 ± 46 MΩ). The small effect of GABA<sub>B</sub> receptors in cesium may reflect a small drift in EPSC amplitude as well as the presence of postsynaptic cesium-permeable GIRK channels (Hommers et al. 2003).

Another characteristic of the piriform cortex is the selective suppression of recurrent excitatory synapses by GABA<sub>B</sub> receptors (Tang and Hasselmo 1994). We observed a similar pattern in AON. Baclofen markedly reduced polysynaptic activity evoked by LOT stimulation in the presence of GABA<sub>A</sub> and NMDA receptor antagonists, which were used to unmask recurrent fast excitation (Supplementary Fig. 2A; unpublished observations). The polysynaptic bursts of activity were restored in CGP55845 (Supplementary Fig. 2A). Paired pulse stimulation of the LOT (ISI, 0.2–5 s) resulted in paired pulse depression of bursts for short ISIs (Supple-
endogenous GABA_B receptors suppress associational excitation in AON.

Intrinsic membrane and synaptic properties influence the circuit dynamics of cortical networks. Our results indicate that AON pyramidal neurons share many of the properties of principal neurons in the piriform cortex as well as other areas of cerebral cortex. However, there were regional differences that may provide insight into region-specific functions in the olfactory system. In particular, AON neurons showed enhanced excitability, whereas the epileptogenic pEN showed diminished excitability compared with piriform cortex. Lateral olfactory tract inputs from olfactory bulb to AON and ventral anterior piriform cortex were weak, suggesting that afferent activation of these regions requires highly synchronous sensory input.

Fig. 6. Recurrent excitatory connections between AON principal neurons. A: a short current pulse (middle, 1 ms, 2 nA) triggers a single action potential (top) in a presynaptic AON principal neuron, triggering a short-latency fast inward current in a postsynaptic AON neuron (bottom). B: responses of the AON neurons from A (presynaptic, top; postsynaptic, bottom) to long current steps at rheobase (black traces) and −2.5 or +2.5 rheobase (gray traces). Short horizontal black lines in A and B indicate a membrane potential of −80 mV.

Fig. 7. Afferent synapses in AON are facilitating and unmodulated by GABA_B receptors. A: the paired pulse ratio of voltage clamp responses to LOT stimulation is plotted as a function in the interstimulus interval (ISI). Black circles and gray lines show individual neurons; open triangles and black lines show the average response. LOT synapses onto AON principal neurons were facilitating for ISIs ranging from 50–200 ms. Inset shows an example response to paired pulse stimulation. B: bath application of baclofen, followed by CGP55845, did not change the paired pulse ratio of LOT stimulation. C: bath application of baclofen reversibly reduced the EPSC amplitude by almost 50% with a potassium-based pipette solution (left), whereas it irreversibly reduced the EPSC amplitude by <25% with a cesium-based electrode (right). D: bath application of baclofen caused a reversible shift in the holding current with a potassium-based pipette solution (left) that was abolished with a cesium-based solution (right), suggesting activation of GABA_B receptors in the recorded (postsynaptic) neuron. Grayscale code for pharmacological condition is shown in C and D. *P < 0.05.
piriform pyramidal neurons do not express Ih
inputs (Williams and Stuart 2000). Although it has been
thought that Ih is thought to normalize or scale synaptic
activity, a small Ih, as well as the results of our passive cable
modeling, suggests that detailed study of dendrite biophysics is
warranted in each of these regions.

The olfactory cortex is shaped like a long ribbon, ex-
tended in the rostro-caudal direction, with sensory input
arriving at the rostral end. As a result, there has long been
interest in rostral-caudal gradients in physiological proper-
ties in olfactory cortex. For example, there is a rostral-to-
caudal gradient in the number of afferent vs. associational
fibers in layer I (Luskin and Price 1983b), as well as a
rostral-to-caudal decrease in the contribution of tufted cell
fibers to the LOT afferent pathway (Matsutani et al. 1989).
Tufted cells are more strongly excited by odors (Sch-
neider and Scott 1983; Christie et al. 2001; Nagayama et al.
2004), so the gradient in tufted cell innervation should result in
relatively less activity in caudal regions. Our results also
indicate a rostral-caudal gradient, extending across AON
and ventral anterior piriform cortex, in intrinsic membrane
properties of pyramidal neurons. These patterns would be
expected to create a gradient in temporal integration and
input-output transformation, which may help spread and
synchronize activity in response to sensory stimuli.

