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Kainate receptor-mediated synaptic transmissions in the adult rodent insular cortex

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Koga K, Sim SE, Chen T, Wu LJ, Kaang BK, Zhuo M. Kainate receptor-mediated synaptic transmissions in the adult rodent insular cortex. J Neurophysiol 108: 1988–1998, 2012. First published July 11, 2012; doi:10.1152/jn.00453.2012.—Kainate (KA) receptors are expressed widely in the central nervous system and regulate both excitatory and inhibitory synaptic transmission. KA receptors play important roles in fear memory, anxiety, and pain. However, little is known about their function in synaptic transmission in the insular cortex (IC), a critical region for taste, memory, and pain. Using whole cell patch-clamp recordings, we have shown that KA receptors contribute to fast synaptic transmission in neurons in all layers of the IC. In the presence of the GABAA receptor antagonist picrotoxin, the NMDA receptor antagonist AP-5, and the selective AMPA receptor antagonist GYKI 53655, KA receptor-mediated excitatory postsynaptic currents (KA EPSCs) were revealed. We found that KA EPSCs are ~5–10% of AMPA/KA EPSCs in all layers of the adult mouse IC. Similar results were found in adult rat IC. KA EPSCs had a significantly slower rise time course and decay time constant compared with AMPA receptor-mediated EPSCs. High-frequency repetitive stimulations at 200 Hz significantly facilitated the summation of KA EPSCs. In addition, genetic deletion of GluK1 or GluK2 subunit partially reduced postsynaptic KA EPSCs, and exposure of GluK2 knockout mice to the selective GluK1 antagonist UBP 302 could significantly reduce the KA EPSCs. These data suggest that both GluK1 and GluK2 play functional roles in the IC. Our study may provide the synaptic basis for the physiology and pathology of KA receptors in the IC-related functions.

kainate receptor; GluK1; GluK2; pyramidal neuron

cortical areas such as prefrontal cortex (PFC), the anterior cingulate cortex (ACC), and insular cortex (IC) are important for pain, emotion, and memory processing (Frankland et al. 2004; LaBar and Cabeza 2006; Zhuo 2008). Animal and human studies consistently suggest that neurons in the IC play important roles in pain and memory (Craig 2002; Jasmin et al. 2003; Shema et al. 2011). Electrophysiological recordings in vivo from animals have shown that insular cortical neurons respond to noxious gustatory, visceral, and nociceptive stimuli (Benison et al. 2011; Gauriau and Bernard 2004; Hanamori et al. 1998). Human imaging data have shown that IC is activated by different pain conditions (Apkarian et al. 2005; Craig 2003; Eder et al. 2003; Henderson et al. 2007). In addition to sensory pain processing, the IC has also been implicated in taste-related learning and memory (Shema et al. 2011). Despite the important roles of the IC in sensory processing and cognition, few studies have been reported about synaptic transmission in the IC.

Glutamate, the major excitatory neurotransmitter in the central nervous system, activates three different receptors that directly gate ion channels, namely, receptors for a-amino-3-hydroxy-5-methyl isoxozole propionic acid (AMPA), N-methyl-D-aspartate (NMDA), and kainate (KA). The KA receptor family is composed of five different subunits, namely, GluK1, GluK2, GluK3, GluK4, and GluK5, which can form a variety of homomeric and heteromeric receptors. Most basal synaptic responses are mediated by AMPA receptors, whereas NMDA and KA receptors are activated by repetitive synaptic stimulation (Hollmann and Heinemann 1994; Jane et al. 2009; Lerma 2003). However, in certain sensory and central synapses, KA receptors have also been reported to contribute to synaptic transmission. In the spinal cord dorsal horn, Li et al. (1999) reported that KA receptors contribute to sensory synaptic transmission activated by high-threshold nociceptive fibers. In “off” bipolar cells of mammalian retina, KA receptors mediate synaptic transmission from cones (DeVries and Schwartz 1999). Wu et al. (2005) reported that both GluK1 and GluK2 receptors contribute to KA transmission in layer II/III pyramidal neurons of the adult mouse ACC (Wu et al. 2005) (see Table 1). Although KA receptor-mediated currents are much smaller than those of AMPA receptor, behavioral studies using gene knockout mice or pharmacological inhibitors have shown that different types of KA receptors participate in distinct physiological functions and pathological conditions (Jane et al. 2009; Ko et al. 2005; Wu et al. 2007). For example, selective and potent GluK1 antagonists inhibit formalin-induced paw licking, carrageenan-induced thermal hyperalgesia, and capsaicin-induced mechanical hyperalgesia (Domínguez et al. 2005; Jones et al. 2006). In gene knockout mice lacking GluK1, behavioral responses to capsaicin or formalin injection were significantly reduced (Ko et al. 2005). In addition, the mice lacking GluK2 showed a significant impairment in fear memory (Ko et al. 2005).

