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Calcium-Permeable AMPA Receptors Mediate Glutamatergic Signaling in Neural Precursor Cells of the Postnatal Olfactory Bulb

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Darcy DP, Isaacson JS. Calcium-permeable AMPA receptors mediate glutamatergic signaling in neural precursor cells of the postnatal olfactory bulb. *J Neurophysiol* 103: 1431–1437, 2010. First published January 20, 2010; doi:10.1152/jn.00821.2009. Neural precursor cells (NPCs) in the mammalian olfactory bulb give rise to local inhibitory neurons that integrate into existing circuitry throughout adult life. However, the functional properties of neurotransmitter receptors expressed by NPCs are not well understood. In this study, we use patch-clamp recording and calcium imaging to explore the properties of glutamate receptors expressed by NPCs in the olfactory bulb subependymal layer. We find that calcium-permeable AMPA receptors (AMPARs) are the major receptor type underlying glutamatergic signaling in olfactory bulb NPCs. We also show that when transmitter uptake is reduced, glutamate spillover from distant nerve terminals in the olfactory bulb can activate nonsynaptic NPC AMPARs and generate increases in intracellular calcium. Together, these results suggest that Ca^{2+} influx via AMPARs may contribute to calcium-dependent processes that govern NPC differentiation and maturation.

INTRODUCTION

In the mammalian brain, the dentate gyrus and olfactory bulb are two major brain regions in which neurogenesis contributes to the maintenance of neural circuits throughout adulthood. In the olfactory bulb, sensory experience influences the survival and properties of newborn neurons (Alonso et al. 2006; Magavi et al. 2005; Petreanu and Alvarez-Buylla 2002), suggesting that neural precursor cells (NPCs) may be sensitive to external factors in their environment. NPCs in the olfactory bulb are thought to express a variety of membrane channels and neurotransmitter receptors prior to synaptic integration, but the functional properties of these signaling systems are not well understood.

Neurogenesis in the olfactory bulb provides an attractive system for studying the intrinsic signaling properties of NPCs and the functional contributions of these newborn neurons to existing circuitry. NPCs in the olfactory bulb develop into local inhibitory neurons throughout life in many vertebrate species including mammals (Lois and Alvarez-Buylla 1994). This process of maturation requires NPC migration to target destinations (Luskin 1998), morphological development, expression of ion channels and neurotransmitter receptors (Carleton et al. 2003), and eventual synaptic integration (Belluzzi et al. 2003; van Praag et al. 2002).

In the olfactory system, NPCs are mainly generated in the subventricular zone (SVZ) (Lois and Alvarez-Buylla 1994) and migrate along the rostral migratory stream (RMS) to the olfactory bulb (Luskin 1998). NPCs are densely concentrated in the inner core of the olfactory bulb within a region termed the subependymal layer (SEL). While SVZ-derived progenitor cells in the RMS retain the capacity to divide, relatively few mitotic cells are found in the SEL (Coskun and Luskin 2002). Postmitotic NPCs migrate radially from this cell layer and mature into local GABAergic interneurons (Baker et al. 2001; Belluzzi et al. 2003; Carleton et al. 2003) and granule and periglomerular cells, which provide inhibition onto principal mitral and tufted cells and shape sensory processing in the bulb.

In contrast to mature olfactory bulb interneurons, the membrane properties and signaling capabilities of NPCs are less well understood. NPCs express voltage-dependent potassium currents but relatively small amounts of small voltage-gated Na^+ channels (Darcy and Isaacson 2009; Wang et al. 2003a) that are insufficient to generate Na^+-dependent action potentials. Although their lack of action potentials suggests that they are "nonexcitable" cells, NPCs generate spontaneous intracellular Ca^{2+} transients mediated by L-type Ca^{2+} channels (Darcy and Isaacson 2009).

Recent studies have also examined the properties of neurotransmitter receptors expressed by NPCs. Activation of $GABA_A$ receptors ($GABA_A$Rs) depolarizes olfactory neuroblasts in the SVZ and RMS (Wang et al. 2003b) and causes an increase in intracellular Ca^{2+} (Platel et al. 2008a). However, the functional properties of NPC glutamate receptors are less clear. NPCs in the SVZ are reported to generate metabotropic glutamate receptor (mGluR)-evoked elevations in intracellular Ca^{2+} and kainate receptor-mediated currents (Platel et al. 2008b). In contrast, kainate has been suggested to activate GluR2-containing AMPARs in olfactory bulb NPCs (Carleton et al. 2003).

In this study, we take advantage of the fact that virtually all cells in the olfactory bulb SEL are migratory, newborn NPCs (Darcy and Isaacson 2009). We use patch-clamp recording and calcium imaging to characterize the properties of NPC glutamate receptors. Our results indicate that Ca^{2+}-permeable, GluR2-lacking AMPARs are a major feature of olfactory NPCs. Furthermore, under conditions that reduce transmitter uptake, nonsynaptic AMPARs on SEL NPCs can be activated via spillover of glutamate from synapses in the granule cell layer. These results suggest that Ca^{2+} influx via AMPARs could contribute to calcium-dependent processes that govern NPC differentiation and maturation.

