Published in final edited form as:

Neurosci Lett. 2010 January 14; 468(3): 186. doi:10.1016/j.neulet.2009.10.071.

Deletion of the GluR5 subunit of kainate receptors affects cocaine sensitivity and preference

Ann M. Gregus¹, Thomas F. Tropea², Yanran Wang¹, Stefanie C. R. Hauck², Alberto C.S. Costa⁴, Anjali M. Rajadhyaksha^{2,3}, and Charles E. Inturrisi^{1,3,*}

¹Department of Pharmacology, Weill Cornell Medical College, New York, NY 10065, U.S.A.

²Department of Pediatric Neurology/Pediatrics, Weill Cornell Medical College, New York, NY 10065, U.S.A.

³Department of Neuroscience, Weill Cornell Medical College, New York, NY 10065, U.S.A.

⁴Departments of Medicine and Psychiatry and Neuroscience Training Program, University of Colorado at Denver and the Health Sciences Center, Denver, Colorado, U.S.A.

Abstract

We have demonstrated previously that mice expressing a constitutive deletion of the kainate receptor subunit GluR5 (GluR5 KO) do not differ from wildtype (WT) littermates of a congenic C57BL/6 background with regard to both the development of morphine physical dependence as measured by naloxone-precipitated withdrawal signs and to morphine reward as revealed by the expression of conditioned place preference (CPP). However, unlike WT, GluR5 KO mice fail to develop antinociceptive tolerance following repeated systemic morphine administration. In this report, we examined the impact of GluR5 deletion on cocaine-mediated CPP and locomotor sensitization. Expression of CPP was evident in WT mice following repeated daily administration of 20 mg/kg (but not 10 mg/kg) i.p. cocaine. Interestingly, GluR5 KO mice exhibited enhanced cocaine preference as compared with WT mice at both 10 and 20 mg/kg doses. In addition, while GluR5 KO mice did not differ from WT with respect to baseline locomotor activity, mutant mice demonstrated increased locomotor hyperactivity versus WT mice after repeated injection of 15 mg/kg i.p. cocaine. Collectively, these data indicate that GluR5 appears to negatively modulate some psychostimulant and rewarding properties of cocaine, as demonstrated by heightened sensitization and salience in mutant mice.

Keywords

Cocaine; Kainate receptor knockout mouse; ionotropic GluR5 subunit; conditioned place preference; locomotor sensitization

© 2009 Elsevier Ireland Ltd. All rights reserved.

*Corresponding author: Department of Pharmacology, Weill Cornell Medical College, Room LC-524, 1300 York Avenue, New York, NY, 10065 Phone (212) 746-6235 Fax (212) 746-8835 ceintur@med.cornell.edu. Reprint requests to: Dr. Charles E. Inturrisi, Department of Pharmacology, Weill Cornell Medical College, Room LC-524, 1300 York Avenue, New York, NY 10065.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

GluR5 is a subunit of the kainate subtype of ionotropic glutamate receptors (iGluR), which participate in fast excitatory neurotransmission. Kainate receptors occur either as homomeric or heteromeric combinations of GluR5, GluR6 and GluR7 or as heteromers of GluR5/6/7 with KA1 or KA2 subunits [13]. Several reports suggest the potential involvement of KAR in cocaine-mediated locomotor sensitization and reward. For example, pretreatment with the AMPA/KA receptor antagonist DNQX blocked the induction, but not the expression, of cocaine locomotor sensitization in rodents [8]. The development of cocaine-induced hyperlocomotion in sensitized rats also was prevented by prior treatment with the AMPA/KA receptor antagonist CNQX microinjected into the nucleus accumbens (NAc) core [21]. In addition, DNQX inhibited expression, but not induction, of CPP to cocaine in rats when administered just before the test phase [7].

Subsequent studies implicate GluR2/GluR5-containing KAR in particular with regard to the manifestation of cocaine-induced addictive behaviors [12], demonstrating that infusion of LY293558 into the NAc core of rats produced a dose-dependent reduction of cocaine self-administration. Support for a specific role of GluR5 in cocaine-mediated plasticity was provided by the observation that mRNA and protein of the GluR5 subunit of KAR are increased in the dorsal prefrontal cortex (PFC) of rats following 3 weeks of withdrawal from chronic cocaine treatment [28].

