Published in final edited form as:

Neuroscience. 2010 August 25; 169(2): 559–573. doi:10.1016/j.neuroscience.2010.05.056.

Acute and chronic cocaine differentially alter the subcellular distribution of AMPA GluR1 subunits in region-specific neurons within the mouse ventral tegmental area

D.A. Lane¹, A. Jaferi¹, M.J. Kreek², and V.M. Pickel^{1,2}

¹Dept. of Neurology and Neuroscience, Weill Medical College of Cornell University; New York, NY 10065

²Laboratory of the Biology of Addictive Diseases, The Rockefeller University; New York, NY 10065

Abstract

Cocaine administration increases AMPA GluR1 expression and receptor-mediated activation of the ventral tegmental area (VTA). Functionality is determined, however, by surface availability of these receptors in transmitter- and VTA-region-specific neurons, which may also be affected by cocaine. To test this hypothesis, we used electron microscopic immunolabeling of AMPA GluR1 subunits and tyrosine hydroxylase (TH; the enzyme needed for dopamine synthesis), in the cortical-associated parabrachial (PB) and in the limbic-associated paranigral (PN) VTA of adult male C57BL/6 mice receiving either a single injection (acute) or repeated escalating-doses for 14 days (chronic) of cocaine. Acute cocaine resulted in opposing VTA-region-specific changes in TH-containing dopaminergic dendrites. TH-labeled dendrites within the PB VTA showed increased cytoplasmic GluR1 immunogold particle density consistent with decreased AMPA receptor-mediated glutamatergic transmission. Conversely, TH-labeled dendrites within the PN VTA showed greater surface expression of GluR1 with increases in both synaptic and plasmalemmal GluR1 immunogold density after a single injection of cocaine. These changes diminished in both VTA subregions after chronic cocaine administration. In contrast, non-TH-containing (presumably GABAergic) dendrites showed VTA-region-specific changes only after repeated cocaine administration such that synaptic GluR1 decreased in the PB, but increased in the PN VTA. Taken together, these findings provide ultrastructural evidence suggesting that chronic cocaine not only reverses the respective depression and facilitation of mesocortical (PB) and mesolimbic (PN) dopaminergic neurons elicited by acute cocaine, but also differentially affects synaptic availability of these receptors in non-dopaminergic neurons of each region. These adaptations may contribute to increased cocaine seeking/relapse and decreased reward that is reported with chronic cocaine use.

Keywords

synaptic plasticity; dopamine; tyrosine hydroxylase; ultrastructure; reward

© 2010 IBRO. Published by Elsevier Ltd. All rights reserved.

Corresponding Author: Diane Lane, Ph.D., Department of Neurology and Neuroscience, Division of Neurobiology, Weill-Cornell Medical College, 407 E. 61st St, New York, NY 10065, dal2021@med.cornell.edu, Phone: (646) 962-8257.

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.

Dopaminergic neurons originating in the ventral tegmental area (VTA) play a critical role in the rewarding effects of cocaine and other drugs of abuse (Koob and Bloom, 1988, Le Moal and Simon, 1991). Changes in AMPA receptor-mediated glutamatergic activation of dopaminergic neurons are thought to contribute to the development of addiction (Kalivas and Stewart, 1991, Wolf, 2003, Kessels and Malinow, 2009). Stimuli that induce long term potentiation (LTP) increase the number of functional AMPA receptors at the synapse (Liao et al., 1995, Malinow and Malenka, 2002, Park et al., 2004). Additionally, LTP can be elicited from neurons within the VTA (Bonci and Malenka, 1999) suggesting that AMPA receptor-mediated plasticity in the VTA may be important for the development of addiction to cocaine. This is supported by findings that both acute and chronic systemic cocaine administration increase AMPA-mediated glutamatergic activation of dopaminergic neurons in the VTA (Ungless et al., 2001, Saal et al., 2003, Borgland et al., 2004, Bellone and Luscher, 2006, Chen et al., 2008) but see (Chen et al., 2008). Moreover, chronic cocaine administration increases both AMPA GluR1 protein expression (Fitzgerald et al., 1996) and responsiveness to local AMPA infusion (White et al., 1995, Zhang et al., 1997) demonstrating changes in AMPA receptor-mediated transmission in VTA neurons after long-term cocaine administration.

Little is known, however, regarding the effects of cocaine administration on GluR1-containing AMPA receptors in the diverse neuronal subtypes and/or subregions of the VTA. Functional differences in the VTA are highly dependent on anatomical connectivity and the transmitter phenotype of neurons, the majority of which are dopaminergic or GABAergic (Johnson and North, 1992). Changes in glutamate activation have been shown to affect dopamine release in both the medial prefrontal cortex (mPFC) and nucleus accumbens (NAc) (Kalivas et al., 1989, Taber and Fibiger, 1995, Karreman et al., 1996, Meltzer et al., 1997, Kretschmer, 1999), the respective primary targets of parabrachial (PB) and paranigral (PN) projection neurons within the VTA (Carr and Sesack, 2000, Lane et al., 2008). Dopaminergic neurons of the PB VTA receive monosynaptic glutamatergic inputs from the mPFC, whereas these glutamatergic efferents target mainly GABAergic neurons in the PN VTA (Carr and Sesack, 2000, Sesack and Carr, 2002, Sesack et al., 2003, Geisler and Zahm, 2005). These differences in circuitry have opposing effects on dopamine release when AMPA receptor antagonist are administered into the VTA (Takahata and Moghaddam, 2000) and result in opposing behavioral output as measured by locomotor activity (Tzschentke and Schmidt, 2000b, a). Consequently, cocaine administration may produce cell type- and VTA region-specific changes in the availability and functional assembly of GluR1-containing AMPA receptors at the synapse. Moreover, these changes may differ depending on the duration of cocaine exposure. To test this hypothesis, we used electron microscopic immunogold labeling of the AMPA receptor GluR1 subunit in the PB and PN VTA regions of mice receiving either acute (single injection) or chronic (14 days of intermittent escalating-dose) systemic cocaine administration. Dopaminergic neurons were identified by immunoperoxidase labeling of tyrosine hydroxylase (TH), a mandatory enzyme for dopamine synthesis not present in other neurons of the VTA, the majority of which are GABAergic (Johnson and North, 1992). The results provide evidence that acute cocaine administration produces region-specific subcellular relocation of AMPA GluR1 subunits consistent with decreased AMPA-mediated activation of mesocortical-but increased activation of mesolimbic-projecting dopamine neurons, an effect that disappears with chronic cocaine administration. In contrast, AMPA GluR1 distributions within presumably GABAergic dendrites show little change following acute cocaine, but are more profoundly influenced by chronic cocaine administration.

Methods

Subjects

Twenty-one naïve adult male C57/BL6 mice (Jackson Labs, Bar Harbor), weighing 19–25 g (starting weight) were individually housed and maintained on a 12-hour light cycle (lights out at 6:00 pm). Food and water were available *ad libitum*. Experimental protocols involving animals followed NIH guidelines to the care and use of research animals and were approved by the Institutional Animal Care and Use Committee (IACUC) at Weill Cornell Medical College.

Cocaine Administration Paradigm

Mice were randomly assigned to one of five treatment groups: Acute cocaine (n=5), chronic cocaine (n=5), single (n=3) or repeated (n=5) saline, and non-injected (n=3) controls. Acute cocaine mice received a single injection of cocaine (15 mg/kg i.p.). Chronically cocaine treated mice received 3 injections per day (at 10:00 am, 4:00 pm, and 10:00 pm) for 14 consecutive days of an escalating dose paradigm of cocaine (ranging from 7.5 mg/kg to 60 mg/kg, i.p. cumulative dose per day). This paradigm mimics the increasing drug intake over time as witnessed with humans addicted to cocaine (Zhang et al., 2002). Control mice were injected with saline in the same volume and intervals as mice receiving acute (1 i.p. injection) or chronic cocaine (3 i.p. injections per day for 14 consecutive days). In addition, to assess any changes in subcellular GluR1 related to the stress of injections, tissue from non-injected (normal) control mice were also included in the study.