METHODS

Slice preparation and electrophysiology

Olfactory bulb slices (350 μm) were prepared from 16- to 30-day-old Sprague-Dawley rats in accordance with institutional and national guidelines using standard procedures. Slices were prepared and maintained in artificial cerebrospinal fluid (ACSF) containing (in mM) 83

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NaCl, 2.5 KCl, 3.3 $MgSO_4$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 22 glucose, 72 sucrose, and 0.5 $CaCl_2$ equilibrated with 95% O_2-5% CO_2 at 34°C for 30 min and at room temperature thereafter. In the recording chamber, slices were viewed using infrared-DIC optics (BX-51W1, Olympus) and superfused with ACSF containing (in mM) 119 NaCl, 2.5 KCl, 1.3 $MgSO_4$, 1 NaH_2PO_4, 26.2 $NaHCO_3$, 22 glucose, and 2.5 $CaCl_2$ equilibrated with 95% O_2-5% CO_2. The ACSF was supplemented with tetrodotoxin (TTX, 1 μM), and the $GABA_AR$ blockers bicuculine (20 μM) and picrotoxin (100 μM) to block $GABA_A$ receptors. In experiments examining synaptically released glutamate, TTX was not added to the ACSF. Granule cell synaptic responses were evoked via a stimulating electrode placed in the external plexiform layer to trigger glutamate release from mitral cell dendrites. All experiments were performed at room temperature.

Whole cell electrodes (~6–7 MΩ) for voltage-clamp recordings were filled with a solution containing (in mM) 120 Cs-gluconate, 5 NaCl, 10 HEPES, 10 phosphocreatine, 3 Mg-ATP, 0.5 Na-GTP, and 0.5 EGTA (pH ~7.3, 300 mosM). Spermine (100 μM) was added to this internal solution to prevent polyamine dialysis. Caged glutamate [methyl 1-[5-(4-amino-4-carboxybutanoyl)]-7-nitroindoline-5-acetate, 300 μM] was included in recirculated ACSF. Glutamate uncaging was elicited with a UV flashlamp (T.I.L.L. Photonics) focused through a [toimes]60 water-immersion objective. The holding potential was −80 mV unless otherwise noted. Nonstationary fluctuation analysis (Sah and Isaacson 1995) was performed by measuring the mean and variance of 10–15 consecutive responses recorded in the presence of cyclothiazide. Measurements were made during the falling phase of the evoked currents. For each cell, the mean and variance were plotted against each other and the initial linear region of the current-variance relationship was fit with the equation: $\sigma^2 = iI$, where I is the mean current and i is the single channel amplitude.

Experiments were corrected for a measured junction potential (~10 mV). Responses were recorded with an Axopatch 200B amplifier (Axon Instruments), filtered at 2–5 kHz and digitized at 10–20 kHz (ITC-18; Instrutech). Data acquisition and analysis were performed with AxoGraph 4.9 (Axon) or Axograph X (AxoGraph Scientific) and IGOR Pro 5/6 (Wavemetrics).

Imaging

For imaging of evoked increases in intracellular Ca^{2+}, olfactory bulb slices were bulk loaded with Oregon Green BAPTA-1 AM (30 μM; 1.7% DMSO and 0.05% Pluronic F-127; Molecular Probes) in ACSF at 34°C for 40–60 min. For experiments examining responses to application of glutamate or AMPA, the ACSF was always supplemented with nimodipine (30 μM) or a combination of Cd^{2+} (200 μM) and Ni^{2+} (200 μM) to block Ca^{2+} influx mediated by L-type Ca^{2+} channels in NPCs (Darcy and Isaacson 2009). Image acquisition (494 nm excitation, 2 × 2 binning, 2- to 4-Hz capture) and analysis were carried out with a cooled-CCD camera system (T.I.L.L. Photonics). Regions of interest (ROIs) were small circles centered on the soma of NPCs.

Analysis

Representative traces are the average of five or more consecutive episodes, except where noted. Data are presented as means ± SE. Student's *t*-test was used to determine statistical significance.

RESULTS

Previous studies suggest that the majority of cells in the SEL of the olfactory bulb are migrating NPCs (Luskin 1998). Indeed virtually all SEL cells have been shown to express doublecortin (Darcy and Isaacson 2009), a microtuble-associated protein found selectively in migrating newborn neurons (Brown et al. 2003; Gleeson et al. 1999; Nacher et al. 2001). We first examined the properties of glutamate receptors in SEL NPCs using whole cell recording and flash photolysis of caged glutamate (300 μM). All voltage-clamped SEL cells had a small cell body (~5 μm diameter) and high-input resistance ($R_{in} > 3$ GΩ). Previous studies have found that high membrane resistance is a characteristic feature of NPCs (Belluzzi et al. 2003; Darcy and Isaacson 2009; Wang et al. 2003a) and rules out the possibility that we were recording from glial cells.

