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Regulation of Locomotor Activity by Metabotropic Glutamate Receptors in the Nucleus Accumbens and Ventral Tegmental Area[1]

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ABSTRACT

Glutamatergic innervation of the ventral tegmental area (VTA) and the nucleus accumbens (NA) regulates locomotor activity. The present study was designed to evaluate the involvement of metabotropic glutamate receptors (mGluRs) in motor activity. Agonists selective for each of the three subgroups of mGluRs were microinjected into the VTA or NA, and motor activity was monitored. The group I agonist (*S*)-3,5-dihydroxyphenylglycine elicited a dose-dependent elevation in motor activity after microinjection into either the VTA or NA. The effect in the NA was blocked by the mGluR1-specific antagonist 7-(hydroxyimino)cyclopropa[*b*]chromen-1a-carboxylate ethyl ester. The group II agonist (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine also elicited a short-duration motor activation after microinjection into either structure. The dose response in the VTA was biphasic, and the coadministration of the group II/III-specific antagonist (*RS*)-α-methyl-4-phosphonophenylglycine partially blocked motor activation in both the NA and VTA. Although the group III agonist L-(+)-2-amino-4-phosphonobutyric acid produced a relatively modest behavioral stimulation after microinjection into the NA, it was without effect in the VTA. These data indicate a role for mGluR subgroups in the regulation of motor activity in the VTA and NA.

The nucleus accumbens (NA) is located within the ventral striatum and is an important neural substrate in motivation, reward, and behavioral activation (Mogenson et al., 1980; Kalivas et al., 1993). Within the NA, emphasis has been placed on the mesoaccumbens dopaminergic afferents as a primary regulator of the motor activation frequently associated with motivation and reward. The ventral tegmental area (VTA) and medial substantia nigra are the sources of dopamine projections to the accumbens (Fallon and Moore, 1978). Supporting a role by these projections in motor activation, the administration of dopamine receptor agonists into the NA elicits locomotor activity (Swanson et al., 1997), and indirect dopamine agonists such as cocaine and amphetamine have been shown to produce their behavioral activating effects by enhancing extracellular dopamine levels in accumbens (Kuczenski and Segal, 1989).

In addition to dopaminergic innervation, the NA receives glutamatergic input from prefrontal cortex, amygdala, and hippocampus (Meredith et al., 1993), and a growing body of evidence suggests that these two neurotransmitter systems may converge to regulate motor activity. For instance, microinjection of glutamate agonists into the NA increases locomotor activity, and antagonists of glutamate or dopamine receptors can inhibit this response (Donzanti and Uretsky, 1983; Pulvirenti et al., 1994). Moreover, glutamate agonists modulate extracellular dopamine levels in the NA, and locomotor activation produced by psychostimulants can be inhibited by glutamate receptor antagonists (Imperato et al., 1990; Burns et al., 1994). Study of the synaptic ultrastructure in the NA has disclosed that excitatory afferents form synaptic contacts on the same dendritic spines as dopaminergic nerve terminals (Sesack and Pickel, 1992). This juxtaposition supports the likelihood that the dopamine and glutamate neurotransmitter systems not only share common postsynaptic targets but also may interact via a paracrine-like heterosynaptic modulation. The prefrontal cortex sends glutamatergic projections not only to NA but also to the VTA (Sesack et al., 1989). This anatomical organization provides an additional mechanism by which glutamatergic innervation of the mesoaccumbens projection can regulate dopamine transmission in the NA and influence the expression of motor behaviors. For example, stimulation of the projection from prefrontal

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ABBREVIATIONS: NA, nucleus accumbens; CPCCOEt, 7-(hydroxyimino)cyclopropa[*b*]chromen-1a-carboxylate ethyl ester; NMDA, *N*-methyl-D-aspartate; CPP, (*R*)-(−)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid; DCG-IV, (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine; DHPG, (*S*)-3,5-dihydroxyphenylglycine; L-AP4, L-(+)-2-amino-4-phosphonobutyric acid; mGluR, metabotropic glutamate receptor; MPPG, (*RS*)-α-methyl-4-phosphonophenylglycine; VTA, ventral tegmental area.

cortex to the VTA or the administration of glutamate agonists directly into VTA increases extracellular dopamine levels in the NA and increases locomotor activity (Suaud-Chagny et al., 1992).