Tissue Preparation

All mice were deeply anesthetized with sodium pentobarbital (120 mg/kg, i.p.; Sigma, St. Louis, MO). For all injected animals, pentobarbital was administered 30 minutes after either the single (acute) or last of the chronic injections of cocaine or saline. After fully anesthetized, mice were perfused through the right ventricle with 20 ml heparin (1,000 U/ml) in saline (American Pharmaceutical Partners, Schaumburg, IL) followed by 50 ml of 3.75% acrolein (Polysciences, Warrington, PA) in 2% paraformaldehyde in 0.1 M phosphate buffer and finally 100 ml of 2% paraformaldehyde in 0.1 M phosphate buffer (Sigma, St. Louis, MO). The brains were removed from the cranium and cut into 3–4 mm coronal blocks that were postfixed in 2% paraformaldehyde for 30 minutes, then sliced to 40 µm sections on a Leica Vibratome VT1000 (Leica Instruments, Nussloch, Germany) in chilled 0.1 M phosphate buffer. The collected vibratome sections were placed in a storage solution (30% sucrose, 30% ethylene glycol in 0.05 M phosphate buffer, pH 7.4) at –20°C until used for immunolabeling of GluR1 and/or TH.

Antisera

GluR1 immunolabeling was achieved using an affinity purified rabbit polyclonal antibody raised against a 13 amino acid peptide sequence corresponding to the C-terminus of rat GluR1 AMPA subunit (Chemicon, Temecula, CA). The GluR1 antiserum is well characterized by immunolabeling and Western blot analysis, which shows that this antiserum recognizes a single band of 110 kDa corresponding to the GluR1 subunit with no cross-reaction with GluR2–4 subunits (Sigel et al. 1995; Aicher et al., 2002; Glass et al., 2005). To the best of our knowledge, it is unknown whether this antiserum recognizes heteromer and/or homomers of the GluR1 subunit. However, both receptor types are most likely detected by this antibody because it was derived from a small 13 amino acid peptide sequence of the C-terminus of the AMPA receptor GluR1 subunit in a portion of the tail that should not be blocked by potential conformational change associated with dimerization. The recognition of both heteromeric and homomeric forms of GluR1-containing AMPA

receptors is further supported by the observed subcellular distribution of GluR1 immunogold particles in association with cytoplasmic organelles as well as synaptic and extrasynaptic portions of the plasma membrane (Lane et al., 2008), neuronal regions with proposed different AMPA receptor subunit compositions (Bellone and Luscher, 2006, Mameli et al., 2007, Argilli et al., 2008).

A mouse monoclonal antibody raised against the rate-limiting enzyme, tyrosine hydroxylase (TH), was commercially obtained from Immunostar (Hudson, WI). The TH immunogen was purified to homogeneity from PC12 cells of rat origin. Western blot analysis shows that this antiserum identifies a single 60 kDa band exclusively in TH transfected, but not non-transfected, HEK293 cells (Immunostar and (Lessard et al.)). Moreover, in brainstem catecholaminergic cell groups the TH-immunoreactivity seen using this antibody is comparable to the distribution of TH mRNA as seen by *in situ* hybridization (Rusnak and Gainer, 2005).

Immunocytochemistry

Coronal sections of tissue containing the VTA of cocaine and saline injected animals were co-processed using a dual immunogold-silver method for the detection of the antiserum against GluR1 subunit of the AMPA glutamate receptor and immunoperoxidase for detection of TH antibody. Co-processing eliminated potential variability in labeling between experimental groups. The pre-embedding dual-labeling protocol used in the present study was adapted from that of Chan and colleagues (Chan et al., 1990).

In preparation for immunolabeling, sections of tissue containing the VTA were placed in 1% sodium borohydride in 0.1 M phosphate buffer to remove excess aldehydes, then freeze-thawed to enhance penetration of immunoreagents. For this, the tissue was incubated in a cryoprotectant solution (25% sucrose and 2.5% glycol in 0.05 M phosphate buffer), then immersed successively in liquid Freon, liquid nitrogen, and room temperature 0.1 M phosphate buffer. Tissue sections were rinsed (0.1 M phosphate buffer) followed by 0.1 M Tris-buffered saline (TBS, pH 7.4) and incubated in 0.5% bovine serum albumin (BSA) in TBS to minimize non-specific labeling. The tissue was then incubated for 42 hours (24 hours at room temperature and 18 hours at 4°C) in a primary antibody solution containing mouse anti-TH (1:50,000) and rabbit anti-GluR1 (1:150) antisera in 0.1% BSA in TBS. For immunoperoxidase detection of TH, the avidin-biotin complex (ABC) method was used (Hsu, 1990). Sections were incubated for 30 minutes in biotinylated horse anti-mouse IgG (1:400) and then a 1:200 ABC solution (Vector Laboratories, Burlingame, CA). The peroxidase reaction product was visualized with 0.022% 3,3'-diaminobenzidine (Aldrich, St. Louis, MO) and 0.003% H₂O₂ in TBS for 6 minutes. For immunogold-silver visualization of GluR1 immunoreactivity, the tissue sections were incubated for 2 hours in goat anti-rabbit colloidal gold (1nm) IgG (1:50; Amersham Pharmacia, Piscataway, NJ). The gold particles were further adhered to the tissue by glutaraldehyde (2%) and enhanced with a silver solution (IntenS-EM kit, Amersham, Arlington Heights, IL) for 7 minutes.

The tissue was post-fixed with 2% osmium tetroxide in 0.2 M phosphate buffer for one hour and then dehydrated through a series of ethanols (30, 50, 70, 95, 100%), which was followed by 100% propylene oxide, and an overnight incubation in a 50/50 mixture of propylene oxide and EMbed 812 Epon substitute (Electron Microscopy Supply, Hatfield, PA). The following day, the sections were incubated in the Epon substitute for two hours, then embedded between two sheets of Aclar plastic and placed in an oven (60°C) for 24 hours.

Selection and sectioning of VTA tissue

The parabrachial (PB) and paranigral (PN) subdivisions of the VTA were separately isolated from the flat-embedded coronal sections of tissue (see trapezoid in Figure 1A) by comparing the distributions of TH-immunoreactivity in reference to landmark structures identified in a mouse brain atlas (Hof et al., 2000). A medial/caudal region of the VTA (ranging from -3.2 to -3.5 mm from Bregma) was selected based on the greatest density of TH-immunoreactive cells and the prevalence of both VTA regions within the same coronal plane. Differences in the ratio of dopaminergic and GABAergic neurons exist throughout the rostral to caudal aspects of VTA (Olson and Nestler, 2007), which may affect changes in GluR1 localization. However, the VTA level selected for the present study shows abundant distributions of both TH- and non-TH labeled cells allowing for sufficient investigation of both cell populations.

Ultrathin sections (70 nm) were cut from the isolated tissue using an ultratome (Leica Instruments, Nussloch, Germany) and a diamond knife (Diatome, Fort Washington, PA). The thin sections were obtained from the surface (1–2 μm) of the flat-embedded tissue, where there is optimal penetration of immunoreagents. The sections were collected on 400-mesh copper grids (Electron Microscopy Science, Fort Washington, PA), counterstained with uranyl acetate and lead citrate (Reynolds, 1963) and examined with a Phillips CM-10 electron microscope (FEI, Hillsboro, OR). The microscopic images were captured with an AMT Advantage HR/HR-B CCD Camera System (Advanced Microscopy Techniques, Danvers MA).

We chose pre-embedded gold-silver immunocytochemistry to analyze the subcellular distribution of GluR1 synaptic labeling because it preserves the fine structural detail necessary for valid quantification of receptor proteins on extrasynaptic membranes that can be lost during the process of plastic embedding (Adams et al., 2002). The pre-embedding method for immunogold also avoids spurious attachment of gold particles to the plastic, which often results in significant background labeling with post-embedding immunogold labeling (Leranth, 1989). Despite these advantages, the pre-embedding method may underestimate receptor proteins within postsynaptic membrane specializations, where there is a known impediment to penetration (Adams et al., 2002). This limitation, however, would be expected to apply equally to both saline- and cocaine-treated tissue making comparisons of synaptic labeling appropriate.

Data Analysis

A total tissue area of $40,237 \mu\text{m}^2$ (1,459 micrographs at 19,000 to 34,000 $\times$ magnification) was examined from the VTA of 21 mice in five treatment groups. Micrographs of the neuropil were collected throughout each region of the VTA to eliminate the possibility of biasing the data. In individual profiles, GluR1 immunogold labeling was evident as small punctuate electron-dense particles and were characterized by their location within each profile (see below). Peroxidase immunoreactive profiles were defined as having an electron density considerably greater than that seen in comparable structures in the surrounding neuropil. Classification of all the labeled profiles was determined by criteria from Peters and colleagues (Peters, 1991).

GluR1 immunogold labeling was relatively sparse, thus profiles containing one or more gold particles were considered to be immunogold labeled. To ensure specificity of this labeling criterion, the percentage of gold particles overlying myelin, which is not known to express AMPA GluR1 subunits thus serving as a control for spurious gold-silver deposits, was examined. Less than 0.01% of observed gold particles were seen on myelin. On the contrary, GluR1 immunogold particles displayed a high degree of specificity, with 94% of all immunogold particles found in dendritic profiles within the neuropil (Figure 1).