Glutamate uncaging evoked brief, inward currents (Fig. 1*A*) in all cells voltage-clamped at −80 mV (peak current: 20.9 ± 9.5 pA; $n = 6$). Bath application of the selective AMPAR antagonist GYKI 53655 (100 μM) (Lerma et al. 2001; Wilding and Huettner 1995) abolished glutamate-evoked currents (5.6 ± 7.9% of control; Fig. 1*A*; $n = 4$), indicating that AMPARs rather than kainate receptors govern glutamate-evoked currents in NPCs. We further explored the possibility that kainate receptors contributed to glutamate-evoked responses by examining the action of concanavalin A (ConA, 0.3 mg/ml), a drug that reduces kainate receptor desensitization and enhances kainate-evoked responses (Partin et al. 1993). Bath application of ConA had no effect on glutamate-evoked currents (Fig. 1*B*). However, subsequent application of cy-

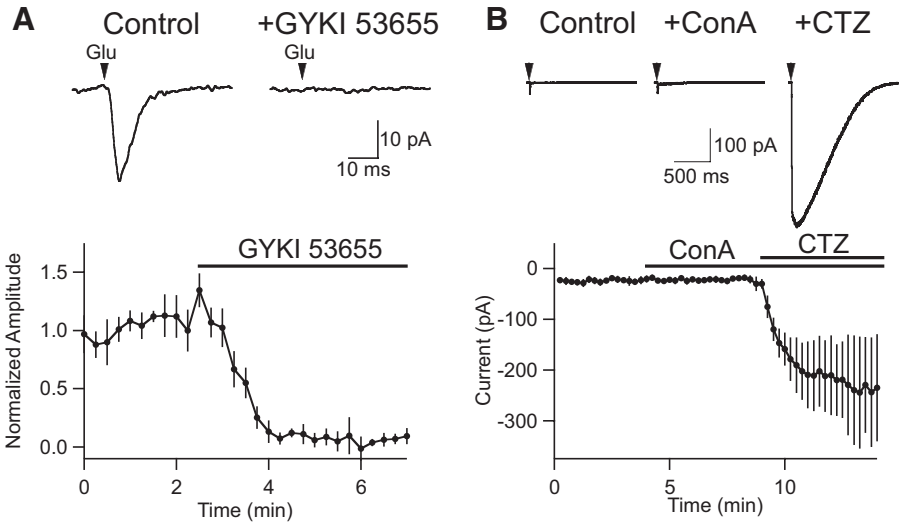

FIG. 1. Glutamate activates AMPA receptors (AMPARs) on olfactory bulb neural precursor cells (NPCs). *A*: the AMPAR antagonist GYKI 53655 blocks glutamate-evoked currents in NPCs. *Top*: traces from a representative cell voltage-clamped at −80 mV. *Bottom*: summary plot of the actions of GYKI 53655 (100 μM) on glutamate-evoked currents ($n = 4$). *B*: concanavalin A (ConA, 0.3 mg/ml) has no effect on glutamate-evoked currents, but subsequent application of cyclothiazide (CTZ, 100 μM) enhances responses in the same cells. *Top*: representative traces from 1 cell. *Bottom*: summary plot of glutamate-evoked currents (−80 mV) in the presence of ConA and CTZ ($n = 5$).