It is tempting to speculate from these reports that neuroadaptive responses to repeated cocaine are mediated by KAR, particularly those comprised of GluR5 subunits. However, the majority of the studies cited above utilized quinoxalinedione antagonists that do not distinguish AMPAR and KAR. Furthermore, although LY293558 is selective for GluR5, it also binds GluR2-containing AMPA iGluR [3,27]. We have shown previously that constitutive deletion of the GluR5 subunit prevents the development of antinociceptive tolerance without altering morphine physical dependence, locomotor activity or reward [4]. To avoid confounds associated with the use of antagonists, we utilized the GluR5 KO mouse model to assess the role of GluR5 in cocaine-mediated locomotor sensitization and reward.

Materials and Methods

Generation and backcrossing of GluR5 knockout (GluR5 KO) mice

GluR5 KO mice were produced on a 129/SvEv background [19] and backcrossed with C57BL/6 mice in parallel with their WT littermates as described [4].

Mice

Male WT and GluR5 KO mice (n = 8-12, 8-12 weeks old, 25-30g) were maintained under climate-controlled conditions on a 12 h light/dark cycle with free access to food and water. Our studies were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. For behavioral experiments, mice were handled and weighed daily and acclimated to the testing environment. The investigator was blinded to the identity and treatment of the mice.

Drugs

Cocaine hydrochloride was obtained from the Research Triangle Institute (Research Triangle Park, NC) through the National Institute on Drug Abuse (Rockville, MD). The doses of cocaine (10, 15 or 20 mg/kg i.p.) were calculated as free base, dissolved in saline with the pH adjusted to 7.0, then injected in a volume of 0.1 ml/10 g of body weight.

Conditioned Place Preference (CPP)

Cocaine preference was assessed as described previously [29] using a three-chamber place preference apparatus (Med Associates Inc., St. Albans, VT). On day 1 (preconditioning), naïve WT or GluR5 KO mice were placed in the central gray chamber for acclimation, then allowed unobstructed access to all three chambers for 20 minutes. Mice were screened such that those exhibiting apparatus bias (initial preference for any chamber in the absence of treatment) were removed from the study, while those remaining (about 60%) were included in subsequent analyses. On days 2-4 (conditioning), these mice were injected with cocaine (10 or 20 mg/kg i.p.) and then randomly confined to either the black or the white chamber for 20 minutes (a.m. session). Four hours later, mice were given saline i.p. and confined to the chamber where they had not received cocaine (p.m. session). On day 5 (test), in the absence of drug treatment, mice were again placed in the central chamber for acclimation, followed by 20 minutes of free exploration, in which time spent in each chamber was recorded. Preference was defined as time spent in the cocaine-paired chamber on the test day minus time spent in the cocaine-paired side on the preconditioning day, reported as the difference score. We included the higher dose of cocaine (20 mg/kg i.p.) because 10 mg/kg did not induce place preference in WT mice. In an effort to minimize the number of animals used in the study, we selected this dose based on the prediction that it would produce more robust CPP than 15 mg/kg.

Locomotor Sensitization

On each of days 1-5, WT and GluR5 KO mice were allowed to acclimate to open-field locomotor activity chambers (Med Associates Inc., St. Albans, VT, USA) for 15 minutes. Mice were removed from the chamber for injection with 15 mg/kg i.p. cocaine, and then returned to the chamber for measurement of locomotor activity for 30 minutes as total distance traveled in cm. Following a 2-week period of abstinence from cocaine, on day 19 (challenge), mice were again placed in the open field chamber for acclimation, removed for injection with cocaine 15 mg/kg i.p., and then reintroduced into the chamber for 30 minutes to monitor locomotor behavior. This dosing regimen was utilized because it produced the most consistent locomotor sensitization in WT mice as revealed by dose-response studies with 10, 15 and 20 mg/kg i.p. cocaine.

Statistical analysis

Data were expressed as the mean $\pm$ S.E.M. and graphed using GraphPad Prism 4 software (GraphPad Inc., La Jolla, CA). p values were determined in Statview (Adept Scientific Plc., Acton, MA) as follows: two-way ANOVA (CPP difference scores) and two-way repeated measures ANOVA (locomotor sensitization). Bonferroni/Dunn post-hoc analysis was used in conjunction with all forms of ANOVA. A value of $p < 0.05$ was considered significant.