Glutamatergic neurotransmission is mediated by both ionotropic and metabotropic receptors (mGluRs). Most information accrued regarding glutamatergic involvement in behavioral activation involves ionotropic glutamate receptors (Burns et al., 1994; Pulvirenti et al., 1994; Pap and Bradbury, 1995). As such, relatively few studies have evaluated the role of mGluRs in the mesoaccumbens projection to modulate motor activity. The few studies that have been conducted reveal that mGluR agonists stimulate motor activity when microinjected into the NA; however, these studies have used the nonselective mGluR agonist (Attarian and Amalric, 1997; Vezina and Kim, 1998, for review), which has been shown to display nearly equal affinity for both group I and group II mGluRs (Conn and Pin, 1997). Eight mGluR genes have been identified, and the protein products are divided into three subgroups based on sequence homology, pharmacology, and coupling to intracellular transduction systems (Conn and Pin, 1997). Group I receptors consist of mGluR1 and mGluR5, group II consists of mGluR2 and mGluR3, and group III includes mGluR4 and mGluR6 through mGluR8. Over the past 5 years, drugs have been developed that are relatively selective for the mGluR subgroups, making it possible to discern which subgroup or subgroups of receptor may be mediating the motor stimulation produced by the less selective mGluR agonists. In the present report, we investigate a role for each mGluR subgroup in motor behavior and offer an overview of their action in two important nuclei within the motive circuit. It is important to note that although the compounds used in this study have shown in vitro selectivity, there are no existing data on the nature of their actions in vivo.

Materials and Methods

Animals and Surgery. Male Sprague-Dawley rats weighing between 250 and 300 g (Simonsen Laboratories, Gilroy, CA) were individually housed with food and water available ad libitum. A 12-h light/dark cycle (7 AM to 7 PM lights on) was used to regulate the animal photocycle. All experimentation was carried out during the light cycle.

Surgeries were performed 5 to 7 days after the arrival of the subjects at the ALAC-approved housing facility, and all experimentation began 1 week after the operative procedure. Animals were anesthetized with Equithesin (3.0 ml/kg), and chronic indwelling guide cannulas (26 gauge, 14 mm; Small Parts, Roanoke, VA) were aimed 1 mm above the injection site in the NA and VTA. The NA was targeted to coordinates +1.2 mm anteroposterior, ±1.5 mm lateromedial, and −6.5 mm dorsoventral from bregma with nose-bar at −3.3 mm according to the atlas of Paxinos and Watson (1986). The stereotaxic coordinates for cannula implantation into the VTA were +2.5 mm anteroposterior, ±0.6 mm lateromedial, and −1.5 mm dorsoventral from interaural zero angled 6 degrees from the midline, in accordance with Pellegrino et al. (1979). The guide cannulas were fixed to the skull with three stainless steel screws (Small Parts) and dental acrylic and were fit with obturators (33 gauge, 14 mm; Small Parts) between testing periods to prevent blockage by debris.

Drugs. All mGluR agonists and antagonists used in this study were purchased from Tocris Cookson (Ballwin, MO). (*S*)-3,5-Dihydroxyphenylglycine (DHPG) and (2*S*,2′*R*,3′*R*)-2-(2′,3′-dicarboxycyclopropyl)glycine (DCG-IV) were dissolved in 0.9% sterile saline. 7-(Hydroxyimino)cyclopropa[*b*]chromen-1a-carboxylate ethyl ester (CPCCOEt) was dissolved in 50% dimethyl sulfoxide and sterile water. The control injection for the CPCCOEt experiment consisted of 50% dimethyl sulfoxide in water. L-(+)-2-Amino-4-phosphonobutyric acid (L-AP4) and (*RS*)-α-methyl-4-phosphonophenylglycine (MPPG) were dissolved in 1 Eq NaOH, neutralized with 0.1 N HCl, and diluted with sterile water (Sigma Chemical Co., St. Louis, MO). All drugs were made up in bulk volume and stored at −80°C. For all drugs, nanomole doses represent the total amount administered per bilateral injection (i.e., 5 nmol = 2.5 nmol/0.5 μl/side).