These findings suggest a possible contribution of KA receptors to sensory and memory-related brain areas such as the IC. In the present study, we employed integrative methods including whole cell patch-clamp recording, pharmacology, and gene knockout mice. We characterized the function of KA receptors in glutamatergic synaptic transmissions. Electrophysiological mappings showed the KA functions in layers I, II/III, and V/VI. In addition, genetically modified mice with deletions of GluK1 or GluK2 were used to show that both GluK1 and GluK2 are involved in synaptic transmission in the IC.
METHODS

Animals. Adult C57Bl/6 mice and Sprague-Dawley rats were purchased from Charles River (8–14 wk old). GluK1 and GluK2 KO mice were obtained as gifts from Stephen F. Heinemann (Salk Institute, San Diego, CA) (Mulle et al. 1998; Sailer et al. 1999). For experiments using knockout (KO) mice, GluK1 and GluK2 were maintained on a mixed 129Sv/C57LJ (SJL) background. For experiments related to mutant mice were performed blind to the genotype.

Whole cell patch-clamp recordings in adult IC slices. Rodents were anesthetized with 1–2% isoflurane. Transverse brain slices of the IC (300 μm) were prepared using standard methods (Li et al. 2010; Wei et al. 2001, 2002, 2005). Slices were transferred to a room temperature-submerged recovery chamber with an oxygenated (95% O2-5% CO2) solution containing (in mM) 124 NaCl, 25 NaHCO3, 2.5 KCl, 1 CaCl2, 2 MgSO4, and 10 glucose. After a 1-h recovery period, slices were transferred into a recording chamber on the stage of an Axioskop 2FS microscope (Carl Zeiss) equipped with infrared differential interference contrast optics for visualizing whole cell recordings in mouse neurons.

Excitatory postsynaptic currents (EPSCs) were recorded from layer II/III and V/VI pyramidal neurons and layer I recording, respectively (see Fig. 7 A and Fig. 7A). Control test pulses were given every 30 s. For frequency facilitation, repetitive stimulation was delivered at 200 Hz (5, 10, or 20 shocks). In the voltage-clamp configuration, recording electrodes (2–5 MΩ) contained the pipette solution composed of (in mM) 120 Cs-glucuronate, 5 NaCl, 1 MgCl2, 0.5 EGTA, 2 Mg-ATP, 0.1 NaGTP, 10 HEPES, and 2 lidocaine-β-methyl bromide quaternary salt (QX-314), pH 7.2, 280–300 mosmol/l. The initial access resistance was 15–30 MΩ, and it was monitored throughout the experiment. Data were discarded if the access resistance changed >15% during experiment. Data were filtered at 1 kHz and digitized at 10 kHz. The membrane potential was held at −60 mV throughout the experiment.

Chemicals and drug application. All chemicals and drugs were obtained from Tocris (St. Louis, MO). All experiments were conducted in the presence of picrotoxin (PTX; 100 μM) and d-2-amino-5-phosphonopentoic acid (AP-5; 50 μM).