Results

Enhancement of Cocaine-mediated Conditioned Place Preference and Locomotor Sensitization in GluR5 KO mice

First, we assessed the development of CPP in WT and GluR5 KO mice as a function of i.p. cocaine dose (10 or 20 mg/kg). Difference scores for each dose in WT and GluR5 KO mice were calculated as the amount of time spent in the cocaine-paired chamber on test day minus that of the preconditioning day and were subjected to two-way ANOVA analysis (Figure 1). Comparison of difference scores (Figure 1) by two-way ANOVA revealed a main effect of genotype ($F_{1,48} = 12.542$, $***p < 0.001$) but not of cocaine dose ($F_{1,48} = 0.086$, $p > 0.05$) and no interaction of genotype and dose ($F_{1,48} = 1.477$, $p > 0.05$). Bonferroni/Dunn post-hoc analysis revealed that GluR5 KO mice exhibited a significantly greater chamber preference than WT mice at 10 mg/kg, i.p. cocaine (WT 117.0 ± 73.8 seconds, GluR5 KO 241.1 ± 58.0

seconds, $**p < 0.01$) and 20 mg/kg, i.p. cocaine (WT 108.8 ± 60.8 seconds, GluR5 KO 230.6 ± 29.1 seconds, $*p < 0.05$).

Assessment of locomotor activity revealed that pre-drug baseline activity measured on days 1-5 and on challenge day 19 did not differ between WT and GluR5 KO mice (Table 1). A two-way repeated measures ANOVA revealed a main effect of day ($F_{1,95} = 14.038$, $****p < 0.0001$) and genotype ($F_{1,95} = 6.476$, $*p < 0.05$) but no interaction of day and genotype ($F_{1,95} = 1.439$, $p > 0.05$) on cocaine-induced locomotor sensitization. Figure 2 depicts distance traveled over a 30-minute interval following 15 mg/kg i.p. cocaine on days 1 and 5 of a repeated daily administration regimen and on day 19 after a two week withdrawal. Comparison of acute locomotor response to cocaine on day 1 between WT and GluR5 KO mice revealed no significant difference between genotypes ($p > 0.05$). Following repeated cocaine treatment, WT mice exhibited an increase in distance traveled on day 5 (11612 ± 564 cm) relative to day 1 (2874 ± 214 cm, $*p < 0.05$), signifying induction of sensitization. Similarly on day 19, following a two-week withdrawal period, WT mice exhibited significantly greater distance traveled (11983 ± 1243 cm; $*p < 0.05$) compared to day 1, indicating sustained expression of sensitization in WT mice. Like WT littermates, GluR5 KO mice exhibited both induction of cocaine sensitization, as demonstrated by elevated locomotor activity following 15 mg/kg i.p. cocaine on day 5 (15336 ± 875 cm) versus day 1 (2877 ± 163 cm, $****p < 0.001$) and expression of sensitization, as demonstrated by significantly greater locomotor activity on day 19 (17095 ± 826 cm; $****p < 0.0001$) compared to day 1. Post-hoc comparisons between genotypes revealed that GluR5 KO mice demonstrated significantly greater locomotor activity compared with WT mice on day 5 ($^{\dagger\dagger}p < 0.01$) and on challenge day 19 ($^{\dagger}p < 0.01$) (Figure 2).

