Published in final edited form as:

Psychopharmacology (Berl). 2010 March ; 208(4): 511–519. doi:10.1007/s00213-009-1753-7.

Gamma-vinyl GABA increases nonvesicular release of GABA and glutamate in the nucleus accumbens in rats via action on anion channels and GABA transporters

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Abstract

Rationale— γ -Amino butyric acid (GABA) is a well-characterized inhibitory neurotransmitter in the central nervous system, which may also stimulate nonvesicular release of other neurotransmitters under certain conditions. We have recently reported that γ -vinyl GABA (GVG), an irreversible GABA transaminase inhibitor, elevates extracellular GABA but fails to alter dopamine release in the nucleus accumbens (NAc).

Objectives—Here, we investigated the mechanism(s) by which GVG elevates extracellular GABA levels and whether GVG also alters glutamate release in the NAc.

Materials and methods—In vivo microdialysis was used to simultaneously measure extracellular NAc GABA and glutamate before and after GVG administration in freely moving rats.

Results—Systemic administration of GVG or intra-NAc local perfusion of GVG significantly increased extracellular NAc GABA and glutamate. GVG-enhanced GABA was completely blocked by intra-NAc local perfusion of 5-nitro-2, 3-(phenylpropylamino)-benzoic acid (NPPB), a selective anion channel blocker and partially blocked by SKF89976A, a type 1 GABA transporter inhibitor. GVG-enhanced glutamate was completely blocked by NPPB or SKF89976A. Tetrodotoxin, a voltage-dependent Na^+ -channel blocker, failed to alter GVG-enhanced GABA and glutamate.

Conclusions—These data suggest that GVG-enhanced extracellular GABA and glutamate are mediated predominantly by the opening of anion channels and partially by the reversal of GABA transporters. Enhanced extracellular glutamate may functionally attenuate the pharmacological action of GABA and prevent enhanced GABA-induced excess inhibition.

Keywords

γ -Vinyl GABA; GABA; Glutamate; Anion channel; GABA transporter; Nucleus accumbens

Introduction

γ -Amino butyric acid (GABA) is a major inhibitory neurotransmitter in the central nervous system (CNS). After synaptic release, GABA activates GABA_A and/or GABA_B receptors and inhibits neuronal activity and neurotransmitter release (Kalivas 1993; Huang 2009). In addition to being released synaptically, GABA is also released from neurons or glial cells by

GABA transporter reversal and other non-vesicular release mechanisms (Attwell et al. 1993; Levi and Raiteri 1993; Koch and Magnusson 2009). GABA transporters and glutamate transporters have been shown to be co-localized on the same cells in the CNS (Raiteri et al. 2002; Bonanno et al. 2006). An increase in extracellular GABA may, by activating GABA transporters, provoke glutamate release by opening anion channels or by reversing glutamate transporters (Raiteri et al. 2005; Liu et al. 2006). Similarly, an increase in extracellular glutamate may stimulate nonvesicular GABA release by activating glutamate transporters located on GABAergic cells (Bonanno and Raiteri 1994). These data suggest that brain GABA and glutamate transmission maintain a functional balance (homeostasis) by reciprocal modulation of excitatory and inhibitory neurotransmitter release (Bonanno and Raiteri 1994; Bonanno et al. 1993, 2006).

γ -Vinyl GABA (GVG), a commonly used anticonvulsant, is an irreversible inhibitor of GABA transaminase, an enzyme responsible for the metabolic degradation of GABA (Cubells et al. 1986). Since GABA transaminase is located on intracellular mitochondria, inhibition of this enzyme causes an increase in cytoplasmic GABA, which leads to GABA efflux via reversal of GABA transporters (Attwell et al. 1993; Levi and Raiteri 1993; Wu et al. 2001, 2003, 2007). Recent studies suggest that GVG is also effective in attenuating the acute rewarding and incentive motivational effects of cocaine and other addictive drugs in a number of animal models of addiction (Dewey et al. 1998; Gardner et al. 2002; Kushner et al. 1997; 1999; Peng et al. 2008a). The underlying mechanism for these behavioral effects has long been thought to be GVG-enhanced extracellular GABA with consequent inhibition of addictive drug-induced increases in extracellular dopamine (DA) in the nucleus accumbens (NAc) (Dewey et al. 1997; Morgan and Dewey 1998; Schiffer et al. 2000). Contrary to this view, we have recently reported that GVG or gabapentin, whether administered systemically or locally into the NAc, fails to alter either basal or cocaine-enhanced extracellular DA (Peng et al. 2008a,b), suggesting that a non-DA mechanism may underlie the action of GVG or gabapentin in animal models of drug addiction (Peng et al. 2008a, b; Xi and Gardner 2008).