Data analysis. Data are means ± SE. Statistical comparisons between two groups were performed using two-tailed paired or unpaired t-tests, and comparisons between more than three groups were performed using Dunnett’s test to identify significant differences. In layers I, II, III, and V/VI of WT, GluK1 KO, GluK2 KO, and GluK2 KO mice with UBP 302, the recordings by repetitive stimulations and input-output were analyzed using both analysis of variance between groups (1-way repeated-measures ANOVA, within each group) and 2-way repeated-measures ANOVA (Student-Newman-Keuls test to compare groups). In all cases, *P < 0.05 was considered statistically significant. Time constants for EPSCs were obtained by fitting one exponential function to the falling phase of the currents.

RESULT

KA receptor-mediated synaptic transmission in adult IC mouse neurons. Whole cell patch-clamp recordings were performed from visually identified pyramidal cells in layers II and III of IC slices in adult WT mice, and a bipolar stimulation electrode was placed in layer V/VI of IC (n = 23 mice; Fig. 1A). The stimulation in the deeper layers may activate passing fibers from thalamocingulate pathway, as well as local fibers (Lee et al. 2007). We recorded the neurons that receive pure monosynaptic synaptic input. We tested monosynaptic responses by delivering 20 shocks at 20 Hz (holding at −60 mV; Fig. 1B). Neurons with monosynaptic inputs followed the repetitive stimulation without failure. To test whether excitatory synaptic transmission is mediated by glutamate, we bath applied an AMPA/KA receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 20 μM). The evoked EPSCs were completely

Table 1. KA receptors in mammalian central synaptic transmission and regulation

<table>
<thead>
<tr>
<th>Area</th>
<th>Animal</th>
<th>Layer</th>
<th>Postneurons</th>
<th>KA Receptors</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal root ganglion</td>
<td>Rats</td>
<td>Lamina II</td>
<td>Cultured neuron</td>
<td>GluK1, GluK2</td>
<td>Kerchner et al. 2001</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>Mice</td>
<td>CA3</td>
<td>Primary afferent pathway</td>
<td>GluK2</td>
<td>Li et al. 1999, Youn et al. 2004</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>Mice</td>
<td>Golgi</td>
<td>GluK2</td>
<td>Bureau et al. 2000</td>
<td></td>
</tr>
<tr>
<td>Nucleus accumbens</td>
<td>Mice</td>
<td>Medium size</td>
<td>GluK2</td>
<td>Casassus et al. 2002</td>
<td></td>
</tr>
<tr>
<td>Motor cortex</td>
<td>Rats</td>
<td>II/III, V</td>
<td>Fast spiking</td>
<td>GluK1, GluK2</td>
<td>Wu et al. 2005</td>
</tr>
<tr>
<td>Anterior cingulate cortex</td>
<td>Adult mice</td>
<td>II/III</td>
<td>Pyramidal</td>
<td>GluK1, GluK2</td>
<td>Present study</td>
</tr>
<tr>
<td>Insular cortex</td>
<td>Adult mice</td>
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<td>Pyramidal</td>
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KA, kainate; GluK1 and GluK2, KA receptor subunits.
blocked by bath application of CNQX \((n = 6 \text{ neurons/6 mice; Fig. 1C})\), indicating that glutamate is the major excitatory transmission in the IC.

Next, we examined whether KA receptors contribute to synaptic responses in the IC neurons of adult mice (Fig. 2A). In the presence of the GABA\(_A\) antagonist, PTX (100 \(\mu M\)) and \(N\)-2-amino-5-phosphonopentanoic acid (AP-5; 50 \(\mu M\)) (baseline; \(a\)). The evoked EPSCs were completely blocked by a AMPA/KA antagonist, 6-cyano-7-nitroquinazoxalene-2,3-dione (CNQX; 20 \(\mu M\)) \((b)\). In all example EPSCs, each trace represents an average of 5–10 consecutive recordings.