Discussion

In this report, we describe behavioral phenotypes of GluR5 KO mice with respect to cocaine-mediated conditioned place preference and locomotor sensitization. WT mice clearly demonstrate CPP following repeated daily i.p. administration of cocaine at a dose of 20 mg/kg. Interestingly, GluR5 KO mice exhibit a phenotype of enhanced cocaine-mediated CPP compared with WT at both 10 and 20 mg/kg (Figure 1). Furthermore, GluR5 KO mice also demonstrate increased locomotor sensitivity to cocaine versus WT mice in the absence of any difference in baseline locomotor activity (Figure 2 and Table 1). These findings are surprising given prior accounts demonstrating inhibition by antagonists of AMPA/KA receptors of both expression of CPP and initiation of locomotor sensitization after repeated administration of cocaine [7,8,12,14,16,21,28]. The major excitatory circuit involved in the rewarding and psychomotor effects of cocaine is comprised of corticofugal glutamatergic afferents from the prefrontal cortex (including the anterior cingulate cortex, or ACC), hippocampus and the basolateral amygdala (BLA), which innervate the nucleus accumbens (NAc) and the ventral tegmental area (VTA) [30]. If the observed effects of AMPA/KA receptor antagonists on neuroadaptive responses to cocaine resulted from inhibition of GluR5 KAR located in the relevant mesocorticolimbic brain regions, one might expect that the deletion of GluR5 would result in decreased sensitivity to cocaine. However, GluR5 KO mice instead exhibit greater cocaine sensitivity, indicating that the attenuation observed with these mixed inhibitors more likely reflects consequences of AMPAR blockade.

The mechanisms by which GluR5-containing KAR contribute to the observed increase in behavioral sensitivity to cocaine are currently undefined. Given the current findings, it is surprising that GluR5 expression is negligible both in the NAc [1] and in the VTA [2], brain regions that are critically involved in the generation of adaptive behavioral responses to psychostimulants [15]. However, GluR5-containing KAR are present in other brain areas believed to mediate cocaine reward including (but not limited to) pyramidal neurons of the ACC [35], interneurons of the BLA [34], cortical afferents projecting to the NAc [1,6], CA1

region of the hippocampus [10,11] and discrete areas of the ventral basal ganglia such as the ventral pallidum [2,33]. Notably it has been demonstrated that in some of these regions, pharmacological antagonism or deletion of GluR5 reduces spontaneous GABA release [6,10,34-36]. These observations suggest that at certain synapses, presynaptically-localized GluR5 modulates GABAergic neurotransmission, in contrast to its classical role of mediating postsynaptic glutamatergic signaling. The consequences of GluR5 activation in these areas are unknown, yet these synaptic arrangements are suggestive of functional interactions between glutamatergic, GABAergic and dopaminergic systems that may be modified by recurrent exposure to cocaine.

While the nature of these interactions remain to be determined, it is possible that with repeated cocaine administration followed by subsequent withdrawal, the absence of GluR5 may accelerate the loss of inhibitory tone which, under conditions of dopamine subsensitivity, may result in reduced opposition to glutamatergic transmission. The cocaine-induced increase in excitatory drive may be augmented in GluR5 KO mice, potentially contributing to their heightened sensitivity to cocaine. As the present studies were conducted with systemic administration of cocaine, future experiments utilizing targeted delivery of cocaine into specific brain regions in conjunction with spatial-temporal transgenic approaches may further elucidate the role of GluR5 in cocaine sensitivity. Moreover, in light of the presynaptic localization of GluR5 in several brain areas associated with drug reward, it would be of interest to examine the effect of repeated cocaine on glutamate and GABA release in a region-specific manner.

Also noteworthy is our observation that while cocaine reward and preference are augmented in GluR5 KO compared with WT mice, behavioral sensitization to morphine is unaffected by the deletion of GluR5 [4]. However, the mechanisms contributing to the differential sensitivity of GluR5 KO mice to repeated administration of morphine or cocaine remain to be determined. Drug-specific expression of reward has been described in certain knockout mouse lines, such as those lacking CB1 cannabinoid receptors [18], β arrestin-2 [5], μ opioid receptors [37], downstream regulatory element antagonistic modulator (DREAM) [9], Neurokinin 1 (NK1) receptors [22] or cAMP response element binding protein (CREB) $\alpha\Delta$ isoforms [31,32]. In addition, Δ FosB induction has been observed in VTA following chronic administration of cocaine but not morphine [20]. An alternative explanation for these phenotypes may be derived from the divergent structural changes produced by cocaine and morphine, for example with regard to dendritic spine density and branching [23-26]. As glutamate is known to produce changes in spine morphology [17], it is possible that such processes may be modulated by GluR5 and that its absence may amplify the structural modifications observed following repeated exposure to cocaine. However, further investigation is needed in order to establish a causal link between these molecular changes and compulsive behaviors associated with drug addiction.