Microinjection and Experimental Design. Immediately before testing, the obturators were removed and the injection cannulas (33 gauge, 15 mm) fitted to a 1-μl Hamilton syringe via PE-20 tubing were inserted to a depth 1 mm below the tip of the guide cannula. Bilateral infusions were made over 60 s in a total volume of 0.5 μl/side. The infusion pump was turned off, and the injection cannulas were left in place for an additional 60 s to prevent backflow of drug, at which time animals were placed into the photocell cages (Omnitech, Columbus, OH). Animals were allowed to recover 2 days after each test day to ensure clearance of drug and recovery from the microinjection procedure. For all experiments, we used a counterbalanced design across days over the complete test period, resulting in each animal receiving a maximum of five microinjections.

Motor activity was monitored in clear Plexiglas boxes measuring 22 × 43 × 33 cm. A series of 16 photobeams (8 on each horizontal axis) tabulated horizontal movements, whereas a series of 8 beams located 8 cm above the floor spanned each box to estimate vertical activity (rearing). Photobeam breaks were recorded by computer interface, and the data were stored after each test day. Total horizontal activity, distance traveled, and vertical activity were monitored during each test period. Each period consisted of a 1-h habituation during which animals were placed in photocell cages before testing. After microinfusion, motor activity was monitored every 15 min for 2 h. Animals were returned to their home cages at the close of each session.

Histology and Data Analysis. The rats were administered an overdose of pentobarbital (>100 mg/kg i.p.) and transcardially perfused with 0.9% saline, followed by a 10% formalin solution. The brain was removed and placed in 10% formalin for at least 1 week to ensure proper fixation. Brains were then blocked, and coronal sections (100 μm) were made through the site of cannula implantation with a vibratome. The brains were subsequently stained with cresyl violet, and anatomical placement was verified by an individual unaware of the animal's behavioral response. The StatView statistics package was used to conduct one-way repeated measures ANOVAs within this study. On the discovery of statistical significance, post hoc analyses were performed on dose-response data with Dunnett's test to compare each dose to saline controls. For antagonist studies, pair-wise post hoc comparisons were made with Fisher's Protected Least Significant Difference. All evaluations of horizontal time course data included a two-way ANOVA with repeated measures over time followed by inspection of individual time points with use of Fisher's Protected Least Significant Difference.

Results

Effect of Group I mGluR Agonist on Motor Activity. Figure 1 illustrates the behavioral activation produced by the microinjection of the group I agonist DHPG into the NA and VTA. A significant effect of dose by DHPG in the NA was measured for horizontal ($F_{4,21} = 4.085$; $P = .013$) counts and total distance traveled ($F_{4,21} = 3.568$; $P = .023$). Analysis of vertical counts revealed only a trend toward a significant effect ($F_{4,21} = 2.33$; $P = .089$). Post hoc comparison with Dunnett's test showed a significant difference from controls at the 5-nmol dose for horizontal ($t_{21} = 3.763$) and vertical counts ($t_{21} = 2.889$), as well as for total distance traveled (t_{21}