that the current was mediated by KA receptors. The pooled data of KA EPSCs are shown in Fig. 2D. The averaged components of KA EPSCs were 10.9 \(\pm 2.0\%\) of the averaged AMPA/KA EPSCs as a baseline (averaged AMPA/KA EPSCs: 149.9 \(\pm 7.7\) pA; averaged KA EPSCs: 16.2 \(\pm 3.2\) pA, \(n = 14\) neurons/10 mice). We also confirmed that the AMPA/KA receptor antagonist CNQX can block the KA EPSCs (2.1 \(\pm 0.8\%\), \(n = 5\) neurons/5 mice; Fig. 2). Another AMPA receptor antagonist, SYM 2206 (100 \(\mu M\)), was also used to dissect the KA EPSCs in mice (Wu et al. 2005). Residual currents were observed 10 min after SYM 2206 perfusion. The averaged KA EPSCs of AMPA/KA EPSCs were 21.8 \(\pm 4.2\%\) \((n = 4\) neurons/4 mice). The discrepancy between the results obtained from GYKI 53655 and SYM 2206 may result from differences in the selectivity of the compounds (Wu et al. 2005). To test whether GYKI 53655 at a higher dose might cause more inhibition, we applied 200 \(\mu M\) of GYKI 53655 for 10 min after 10-min GYKI (100 \(\mu M\)) treatment in the
same neurons. GYKI 53655 (200 μM) did not cause any further inhibition (n = 5 neurons/3 mice; Fig. 2E).

KA receptor-mediated synaptic transmission in adult IC rat neurons. We further recorded pyramidal cells in layers II and III of adult rats (n = 5 rats; Fig. 2, A and C). Following mouse recordings in Fig. 2, A and B, we examined whether KA receptors contribute to synaptic responses in the IC neurons of adult rats (Fig. 2). As shown in Fig. 2C, GYKI 53655 had a fairly rapid and rigorous inhibitory effect on EPSCs in adult rats. A small residual EPSC persisted in the presence of GYKI 53655 10 min after perfusion. Perfusion of CNQX entirely blocked the residual GYKI 53655-resistant current (Fig. 2C), suggesting that the currents in adult rats were also mediated by KA receptors. The pooled data of KA EPSCs are shown in Fig. 2D. In rats, the summarized KA EPSCs were 7.0 ± 1.4% of the baseline (averaged AMPA/Kainate EPSCs: 117.7 ± 20.0 pA; KA EPSCs: 7.2 ± 0.5 pA, n = 5 neurons/5 rats). Compared with KA currents in adult mice, there was not a statistically significant difference (P > 0.05). These results suggest that a relatively small component of fast excitatory synaptic transmission is mediated by synaptic KA receptors in the adult rodent IC.

Slow kinetics and voltage dependence of KA receptor-mediated EPSCs. Despite the rapid desensitization and deactivation of heterologously expressed KA receptors (Paternain 1998; Swanson and Heinemann 1998), most studies report that KA receptor-mediated EPSCs have slow kinetics (Ali 2003; Bureau et al. 2000; Cossart et al. 1998; DeVries and Schwartz 1999; Frerking et al. 1998; Kidd and Isaac 1999; Li et al. 1999; Wu et al. 2005). We
performed a quantitative study of the kinetics of KA EPSCs in adult mouse IC slices. As shown in Fig. 3A, KA EPSCs displayed slower kinetics than AMPA EPSCs. Both the decay time (13.5 ± 1.5 ms in AMPA vs. 85.2 ± 8.2 ms in KA, n = 14, *P < 0.01) and the rise time (10–90%) (3.8 ± 0.4 ms in AMPA vs. 10.0 ± 2.3 ms in KA, n = 14, *P < 0.05) of KA EPSCs were significantly slower than those of the AMPA EPSCs (Fig. 3A–C). The slow kinetics of KA EPSCs in IC pyramidal neurons are similar to those reported in many brain areas, including spinal dorsal horn neurons (Li et al. 1999), hippocampal CA3 neurons (Castillo et al. 1997), thalamocortical neurons (Kidd and Isaac 1999), and ACC neurons (Wu et al. 2005).