In summary, the current findings suggest specific involvement of GluR5 in negative modulation of cocaine sensitivity and preference. Future studies with GluR5 KO mice could provide information about the mechanisms of cocaine-mediated behavioral sensitization and may lend insight into drug-specific reward pathways.

Acknowledgments

This work was supported in part by NIDA grants DA001457 and DA000198 (CEI), NIDA training grant DA007274 (AG), and NIDA center grant DA005130 (CEI).

References

- [1]. Bettler B, Boulter J, Hermans-Borgmeyer I, O'Shea-Greenfield A, Deneris ES, Moll C, Borgmeyer U, Hollmann M, Heinemann S. Cloning of a novel glutamate receptor subunit, GluR5: expression in the nervous system during development. *Neuron* 1990;5:583–595. [PubMed: 1977421]
- [2]. Bischoff S, Barhanin J, Bettler B, Mulle C, Heinemann S. Spatial distribution of kainate receptor subunit mRNA in the mouse basal ganglia and ventral mesencephalon. *J Comp Neurol* 1997;379:541–562. [PubMed: 9067842]
- [3]. Bleakman R, Schoepp DD, Ballyk B, Bufton H, Sharpe EF, Thomas K, Ornstein PL, Kamboj RK. Pharmacological discrimination of GluR5 and GluR6 kainate receptor subtypes by (3S,4aR,6R,8aR)-6-[2-(1(2)H-tetrazole-5-yl)ethyl]decahydroisdoquinoline-3 carboxylic-acid. *Mol Pharmacol* 1996;49:581–585. [PubMed: 8609884]
- [4]. Bogulavsky JJ, Gregus AM, Kim PT, Costa AC, Rajadhyaksha AM, Inturrisi CE. Deletion of the glutamate receptor 5 subunit of kainate receptors affects the development of morphine tolerance. *J Pharmacol Exp Ther* 2009;328:579–587. [PubMed: 18957577]
- [5]. Bohn LM, Gainetdinov RR, Sotnikova TD, Medvedev IO, Lefkowitz RJ, Dykstra LA, Caron MG. Enhanced rewarding properties of morphine, but not cocaine, in beta(arrestin)-2 knock-out mice. *J Neurosci* 2003;23:10265–10273. [PubMed: 14614085]
- [6]. Casassus G, Mulle C. Functional characterization of kainate receptors in the mouse nucleus accumbens. *Neuropharmacology* 2002;42:603–611. [PubMed: 11985817]
- [7]. Cervo L, Samanin R. Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. *Brain Res* 1995;673:242–250. [PubMed: 7606438]
- [8]. Cervo L, Samanin R. Effects of dopaminergic and glutamatergic receptor antagonists on the establishment and expression of conditioned locomotion to cocaine in rats. *Brain Res* 1996;731:31–38. [PubMed: 8883851]
- [9]. Cheng HY, Laviolette SR, van der Kooy D, Penninger JM. DREAM ablation selectively alters THC place aversion and analgesia but leaves intact the motivational and analgesic effects of morphine. *Eur J Neurosci* 2004;19:3033–3041. [PubMed: 15182311]
- [10]. Christensen JK, Paternain AV, Selak S, Ahring PK, Lerma J. A mosaic of functional kainate receptors in hippocampal interneurons. *J Neurosci* 2004;24:8986–8993. [PubMed: 15483117]
- [11]. Clarke VR, Collingridge GL. Characterisation of the effects of ATPA, a GLU(K5) kainate receptor agonist, on GABAergic synaptic transmission in the CA1 region of rat hippocampal slices. *Neuropharmacology* 2004;47:363–372. [PubMed: 15275825]
- [12]. Di Ciano P, Everitt BJ. Dissociable effects of antagonism of NMDA and AMPA/KA receptors in the nucleus accumbens core and shell on cocaine-seeking behavior. *Neuropsychopharmacology* 2001;25:341–360. [PubMed: 11522463]
- [13]. Dingledine R, Borges K, Bowie D, Traynelis SF. The glutamate receptor ion channels. *Pharmacol Rev* 1999;51:7–61. [PubMed: 10049997]
- [14]. Gass JT, Olive MF. Glutamatergic substrates of drug addiction and alcoholism. *Biochem Pharmacol* 2008;75:218–265. [PubMed: 17706608]
- [15]. Kalivas PW, Volkow ND. The neural basis of addiction: a pathology of motivation and choice. *Am J Psychiatry* 2005;162:1403–1413. [PubMed: 16055761]
- [16]. Karler R, Calder LD, Bedingfield JB. Cocaine behavioral sensitization and the excitatory amino acids. *Psychopharmacology (Berl)* 1994;115:305–310. [PubMed: 7871069]
- [17]. Lippman J, Dunaevsky A. Dendritic spine morphogenesis and plasticity. *J Neurobiol* 2005;64:47–57. [PubMed: 15884005]
- [18]. Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O. Cocaine, but not morphine, induces conditioned place preference and sensitization to locomotor responses in CB1 knockout mice. *Eur J Neurosci* 2000;12:4038–4046. [PubMed: 11069600]
- [19]. Mulle C, Sailer A, Swanson GT, Brana C, O'Gorman S, Bettler B, Heinemann SF. Subunit composition of kainate receptors in hippocampal interneurons. *Neuron* 2000;28:475–484. [PubMed: 11144357]