The parameters used for between-group comparison include: (A) profile size, (B) GluR1 immunogold distribution, (C) GluR1 synaptic analysis, and (D) TH immunoreactivity. A power analysis of the significant parametric tests was completed to evaluate confidence of the results and to ensure that adequate sample sizes were used. Significant findings had power values ranging from 0.634 to 0.995.

Profile Size—The profile area, perimeter, average diameter, and form factor were measured for each profile [total of 4,618 profiles (4,376 dendrites); 2,222 in the parabrachial VTA and 2,396 in the paranigral VTA] using Microcomputer Image Device software (MCID; Imaging Research, St. Catharines, Ontario). A cluster analysis was performed to statistically sort the profiles by size and Students' t-tests were conducted to evaluate size differences between the PB and PN VTA regions.

GluR1 Immunogold Labeling—GluR1 gold particles were characterized by their location within the profile, such as whether they were in contact with the plasma membrane, post-synaptic density, or other cytoplasmic structures. These numbers were combined with size measurements obtained from the MCID program to give the following measures of gold particle density: the number of GluR1 gold particles on the plasma membrane divided by the perimeter of the profile (PM/perim), the cytoplasmic gold particles divided by the area of the profile (cyt/area), and the total number of gold particles divided by the area of the profile (total/area). The data for each neuronal population (either TH or non-TH labeled dendrites) were analyzed separately using a two factor (drug treatment group X dendritic size) ANOVA followed by posthoc analysis using a Tukey test.

GluR1 Synaptic Analysis—Synaptic labeling was examined by quantifying the ratio of GluR1-labeled synapses out of the total number of synapses per experimental group. In addition, the number of GluR1 gold particles at post-synaptic densities was counted. The ratio of synaptic labeling was evaluated using Chi-Squared analyses and differences in the number of synaptic GluR1 gold particles were analyzed with a single factor ANOVA.

Results

Visualization of GluR1 immunogold distribution

The distribution of GluR1 immunogold particles in the mouse VTA of the present study was similar to that previously reported in the rat VTA (Fig. 1; (Lane et al., 2008)). In both the PB and PN subdivisions of the normal mouse VTA, GluR1 immunogold labeling was most evident post-synaptically in somatodendritic profiles with or without TH immunoreactivity. GluR1 immunogold labeling was also sometimes detected in axons and their terminals and in glia (Figure 1B and C). The labeling was highly selective for individual dendritic segments seen in coronal sections and absent from other similar profiles within the same neuropil.

In both TH and non-TH labeled dendrites, GluR1 immunogold particles were primarily found within the cytoplasm (75%; n= 4533) and associated with cytoplasmic endomembranes (Figure 1B). The remaining GluR1 immunogold particles (25%; n= 1,507) were in contact with the dendritic plasma membrane. Plasmalemmal GluR1 immunogold particles were roughly equally distributed between post-synaptic densities (PSDs; 43%) as non-synaptic portions of the membrane (57%). All PSD-associated GluR1 immunogold particles (n=460) were located at asymmetric excitatory-type synapses (Carlin et al., 1980; Rollenhagen and Lubke, 2006).

Dendritic Size

The majority of GluR1-labeled dendrites, compiled of all treatment groups, were small to medium sized as measured by the average diameter of each dendrite. Small dendrites (ranging from 0.1 to 0.6 μm) composed 51%, whereas, medium dendrites (ranging from 0.6 to 1.0 μm) composed 41% of the total number of GluR1 containing dendrites sampled. Only 8% of the dendrites were greater than 1.0 μm in average diameter. These larger dendrites were the most variable in size (ranging from 1.0 – 2.51 μm), and at least some of them may include portions of labeled somata. Experimentally induced changes in GluR1 immunogold labeling were seen only in small dendrites. These smaller dendrites are, most likely, more distal from the soma, where there are a higher proportion of excitatory-type inputs (Peters, 1991, Lane et al., 2008).

Effects of IP injections on subcellular GluR1 localization

There was a significant increase in both cytoplasmic ($F(2,225) = 13.44, p < 0.05$) and plasmalemmal ($F(2,225) = 5.51, p < 0.05$) GluR1 immunogold density in small TH-labeled dendrites within the PB VTA of mice receiving saline injections (either a single or multiple) compared to non-injected controls. There were no significant changes in GluR1 immunogold labeling between non-injected and saline injected mice in TH-labeled dendrites of the PN VTA or non-TH-labeled dendrites of either VTA region. Further, there were no significant differences between a single (acute) or repeated (chronic) saline injections in either cell type or VTA region.

Effects of cocaine administration on subcellular GluR1 localization

TH-labeled Dendrites—Cocaine administration resulted in opposing changes, dependent upon VTA region, in the subcellular localization of AMPA-receptor GluR1 subunits in TH-labeled dendrites of putative dopaminergic neurons. Small TH-labeled dendrites within the PB VTA showed an increase in cytoplasmic density of AMPA-receptor GluR1 subunits, whereas, small TH-labeled dendrites within the PN VTA demonstrated an increase in surface expression (both synaptic and plasmalemmal density) of GluR1 subunits after a single cocaine injection. Following chronic cocaine administration, however, the subcellular distribution of AMPA-receptor GluR1 subunits in these dendrites was not distinguishable from saline controls in either region of the VTA.

Parabrachial VTA: Dopaminergic dendrites of the PB VTA showed a significant increase in cytoplasmic GluR1 immunogold labeling ($F(4, 1285) = 3.22, p < 0.05$) after a single injection of cocaine (Fig. 2B and D). A small decrease in the number of synapses labeled with GluR1 immunogold particles ($\chi^2(4) = 2.63, p > 0.05$; Fig. 2B and F) and plasmalemmal GluR1 labeling ($F(4, 1285) = 1.82, p > 0.05$; Fig. 2B and E) as well as an increase in total GluR1 density ($F(4, 1285) = 4.02, p < 0.05$) were also observed following acute cocaine administration. Taken together, the findings demonstrate that a single injection of cocaine results in internalization and/or cytoplasmic retention of GluR1-containing AMPA receptors.

Following chronic cocaine administration, the subcellular distribution of immunolabeled GluR1 subunits reverted to a level similar to that of saline control animals (Fig. 2C). Tukey posthoc tests confirm that the cytoplasmic density of GluR1 immunogold particles (# of particles/dendritic area) significantly decreased compared to acute cocaine administration, while both plasmalemmal and synaptic GluR1 labeling slightly increased.

Paranigral VTA: TH-containing dopaminergic dendrites of the PN VTA showed a significant increase in the percentage of synapses with GluR1 labeling ($\chi^2(4) = 13.86, p < 0.05$, Fig 3C and G) following a single injection of cocaine. In addition, there was a

significant increase in plasmalemmal ($F(4, 1476) = 3.14, p < 0.05$; Fig. 3B and F) and total ($F(4, 1476) = 2.77, p < 0.05$) GluR1 immunogold particle density in these dendrites. These data provide ultrastructural evidence suggesting that the surface/synaptic availability of GluR1-containing AMPA receptors in TH-containing dendrites of the PN VTA is increased after a single injection of cocaine.

Following chronic cocaine administration, the percentage of synapses labeled with GluR1 immunogold particles decreased ($\chi^2(4) = 13.86, p < 0.05$; Fig. 3D and G) and plasmalemmal GluR1 distributions in TH-containing dendrites returned to levels similar to saline controls (3D, E, and F). These changes suggest a loss of cocaine effect on mesolimbic dopaminergic neurons of the PN VTA with repeated cocaine administration.

Non-TH Labeled Dendrites—Acute cocaine produced no significant changes in AMPA GluR1 immunogold labeling in dendrites without TH immunoreactivity in either PB or PN VTA (see discussion). There was, however, a slight increase in cytoplasmic GluR1 immunogold density in these dendrites in both VTA subregions after a single injection of cocaine. The most pronounced changes in GluR1 immunogold labeling were seen following chronic cocaine administration when non-TH containing dendrites showed opposing effects on synaptic labeling depending upon VTA region.

Parabrachial VTA: In non-TH labeled dendrites of the PB VTA, acute cocaine administration resulted in a non-significant increase in cytoplasmic GluR1 labeling ($F(4, 814) = 2.26, p > 0.05$; Fig 4B and D) and slight decrease in the percentage of GluR1-labeled synapse (Fig 4B and F). Although, not statistically significant, this decrease in synaptic GluR1 labeling was greater with repeated cocaine administration ($\chi^2(4) = 2.39, p > 0.05$; Figure 4C and F).