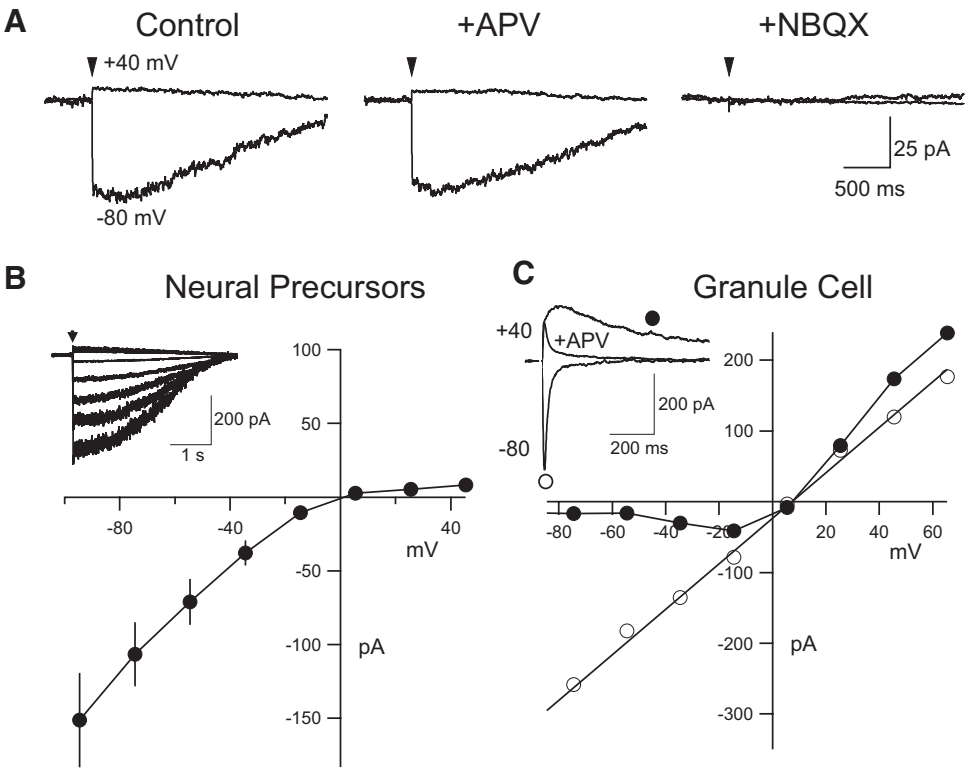

FIG. 2. NPC AMPARs show strong voltage-dependent inward rectification. *A*: glutamate uncaging evokes inward currents at −80 mV and little outward current at +40 mV (control). Blocking *N*-methyl-D-aspartate receptors (NMDARs) has no effect on glutamate-evoked currents [+ 2-amino-5-phosphonovaleric acid (APV)] while subsequent application of an AMPAR antagonist (+NBQX) abolishes glutamate-evoked responses. *B*: summary of the current-voltage (*I-V*) relationship of glutamate-evoked currents in NPCs recorded in the presence of CTZ ($n = 11$). *Inset*: single traces recorded over a range of membrane potentials from a representative cell. *C*: representative *I-V* relationship of excitatory postsynaptic currents (EPSCs) from a granule cell reveals the contribution of NMDARs and nonrectifying AMPARs to synaptic transmission. Inset, traces illustrate the AMPAR-mediated EPSC at −80 mV and synaptic response at +40 mV before and after block of NMDARs (+APV). The *I-V* relationship of the AMPAR component (○) and NMDAR component (●) was measured at the time points indicated on the *inset*.

clothiazide (CTZ, 100 μM), a selective blocker of AMPAR desensitization (Partin et al. 1993) dramatically enhanced the amplitude of glutamate-evoked responses (Fig. 1*B*, control, 25.1 ± 6.8 pA; CTZ, 234.5 ± 100.2 pA, $n = 5$). Together, these pharmacological experiments indicate that SEL NPCs express functional AMPARs, whereas kainate receptors are unlikely to contribute to glutamate-evoked ionotropic responses.

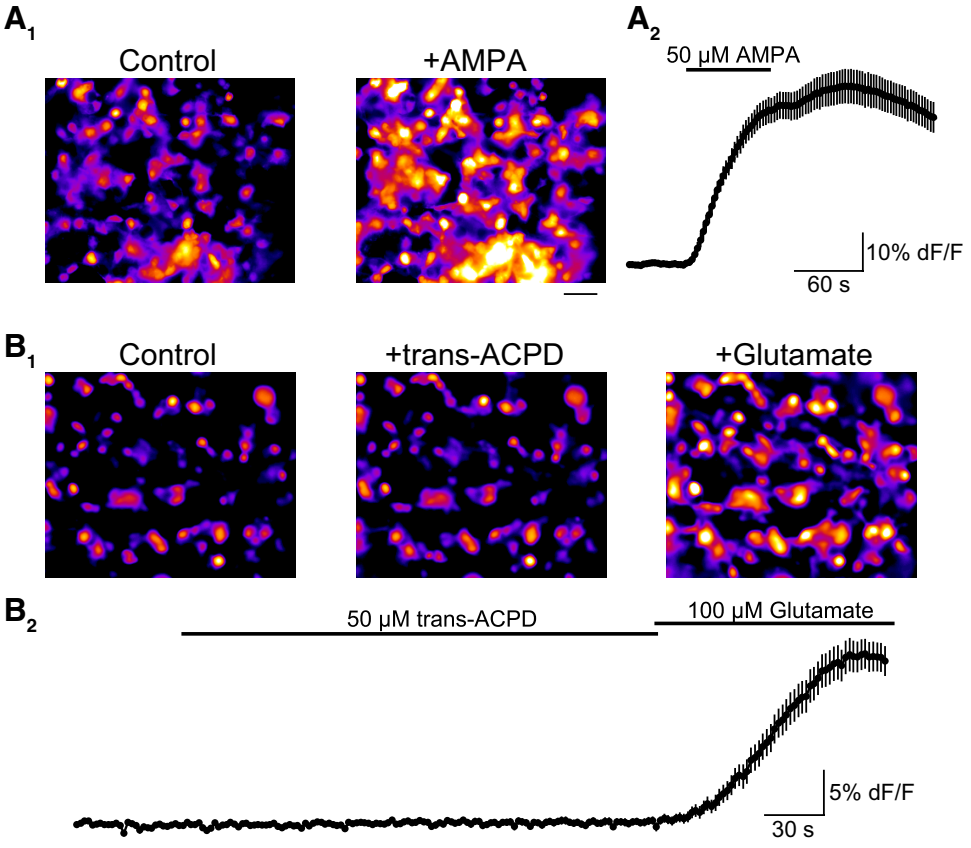

FIG. 3. Activation of AMPARs on increases NPC intracellular Ca^{2+}. *A1*: example raw fluorescence of NPCs in the subependymal layer (SEL) loaded with Oregon Green BAPTA-1 AM before (control) and during application of AMPA (50 μM) and CTZ (100 μM). Scale bar = 20 μm. *A2*: summary of responses to cells randomly selected from *A1* plotted as d*F*/*F* ($n = 10$) *A1*. *B*: glutamate-evoked increases in intracellular Ca^{2+} are not due to mGluRs. *B1*: example raw fluorescence before (control) and after application of a broad-spectrum mGluR agonist (+*trans*-ACPD, 20 μM) followed by subsequent application of glutamate (+Glutamate, 100 μM) with cyclothiazide. *B2*: summary plot showing d*F*/*F* in response to *trans*-ACPD and glutamate ($n = 40$ cells, 5 slices). All experiments were performed in the presence of Cd^{2+} (200 μM) and Ni^{2+} (200 μM) to prevent activation of voltage-gated Ca^{2+} channels.

