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

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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<sub>Β</sub> 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, suggests that the AON can boost and distribute activity in feedforward and feedback circuits throughout the olfactory system.

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 sagittally at the midline. The right hemisphere was mounted on an acrylic block specially machined so that slices contained the AON, APCV, 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 NaHCO<sub>3</sub>, 2.5 KCl, 1 Na<sub>2</sub>HPO<sub>4</sub>, 3.3 MgSO<sub>4</sub>, 0.5 CaCl<sub>2</sub>, 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.

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WITH ITS FEEDBACK CONNECTIONS to the ipsilateral and contralateral olfactory bulbs, and feedback 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 ventro-rostral anterior piriform cortex (APC<sub>V,R</sub>) (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, APC<sub>V,R</sub> 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, APC<sub>V,R</sub>, 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 (APC<sub>V,R</sub> and APC<sub>V,C</sub>) and principal neurons in pEN and compared their properties.
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 ventroposterioris 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. Periformal pyramidal neurons were targeted in the deep half of layer II (layer IIb) to avoid semilunar 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 GABAAergic 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 current fits 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-glutamate, 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, GABAₐ, or GABAₐ 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 circuit was not employed. 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 calculations of firing rates, due to the 800-ms step duration. Action potential threshold was defined visually, as the point with maximum curvature. Slope conductance was calculated as the slope of a linear fit to the current-voltage (I-V) relation at threshold (Fig. 2E). Slope conductance was calculated as the slope of a linear fit to the current-voltage (I-V) relation at threshold (Fig. 2E). The larger conductance in pEN neurons at hyperpolarized potentials (2.5 to 8.5 mV; F = 7.1, P < 0.001), where pEN neurons showed more inward rectification (Fig. 2D). 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 at −105 mV; 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 conductances at −85 and −105 mV; F = 5.8; P < 0.005; Fig. 1D). The inward rectification in pEN probably did not result from I₅, 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 AON neurons (McGinley, unpublished observations) were excluded from analysis.
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 (APC$_{V \cdot C}$, 50%; APC$_{V \cdot C \cdot R}$, 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

Fig. 2. Action potentials in principal neurons across regions have similar shapes, except for AP thresholds and afterhyperpolarization (AHP) in AON. A: voltage response (top) for 3 current steps (bottom) in a typical neuron from the AON. Red line indicates a transmembrane voltage of −80 mV. Gray traces are at rheobase in this neuron (160 pA). B: left, an example of the action potential waveform at rheobase (enlarged from A) illustrating the voltage threshold (c) and action potential half-width (bar). At top right is a further enlargement of the threshold region (same action potential) illustrating the AHP (bar). At bottom right, hyperpolarized step (enlarged from A) showing the calculation of sag (b/a). A single exponential was fit to the sag time course (red dotted curve and tau). C: the amount of current necessary to trigger an action potential (rheobase) was similar for principal neurons across subregions (left 3 bars). pEN neurons had a higher rheobase (right bar). Recording region indicated below the x-axis. D: neurons in AON had consistently hyperpolarized action potential thresholds compared with pyramidal neurons in piriform cortex. E: the AHP measured relative to AP threshold. Pyramidal neurons in AON had consistently smaller AHPs compared with piriform cortex. *P < 0.05; **P < 0.01; ***P < 0.001.

Fig. 3. Regional differences in firing rates and the extent of firing rate adaptation in principal neurons. A: the average firing rate vs. current amplitude (f-I curve) reveals lower firing rates in pEN neurons. B: examples (gray symbols) and average (black) of instantaneous firing rates (1/Δt) for 14 AON pyramidal neurons during current application at 2.5× rheobase. Inset shows an example AON firing pattern (top) in response to a current step at 2.5× rheobase (400 pA, bottom). Red bar indicates the interspike interval (Δt) for the 7th AP interval. Scale bars apply to inset. C: the average firing rate (at rheobase) is smallest in pEN, reflecting a tendency to fire only 1 action potential at threshold. D: the average firing rate at 2.5× rheobase (first 10 action potentials) was lower in pEN neurons. E: principal neurons in all subregions showed firing rate adaptation. Neurons in AON and pEN showed more firing rate adaptation than neurons in APCv-C. Neurons in APCv-R showed intermediate firing rate adaptation. *P < 0.05; **P < 0.02; ***P < 0.01; ****P < 0.005.
1969; Jackson 1992). 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; McNamara 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 GABAB 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 GABAB 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_B 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_B 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; 7.0 ± 0.4 MΩ; baclofen, 4.7 ± 0.3 MΩ; CGP55845, 78.4 ± 9.7 MΩ) but not with a cesium-based pipette solution (n = 3; 7.5 ± 0.5 MΩ; baclofen, 195 ± 42 MΩ; CGP55845, 261 ± 46 MΩ). The small effect of GABA_B 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_B 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_A 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-
DISCUSSION