- [20]. Perrotti LI, Weaver RR, Robison B, Renthal W, Maze I, Yazdani S, Elmore RG, Knapp DJ, Selley DE, Martin BR, Sim-Selley L, Bachtell RK, Self DW, Nestler EJ. Distinct patterns of DeltaFosB induction in brain by drugs of abuse. *Synapse* 2008;62:358–369. [PubMed: 18293355]
- [21]. Pierce RC, Bell K, Duffy P, Kalivas PW. Repeated cocaine augments excitatory amino acid transmission in the nucleus accumbens only in rats having developed behavioral sensitization. *J Neurosci* 1996;16:1550–1560. [PubMed: 8778304]
- [22]. Ripley TL, Gadd CA, De Felipe C, Hunt SP, Stephens DN. Lack of self-administration and behavioural sensitisation to morphine, but not cocaine, in mice lacking NK1 receptors. *Neuropharmacology* 2002;43:1258–1268. [PubMed: 12527475]
- [23]. Robinson TE, Kolb B. Alterations in the morphology of dendrites and dendritic spines in the nucleus accumbens and prefrontal cortex following repeated treatment with amphetamine or cocaine. *Eur J Neurosci* 1999;11:1598–1604. [PubMed: 10215912]
- [24]. Robinson TE, Kolb B. Morphine alters the structure of neurons in the nucleus accumbens and neocortex of rats. *Synapse* 1999;33:160–162. [PubMed: 10400894]
- [25]. Robinson TE, Kolb B. Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology* 2004;47(Suppl 1):33–46. [PubMed: 15464124]
- [26]. Russo SJ, Mazei-Robison MS, Ables JL, Nestler EJ. Neurotrophic factors and structural plasticity in addiction. *Neuropharmacology* 2009;56(Suppl 1):73–82. [PubMed: 18647613]
- [27]. Simmons RM, Li DL, Hoo KH, Deverill M, Ornstein PL, Iyengar S. Kainate GluR5 receptor subtype mediates the nociceptive response to formalin in the rat. *Neuropharmacology* 1998;37:25–36. [PubMed: 9680256]
- [28]. Toda S, McGinty JF, Kalivas PW. Repeated cocaine administration alters the expression of genes in corticolimbic circuitry after a 3-week withdrawal: a DNA macroarray study. *J Neurochem* 2002;82:1290–1299. [PubMed: 12358776]
- [29]. Tropea TF, Kosofsky BE, Rajadhyaksha AM. Enhanced CREB and DARPP-32 phosphorylation in the nucleus accumbens and CREB, ERK, and GluR1 phosphorylation in the dorsal hippocampus is associated with cocaine-conditioned place preference behavior. *J Neurochem* 2008;106:1780–1790. [PubMed: 18554320]
- [30]. Vanderschuren LJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. *Psychopharmacology (Berl)* 2000;151:99–120. [PubMed: 10972458]
- [31]. Walters CL, Blendy JA. Different requirements for cAMP response element binding protein in positive and negative reinforcing properties of drugs of abuse. *J Neurosci* 2001;21:9438–9444. [PubMed: 11717377]
- [32]. Walters CL, Kuo YC, Blendy JA. Differential distribution of CREB in the mesolimbic dopamine reward pathway. *J Neurochem* 2003;87:1237–1244. [PubMed: 14622103]
- [33]. Wisden W, Seeburg PH. A complex mosaic of high-affinity kainate receptors in rat brain. *J Neurosci* 1993;13:3582–3598. [PubMed: 8393486]
- [34]. Wu LJ, Ko SW, Toyoda H, Zhao MG, Xu H, Vadakkan KI, Ren M, Knifed E, Shum F, Quan J, Zhang XH, Zhuo M. Increased Anxiety-Like Behavior and Enhanced Synaptic Efficacy in the Amygdala of GluR5 Knockout Mice. *PLoS ONE* 2007;2:e167. [PubMed: 17245443]
- [35]. Wu LJ, Xu H, Ren M, Zhuo M. Genetic and pharmacological studies of GluR5 modulation of inhibitory synaptic transmission in the anterior cingulate cortex of adult mice. *Dev Neurobiol* 2007;67:146–157. [PubMed: 17443779]
- [36]. Wu LJ, Zhao MG, Toyoda H, Ko SW, Zhuo M. Kainate receptor-mediated synaptic transmission in the adult anterior cingulate cortex. *J Neurophysiol* 2005;94:1805–1813. [PubMed: 15928066]
- [37]. Yoo JH, Yang EM, Lee SY, Loh HH, Ho IK, Jang CG. Differential effects of morphine and cocaine on locomotor activity and sensitization in mu-opioid receptor knockout mice. *Neurosci Lett* 2003;344:37–40. [PubMed: 12781916]