Paranigral VTA: In the PN VTA, the most pronounced changes in GluR1 immunogold labeling occurred with repeated cocaine administration, which resulted in increased numbers of synapses labeled with GluR1 immunogold particles ($\chi^2(4) = 5.58, p > 0.05$; Figure 5C, D, and G). There was also a small decrease in cytoplasmic GluR1 immunogold density after repeated cocaine administration (n.s., $F(4, 797) = 1.02, p > 0.05$, Fig. 5C, D, and E). The findings suggest that repeated cocaine administration may promote synaptic translocation of GluR1-containing AMPA receptors in non-dopaminergic dendrites of the PN VTA.

Discussion

Our results are summarized in Figure 6, which schematically illustrates neuronal phenotype- and VTA region-specific changes in response to cocaine administration. Specifically, we show a significant increase in GluR1 immunogold cytoplasmic density in TH-labeled, putatively dopamine synthesizing, dendrites of the PB VTA after a single, but not repeated, injection of cocaine. In contrast, TH-labeled dendrites of the PN VTA have increased surface expression, both synaptic and plasmalemmal, of GluR1 immunogold particles after a single injection of cocaine, which reverts to distributions similar to saline controls after chronic cocaine. These results, together with evidence that cytoplasmic GluR1 subunits are not available for surface activation, suggest that acute cocaine administration produces trafficking of GluR1-containing AMPA receptors allowing for decreased activation of mesocortical- but increased activation of mesolimbic-projecting dopaminergic neurons, an effect that disappears with chronic cocaine administration. This suggests a homeostatic change in the function of glutamatergic transmission in the VTA that compensates for the ongoing presence of cocaine. The schematic also illustrates our findings showing that non-TH, presumably GABAergic, dendrites have GluR1 distributions that are more profoundly influenced by chronic cocaine administration. In these dendrites, chronic cocaine increased

the proportion of synapses immunolabeled with GluR1 in the PN VTA, but decreased this labeling in the PB VTA. These results are discussed with regard to their implications for the increased locomotor activation and decreasing reward associated with chronic cocaine use.

VTA region-dependent changes in subcellular GluR1 distributions within dopamine neurons after a single cocaine injection

The significant increase in cytoplasmic GluR1 density, with decreased plasmalemmal surface expression, in small TH-labeled dendrites of the PB VTA suggests that acute cocaine administration results in internalization of surface/synaptic GluR1 subunits into the cytoplasm of these dendrites. The decrease in GluR1 surface expression may reflect enhanced glutamate release following acute cocaine administration (Kalivas, 1995) leading to decreased activation of dopaminergic neurons in the PB VTA. In contrast, TH-containing dendrites within the PN VTA show an increase in synaptic and plasmalemmal GluR1 immunogold particles following acute cocaine administration consistent with increased AMPA receptor-mediated glutamatergic transmission. This effect could facilitate bursting activity of these dopaminergic neurons (Grace and Bunney, 1984, Overton and Clark, 1992).

In addition to the observed effects of acute cocaine on GluR1 subcellular distribution, there was also a significant increases in the total amount of GluR1 immunogold density in dopaminergic dendrites of both VTA subregions following a single injection of cocaine. No similar changes were seen after chronic cocaine administration. Previous work shows that blocking mRNA transcription prevents cocaine-induced changes in AMPA receptor GluR1-dependent activation of dopaminergic neurons suggesting that new protein synthesis is required for this type of neuronal activation (Argilli et al., 2008). Similarly, blocking translational activity in the VTA prevents metabotropic glutamate receptor-dependent long term depression, which plays a role in cocaine-induced changes in AMPA receptor subunit composition (Mameli et al., 2007). Our finding of an overall increase in GluR1 density supports the idea that changes in GluR1 immunogold labeling are not only a result of AMPA receptor trafficking, but may also be explained by increased synthesis of AMPA receptor GluR1 subunits after a single injection of cocaine.

AMPA receptors are composed of tetramers (two dimers) of four different types of subunits (GluR1- GluR4). All four subunits are found within the VTA, however, GluR1 and GluR2 are more prevalent than GluR3 and GluR4 (Sato et al., 1993, Paquet et al., 1997, Lu et al., 2001). The structure of AMPA receptors is important because the inclusion of the GluR2 subunit renders the receptor calcium impermeable, whereas AMPA receptors composed of GluR1 subunits allow for calcium permeability into the cell (Hollmann et al., 1991, Cull-Candy et al., 2006). Synaptic insertion of GluR1-containing AMPA receptors is a necessary first step in the development of neuronal changes leading to increased excitability of dopaminergic neurons (Liao et al., 2001, Argilli et al., 2008), a proposed mechanism of cocaine addiction (Bellone and Luscher, 2006). This idea is supported by findings that show VTA GluR1 protein increases after administration of cocaine, as well as other drugs of abuse (Fitzgerald et al., 1996), and increases in VTA GluR1 are linked to the development of behavioral sensitization (Carlezon et al., 1997, Churchill et al., 1999, Grignaschi et al., 2004). As such, our finding of opposing regional changes in subcellular GluR1 provides anatomical evidence suggestive of decreased activation of mesocortical-projecting dopaminergic neurons of the PB VTA, but increased activation of mesolimbic-projecting dopaminergic neurons of the PN VTA after a single injection of cocaine.

The observed regional differences of GluR1 trafficking in dopamine dendrites of the PB and PN VTA may be a reflection of their anatomical connectivity and/or function. The PB VTA contains mesocortical dopaminergic neurons that primarily project to the mPFC (Carr and Sesack, 2000, Lane et al., 2008), a region important for drug seeking behavior and

reinstatement of cocaine self-administration (Goeders and Smith, 1983, Robbins, 1996, McFarland and Kalivas, 2001, Park et al., 2002, Capriles et al., 2003, Sun et al., 2005). The PN VTA contains mesolimbic dopaminergic neurons with principal projections to the nucleus accumbens, a brain region important for mediating reward (and aversion) salience (Jentsch and Taylor, 1999). Further, glutamatergic inputs from the prefrontal cortex (PFC) to these two VTA regions differ, with direct glutamatergic afferents terminating onto mesocortical dopaminergic neurons within the PB VTA and indirect input, via GABAergic neurons, terminating on mesolimbic dopaminergic neurons of the PN VTA (Carr and Sesack, 2000, Geisler et al., 2007). This circuitry has been shown to have an opposing effect on dopamine release in the prefrontal cortex and nucleus accumbens when an AMPA antagonist is applied into the VTA (Takahata and Moghaddam, 2000). Further, depletion of dopamine in the mPFC enhances dopaminergic release in the NAc (Thompson and Moss, 1995), whereas increased mPFC dopamine dampens rewarding effects of natural stimuli (Mitchell and Gratton, 1992) supporting the idea that the mPFC acts as a negative feedback system for DA release in the NAc (reviewed in (Tzschentke and Schmidt, 2000b)). Thus, the regional differences in the subcellular GluR1 localization in TH-containing dendrites following a single systemic injection of cocaine are functionally intuitive. They provide anatomical evidence for decreased activation of mPFC-projecting dopaminergic neurons involved in drug seeking and reinstatement of cocaine self-administration with a concurrent increase in glutamatergic activation of NAc-projecting dopaminergic neurons involved in reward.

Loss of cocaine effect on GluR1-containing AMPA receptors in VTA dopaminergic dendrites after repeated escalating doses of cocaine

Following 14 days of escalating doses of cocaine, dopaminergic dendrites within both regions of the VTA have similar subcellular AMPA receptor GluR1-subunit distributions as cocaine naïve animals. The base-line distribution of AMPA receptor GluR1 subunits was seen despite the presence of cocaine when administered thirty minutes prior to brain perfusion and tissue processing, a time point at which there is a high systemic concentration of cocaine (Pan and Hedaya, 1998) and observed acute effects of cocaine (see above). Conceivably, the comparable subcellular GluR1 distributions seen in both chronic cocaine-treated and saline control animals may reflect a homeostatic feedback mechanism of the glutamatergic system in response to cocaine.