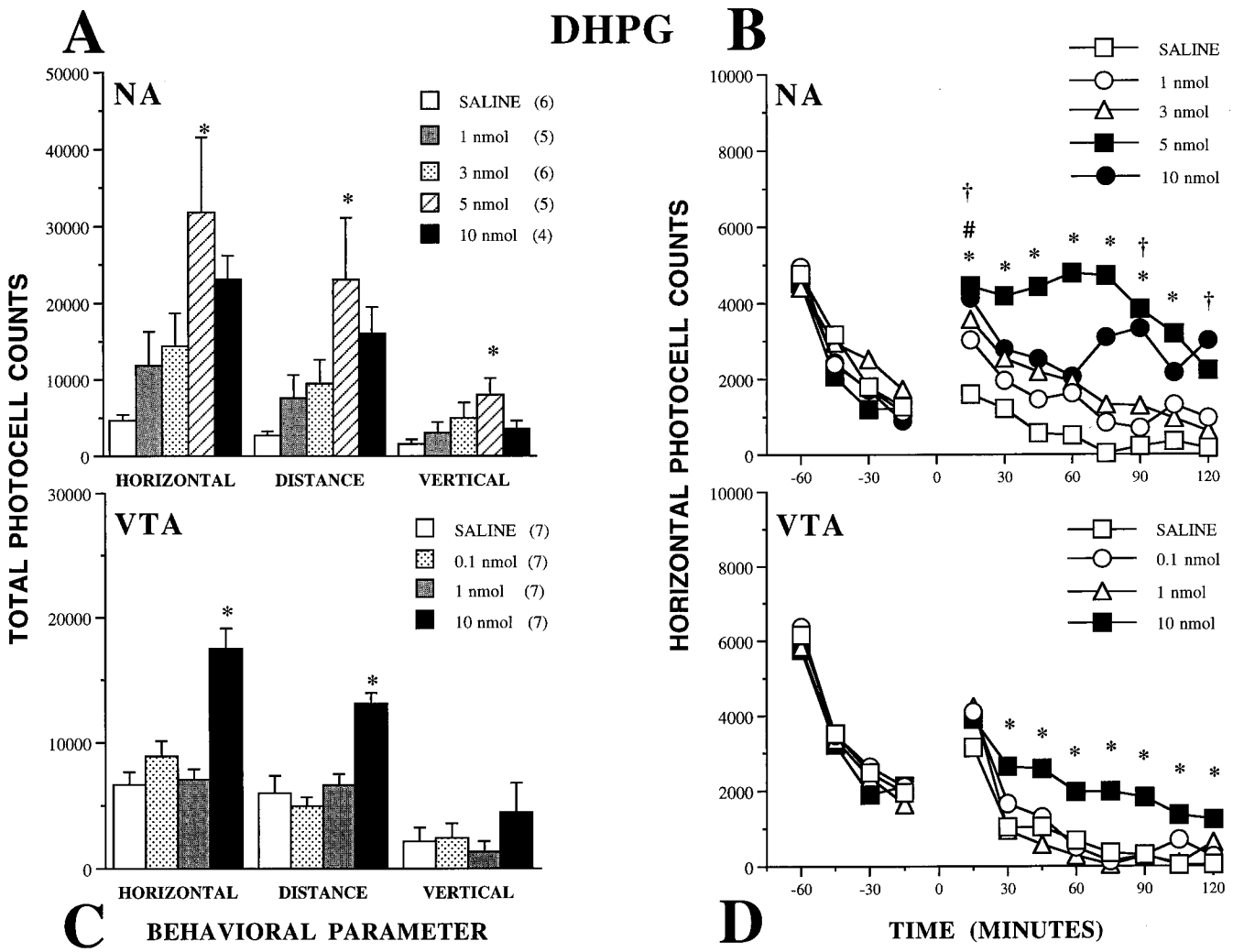

Fig. 1. Stimulation of group I mGluRs with DHPG in the VTA or NA elicits dose-dependent motor activation. A and C, total amount of activity generated during the 2 h after microinjection. The number of vertical counts was multiplied by 1000 for illustrative purposes. B and D, time course of the horizontal data shown on the left. The number of determinations at each dose is shown in parentheses, and the data are shown as mean ± S.E. $^{*}P < .05$, comparing 5 nmol with saline. $^{\dagger}P < .05$, comparing 10 nmol with saline. $^{\#}P < .05$, comparing 3 nmol with saline.