In an attempt to explain why GVG-enhanced extracellular GABA fails to inhibit synaptic DA release, we proposed that GVG-enhanced extracellular GABA may be derived from extrasynaptic, nonvesicular sources. Furthermore, the high densities of GABA transporters located on synapse-associated astrocytes may mediate significant astrocytic GABA uptake, thus blocking GABA diffusion into the synaptic cleft (Schousboe et al. 2004; Héja et al. 2006; Peng et al. 2008a). To test this hypothesis, in the present study, we investigated the cellular mechanisms by which GVG elevates extracellular GABA in the NAc and observed the effects of GVG on extracellular NAc glutamate, another neurotransmitter importantly involved in drug reward and relapse (Kalivas 2004; Wise 2004; Xi and Gardner 2008), using in vivo brain microdialysis.

Materials and methods

Animals

Male Long-Evans rats (Charles River Laboratories, Raleigh, NC, USA) weighing 250–300 g were used. They were housed individually in a climate-controlled animal room on a reversed light–dark cycle with free access to food and water. The animals were maintained in a facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. All experimental procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals of the US National Academy of Sciences and were approved by the Animal Care and Use Committee of the National Institute on Drug Abuse of the US National Institutes of Health.

In vivo microdialysis

Surgery and in vivo brain microdialysis procedures were the same as we have reported previously (Peng et al. 2008a; Xi et al. 2003). Briefly, all rats were bilaterally implanted with intracranial guide cannulae (20 gauge, 14 mm; Plastics One, Roanoke, VA, USA) into the NAc under sodium pentobarbital anesthesia (65 mg/kg i.p.) with standard aseptic surgical and stereotaxic technique. The stereotaxic coordinates for the NAc were AP +1.7, ML $\pm$ 2.0, DV -4.0 mm according to the atlas of Paxinos and Watson (1998) using an approach angle of 6° from vertical.

In vivo microdialysis was performed in freely moving animals 5–7 days after surgery. Microdialysis probes were constructed as previously described (Xi et al. 2003). Probes were inserted into the NAc at least 12 h before experiments to minimize damage-induced neurotransmitter release during the experiment. On the day of the experiment, dialysis buffer (5 mM glucose, 2.5 mM KCl, 140 mM NaCl, 1.4 mM CaCl₂, 1.2 mM MgCl₂, 0.15% phosphate buffered saline, pH 7.4) was perfused through the probe (2.0 μ l/min) via syringe pump (Bioanalytical Systems, Inc. West Lafayette, IN, USA) beginning at least 2 h prior to sample collection. Dialysis samples were collected every 20 min in 10 μ l 0.5 M perchloric acid to prevent degradation of neurotransmitters.

After 1 h of baseline sample collection, one of three doses of GVG (25, 100, and 300 mg/kg, i.p.) or vehicle (1 ml/kg 25% 2-hydroxypropyl- β -cyclodextrin) was administered systemically, or different concentrations of GVG (1, 10, 100, and 1,000 μ M) or GABA (1, 10, 100, and 1,000 μ M) were locally infused into the NAc by reverse microdialysis. To determine the vesicular and/or nonvesicular origins of GVG-induced changes in extracellular GABA and glutamate, we also observed the effects of intra-NAc local perfusion of tetrodotoxin (TTX; a voltage-dependent Na⁺ channel blocker, 1 μ M), SKF89976A (type 1 GABA transporter inhibitor, 300 μ M) or 5-nitro-2, 3-(phenylpropylamino)-benzoic acid (NPPB; an anion channel blocker, 300 μ M) on GVG-induced changes in NAc glutamate and GABA. After collection, all samples were frozen at -80°C until analyzed.

Following the completion of brain microdialysis experiments, rats were given an overdose of pentobarbital (>100 mg/kg i.p.) and transcardially perfused with 0.9% saline followed by 10% formalin solution. Brains were sectioned, and anatomical placements of microdialysis probes were examined (Peng et al. 2008a).