To further characterize synaptic KA receptors, we studied the current-voltage (I-V) relationship of the KA EPSCs. The I-V relationship of KA receptors can reflect the calcium permeability and the subunit composition of channels (Egebjerg and Heinemann 1993; Ruano et al. 1995). In the presence of GYKI 53655, KA EPSCs were induced by single electric shocks. When recorded at various holding potentials ranging from −60 to +40 mV, KA EPSCs reversed at a potential of −0.15 ± 0.82 mV (n = 5 neurons/5 mice; Fig. 3, D and E).

The current recorded at the peak amplitude in relation to the holding potential was then plotted. Figure 3E illustrates the I-V curve of KA EPSCs in adult mouse IC neurons, which displays a strong outward rectification (Fig. 3, D and E). The mean rectification index of the KA EPSCs (ratio of estimated conductance at +40 and −60 mV) was 2.4 ± 0.8 (n = 5 neurons/5 mice). This finding suggests that the KA receptors involved in this response are likely of the edited, calcium-impermeable form, because unedited receptors show strong inward rectification (Burnashev et al. 1996; Seeburg 1996).

**Summation properties of KA receptor-mediated EPSCs during repetitive stimulation.** In most synapses, brief repetitive impulse trains greatly facilitate KA receptor-mediated EPSCs (Castillo et al. 1997; Mulle et al. 1998; Vignes et al. 1997; Wu et al. 2005). To determine the summation properties of KA receptor-mediated synaptic responses in the mouse IC, repetitive stimulations were applied by single, 5, 10, and 20 shocks at 200 Hz. As shown in Fig. 4A, in the presence of GYKI 53655, a small residual KA EPSC was significantly increased in amplitude after repetitive stimulation.
repetitive stimulation (24.8 ± 4.1 pA by 5 shocks, 27.8 ± 3.8 pA by 10 shocks, and 26.6 ± 3.8 pA by 20 shocks compared with 12.6 ± 2.4 pA by single stimulation, n = 15 neurons/12 mice, *P < 0.05; see Fig. 4). Ten and 20 shocks at a 200-Hz train significantly increased KA EPSCs compared with those induced by single shock. No additional increase was observed by 20 shocks compared with those obtained by 10 shocks, suggesting the saturation of the KA EPSCs (Fig. 4, A and B).

KA receptor-mediated EPSCs in WT, GluK1 KO, or GluK2 KO mice. Next, we determined the subunit composition of synaptic KA receptors in adult mouse IC neurons using mutant mice lacking GluK1 and GluK2 subunits. We employed two methods to evaluate the contribution of GluK1 and GluK2 in mediating the postsynaptic currents. First, we detected KA receptor-mediated currents induced by single stimulation in GluK1 and GluK2 KO mice and in GluK2 KO mice with bath application of a selective GluK1 antagonist, UBP 302 (10 μM) (Fig. 5, A–C). As a result, GluK1 or GluK2 KO mice partially reduced KA EPSCs (6.2 ± 1.3%, n = 8 neurons/6 GluK1 KO mice; 8.2 ± 1.5%, n = 9 neurons/7 GluK2 KO mice). Interestingly, KA EPSCs were significantly blocked in GluK2 KO mice with bath applied UBP 302 (4.1 ± 0.4%, n = 7 neurons/7 GluK2 KO mice, compared with WT group, *P < 0.05; Fig. 5D). These results suggest that both GluK1 and GluK2 receptors may play the functional roles in the IC.