= 3.505). No interaction was measured in any of the three parameters tested. Figure 1B illustrates the time course for horizontal counts with DHPG. As can be seen, the motor stimulant response after the 5 nmol DHPG infusion was sustained for the duration of the experiment. Visual inspection might suggest that 3 and 10 nmol of DHPG also produced significant behavioral activation; however, due to the typically large variance observed in the DHPG response and the relatively low number of determinations used in this study, these doses did not prove to be significant when analyzed with the use of Dunnett's test.

Figure 1C shows the effect of DHPG infusion into the VTA. The highest dose of DHPG (10 nmol) elicited a statistically significant increase in horizontal activity ($F_{3,24} = 16.427$; $P = .0001$) and distance traveled ($F_{3,24} = 10.55$; $P = .0001$). Vertical activity was not significantly altered across dose ($F_{3,24} = 0.719$; $P = .550$). No interaction was witnessed for any parameter tested. Figure 1D depicts the time course of horizontal counts elicited by DHPG microinjection into the VTA. The highest dose of 10 nmol of DHPG produced an increase in activity over nearly the entire 2-h postmicroinjection period.

Effect of Group II mGluR Agonist on Motor Activity. The motor effects of local infusion of the specific group II agonist DCG-IV into the NA and VTA are shown in Fig. 2. Figure 2A shows that the microinjection of DCG-IV into the NA produced a dose-dependent elevation in all three parameters of motor behavior (horizontal: $F_{3,23} = 4.837$, $P = .009$; distance: $F_{3,23} = 4.186$, $P = .017$; vertical: $F_{3,23} = 3.751$, $P = .025$). Post hoc analysis with Dunnett's test revealed a significant difference from control for all three parameters at the 0.1-nmol dose. The time course shown in Fig. 2B reveals a significant interaction ($F_{7,,21} = 3.391$; $P = .0001$). Microinjection of 0.1 nmol produced an increase in horizontal counts that endured for roughly the first 45 min after microinjection.

Figure 2, C and D, illustrates dose-response and time course data for DCG-IV microinjection into the VTA, respectively. In contrast to the NA, the dose-response of DCG-IV in the VTA was biphasic. A significant dose effect was seen for horizontal activity ($F_{3,33} = 3.657$; $P = .022$) and vertical activity ($F_{3,35} = 4.579$; $P = .008$), whereas a large variance in the distance traveled prevented statistical significance. Post hoc analysis showed a significant effect for the 0.1-nmol dose.