We next examined the current-voltage relationship of glutamate-evoked currents in SEL NPCs recorded in the presence of CTZ. Although robust currents were evoked by glutamate uncaging at −80 mV, membrane depolarization to +40 mV revealed only small outward, glutamate-evoked currents (Fig. 2*A*). The *N*-methyl-D-aspartate receptor (NMDAR) antagonist D-2-amino-5-phosphonovaleric acid (D-APV; 50 μM) had no effect on glutamate-evoked responses at either potential, indicating that NMDA receptors do not contribute to glutamate-evoked currents in SEL NPCs (−80 mV: 108.9 ± 7.6% of control; +40 mV: 98.1 ± 14.1% of control; $n = 4$). In contrast, application of the AMPAR antagonist 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX) completely abolished glutamate evoked responses at both hyperpolarized and depolarized potentials (20 μM; −80 mV: 1.2 ± 0.4% of control; +40 mV: −0.8 ± 1.6% of control; Fig. 2*A; n* = 6). These results reveal that NPCs express functional AMPARs but not NMDARs. We examined glutamate-evoked responses over a range of membrane potentials and found that the current-voltage relationship of NPC AMPAR currents was strongly inwardly rectifying (Fig. 2*B; n* = 11). The virtual absence of outward currents at positive membrane potentials indicates that the majority of NPC AMPARs lack the GluR2 receptor subtype. To further explore the properties of AMPARs underlying glutamate-evoked currents in NPCs, we used nonstationary fluctuation analysis (see METHODS) to determine the AMPAR single-channel conductance. The relationship between mean current and variance for responses at −80 mV (cf. Fig. 2*B, inset*) indicated an average single-channel conductance of 7.05 ± 1.11 pS ($n = 6$), in close agreement with previously reported values for GluR2-lacking AMPARs (Swanson et al. 1997). Together, these results reveal that GluR2-lacking AMPARs are the major receptor type governing glutamatergic signaling in SEL NPCs.

The absence of NMDARs and presence of GluR2-lacking AMPARs in NPCs is markedly different from the glutamate receptors expressed by mature interneurons in the olfactory bulb. For example, synaptically evoked excitatory postsynaptic