Quantification of glutamate and GABA

Concentrations of glutamate and GABA in the dialysis samples were determined using high-pressure liquid chromatography with fluorometric detection as reported previously (Peng et al. 2008a; Xi et al. 2003). Two sets of different excitation wavelengths (Ex λ) and emission wavelengths (Em λ) were used to simultaneously measure glutamate (Ex λ , 314 nm; Em λ , 394 nm) and GABA (Ex λ , 336 nm; Em λ , 420 nm) levels from the same samples. The areas under the curve (AUCs) of the glutamate and GABA peaks were measured using an “EZChrom Elite” ESA chromatography data system. Glutamate and GABA values were quantified with external standard curves (glutamate, 0.1–5 μ M; GABA, 0.01–1 μ M). The limits of detection for glutamate and GABA were 1–2 and 0.1–1 pM, respectively.

Drugs and chemicals

GVG and NPPB were purchased from Tocris Bioscience (Ellisville, MO, USA). GABA, TTX, SKF89976A and 2-hydroxypropyl- β -cyclodextrin were purchased from Sigma-Aldrich (Saint Louis, MO, USA). GVG was dissolved in 25% (w/v) 2-hydroxypropyl- β -

cyclodextrin for i. p. injections. For intra-NAc local perfusion, all drugs were dissolved in the dialysis buffer described above.

Data analysis

All data are presented as means $\pm$ SEM. Two-way (treatment $\times$ time) analysis of variance (ANOVA) for repeated measures over time was used to analyze drug-induced changes in extracellular GABA and glutamate. Post hoc individual group comparisons were carried out using the Fisher LSD procedure.

Results

Systemic administration of GVG elevates extracellular NAc GABA and glutamate

Figure 1a shows that systemic administration of GVG (25, 100, and 300 mg/kg, i.p.) dose-dependently elevated extracellular NAc GABA levels. Two-way ANOVA for repeated measures over time revealed a statistically significant GVG dose main effect ($F_{3,28}=19.78$, $P<0.001$), time main effect ($F_{17,476}=9.07$, $P<0.001$), and GVG dose $\times$ time interaction ($F_{51,476}=4.74$, $P<0.001$). Individual group comparisons revealed a statistically significant increase in extracellular GABA after 100 mg/kg ($P<0.01$) and 300 mg/kg GVG ($P<0.001$) but not after 25 mg/kg GVG. Figure 1b shows that GVG (25, 100, but not 300, mg/kg, i.p.) significantly elevated extracellular NAc glutamate. Two-way ANOVA for repeated measures over time revealed a significant GVG dose main effect ($F_{3,30}=5.87$, $P<0.01$), time main effect ($F_{17,510}=8.29$, $P<0.001$), and GVG dose $\times$ time interaction ($F_{51,510}=3.93$, $P<0.001$). Individual group comparisons revealed a statistically significant increase in extracellular glutamate levels after 25 and 100 mg/kg but not after 300 mg/kg GVG, compared to the vehicle group.

Intra-NAc local perfusion of GVG elevates extracellular NAc GABA and glutamate

Figure 2a shows that intra-NAc perfusion of GVG (1, 10, 100, and 1,000 μ M) dose dependently elevated extracellular NAc GABA (two-way ANOVA, $F_{1,12}=69.32$, $P<0.001$). Individual group comparisons revealed a statistically significant overall increase in extracellular GABA after GVG administration ($P<0.05$), when compared to the vehicle group (Fig. 2a), as well as significant increases in GABA at the specific time and dose points indicated on Fig. 2a.

Figure 2b shows that intra-NAc local perfusion of the same concentrations of GVG or GABA significantly elevated extracellular glutamate levels (two-way ANOVA, $F_{2,20}=3.23$, $P<0.05$). Individual group comparisons revealed a statistically significant overall increase in extracellular glutamate after GVG ($P<0.05$) or GABA ($P<0.05$) administration, when compared to the vehicle group (Fig. 2b), as well as significant increases in glutamate at the specific time and dose points indicated on Fig. 2b.