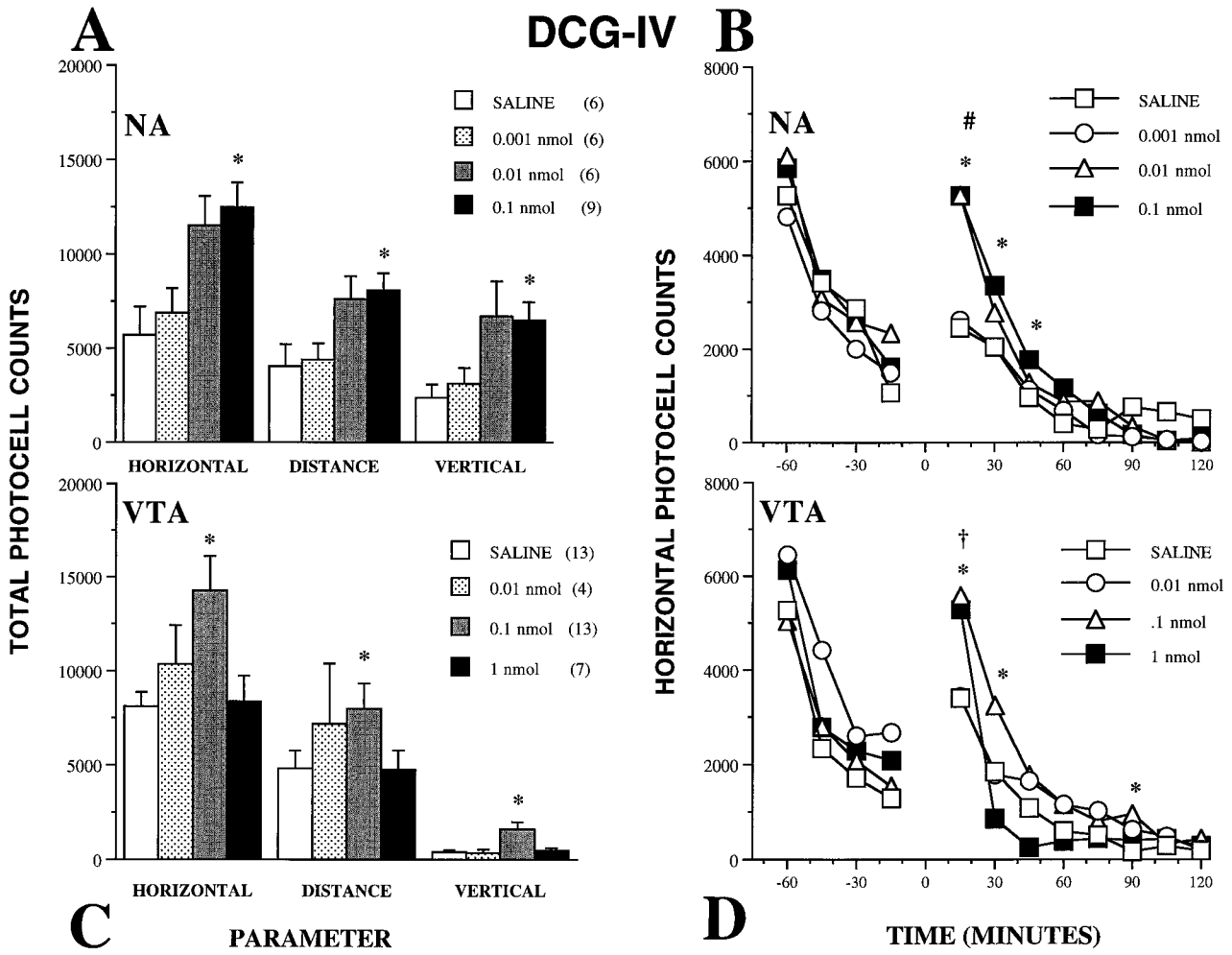

Fig. 2. Dose-dependent elevation of motor activity in the VTA and NA by the group II agonist DCG-IV. A and C, total number of horizontal photocell counts generated during the 2 h after microinjection. The number of vertical counts was multiplied by 1000 for illustrative purposes. B and D, time course of the horizontal data shown on the left. The number of determinations at each dose is shown in parentheses, and the data are shown as mean ± S.E. $^{*}P < .05$, comparing all doses of DCG-IV with saline. $^{\#}P < .05$, comparing 0.01 nmol of DCG-IV with saline. $^{\dagger}P < .05$, comparing 1 nmol of DCG-IV with saline.

Statistical survey of the time course in Fig. 2D revealed a significant interaction for horizontal activity ($F_{7,21} = 2.852$; $P = .0001$), and 0.1 nmol of DCG-IV elicited a significant increase in photocell counts during the first 30 min after microinjection.

Effect of Group III mGluR Agonist on Motor Activity. Figure 3 illustrates that the effect of the group III agonist L-AP4 on motor activation was less robust than that of either the group I or group II agonists. Statistical significance was found only in the NA for horizontal activity and only at the highest dose tested ($F_{3,20} = 3.992$; $P = .022$; Fig. 3). There were no significant effects seen in distance traveled or vertical counts. The time course data in Fig. 3B revealed a near-significant interaction ($F_{7,21} = 1.593$; $P = .0589$); motor activity was elevated by the highest dose of L-AP4 (10 nmol) at 15 and 30 min after microinjection. L-AP4 microinjection into the VTA produced no significant alteration in horizontal, distance traveled, or vertical activity.