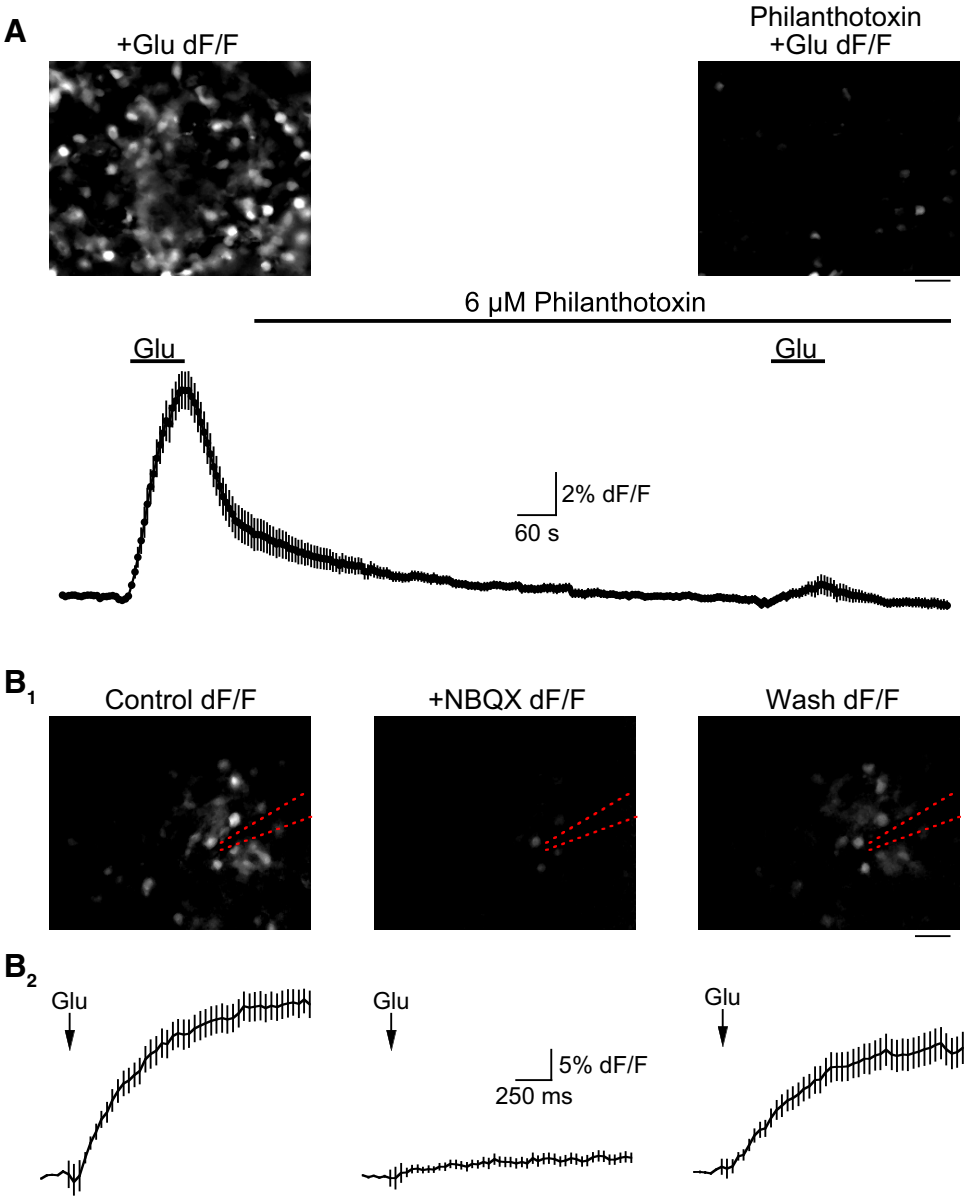

FIG. 4. Glutamate-evoked increases in NPC intracellular Ca^{2+} are mediated by AMPARs. *A, top*: peak d*F*/*F* images of Ca^{2+} responses in Oregon Green-loaded SEL NPCs to bath application of glutamate (50 μM) under control conditions (*left*) and in the presence of philanthotoxin (6 μM, *right*). Scale bar = 20 μm. *Bottom*, time course of the average responses of 15 cells from the same experiment. *B1*: representative d*F*/*F* images of Ca^{2+} responses in Oregon Green-loaded SEL NPCs to puffer pipette (red dotted line), application of glutamate (200 μM, 20 ms, 5 psi) under control conditions (*left*) in the presence of NBQX (*middle*, 20 μM), and following washout of the AMPAR antagonist (*right*). Scale bar = 20 μm. *B2*: summary of the responses of 20 cells from each period of the experiment displayed in *B1*. The same cells were measured under each condition. All experiments were performed in the presence of nimodipine (30 μM) to prevent activation of NPC voltage-gated Ca^{2+} channels.

currents in granule cells display a robust NMDAR component at +40 mV and nonrectifying AMPAR responses (Fig. 2*C*) (Isaacson and Strowbridge 1998). Thus GluR2-lacking AMPARs are initially expressed in newborn olfactory bulb interneurons, while GluR2-containing AMPARs and NMDARs are expressed later to govern synaptic transmission by cells that have integrated into functional olfactory bulb circuits.

AMPARs that lack the edited GluR2 subunit have a high permeability to Ca^{2+} (Burnashev et al. 1992; Hollmann et al. 1991; Hume et al. 1991; Verdoorn et al. 1991), providing a possible route for Ca^{2+} influx in NPCs. To test this idea, we performed Ca^{2+} imaging experiments in olfactory bulb slices loaded with the fluorescent Ca^{2+} indicator Oregon Green BAPTA-1 AM (OG1-AM; 30 μM). We studied Ca^{2+} responses under conditions to pharmacologically isolate Ca^{2+} influx directly via AMPARs. Slices were superfused with ACSF containing D-APV (50 μM) to block NMDARs. The ACSF was always supplemented with nimodipine (30 μM) or a combination of Cd^{2+} (200 μM) and Ni^{2+} (200 μM) to block Ca^{2+} influx mediated by L-type Ca^{2+}channels in NPCs (Darcy and Isaacson 2009). Consistent with previous observations (Darcy and Isaacson 2009), nimodipine or the inorganic Ca^{2+} channels blockers abolished spontaneous and depolarization-evoked Ca^{2+} transients in SEL cells (not shown). Under these conditions, bath application of AMPA (50 μM) and CTZ (100 μM) caused a marked rise in intracellular Ca^{2+} in virtually all cells in the SEL (Fig. 3*A;* average d*F*/*F* increase of 38.3 ± 2.5%; $n = 50$ cells, 5 slices). We next examined the actions of glutamate on SEL intracellular Ca^{2+} under identical conditions. In these experiments, we first applied the broad-spectrum metabotropic glutamate receptor (mGluR) agonist, (±)-1-aminocyclopentane-trans-1,3-dicarboxylic acid (*trans*-ACPD) (50 μM), to explore the possibility that SEL NPCs might possess mGluRs capable of triggering Ca^{2+} release from intracellular stores (Conn and Pin 1997). However, *trans*-ACPD had no effect on SEL NPCs (Fig. 3*B*), whereas the subsequent application of glutamate (100 μM) caused a marked increase in intracellular Ca^{2+} in the same cells (19.4 ± 1.7% d*F*/*F*; $n = 40$ cells, 5 slices). These experiments suggest that ionotropic AMPARs mediate glutamate-evoked Ca^{2+} influx in SEL NPCs.