Alternately, the function of VTA GluR1-containing AMPA receptor in mediating addictive behaviors may be transient. Behavioral sensitization is an animal model of addiction thought to parallel neuroadaptive changes associated with cocaine addiction. The VTA appears to be important in initiating, but not maintaining the neuroplasticity associated with behavioral sensitization (Wolf, 2003). Repeated cocaine administration leads to a progressive enhancement of its stimulant effects that can be measured by increases in locomotor activity across drug treatment trials (Stewart and Badiani, 1993) which persist even after cocaine administration has ceased (Henry and White, 1995). The enhanced locomotor activity, or behavioral sensitization, is associated with an increase in GluR1 in the VTA (Carlezon and Nestler, 2002, Grignaschi et al., 2004, Argilli et al., 2008). Local administration of a glutamate receptor antagonist in the VTA prevents the development of behavioral sensitization (Karler et al., 1989) and conditioned place preference to cocaine (Harris and Aston-Jones, 2003). However, once sensitization develops, VTA injections of glutamate antagonists do not affect locomotor sensitization (Kalivas and Alesdatter, 1993). This suggests that AMPA receptor-dependent activation of VTA dopaminergic neurons is transient and involved largely in initiating, but not maintaining, neuroplasticity associated with addictive behavior. This may explain why there was little change, as compared to

saline controls, in subcellular GluR1 localization in dopaminergic dendrites within both VTA subregions following chronic cocaine treatment.

The observed absence of change in GluR1 distribution seen with chronic cocaine administration may also reflect a change towards NMDA receptor-mediated transmission or a shift in AMPA receptor subunit composition in these neurons following chronic cocaine administration. The recruitment of GluR1-containing AMPA receptors to sites along the plasma membrane is important for activation of the cell and potentially NMDA receptors, which are crucial for the induction of long term potentiation (LTP), a proposed mechanism of addiction. Cocaine has been shown to increase the AMPA:NMDA ratio in VTA dopaminergic neurons facilitating LTP induction (Ungless et al., 2001, Saal et al., 2003, Borgland et al., 2004). AMPA receptors can rapidly move in and out of the synapse to mediate this type of neuronal activation (Heine et al., 2008). However, once activated, NMDA receptors may be more important in mediating neuroplasticity associated with addictive behaviors reducing the need for AMPA receptor activation (Bredt and Nicoll, 2003). Alternately, increases in synaptic activation following cocaine administration are attributed to increased GluR1-containing AMPA receptors at post-synaptic densities in the VTA (Argilli et al., 2008). It has been shown that these GluR1-containing AMPA receptors are displaced by GluR2-containing AMPA receptors 24 hours after a single cocaine injection (Bellone and Luscher, 2006; Mameli et al., 2007) providing a potential rationale for the lack of changes in subcellular GluR1 localization seen following chronic cocaine administration.

Subcellular GluR1 localization and cocaine-induced trafficking in non-TH dendrites

Acute cocaine administration resulted in a small increase in cytoplasmic GluR1 immunogold density in non-TH containing dendrites of both VTA regions. Most non-TH labeled neurons in the VTA are GABAergic (Johnson and North, 1992), and exert tonic inhibition on VTA dopaminergic neurons (Yim and Mogenson, 1980, Xi and Stein, 1998). Thus, internalization of GluR1-containing AMPA receptors, as suggested by the increased cytoplasmic labeling in non-TH dendrites, may result in decreased GABA inhibition of VTA dopaminergic neurons after a single injection of cocaine.

Chronic cocaine administration resulted in VTA region-specific differences in synaptic GluR1 labeling in dendrites without TH-immunoreactivity. In the PB VTA, these dendrites showed a non-significant decrease in synaptic GluR1 labeling, which may allow for disinhibition of VTA dopaminergic neurons that project to mPFC. This is of particular interest because loss of GABAergic inhibition has been shown to facilitate induction of LTP to chronic cocaine (Liu et al., 2005). Conversely, there was a relatively large increase in the number of synapses labeled with GluR1 in non-TH-containing dendrites within the PN VTA, although this change was not significant. Increased inhibition of VTA dopaminergic neurons results in decreased dopamine release in the nucleus accumbens (Kalivas and Duffy, 1990, Xi and Stein, 1998). Together, the findings provide anatomical evidence for decreased GABA inhibition of PB, but increased GABA inhibition of PN dopamine neurons within the VTA following repeated cocaine administration, which may be a potential mechanism for increased drug seeking and declining reward that occurs with repeated cocaine use.

Neither acute nor chronic cocaine administration produced significant changes in AMPA GluR1 distributions in non-TH-containing dendrites of the VTA. Conceivably, the heterogeneity of VTA GABAergic neurons (Bayer and Pickel, 1991, Van Bockstaele and Pickel, 1995, Omelchenko and Sesack, 2009) and potential inclusion of glutamatergic neurons (Yamaguchi et al., 2007) may result in differential GluR1 changes in distinct neuronal subpopulations explaining the muted cocaine-induced changes seen in non-TH-containing dendrites of the VTA.

Effects of injection on subcellular GluR1 localization

Overall, there were no significant changes in GluR1 immunogold labeling between saline injected and non-injected mice, with one exception. Mesocortical projecting TH-labeled dendrites of the PB VTA showed a significant increase in both cytoplasmic and plasmalemmal GluR1 immunogold density following both single and multiple injections of saline, as compared to non-injected mice (Figure 2D and E). These cortical-projecting dopaminergic neurons have been shown to be more vulnerable to stress (Horger and Roth, 1996) and to play an important role in stress-induced reinstatement of cocaine use (McFarland and Kalivas, 2001, Park et al., 2002). Acute stress is also known to increase AMPA mediated synaptic excitability (Saal et al., 2003), and stress from saline injections upregulates VTA GluR1 (Fitzgerald et al., 1996). Our findings support these previous studies and are the first to demonstrate PB VTA-specific changes in AMPA receptor GluR1 subcellular distributions in dopaminergic dendrites potentially induced by stress from intraperitoneal injection of saline. The changes in AMPA GluR1 labeling did not significantly differ with one or multiple saline injections.

Implications

The findings in the present study provide ultrastructural evidence for transient acutecocaine-induced increase in cytoplasmic AMPA GluR1 in mesocortical (PB VTA) and increase in dendritic surface expression in mesolimbic (PN VTA) dopamine neurons. These findings are consistent with the respective differential anatomical connectivity of these regions (Van Bockstaele and Pickel, 1995, Sesack and Carr, 2002, Sesack et al., 2003) and the known differential glutamatergic control of mesocortical and mesolimbic projection neurons (Takahata and Moghaddam, 2000). Moreover, we show that in contrast with dopaminergic neurons, putative GABAergic neurons located preferentially in the PN VTA show enhanced synaptic GluR1 labeling following repeated, but not single, injections of cocaine. These neurons are likely among those that received monosynaptic input from the PFC and project to the NAc (Carr and Sesack, 2000, Omelchenko and Sesack, 2009). The increased activation of these neurons, suggested by the increased surface expression of GluR1, reveals a novel mechanism for enhanced VTA inhibition of the limbic reward circuit such that increasingly higher doses are needed following repeated administration of cocaine.

Acknowledgments

This work was supported by grants NIH-NIDA P60 05130 to MJK and VMP, NIH-NIDA R01 004600 to VMP, and NIH-NIDA T32 007274 to Charles Inturrisi.

List of abbreviations

ABC	Avidin-biotin complex
BSA	Bovine serum albumin
LTP	Long term potentiation
mPFC	Medial Prefrontal Cortex
NAc	Nucleus Accumbens
PB VTA	Parabrachial Ventral Tegmental Area
PN VTA	Paranigral Ventral Tegmental Area
TBS	Tris buffered saline
TH	Tyrosine Hydroxylase