Blockade of Motor Activation with Subgroup-Specific Antagonists. Verification that the effect of DHPG was mediated by mGluR1 is illustrated in Fig. 4, A and B, which shows that the specific mGluR 1 antagonist CPCCOEt (10 nmol; Litschig et al., 1999) abolished the motor activation elicited by DHPG (5 nmol) in the NA (treatment: $F_{3,24} = 4.161$, $P = .0161$; interaction: $F_{7,21} = 1.864$, $P = .0163$). DHPG microinfusion elicited an expected increase in motor activity that lasted the duration of the 2-h test period, whereas the coadministration of CPCCOEt completely abolished the effect.

To eliminate the possibility that the DCG-IV-mediated increase in motor activity may be due to its reported action on *N*-methyl-D-aspartate (NMDA) receptors, the NMDA receptor antagonist (*R*)-(−)-3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) was coadministered with DCG-IV in the NA. Figure 4, C and D, illustrates that the significant increase in horizontal activity (treatment: $F_{3,28} = 18.933$, $P = .0001$; interaction: $F_{7,21} = 8.224$, $P = .0001$) elicited by DCG-IV in the NA was not blocked by a dose of CPP previously shown to be behaviorally effective on i.c. microinfusion (Kalivas and Alesdatter, 1993). Rather, the coinfusion of DCG-IV and CPP results in a slightly increased but statistically insignificant motor activation compared with DCG-IV alone.

The group II/III-specific antagonist MPPG was used to block the increase in activity (treatment: $F_{3,28} = 2.07$, $P = .1268$; interaction: $F_{7,21} = 1.897$, $P = .013$) elicited in the NA