GVG enhances nonvesicular release of NAc GABA

Figure 3a shows the effects of intra-NAc local perfusion of TTX, SKF89976A, or NPPB alone on extracellular GABA. Two-way ANOVA for repeated measures over time revealed a significant treatment main effect ($F_{3,21}=12.59$, $P<0.001$), time main effect ($F_{17,357}=5.27$, $P<0.001$), and treatment $\times$ time interaction ($F_{51,357}=5.21$, $P<0.001$). Individual group comparisons revealed a statistically significant increase in extracellular GABA levels after local perfusion of 300 μ M SKF89976A ($P<0.001$) but not 1 μ M TTX or 300 μ M NPPB. Figure 3b shows the effects of pretreatment with TTX (1 μ M), SKF89976A (300 μ M), or NPPB (300 μ M) on GVG-enhanced NAc GABA. Since SKF89976A pretreatment significantly elevated basal levels of extracellular GABA, we renormalized GVG-induced changes in extracellular GABA over the new baseline before GVG administration (Fig. 3c).

Two-way ANOVA for repeated measures over time for the data shown in Fig. 3c revealed a significant treatment main effect ($F_{4,35}=5.09$, $P<0.01$), time main effect ($F_{14,490}=12.83$, $P<0.001$), and treatment $\times$ time interaction ($F_{56,490}=3.10$, $P<0.001$). Figure 3d shows AUC data derived from the data in Fig. 3c. One-way ANOVA revealed a significant treatment main effect ($F_{4,35}=5.14$, $P<0.01$). Individual group comparisons revealed a significant reduction in GVG-enhanced GABA after NPPB ($P<0.01$) or SKF89976A ($P<0.05$) but not after TTX (Fig. 3d), when compared with the vehicle pretreatment group.

GVG enhances nonvesicular release of NAc glutamate

Figure 4a shows the effects of local perfusion of TTX, SKF89976A, or NPPB alone on basal levels of extracellular glutamate. Two-way ANOVA for repeated measures over time revealed a significant treatment main effect ($F_{5,27}=9.80$, $P<0.001$), a significant time main effect ($F_{17,459}=3.91$, $P<0.001$), and a significant treatment $\times$ time interaction ($F_{51,459}=2.41$, $P<0.001$). Individual group comparisons revealed a significant decrease in extracellular glutamate after local perfusion of 300 μ M NPPB ($P<0.001$) or 300 μ M SKF89976A ($P<0.05$) but not 1 μ M TTX. Figure 4b shows the effects of pretreatment with TTX, SKF89976A, or NPPB on 100 mg/kg GVG-enhanced NAc glutamate. Since both SKF89976A and NPPB pretreatment lowered basal levels of extracellular glutamate, we renormalized GVG-induced changes in glutamate over the new baseline before GVG administration (Fig. 4c). Two-way ANOVA for repeated measures over time for the data shown in Fig. 4c revealed a statistically significant treatment main effect ($F_{4,34}=9.15$, $P<0.001$), time main effect ($F_{14,476}=3.57$, $P<0.001$), and treatment $\times$ time interaction ($F_{56,476}=3.08$, $P<0.001$). Figure 4d shows AUC data derived from the data shown in Fig. 4c. One-way ANOVA revealed a statistically significant treatment main effect ($F_{4,34}=9.22$, $P<0.001$). Individual group comparisons revealed a significant reduction in GVG-enhanced glutamate after SKF89976A ($P<0.001$) or NPPB ($P<0.001$) but not TTX.

Histology

Figure 5a shows a representative microdialysis probe position in the rat brain, demonstrating that the active membrane portion of the probe, below the non-membrane (stainless steel) portion, was located in the NAc. Figure 5b shows a depiction of all microdialysis probe locations (active dialysis membranes) in the NAc. The active membranes of the dialysis probes were located within both the core and shell.

Discussion

The major findings of the present study are that systemic or intra-NAc local administration of GVG significantly elevated extracellular NAc GABA and glutamate, which was completely blocked by intra-NAc local perfusion of NPPB and partially attenuated by intra-NAc SKF89976A. Intra-NAc administration of TTX had no effect. These findings suggest that GVG-enhanced extracellular GABA and glutamate are derived from nonvesicular sources, and both anion channels and type 1 GABA transporters (GAT1) are critically involved in these actions.

As noted above, GABA exerts inhibitory control on neuronal activity and neurotransmitter release by activation of GABA_A and/or GABA_B receptors (Bowery 1993; Rudolph and Mohler 2006). GABA transporters play an important role in regulating synaptic GABA levels and neuronal activity. Four types of GABA transporters (GAT1–GAT4) have been identified in the CNS, with GAT1 being the most abundant (Conti et al. 1998; Schousboe 2000). Physiologically, GABA transporters rapidly move synaptically released GABA into presynaptic terminals and/or surrounding astrocytes (Schousboe et al. 2004). In the present study, we found that blockade of GAT1 with SKF89976A caused a significant increase in

extracellular NAc GABA levels. In contrast, neither TTX nor NPPB significantly altered basal levels of extracellular GABA. These data suggest an important role for GAT1 but not vesicular GABA release or anion channels in maintaining basal levels of extracellular GABA (Timmerman and Westerink 1997).