The strength of afferent inputs. Our results indicate single
fiber inputs to pyramidal neurons in piriform cortex and
AON are weak. However, it has previously been reported
that these inputs to piriform pyramidal neurons are “strong”
(Franks and Isaacson 2006). As our experiments were per-
formed in physiological levels of calcium and magnesium,
this difference could result from the fact that small inputs
are harder to resolve in high divalents (see Supplementary
Fig. 3), the conditions used in the prior experiments in
piriform cortex. Ultrastructural evidence suggests LOT syn-
apses are comparable to CA1, although somewhat larger on
average (Schikorski and Stevens 1999). Integration of many
weak inputs is consistent with the broad and complex
receptive fields of piriform pyramidal neurons (Wilson
2001; Stettler and Axel 2009) and sparse activity in the
piriform cortex (Schoenbaum and Eichenbaum 1995; Stet-
tler and Axel 2009). Weak afferent inputs are the norm in
neocortex (Bruno and Sakmann 2006) and at the analogous
olfactory synapse in the locust (Jortner et al. 2007). As our
results were restricted to AON and APC, single fiber inputs
to posterior piriform cortex and/or APC may be stronger,
possibly to compensate for a reduction in the number of
LOT inputs. Furthermore, a distinct class of principal neu-
ron in piriform cortex, semilunar neurons, receive stronger
LOT input than piriform neurons (Suzuki and Bekkers
2006), perhaps because of stronger single fiber inputs. As
expected, single fiber inputs were larger with a cesium-
compared with a potassium-based electrode solution, reflecting
an improved voltage clamp of dendritic inputs (but see
Williams and Mitchell 2008).

The role of the AON in olfaction. The AON is integrated into
the excitatory circuit of piriform cortex (Luskin and
Price 1983a; unpublished observations) and shares with
piriform cortex and neocortex in exhibiting weak synaptic
strengths as well as pathway-specific, short-term plasticity
and modulation by GABAB receptors (Bruno and Sakmann
2006; Tsodyks and Markram 1997; Bower and Haberly
1986; Hasselmo and Bower 1990, 1992; Tang and Hasselmo
1994; Linster and Hasselmo 2001; McNamara et al. 2004).
The membrane properties of pyramidal neurons in AON were
typical of cerebral cortex (Larkman and Mason 1990; Krahe
and Gabbiani 2004). However, common properties of AON
and piriform cortex are not shared by all targets of the olfactory
bulb (Chiang and Strowbridge 2007; Suzuki and Bekkers
2006), and the role of inhibitory neuronal populations in these
regions remains largely unexplored.

The precise role of olfactory areas in processing information
is still a topic of debate (Kay and Sherman 2007;
Fontanini and Bower 2006; Cleland 2010). The AON was
originally considered a nuclear structure because it has only
two distinct layers (Herrick 1924). However, pars principalis
contains a large population of neurons with pyramidal
morphology (Brunjes et al. 2005; Brunjes et al. 2009), and
recent evidence is more consistent with a contribution of
AON to a range of cortical functions (Haberly 2001; Brunjes
et al. 2005). Individual neurons in AON respond to multiple
distinct odor mixtures as well as several chemically unre-
lated components of individual mixtures (Lei et al. 2006).
Commissural pathways connecting the AON allow con-
trolateral recall of memories (Haberly and Price 1978;
Kucharski and Hall 1987). AON neurons can change their
preferred side following unilateral nares occlusion (Kikuta
et al. 2008), and neurons in pars externa receive excitation
from one bulb and inhibition from the other (Kikuta et al.
2010). These functions, together with the current results, are inconsistent with a view of the AON as a simple relay, but rather as a module of olfactory cortex.

ACKNOWLEDGMENTS

We thank Lew Haberly and Sherry Feig for help in developing the slice preparation and getting started in olfactory cortex.

GRANTS

This work was supported by National Institute of Neurological Disorders and Stroke Grant F31-NS-058196 (M. J. McGlinery), a Max Planck Research Award (G. L. Westbrook), and Grant NS-26494 (G. L. Westbrook).

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