VTA Ventral Tegmental Area

References

- Adams MM, Fink SE, Shah RA, Janssen WG, Hayashi S, Milner TA, McEwen BS, Morrison JH. Estrogen and aging affect the subcellular distribution of estrogen receptor- α in the hippocampus of female rats. *J Neurosci*. 2002; 22:3608–3614. [PubMed: 11978836]
- Argilli E, Sibley DR, Malenka RC, England PM, Bonci A. Mechanism and time course of cocaine-induced long-term potentiation in the ventral tegmental area. *J Neurosci*. 2008; 28:9092–9100. [PubMed: 18784289]
- Bayer VE, Pickel VM. GABA-labeled terminals form proportionally more synapses with dopaminergic neurons containing low densities of tyrosine hydroxylase-immunoreactivity in rat ventral tegmental area. *Brain Res*. 1991; 559:44–55. [PubMed: 1685938]
- Bellone C, Lüscher C. Cocaine triggered AMPA receptor redistribution is reversed in vivo by mGluR-dependent long-term depression. *Nat Neurosci*. 2006; 9:636–641. [PubMed: 16582902]
- Bonci A, Malenka RC. Properties and plasticity of excitatory synapses on dopaminergic and GABAergic cells in the ventral tegmental area. *J Neurosci*. 1999; 19:3723–3730. [PubMed: 10234004]
- Borgland SL, Malenka RC, Bonci A. Acute and chronic cocaine-induced potentiation of synaptic strength in the ventral tegmental area: electrophysiological and behavioral correlates in individual rats. *J Neurosci*. 2004; 24:7482–7490. [PubMed: 15329395]
- Bredt DS, Nicoll RA. AMPA receptor trafficking at excitatory synapses. *Neuron*. 2003; 40:361–379. [PubMed: 14556714]
- Capriles N, Rodaros D, Sorge RE, Stewart J. A role for the prefrontal cortex in stress and cocaine-induced reinstatement of cocaine seeking in rats. *Psychopharmacology (Berl)*. 2003; 168:66–74. [PubMed: 12442201]
- Carlezon WA Jr, Boundy VA, Haile CN, Lane SB, Kalb RG, Neve RL, Nestler EJ. Sensitization to morphine induced by viral-mediated gene transfer. *Science*. 1997; 277:812–814. [PubMed: 9242609]
- Carlezon WA Jr, Nestler EJ. Elevated levels of GluR1 in the midbrain: a trigger for sensitization to drugs of abuse? *Trends Neurosci*. 2002; 25:610–615. [PubMed: 12446127]
- Carr DB, Sesack SR. Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. *J Neurosci*. 2000; 20:3864–3873. [PubMed: 10804226]
- Chan J, Aoki C, Pickel VM. Optimization of differential immunogold-silver and peroxidase labeling with maintenance of ultrastructure in brain sections before plastic embedding. *J Neurosci Methods*. 1990; 33:113–127. [PubMed: 1977960]
- Chen BT, Bowers MS, Martin M, Hopf FW, Guillory AM, Carelli RM, Chou JK, Bonci A. Cocaine but not natural reward self-administration nor passive cocaine infusion produces persistent LTP in the VTA. *Neuron*. 2008; 59:288–297. [PubMed: 18667156]
- Churchill L, Swanson CJ, Urbina M, Kalivas PW. Repeated cocaine alters glutamate receptor subunit levels in the nucleus accumbens and ventral tegmental area of rats that develop behavioral sensitization. *J Neurochem*. 1999; 72:2397–2403. [PubMed: 10349849]
- Cull-Candy S, Kelly L, Farrant M. Regulation of Ca²⁺-permeable AMPA receptors: synaptic plasticity and beyond. *Curr Opin Neurobiol*. 2006; 16:288–297. [PubMed: 16713244]
- Fitzgerald LW, Ortiz J, Hamedani AG, Nestler EJ. Drugs of abuse and stress increase the expression of GluR1 and NMDAR1 glutamate receptor subunits in the rat ventral tegmental area: common adaptations among cross-sensitizing agents. *J Neurosci*. 1996; 16:274–282. [PubMed: 8613793]
- Geisler S, Derst C, Veh RW, Zahm DS. Glutamatergic afferents of the ventral tegmental area in the rat. *J Neurosci*. 2007; 27:5730–5743. [PubMed: 17522317]
- Geisler S, Zahm DS. Afferents of the ventral tegmental area in the rat-anatomical substratum for integrative functions. *J Comp Neurol*. 2005; 490:270–294. [PubMed: 16082674]

- Goeders NE, Smith JE. Cortical dopaminergic involvement in cocaine reinforcement. *Science*. 1983; 221:773–775. [PubMed: 6879176]
- Grace AA, Bunney BS. The control of firing pattern in nigral dopamine neurons: burst firing. *J Neurosci*. 1984; 4:2877–2890. [PubMed: 6150071]
- Grignaschi G, Burbassi S, Zennaro E, Bendotti C, Cervo L. A single high dose of cocaine induces behavioural sensitization and modifies mRNA encoding GluR1 and GAP-43 in rats. *Eur J Neurosci*. 2004; 20:2833–2837. [PubMed: 15548228]
- Harris GC, Aston-Jones G. Critical role for ventral tegmental glutamate in preference for a cocaine-conditioned environment. *Neuropsychopharmacology*. 2003; 28:73–76. [PubMed: 12496942]
- Heine M, Groc L, Frischknecht R, Beique JC, Lounis B, Rumbaugh G, Huganir RL, Cognet L, Choquet D. Surface mobility of postsynaptic AMPARs tunes synaptic transmission. *Science*. 2008; 320:201–205. [PubMed: 18403705]
- Henry DJ, White FJ. The persistence of behavioral sensitization to cocaine parallels enhanced inhibition of nucleus accumbens neurons. *J Neurosci*. 1995; 15:6287–6299. [PubMed: 7666211]
- Hoff, PR.; Young, WG.; Bloom, FE.; Belinchenko, PV.; Celio, MR., editors. *Comparative cytoarchitectonic atlas of the C57BL/6 and 129/Sv mouse brains*. Amsterdam: Elsevier; 2000.
- Hollmann M, Hartley M, Heinemann S. Ca²⁺ permeability of KA-AMPA-gated glutamate receptor channels depends on subunit composition. *Science*. 1991; 252:851–853. [PubMed: 1709304]
- Horger BA, Roth RH. The role of mesoprefrontal dopamine neurons in stress. *Crit Rev Neurobiol*. 1996; 10:395–418. [PubMed: 8978988]
- Hsu SM. Immunohistochemistry. *Methods Enzymol*. 1990; 184:357–363. [PubMed: 1697021]
- Jentsch JD, Taylor JR. Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology (Berl)*. 1999; 146:373–390. [PubMed: 10550488]
- Johnson SW, North RA. Two types of neurone in the rat ventral tegmental area and their synaptic inputs. *J Physiol*. 1992; 450:455–468. [PubMed: 1331427]
- Kalivas PW. Interactions between dopamine and excitatory amino acids in behavioral sensitization to psychostimulants. *Drug Alcohol Depend*. 1995; 37:95–100. [PubMed: 7758408]
- Kalivas PW, Alesdatter JE. Involvement of N-methyl-D-aspartate receptor stimulation in the ventral tegmental area and amygdala in behavioral sensitization to cocaine. *J Pharmacol Exp Ther*. 1993; 267:486–495. [PubMed: 8229779]
- Kalivas PW, Bourdelais A, Abhold R, Abbott L. Somatodendritic release of endogenous dopamine: in vivo dialysis in the A10 dopamine region. *Neurosci Lett*. 1989; 100:215–220. [PubMed: 2761771]
- Kalivas PW, Duffy P. Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. *Synapse*. 1990; 5:48–58. [PubMed: 2300906]
- Kalivas PW, Stewart J. Dopamine transmission in the initiation and expression of drug- and stress-induced sensitization of motor activity. *Brain Res Brain Res Rev*. 1991; 16:223–244. [PubMed: 1665095]
- Karler R, Calder LD, Chaudhry IA, Turkanis SA. Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. *Life Sci*. 1989; 45:599–606. [PubMed: 2671566]
- Karreman M, Westerink BH, Moghaddam B. Excitatory amino acid receptors in the ventral tegmental area regulate dopamine release in the ventral striatum. *J Neurochem*. 1996; 67:601–607. [PubMed: 8764585]
- Kessels HW, Malinow R. Synaptic AMPA receptor plasticity and behavior. *Neuron*. 2009; 61:340–350. [PubMed: 19217372]
- Koob GF, Bloom FE. Cellular and molecular mechanisms of drug dependence. *Science*. 1988; 242:715–723. [PubMed: 2903550]
- Kretschmer BD. Modulation of the mesolimbic dopamine system by glutamate: role of NMDA receptors. *J Neurochem*. 1999; 73:839–848. [PubMed: 10428083]
- Lane DA, Lessard AA, Chan J, Colago EE, Zhou Y, Schlussman SD, Kreek MJ, Pickel VM. Region-specific changes in the subcellular distribution of AMPA receptor GluR1 subunit in the rat ventral tegmental area after acute or chronic morphine administration. *J Neurosci*. 2008; 28:9670–9681. [PubMed: 18815253]