We further analyzed the summated KA receptor-mediated currents induced by high-frequency stimulations (single, 5, 10, and 20 shocks at 200 Hz) in GluK1 and GluK2 KO mice (Fig. 6, A and B). High-frequency stimulations enhanced KA receptor-mediated currents in slices of WT, GluK1, GluK2 KO, and GluK2 KO + UBP 302 mice. As shown in Fig. 6, A and B, the summations of KA EPSCs induced by repetitive stimulations in GluK1 or GluK2 KO mice were partially reduced compared with those of WT mice, although there was not a significant difference in KA EPSCs between GluK1 and GluK2 KO mice (14.5 ± 2.1 pA by 5 shocks, 16.4 ± 2.8 pA by 10 shocks, and 16.7 ± 4.4 pA by 20 shocks, n = 6 neurons/GluK1 KO mice; 14.7 ± 3.1 pA by 5 shocks, 16.0 ± 3.9 pA by 10 shocks, and 17.6 ± 4.2 pA by 20 shocks, n = 7 neurons/GluK2 KO mice, *P > 0.05). On the other hand, in GluK2 KO mice with UBP 302, a dramatic decrease in KA receptor-mediated currents was also observed (6.6 ± 1.5 pA by 5 shocks, 6.9 ± 1.0 pA by 10 shocks, and 6.4 ± 0.6 pA by 20 shocks, n = 6 neurons/GluK2 KO mice with UBP 302, *P < 0.05 compared with WT), suggesting that both GluK1 and GluK2 receptors mediate the KA EPSCs. To further confirm the results, we compared the input (stimulation intensity)-output (KA EPSC amplitude) relationship of KA EPSCs in WT and KO mice. As shown in Fig. 6C, both GluK1 and GluK2 KO mice showed slightly decreased KA EPSCs (P > 0.05, n = 6 neurons/GluK1 KO, n = 6 neurons/GluK2 KO). Furthermore, GluK2 KO mice with UBP 302 significantly decreased KA EPSCs compared with WT (n = 10 neurons/10 WT mice, n = 6 neurons/GluK2 KO mice with UBP 302, *P < 0.05). Taken together, these results indicate that both GluK1 and GluK2 underlie the synaptic KA receptor-mediated current in the IC.

Comparison of KA receptor-mediated EPSCs in layers I, II/III, and V/VI in mice IC neurons. To further study KA receptor function in other layers of the IC, we next recorded neurons in layer I and V/VI (Fig. 7). By applying local stimulation in layer II/III of the IC, we observed fast EPSCs recorded at baseline (a) and after application of GYKI 53655 (b) in GluK1 KO mice (A). GluK2 KO mice (B), and GluK2 KO mice with UBP 302 (10 μM), a selective GluK1 antagonist (C). D: pooled data of KA-EPSCs in WT (n = 14), GluK1 KO (n = 8), GluK2 KO (n = 9), and GluK1 KO + UBP 302 mice (n = 7). The averaged amplitude of KA-mediated currents in WT and GluK2 KO + UBP 302 mice were significantly different (Dunnett’s test, *P < 0.05).


DISCUSSION

To our knowledge, the current work represents the first demonstration that KA subtype receptors contribute to fast excitatory synaptic transmission in IC neurons of adult rodents. Using whole cell patch-clamp recordings from IC neurons of brain slices, we studied functional KA receptors using single or train stimulation-evoked EPSCs. We mainly recorded neurons in layer II/III, as well as in layers I and V/VI, to detect electrophysiological mapping of postsynaptic KA functions in the IC. IC neurons in all layers have the functional KA receptors. Furthermore, using GluK1 or GluK2 KO mice, we provide the first evidence that both GluK1 and GluK2 contribute to functional synaptic KA receptors in layer II/III neurons. Considering the cumulative physiological evidence for the role of the IC in pain and higher brain functions, the present study provides important synaptic mechanisms for KA receptors in the IC-related physiological and pathological functions.