Figure 1. GluR5 KO mice display enhanced conditioned place preference to cocaine

Conditioned Place Preference (CPP) was examined in WT and GluR5 KO mice after a 4-day conditioning period with cocaine 10 or 20 mg/kg i.p.. After 10 mg/kg i.p. cocaine, GluR5 KO (but not WT) mice exhibited a significant increase (* $p < 0.05$) in difference score calculated as time spent on the cocaine-paired side on the test day minus preconditioning, indicating the expression of CPP in GluR5 KO but not WT mice at this dose. Cocaine 20 mg/kg i.p. induced CPP in both WT (* $p < 0.05$) and GluR5 KO mice (* $p < 0.05$), but preference was enhanced in GluR5 KO compared with WT (** $p < 0.01$).

Figure 2. GluR5 KO mice display enhanced locomotor sensitization to cocaine

Locomotor sensitivity to cocaine 15 mg/kg was examined in WT and GluR5 KO mice. Following administration of 15 mg/kg i.p. cocaine, WT mice exhibited an increase in distance traveled on day 5 (* $p < 0.05$) and on day 19 (* $p < 0.05$) compared with day 1, signifying induction and sustained expression of sensitization. GluR5 KO mice also exhibited an enhancement of cocaine-mediated locomotor activity on day 5 (*** $p < 0.001$) and on day 19 (**** $p < 0.0001$) versus day 1, which was significantly greater than WT on day 5 (†† $p < 0.01$) and on challenge day 19 (†† $p < 0.01$).

TABLE 1
WT and GluR5 KO mice do not differ with respect to baseline locomotor activity

Baseline distance traveled (cm) during a 15 minute period (mean $\pm$ S.E.M.) was measured by open-field activity monitors in WT and GluR5 KO mice on each of days 1-5 and challenge (day 19) immediately prior to cocaine administration. There was no significant difference between WT and GluR5 KO mice ($p > 0.05$) with respect to baseline locomotor activity at any timepoint examined.

Timepoint (baseline)	Distance traveled in cm (mean $\pm$ S.E.M.)	
	WT	GluR5 KO
Day 1	2874 $\pm$ 214	2877 $\pm$ 163
Day 2	2178 $\pm$ 230	2132 $\pm$ 158
Day 3	2207 $\pm$ 137	1840 $\pm$ 175
Day 4	1957 $\pm$ 224	2119 $\pm$ 163
Day 5	2284 $\pm$ 175	2151 $\pm$ 149
challenge	2396 $\pm$ 252	2356 $\pm$ 134