- Le Moal M, Simon H. Mesocorticolimbic dopaminergic network: functional and regulatory roles. *Physiol Rev.* 1991; 71:155–234. [PubMed: 1986388]
- Leranth, CaP; VM. Electron Microscopic Pre-embedding Double Immunostaining Methods. In: Heimer; L. a, Z.; L, editors. *Tract Tracing Methods II: Recent Progress*. New York: Plenum; 1989.
- Lessard A, Coleman CG, Pickel VM. Chronic intermittent hypoxia reduces neurokinin-1 (NK(1)) receptor density in small dendrites of non-catecholaminergic neurons in mouse nucleus tractus solitarius. *Exp Neurol.* 2010
- Liao D, Hessler NA, Malinow R. Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature.* 2005; 375:400–404. 1995. [PubMed: 7760933]
- Liao D, Scannevin RH, Haganir R. Activation of silent synapses by rapid activity-dependent synaptic recruitment of AMPA receptors. *J Neurosci.* 2001; 21:6008–6017. [PubMed: 11487624]
- Liu QS, Pu L, Poo MM. Repeated cocaine exposure in vivo facilitates LTP induction in midbrain dopamine neurons. *Nature.* 2005; 437:1027–1031. [PubMed: 16222299]
- Lu W, Man H, Ju W, Trimble WS, MacDonald JF, Wang YT. Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. *Neuron.* 2001; 29:243–254. [PubMed: 11182095]
- Malinow R, Malenka RC. AMPA receptor trafficking and synaptic plasticity. *Annu Rev Neurosci.* 2002; 25:103–126. [PubMed: 12052905]
- Mameli M, Balland B, Lujan R, Luscher C. Rapid synthesis and synaptic insertion of GluR2 for mGluR-LTD in the ventral tegmental area. *Science.* 2007; 317:530–533. [PubMed: 17656725]
- McFarland K, Kalivas PW. The circuitry mediating cocaine-induced reinstatement of drug-seeking behavior. *J Neurosci.* 2001; 21:8655–8663. [PubMed: 11606653]
- Meltzer LT, Christoffersen CL, Serpa KA. Modulation of dopamine neuronal activity by glutamate receptor subtypes. *Neurosci Biobehav Rev.* 1997; 21:511–518. [PubMed: 9195610]
- Mitchell JB, Gratton A. Partial dopamine depletion of the prefrontal cortex leads to enhanced mesolimbic dopamine release elicited by repeated exposure to naturally reinforcing stimuli. *J Neurosci.* 1992; 12:3609–3618. [PubMed: 1527600]
- Olson VG, Nestler EJ. Topographical organization of GABAergic neurons within the ventral tegmental area of the rat. *Synapse.* 2007; 61:87–95. [PubMed: 17117419]
- Omelchenko N, Sesack SR. Ultrastructural analysis of local collaterals of rat ventral tegmental area neurons: GABA phenotype and synapses onto dopamine and GABA cells. *Synapse.* 2009; 63:895–906. [PubMed: 19582784]
- Overton P, Clark D. Iontophoretically administered drugs acting at the N-methyl-D-aspartate receptor modulate burst firing in A9 dopamine neurons in the rat. *Synapse.* 1992; 10:131–140. [PubMed: 1533955]
- Pan WJ, Hedaya MA. An animal model for simultaneous pharmacokinetic/pharmacodynamic investigations: application to cocaine. *J Pharmacol Toxicol Methods.* 1998; 39:1–8. [PubMed: 9596142]
- Paquet M, Tremblay M, Soghomonian JJ, Smith Y. AMPA and NMDA glutamate receptor subunits in midbrain dopaminergic neurons in the squirrel monkey: an immunohistochemical and in situ hybridization study. *J Neurosci.* 1997; 17:1377–1396. [PubMed: 9006980]
- Park M, Penick EC, Edwards JG, Kauer JA, Ehlers MD. Recycling endosomes supply AMPA receptors for LTP. *Science.* 2004; 305:1972–1975. [PubMed: 15448273]
- Park WK, Bari AA, Jey AR, Anderson SM, Spealman RD, Rowlett JK, Pierce RC. Cocaine administered into the medial prefrontal cortex reinstates cocaine-seeking behavior by increasing AMPA receptor-mediated glutamate transmission in the nucleus accumbens. *J Neurosci.* 2002; 22:2916–2925. [PubMed: 11923456]
- Peters, A.; Palay, SL.; Webster, HD. *The Fine Structure of the Nervous System*. New York: Oxford University Press; 1991.
- Reynolds ES. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol.* 1963; 17:208–212. [PubMed: 13986422]
- Robbins TW. Dissociating executive functions of the prefrontal cortex. *Philos Trans R Soc Lond B Biol Sci.* 1996; 351:1463–1470. discussion 1470-1461. [PubMed: 8941958]

- Rusnak M, Gainer H. Differential effects of forskolin on tyrosine hydroxylase gene transcription in identified brainstem catecholaminergic neuronal subtypes in organotypic culture. *Eur J Neurosci.* 2005; 21:889–898. [PubMed: 15787695]
- Saal D, Dong Y, Bonci A, Malenka RC. Drugs of abuse and stress trigger a common synaptic adaptation in dopamine neurons. *Neuron.* 2003; 37:577–582. [PubMed: 12597856]
- Sato K, Kiyama H, Tohyama M. The differential expression patterns of messenger RNAs encoding non-N-methyl-D-aspartate glutamate receptor subunits (GluR1–4) in the rat brain. *Neuroscience.* 1993; 52:515–539. [PubMed: 8450957]
- Sesack SR, Carr DB. Selective prefrontal cortex inputs to dopamine cells: implications for schizophrenia. *Physiol Behav.* 2002; 77:513–517. [PubMed: 12526992]
- Sesack SR, Carr DB, Omelchenko N, Pinto A. Anatomical substrates for glutamate-dopamine interactions: evidence for specificity of connections and extrasynaptic actions. *Ann N Y Acad Sci.* 2003; 1003:36–52. [PubMed: 14684434]
- Stewart J, Badiani A. Tolerance and sensitization to the behavioral effects of drugs. *Behav Pharmacol.* 1993; 4:289–312. [PubMed: 11224198]
- Sun W, Akins CK, Mattingly AE, Rebec GV. Ionotropic glutamate receptors in the ventral tegmental area regulate cocaine-seeking behavior in rats. *Neuropsychopharmacology.* 2005; 30:2073–2081. [PubMed: 15841101]
- Taber MT, Fibiger HC. Electrical stimulation of the prefrontal cortex increases dopamine release in the nucleus accumbens of the rat: modulation by metabotropic glutamate receptors. *J Neurosci.* 1995; 15:3896–3904. [PubMed: 7751954]
- Takahata R, Moghaddam B. Target-specific glutamatergic regulation of dopamine neurons in the ventral tegmental area. *J Neurochem.* 2000; 75:1775–1778. [PubMed: 10987862]
- Thompson TL, Moss RL. In vivo stimulated dopamine release in the nucleus accumbens: modulation by the prefrontal cortex. *Brain Res.* 1995; 686:93–98. [PubMed: 7583276]
- Tzschentke TM, Schmidt WJ. Differential effects of discrete subarea-specific lesions of the rat medial prefrontal cortex on amphetamine- and cocaine-induced behavioural sensitization. *Cereb Cortex.* 2000a; 10:488–498. [PubMed: 10847599]
- Tzschentke TM, Schmidt WJ. Functional relationship among medial prefrontal cortex, nucleus accumbens, and ventral tegmental area in locomotion and reward. *Crit Rev Neurobiol.* 2000b; 14:131–142. [PubMed: 11513242]
- Ungless MA, Whistler JL, Malenka RC, Bonci A. Single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. *Nature.* 2001; 411:583–587. [PubMed: 11385572]
- Van Bockstaele EJ, Pickel VM. GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. *Brain Res.* 1995; 682:215–221. [PubMed: 7552315]
- White FJ, Hu XT, Zhang XF, Wolf ME. Repeated administration of cocaine or amphetamine alters neuronal responses to glutamate in the mesoaccumbens dopamine system. *J Pharmacol Exp Ther.* 1995; 273:445–454. [PubMed: 7714800]
- Wolf ME. LTP may trigger addiction. *Mol Interv.* 2003; 3:248–252. [PubMed: 14993438]
- Xi ZX, Stein EA. Nucleus accumbens dopamine release modulation by mesolimbic GABAA receptors—an in vivo electrochemical study. *Brain Res.* 1998; 798:156–165. [PubMed: 9666112]
- Yamaguchi T, Sheen W, Morales M. Glutamatergic neurons are present in the rat ventral tegmental area. *Eur J Neurosci.* 2007; 25:106–118. [PubMed: 17241272]
- Yim CY, Mogenson GJ. Effect of picrotoxin and nipecotic acid on inhibitory response of dopaminergic neurons in the ventral tegmental area to stimulation of the nucleus accumbens. *Brain Res.* 1980; 199:466–473. [PubMed: 7417797]
- Zhang XF, Hu XT, White FJ, Wolf ME. Increased responsiveness of ventral tegmental area dopamine neurons to glutamate after repeated administration of cocaine or amphetamine is transient and selectively involves AMPA receptors. *J Pharmacol Exp Ther.* 1997; 281:699–706. [PubMed: 9152375]
- Zhang Y, Mantsch JR, Schlussman SD, Ho A, Kreek MJ. Conditioned place preference after single doses or "binge" cocaine in C57BL/6J and 129/J mice. *Pharmacol Biochem Behav.* 2002; 73:655–662. [PubMed: 12151041]

Figure 1.