KA-mediated current in insular cortex. In the present study, the contribution of KA EPSCs evoked by single stimulations was very small in adult mouse IC neurons (~5–10% of AMPA KA-mediated currents in all layers of the IC in mice and in layer II/III in rats). The contribution of KA EPSCs in adult mice was similar in adult rats (Fig. 2). Moreover, saturated KA-mediated EPSCs were nearly 30 pA after high-frequency repetitive stimulations (Fig. 4). A small percentage of KA EPSCs compared with AMPA KA EPSCs has been reported in many preparations: for example, Golgi cells in the cerebellum (Bureau et al. 2000) and layer V pyramidal neurons of the IC. IC neurons in all layers have the functional KA receptors. Furthermore, using GluK1 or GluK2 KO mice, we provide the first evidence that both GluK1 and GluK2 contribute to functional synaptic KA receptors in layer II/III neurons. Considering the cumulative physiological evidence for the role of the IC in pain and higher brain functions, the present study provides important synaptic mechanisms for KA receptors in the IC-related physiological and pathological functions.
Rogawski 1998). Possible explanations are that calcium permeability of GluK receptors in the IC may be different from that in other areas (Burnashev et al. 1996; Seeburg 1996). RNA editing generates different forms for KA receptor subunits GluK1 and GluK2. These subunits carry alternative residues in the Q/R site of their M2 region (Sommer et al. 1991). The Q/R site in GluK1 and GluK2 is important for calcium permeability of the glutamate-activated channel (Egebjerg and Heinemann 1993; Köhler et al. 1993). Indeed, strong outward rectification of the I-V curve for KA EPSC was observed in adult IC neurons (Fig. 3). Similar results were reported in pyramidal neurons in layer II/III and layer V in motor cortex (Ali 2003) and in layer II/III in the ACC (Wu et al. 2005). The use of adult mice in the present study may partially explain the small amplitude of KA EPSCs. The Q/R site in GluK1 and GluK2 is subject to developmentally controlled pre-mRNA editing (Seeburg 1996). For example, GluK1 transcripts are edited by shifting 6–50% during embryonal day 15 in adult rats in hippocampus. For GluK2, 30–70% of Q/R site in GluK2 KO is edited in the embryonal day 15 to adult rat in hippocampus. KA receptors at neocortical synapses during this developmental period also exhibit a strongly inward rectifying I-V rela-

Fig. 7. Recordings of KA EPSCs from layers I, V/VI, and II/III in IC. A: recording diagram of layer I neurons and stimulation in layer II/III of adult mouse IC. B: recording diagram of layer V/VI pyramidal neurons and stimulation in layer II/III of adult mouse IC. C and D: in layer I (C) and layer V/VI (D), AMPA/KA EPSCs were recorded in the presence of PTX (100 μM) and AP-5 (50 μM) (baseline; a). After the perfusion of GYKI 53655 (100 μM), a small residual current remained (b) that could be totally blocked by CNQX (20 μM). Sample points show the time course of GYKI 53655 and CNQX effects on this neuron (c). E: pooled data of KA EPSCs in layer I (n = 7), V/VI (n = 8), and II/III neurons (n = 15).
KA receptor-mediated EPSC kinetics. In adult IC slices, we observed slow kinetics of KA EPSCs compared with AMPA EPSCs in the same cells. This result is similar to those in other central nervous systems (Ali 2003; Bureau et al. 2000; Cossart et al. 1998; DeVries and Schwartz 1999; Frerking et al. 1998; Kidd and Isaac 1999; Li et al. 1999; Wu et al. 2005). It is generally accepted that the kinetics of KA EPSCs might be due to intrinsic properties of these postsynaptic receptors (Lerma 2003). What other mechanisms might explain the slow kinetics? One possibility is that KA receptors interact with proteins at the synapse and that this causes a change in their kinetic properties (Bowie et al. 2003; Garcia et al. 1998; Straub et al. 2011a, 2011b; Zhang et al. 2009). Recently, neuropilin and tolloid-like (Neto) family were identified as a novel accessory subunit of KA receptors (Zhang et al. 2009). The KA receptor auxiliary subunit Neto1 regulates the slow kinetics in hippocampus (Straub et al. 2011a). Neto2 interacts specifically with recombinant and native GluK1 and GluK2 and modulates KA receptor currents in vitro and in vivo (Straub et al. 2011b). Furthermore, a study has provided evidence for this by showing that interactions of GluK2 homomers or GluK2/GluK5 heteromers with the postsynaptic density protein SAP 90 produced receptors that did not fully desensitize in response to glutamate (Bowie et al. 2003; Garcia et al. 1998).