Calcium-permeable AMPARs lacking the GluR2 subunit are selectively blocked by the noncompetitive antagonist philanthotoxin (Toth and McBain 1998; Washburn and Dingledine 1996). We next explored the actions of this selective antagonist on glutamate-evoked Ca^{2+} influx in NPCs. In these experiments, glutamate was first bath-applied under control conditions and then again in the presence of philanthotoxin. Following bath application of philanthotoxin (6 μM), glutamate-evoked Ca^{2+} responses (d*F*/*F*) were markedly reduced to 18 ± 3.0% of those under control conditions in the same cells (Fig. 4*A*, $n = 25$ cells, 2 slices). In separate experiments in the absence of the blocker, we confirmed that this double application of glutamate resulted in identical increases in intracellular calcium in the same SEL cells (106 ± 7.0%, $n = 20$ cells, 2 slices). Furthermore, focal application of glutamate (200 μM, 20 ms, 5 psi) via a puffer pipette evoked rapid increases in intracellular Ca^{2+} in SEL NPCs that were reversibly blocked by the selective antagonist NBQX (Fig. 4*B;* control: 67.5 ± 3.5% d*F*/*F*, NBQX: 5.2 ± 1.0% d*F*/*F*, wash: 54.8 ± 4.6% d*F*/*F*; $n = 50$ cells, 3 slices). Taken together, these results provide strong evidence that GluR2-lacking AMPARs mediate direct Ca^{2+} influx in SEL NPCs.

NPCs do not receive direct synaptic contacts; however, we next tested whether extrasynaptic glutamate due to spillover from synapses in the granule cell layer of the olfactory bulb might activate NPC AMPARs. To address this question, we placed a stimulating electrode in the granule cell layer to activate centrifugal glutamatergic inputs that synapse onto granule cell dendrites (Balu et al. 2007). We delivered a train of stimuli (10 Hz, 30 s) to this pathway and used calcium imaging to monitor the activity of NPCs in the adjacent SEL of the olfactory bulb. Glutamate spillover is strongly regulated by active glutamate transporters (Isaacson 1999; Kanai and Hediger 1992; Storck et al. 1992); indeed stimulation under control conditions did not evoke any response in NPCs in the SEL (data not shown, $n = 5$ slices). However, in the presence of the glutamate uptake blocker d-threo-b-benzyloxyaspartic acid (D-TBOA) (50 μM), stimulation evoked clear increases in intracellular Ca^{2+} in NPCs (Fig. 5*A; n* = 3 slices). The kinetics of the Ca^{2+} responses were slowly rising during stimulation and slowly decaying following stimulation (Fig. 5*B*), consistent with the gradual accumulation of glutamate around NPCs from a distant source. Spillover-evoked responses of NPCs were unaffected by APV (50 μM) but reversibly blocked by NBQX (20 μM), confirming that AMPARs underlie the Ca^{2+} signals (Fig. 5*B; n* = 15 cells). These data indicate that when uptake

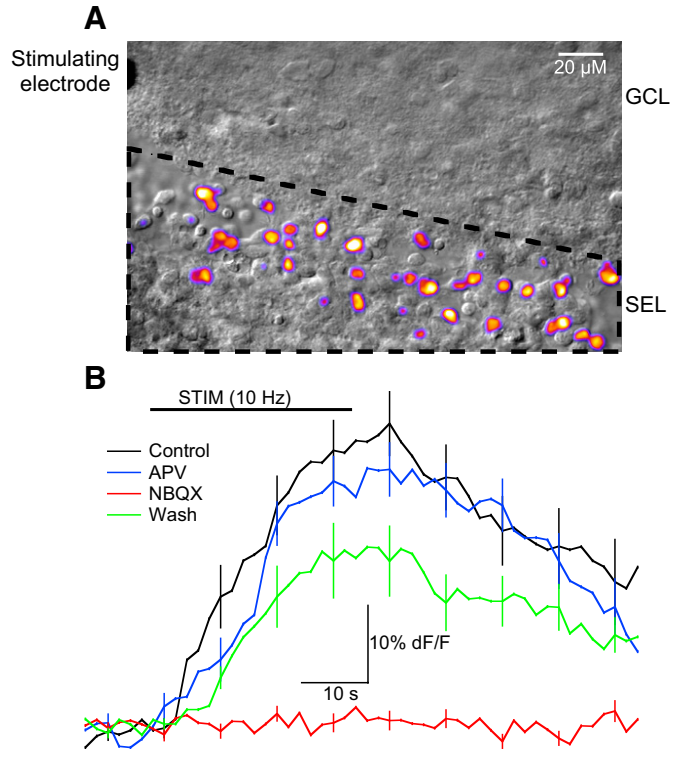

FIG. 5. Spillover of synaptically released glutamate activates AMPARs on SEL NPCs. *A*: DIC image shows the placement of a bipolar stimulating electrode in the granule cell layer (GCL) of a slice loaded with Oregon Green. Only the region of the slice delineated by the dashed line was imaged for fluorescence. The overlaid d*F*/*F* image shows SEL cells (pseudocolored) that responded to synaptic stimulation. *B*: average time course of d*F*/*F* responses of 15 SEL NPCs from one representative experiment. Cells responded under control conditions (black trace) and after application of APV (blue, 50 μM). Responses were abolished by application of NBQX (red, 20 μM) and returned following washout of the AMPAR antagonist (green).

is reduced, glutamate released from nerve terminals in the nearby granule cell layer can spillover from active synapses and activate AMPARs on SEL NPCs.