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.

Regional differences in excitability. Only a few physiological studies have included the AON (Boulet et al. 1978; Kucharski and Hall 1987; McNamara et al. 2004; Lei et al. 2006; Kikuta et al. 2008, 2010) and none have explored their physiology with whole cell recording. AON pyramidal neurons had enhanced membrane excitability at threshold, and at 2.5× rheobase, compared with piriform pyramidal neurons. Pyramidal neurons in neocortex and hippocampus show a range of excitable properties, consistent with the differences we observed between AON and subregions of piriform cortex (Larkman and Mason 1990; Krahe and Gabbiani 2004). Burst firing in AON was not as pronounced as for thalamic relay neurons (Jahnsen and Llinas 1984). Afterhyperpolarization (AHP) amplitudes in piriform pyra-
midal neurons are affected by learning (Cohen-Matsliah et al. 2010), thus the smaller AHP in AON may reflect different intrinsic plasticity (Liraz et al. 2009; Cohen-Matsliah et al. 2010). The membrane properties were surprisingly uniform for pars principalis pyramidal neurons, which project to piriform. Other subregions (pars externa and pars medi-
alis) that project exclusively to the olfactory bulb were not explored, and thus could exhibit different membrane properties. It is also possible that there are differences between subregions within pars principalis (Meyer et al. 2006), as our recordings were focused primarily on pars lateralis. It should also be noted that our experiments were performed on juvenile animals (P22–28) because of the limits of whole cell recording, thus it is possible that there will be differences in older animals.

Perhaps surprisingly, pEN neurons were less excitable than the other regions we explored. The pEN, also called the “area tempestas,” is a known epileptogenic locus within the olfactory system (Piredda and Gale 1985; Ekstrand et al. 2001). pEN principal neurons had a pronounced inward rectification, consistent with a higher expression of IRK and/or GIRK channels. In olfactory cortex, neurons, rather than glia, may buffer extracellular potassium, during local increases in extracellular potassium such as seizures (Howe et al. 2008). Therefore, a high potassium buffering capacity of pEN neurons could be important in controlling excitation. Pyramidal neurons in the neocortex and hippocampus have a large \( I_h \), particularly in dendrites where \( I_h \) is thought to normalize or scale synaptic inputs (Williams and Stuart 2000). Although it has been reported that piriform pyramidal neurons do not express \( I_h \) (e.g., Howe et al. 2008), we observed small, but consistent sags in AON, piriform, and pEN neurons, most likely resulting from \( I_h \). A small \( I_h \), as well as the results of our passive cable analysis, is consistent with a more passive and compact dendritic tree in piriform pyramidal neurons compared with neocortex (Bathellier et al. 2009; Spruston 2008). Our differences in estimates of basic cable properties between AON, pEN, and APC suggest that detailed study of dendrite biophysics is warranted in each of these regions.

The olfactory cortex is shaped like a long ribbon, extended 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 properties in olfactory cortex. For example, there is a rostral-to-caudal gradient in the number of afferent versus associational fibers in layer I (Luskin and Price 1983a), 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 (Schneider 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 performed 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 synapses 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; Stettler 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 neuron in piriform cortex, semilunar neurons, receive stronger LOT input than pyramidal 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 GABA \(_B\) 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 unrelated components of individual mixtures (Lei et al. 2006). Commisural pathways connecting the AON allow contralateral 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.
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DISCLOSURES

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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.