However, KA EPSCs evoked by single, repetitive stimulations at 200 Hz and their input-output relationships were not...
completely blocked by genetic deletions of GluK2 receptor combined with the application of GluK1 antagonist. We consider that the residual components of KA EPSCs may be mediated by other KA receptor subunits or heteromeric KA receptors, since GluK1–5 receptors mRNA subunit widely express in many cortical regions and hippocampus (Bahn et al. 1994). KA receptors are known to be composed of both homomeric and heteromeric coassemblies such as GluK1, GluK2, GluK3, GluK1/GluK5, GluK2/GluK5, and GluK1/ GluK2 (Hollmann and Heinemann 1994; Jane et al. 2009; Lerma et al. 2001). Coassemblies with GluK1 and GluK2 or GluK1/GluK2 combined with other subunits in the IC still remain unclear. To dissect the exact composition of KA receptors in the IC, KO mice selective for the other KA receptor subunits as well as selective pharmacological tools are needed in future studies. Another possibility is that synaptic KA receptors are heteromers that have novel properties and that these combinations of subunits are not found for the native extrasynaptic or heterologously expressed KA receptors studied so far. The properties of GluK2 homomers are well known; they exhibit rapid kinetics similar to AMPA receptors (Herb et al. 1992; Swanson et al. 1996; Swanson and Heinemann 1998). Although there is no information on GluK1(Q)/GluK3/GluK5 heteromers, it is known that GluK1(Q)/GluK5 also exhibits rapid kinetics (Herb et al. 1992). However, coassembly of different subunits can cause alterations in the properties of the receptor complex (Cui and Mayer 1999; Herb et al. 1992), and GluK1-containing receptors exhibit variations in kinetics, for example, in the rate of recovery from desensitization (Swanson and Heinemann 1998). These properties could provide potential mechanisms for the slow kinetics of synaptic KA receptors.

Functional roles of IC and possible involvement of KA receptors. Recent studies from both humans and animals suggest that the IC is important for the processing of sensory information, memory, emotion, and other higher order brain functions (Calejesan et al. 2000; Casey et al. 1996; Craig 2009; Davis et al. 1997, 2000; Devinsky et al. 1995; Donahue et al. 2001; Eisenberger et al. 2003). Previous results have shown that the IC is involved in pain and fear memory in rodents (Shema et al. 2011; Wei et al. 2001, 2002). In addition, a previous anatomical study showed that the IC receives input from the thalamus, amygdala, hippocampus, and the ACC and communicates with these brain nuclei (Jasmin et al. 2004). It is well known that the amygdala, hippocampus, and ACC play important roles in pain and fear memory (Johansen et al. 2011; Zhuo 2008). Our previous studies have indicated that different subunits of KA receptors show distinct behavioral phenotypes in pain and fear memory (Ko et al. 2005). GluK2 KO mice showed a significant impairment in synaptic plasticity within the amygdala and auditory cortex and a deficit in fear memory testing (Ko et al. 2005). On the other hand, mice lacking GluK1 had significantly reduced responses to capsaicin or inflammatory pain (Ko et al. 2005). Combined with evidence from these previous studies, the findings of this study indicate that the functional GluK1 and GluK2 receptors may underlie the synaptic basis for IC-related brain functions, such as pain and memory.

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DISCLOSURES
The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS
K.K. and M.Z. conception and design of research; K.K., S.-E.S., T.C., and L.-J.W. performed experiments; K.K., S.-E.S., T.C., and L.-J.W. analyzed data; K.K. and L.-J.W. prepared figures; K.K. and M.Z. drafted manuscript; L.-J.W. and M.Z. interpreted results of experiments; B.-K.K. and M.Z. edited and revised manuscript; M.Z. approved final version of manuscript.

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