In addition to reuptake of synaptic GABA, GABA transporters can also work in reverse mode to release GABA from neurons or astrocytes (Bonanno and Raiteri 1994; Levi and Raiteri 1993; Overstreet and Westbrook 2001; Wu et al. 2001, 2003, 2007). In the present study, we found that systemic or intra-NAc local administration of GVG significantly increased intracellular GABA levels, an effect that was partially attenuated by intra-NAc administration of SKF89976A, but not TTX, suggesting an important role for GAT1 in mediating GVG-enhanced extracellular GABA.

Surprisingly, we found that blockade of anion channels by NPPB did not alter basal levels of, but completely blocked GVG-induced increases in, extracellular NAc GABA. The mechanisms underlying this action are unclear. Since GVG is an amino acid analog that cannot freely penetrate cell membranes, one possibility is that GVG may enter cells via anion channels. Thus, blocking anion channels may prevent GVG from entering cells and therefore block its pharmacological action. A second possibility is that increased intracellular GABA may increase intracellular osmotic pressure, causing cell swelling and activation of anion channels (Kimelberg et al. 2006; Fisher et al. 2008). Since activated anion channels are permeable to amino acid neurotransmitters (Kimelberg et al. 1990; Saransaari and Oja 2005; Malarkey and Parpura 2008), a sharp increase in intracellular GABA concentration may cause GABA efflux through activated anion channels (Nicholls and Attwell 1990; Kimelberg et al. 2006) and/or reversal of GABA transporters as shown previously (Wu et al. 2001, 2003, 2007).

Glutamate is the major excitatory neurotransmitter in the CNS, playing an important role in various brain functions. Glutamate can be released from neurons and/or glial cells by several distinct mechanisms, including Ca^{2+} -dependent vesicular release, reversal of glutamate transporters, and opening of anion channels induced by cell swelling (Malarkey and Parpura 2008). An important finding of the present study is that NPPB-induced blockade of anion channels significantly attenuated, while TTX-induced blockade of voltage-dependent Na^+ channels failed to alter, basal extracellular NAc glutamate levels, suggesting an important role for anion channels in maintaining basal levels of extracellular glutamate in vivo. We note recent reports suggesting that NPPB may not be highly selective for membrane anion channels. It may also block vesicular anion channels, thereby affecting vesicular loading (Pothos et al. 2002) and may also block hemichannel function (Eskandari et al. 2002). Given that TTX alone failed to alter extracellular glutamate levels, we suggest that NPPB-induced reduction in extracellular glutamate is unlikely to have been mediated by altering vesicular loading and/or trafficking. Although recent studies suggest that hemichannels may mediate glutamate release from astrocytes in vitro (Ye et al. 2003), in vivo evidence is lacking. In addition to NPPB, the GAT1 inhibitor SKF89976A also lowered basal glutamate levels. We interpret this as secondary to SKF89976A-enhanced extracellular GABA. That is, under basal physiological conditions, SKF89976A blocks GABA reuptake—elevating synaptic GABA levels, which may then spill over to inhibit neighboring glutamatergic neurons.

We also found, unexpectedly, that systemic or intra-NAc administration of GVG significantly increased extracellular NAc glutamate, an effect that was blocked by intra-NAc local perfusion of NPPB but not TTX. These findings suggest that non-vesicular, anion channel-mediated mechanisms may underlie GVG-enhanced extracellular glutamate, as well as GVG-enhanced extracellular GABA (as discussed above). There are several possible explanations for this action. First, blockade of anion channels may prevent GVG from