(A) Light micrograph of plastic embedded coronal section of tissue (-3.40 mm Bregma) showing the area used for electron microscopic analysis (trapezoid). The sampled area contains both the PB and PN VTA. (B) A representative electron micrograph of the VTA showing GluR1 immunogold labeling in both dopaminergic (TH + GluR1 den) and non-dopaminergic (Non-TH + GluR1 den) dendrites. TH is identified by dense immunoperoxidase reaction product. Within these dendrites, GluR1 immunogold particles are most evident in the cytoplasm (gray arrows), but are also seen on the plasma membrane (white arrow). GluR1 labeling is highly selective for individual dendritic segments seen in coronal sections and absent from other similar profiles within the same neuropil (ul den). An axon terminal containing GluR1 immunogold (black arrows, Glu ter) is also seen in the electron micrograph. Both the electron micrograph and quantitative analysis of labeled profiles shown in the bar graph (C) demonstrate the predominantly post-synaptic (dendritic) distribution of GluR1 labeling. Quantitative analysis indicates that only a few soma, axons, or glia contain detectable GluR1 and/or TH labeling. PB VTA, parabrachial ventral tegmental area; PN VTA, paranigral ventral tegmental area; SNC, substantia nigra compacta; SN, substantia nigra; ML, medial lemniscus; IP, intrapeduncular nucleus; IF, intrafascicular nucleus; Glu te, GluR1-labeled axon terminal; ul te, unlabeled axon terminal; em, endomembrane; scale bar = $500\text{ }\mu\text{m}$ (A) and 500 nm (B).

Figure 2.

Electron micrographs (A–C) and quantitative analysis (bar graphs, D–F) of GluR1 immunogold distributions in dendrites showing immunoperoxidase labeling for TH in the mouse PB VTA. GluR1-gold particles in dopaminergic dendrites (TH + GluR1 den) have a predominantly cytoplasmic distribution (gray arrows) with some plasmalemmal labeling (white arrows) in saline controls (A). Following a single injection of cocaine (B), GluR1 immunogold particles are more readily seen in the cytoplasm (gray arrows) of small dendrites containing peroxidase labeling for TH. However, after repeated cocaine administration (C) GluR1 immunogold is once again located both in the cytoplasm (gray arrow) and on the plasma membrane (white arrows). Bar graphs show a significant increase in cytoplasmic with non-significant decreases in plasmalemmal and synaptic GluR1 labeling after acute cocaine, as compared to saline controls. These acute cocaine-induced changes in GluR1 distribution diminish with repeated cocaine administration. Ul te, unlabeled axon terminal; curved black arrow = synapse; * = $p < 0.05$; scale bar = 500 nm.

Figure 3.

Electron micrographs (A–D) and quantitative analysis (bar graphs, E–G) showing GluR1 immunogold particles in dendrites containing immunoperoxidase labeling for TH in the mouse PN VTA. Dopaminergic dendrites (TH + GluR1 den) of the PN VTA have a predominantly cytoplasmic distribution of GluR1 immunogold particles in saline treated mice (A). After a single injection of cocaine, GluR1 immunogold particles are more often seen on the plasma membrane (white arrows, B) and near (gray arrow, C) or at (white triangle, C) asymmetric excitatory-type synapses. Following repeated cocaine administration (D), GluR1 gold particles are, once again, observed mostly within the cytoplasm (gray arrows) of dendrites containing peroxidase labeling for TH. The bar graphs show no significant differences between any of the cocaine treatment group and saline controls in mean cytoplasmic GluR1 density (E). However, there is a significant increase in mean plasmalemmal GluR1 immunogold density (F) and a significant increase in the proportion of GluR1-labeled synapses (G) after a single injection of cocaine. These changes are not seen with repeated cocaine administration. MA, myelinated axon; ul te, unlabeled axon terminals; curved black arrow = synapse; white triangle = GluR1 labeling at the post-synaptic density; * = $p < 0.05$; scale bar = 500 nm.

Figure 4.

Electron micrographs (A–C) and quantitative analysis (bar graphs, D–F) showing GluR1 immunogold in dendrites without immunoperoxidase labeling for TH in the mouse PB VTA. Non-TH dendrites (non-TH + GluR1 den) have a predominantly cytoplasmic (gray arrow) GluR1 immunogold distribution following saline administration (A). However, GluR1 immunogold particles are also observed on the plasma membrane (white arrow) and near (black arrow) or at a synapse (white triangle) on these dendrites (A). After a single injection of cocaine (B), GluR1 immunogold is more evident in the cytoplasm (gray arrows), but still found near asymmetric, excitatory-type, post-synaptic densities (black arrow). This pattern in GluR1 labeling is not seen with repeated cocaine administration (C), where there are

fewer synapses (black curved arrows) labeled with GluR1 immunogold particles. Quantitative analysis in the bar graphs (D–F) show no significant differences between control and drug treatment groups in the cytoplasmic (E), plasmalemmal (F), or synaptic (G) density of GluR1 immunogold particles. However, there is a trend for increased cytoplasmic GluR1 labeling after a single cocaine injection and decreased synaptic GluR1 labeling following chronic cocaine administration, as compared to saline controls. UI te, unlabeled axon terminals; curved black arrow = synapse; white arrow = GluR1 immunogold particle at the post-synaptic density; scale bar = 500 nm.

Figure 5.

Electron micrographs (A–D) and quantitative analysis (bar graphs, E–G) showing GluR1 immunogold in small dendrites without immunoperoxidase labeling for TH in the mouse PN VTA. Dendrites containing GluR1, but no peroxidase labeling of TH, (non-TH + GluR1 den) have a predominantly cytoplasmic (gray arrow) GluR1 immunogold distribution following saline administration (A). Immunogold particles are also seen occasionally on the plasma membrane (white arrows). The prevalent cytoplasmic distribution of GluR1 immunogold particles is also seen in VTA sections from mice receiving acute cocaine administration (B), where GluR1 immunogold labeling is most evident in the cytoplasm (gray arrows) and more rarely seen on the plasma membrane (white arrow). In contrast, after chronic cocaine administration (C and D), GluR1 immunogold particles are more evident at synapses (white triangles in both C and D), although GluR1 immunogold particles are still seen in the cytoplasm (gray arrows) of these dendrites. Quantitative analyses (bar graphs) show no significant cocaine-induced changes in cytoplasmic (E), plasmalemmal (F), or synaptic (G) GluR1 labeling as compared to saline controls. However, there is a small trend for increased cytoplasmic/decreased plasmalemmal GluR1 density in non-TH dendrites after acute cocaine administration. Further, there is a large, but non-significant, increase in the proportion of synapses labeled with GluR1 immunogold particles following chronic cocaine administration (G), as compared to animals receiving repeated injections of saline. Glu te, GluR1 labeled axon terminal; ul te, unlabeled axon terminal; gray arrow = cytoplasmic GluR1 immunogold particle; curved black arrow = synapse; white triangle = GluR1 immunogold labeling at the synapse; black block arrow = presynaptic GluR1 immunogold particle; scale bar = 500 nm.

Figure 6.

Schematic diagram summarizing changes in AMPA receptor GluR1 immunogold labeling in TH and non-TH containing dendrites of the mouse PB and PN VTA following acute and chronic cocaine administration. TH-labeled dendrites of the PB VTA show a significant increase in GluR1 immunogold cytoplasmic density (gold circles) after a single (acute), but not repeated (chronic), injection of cocaine. In contrast, TH-labeled dendrites of the PN VTA have increased surface expression, both synaptic (red circles) and plasmalemmal (green circles), of GluR1 immunogold particles after a single injection of cocaine, which reverts to basal distributions (bottom left corner) similar to saline controls after chronic cocaine. Non-TH containing dendrites show little change in GluR1 immunogold labeling

following acute cocaine administration and are more influenced by repeated cocaine administration. Chronic cocaine decreases the proportion of synapses (darkened segments on the rim of the conical dendrites) immunolabeled with GluR1 in the PB VTA, but increases this labeling in the PN VTA. The drug dose paradigm used for each condition is illustrated in the graph at the bottom of the diagram. Doses for the chronic cocaine group are reported as the total dose per day (the sum of 3 daily injections). Three-dimensional cones = transversely cut dendrites whose plasmalemmal and cytoplasmic components are defined by the dark brown rim and lighter center; gold circles = cytoplasmic GluR1 labeling; green circles = plasmalemmal GluR1 labeling; red circles = synaptic GluR1 labeling; tear drop containing small clear circles = presynaptic terminals; thickened dark portion of dendritic circles = synapses.