DISCUSSION

In this study, we show that Ca^{2+}-permeable, GluR2-lacking AMPARs are the major subtype of glutamate receptors functionally expressed by newborn neural precursors in the olfactory bulb. We also find that NPC AMPARs can be activated via synaptically released glutamate under conditions maximizing endogenous neurotransmitter spillover, providing a nonsynaptic signaling mechanism for glutamate-evoked Ca^{2+} influx. Given the important role of Ca^{2+} as an intracellular signaling molecule, AMPARs may have important functional consequences for the migration, maturation, and early signaling of olfactory bulb NPCs.

It has previously been suggested that neuroblasts within the mouse subventricular zone express functional kainate receptors containing the GLU_{K5} subunit (Platel et al. 2008b). This was based on findings that selective kainate receptor agonists led to small increases in membrane current noise and generated elevations in intracellular Ca^{2+}. In addition, mGluR5 receptor agonists led to rises in intracellular Ca^{2+}, suggesting that mGluRs also mediate signaling in subventricular zone neuroblasts. In contrast, our results indicate that AMPARs are the major receptor governing glutamate-evoked responses in olfactory bulb NPCs. We show that glutamate-evoked currents are blocked by the AMPAR-selective antagonist GYKI 53655 and insensitive to the kainate receptor modulator concanavilin A. We also find that when voltage-gated Ca^{2+} channels are blocked, both glutamate and AMPA, but not a broad spectrum mGluR agonist, trigger Ca^{2+} influx in SEL NPCs. Furthermore, philanthotoxin, the selective antagonist of GluR2-lacking AMPARs, blocks glutamate-evoked Ca^{2+} influx in NPCs. Given that NPCs in the olfactory bulb SEL originate as neuroblasts and initially migrate from the subventricular zone, differences in glutamate receptors may reflect different maturational states of these migrating cells as they progress from their site of origin to their near-final destination.

Our results indicating the lack of NMDARs on SEL NPCs are consistent with a previous electrophysiological study of newborn migrating "class 1" cells in the RMS that expressed GFP following retroviral infection of the SVZ (Carleton et al. 2003). In that study of the properties of newborn neurons at various stages of maturation, functional NMDARs were only first expressed by radially migrating neuroblasts in the granule cell layer ("class 2" cells). During the development of many brain regions, GluR2-lacking AMPARs are typically expressed at early developmental stages while GluR2-containing AMPARs are expressed later in development (Aizenman et al. 2002; Ho et al. 2007; Kumar et al. 2002). Our results indicating a marked inward rectification of AMPAR-mediated currents and AMPA-evoked Ca^{2+} influx provide strong evidence that GluR2-lacking AMPARs are a major feature of SEL NPCs. It has been proposed that class 1 neuroblasts express Ca^{2+}-impermeable, GluR2 containing AMPARs (Carleton et al. 2003). This was based on the claim that AMPAR-mediated currents during depolarizing voltage ramps (from -105 to $+95$ mV) displayed a linear current-voltage relationship. However, the intracellular polyamine block of GluR2-lacking AMPARs that generates rectifying *I-V* relationships only occurs at voltages between 0 to $+40$ mV and is relieved at more depolarized potentials (Koh et al. 1995). Given this voltage dependence of polyamine block, the prominent inward rectification of AMPARs in NPCs may have been obscured by the large voltage ramps used previously to measure AMPAR *I-V* relationships (Carleton et al. 2003).

Olfactory experience influences NPC survival and responses (Alonso et al. 2006; Magavi et al. 2005; Petreanu and Alvarez-Buylla 2002), suggesting NPCs may be sensitive to external factors in their environment. We show that when transmitter uptake is reduced, glutamate spillover from active synapses can activate Ca^{2+}-permeable AMPARs on NPCs. Our experiments reveal extrasynaptic actions of glutamate over a large distance, from the granule cell layer to the SEL, under conditions that maximize the detection of spillover. Given that these results were only obtained under conditions in which transmitter uptake was reduced, it is unclear if AMPARs on migrating NPCs would be activated by endogenous-released glutamate under physiological conditions. However, during radial migration through the granule cell layer, NPCs may come into close proximity to centrifugal glutamatergic synapses made by cortical inputs onto granule cell dendrites. One possibility is that migrating NPCs in the granule cell layer transiently come close enough to local sources of glutamate spillover for AMPARs to be activated.

Intracellular Ca^{2+} is thought to play an important role in regulating the migration and maturation of newborn neurons (Gomez and Spitzer 1999; Komuro and Kumada 2005; Komuro and Rakic 1996; Platel et al. 2008a; Zheng 2000). L-type voltage-gated Ca^{2+} have recently been shown to underlie spontaneous and depolarization-evoked Ca^{2+} transients in SEL cells (Darcy and Isaacson 2009). Our results suggest that Ca^{2+}-permeable AMPARs may also contribute to Ca^{2+} signaling in olfactory bulb NPCs.

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