entering cells and, therefore, block the pharmacological action of GVG on extracellular GABA and glutamate. However, this hypothesis fails to explain how GVG elevates extracellular glutamate. Second, GVG may initially increase both intracellular GABA and glutamate, causing GABA and glutamate co-release, via activated anion channels. We believe this to be likely because inhibition of intracellular GABA transaminase by GVG leads to an increase in intracellular GABA levels, which may subsequently inhibit glutamate metabolism or GABA synthesis from its precursor glutamate (Madsen et al. 2008; Buddhala et al. 2009). Given that both GABA and glutamate are osmotically active solutes, increased intracellular GABA and glutamate may induce cell swelling and activation of anion channels, producing GABA and glutamate release via activated anion channels. A third possibility is that increased extracellular GABA may indirectly activate anion channels via a GABA transporter-mediated mechanism (Bonanno et al. 2006). This is supported by our findings that intra-NAc perfusion of SKF89976 partially attenuated GVG-enhanced extracellular glutamate and that intra-NAc perfusion of GABA dose-dependently increased extracellular glutamate levels.

We note that GVG, at the highest dose tested (300 mg/kg, i.p.), failed to alter extracellular glutamate (Fig. 1b), while intra-NAc local perfusion of GVG dose-dependently increased extracellular NAc glutamate (Fig. 2b). The mechanisms underlying these conflicting findings are unclear. It has been reported that systemic administration of GVG not only increases extracellular GABA but also decreases extracellular glutamate and aspartate levels in the prefrontal cortex and hippocampus (Halonen et al. 1991; Löscher and Hörstermann 1994; Tong et al. 2009). Thus, it is likely that high doses of GVG may produce an inhibitory effect on cortical or hippocampal glutamatergic neurons, decreasing glutamatergic inputs to the NAc. This action may counteract GVG-enhanced extracellular glutamate release in the NAc.

In conclusion, the present study demonstrates that systemic or local intra-NAc administration of GVG elevates both extracellular GABA and glutamate levels. We suggest that GVG elevates intracellular GABA and glutamate levels by inhibiting GABA transaminase, causing a co-release of GABA and glutamate via activated anion channels. Such an increase in extracellular glutamate may attenuate GABA's pharmacological action and prevent GABA-induced excessive inhibition of neuronal function, thus constituting a homeostatic regulatory mechanism.

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Fig. 1.

Effects of systemic administration of GVG (25, 100, and 300 mg/kg, i.p.) on extracellular GABA (**a**) and glutamate (**b**) in the NAc. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to the vehicle control group at each time point

Fig. 2.

The effects of intra-NAc local perfusion of GVG or GABA (1, 10, 100, and 1,000 μM) on extracellular GABA (a) and glutamate (b) in the NAc. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, compared to the vehicle group at each time point

Fig. 3.

a Effects of intra-Nac local perfusion of SKF89976A (300 μ M), TTX (1 μ M), or NPPB (300 μ M) alone on extracellular Nac GABA. **b–d** Effects of 300 mg/kg GVG on extracellular GABA in the absence or presence of intra-Nac local perfusion of SKF89976A (300 μ M), TTX (1 μ M), or NPPB (300 μ M). **b** Percent (%) change in extracellular GABA over pre-intra-Nac drug perfusion baselines. **c** Renormalized data from **b**. **d** AUC data from **c**. * P <0.05, ** P <0.01, *** P <0.001, compared with the vehicle (Veh) group (**a**), compared with the Veh + Veh group (**c**). # P <0.05, ## P <0.01, compared with the Veh + GVG group (**c**). + P <0.05, ++ P <0.01, inter-group comparisons (**d**)

Fig. 4.

a Effects of intra-NAc local perfusion of SKF89976A (300 μ M), TTX (1 μ M), or NPPB (300 μ M) alone on extracellular NAc glutamate. **b–d** Effects of 100 mg/kg GVG on extracellular glutamate in the absence or presence of intra-NAc local perfusion of SKF89976A (300 μ M), TTX (1 μ M), or NPPB (300 μ M). **b** Percent change (%) in extracellular glutamate over pre-intra-NAc drug perfusion baselines. **c** Renormalized data from **b**. **d** AUC data from **c**. * P <0.05, ** P <0.01, *** P <0.001, compared with the Veh group (**a**) or compared with the Veh + Veh group (**c**). + P <0.05, ++ P <0.01, inter-group comparisons (**d**)

Fig. 5.

a Photomicrograph of a stained coronal brain section, demonstrating the position and extent of a representative guide cannula and representative microdialysis probe, including the non-membrane portion (~1 mm beyond the tip of the guide cannula) and the active membrane portion. **b** Schematic reconstructions of positions of active microdialysis membranes within the NAc. Dialysis membranes spanned the length of the core and shell compartments of the NAc