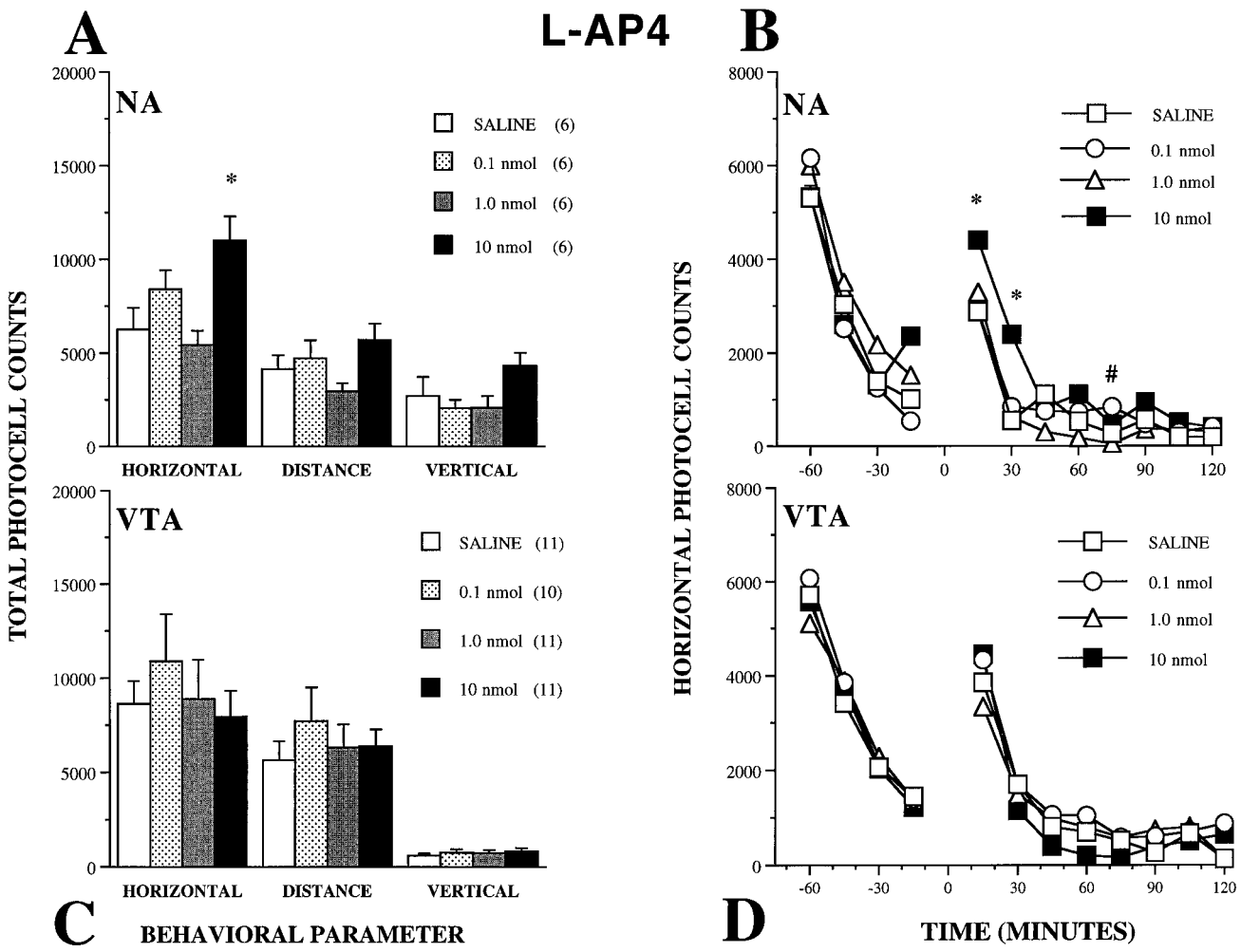

Fig. 3. Effect on motor activity of the group III agonist L-AP4 microinjection into the VTA or NA. Left, total number of horizontal photocell counts generated during the 2 h after microinjection. The number of vertical counts was multiplied by 1000 for illustrative purposes. Right, time course of the horizontal data shown on the left. The number of determinations at each dose is shown in parentheses, and the data are shown as mean ± S.E. $^{*}P < .05$, comparing all doses of L-AP4 with saline. $^{\#}P < .05$, comparing 0.1 nmol of L-AP4 with saline.

by the group II agonist DCG-IV (Fig. 5, A and B). MPPG partially blocked the stimulant effects of DCG-IV in NA in that the mixture of DCG-IV and MPPG was not significantly different from DCG-IV alone or saline. The coinfusion of MPPG plus DCG-IV produced a slight increase in motor activity early in the test period.

The group II/III antagonist MPPG (10 nmol) was also coinfused with a behavioral activating dose of DCG-IV (0.1 nmol) into the VTA. The results of this experiment are shown in Fig. 5, C and D; DCG-IV microinjection into the VTA elicited an increase in horizontal photocell counts (treatment: $F_{3,35} = 3.86$, $P = .01531$; interaction: $F_{7,21} = 1.821$, $P = .0174$) that was partially inhibited by MPPG. MPPG alone caused an increase in photocell counts, and statistical analysis demonstrated it to be intermediate between those of saline and DCG-IV alone. Note that the increase in photocell counts with 10 nmol of MPPG occurred late in the test period (90–120 min; see Fig. 5D).

Histology. Figure 6 illustrates the location of cannulas tips placed into the VTA and NA. Guide cannula placements were located throughout the rostrocaudal and mediolateral extent of the VTA. In the NA, the cannulas tended to terminate near the medial border between the shell and core. No overt differences in behavior were detected with respect to rostrocaudal placement of injection cannulas.

Discussion

Previous research reveals that the stimulation of mGluR receptors in the NA elicits a dose-dependent elevation in motor activity (Attarian and Amalric, 1997; Vezina and Kim, 1999). These studies were conducted with the nonselective mGluR agonist (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid. The present research shows that stimulation of either group I or group II mGluR subgroups produced a motor stimulant response in either the VTA or NA, whereas the stimulation of group III receptors was relatively ineffective.

Motor Activity Elicited by mGluR Receptor Stimulation in NA. The group I agonist DHPG elicited a robust and enduring motor stimulant response when administered into the NA. Both of the group I mGluR subtypes are found in the NA, with mGLuR5 being in particularly high abundance (Testa et al., 1994). In general, both group I receptor subtypes are located perisynaptically, where mGuR1 is located at both presynaptic and postsynaptic sites and mGLuR5 appears to be more postsynaptic (Fotuhi et al., 1993; Lujan et

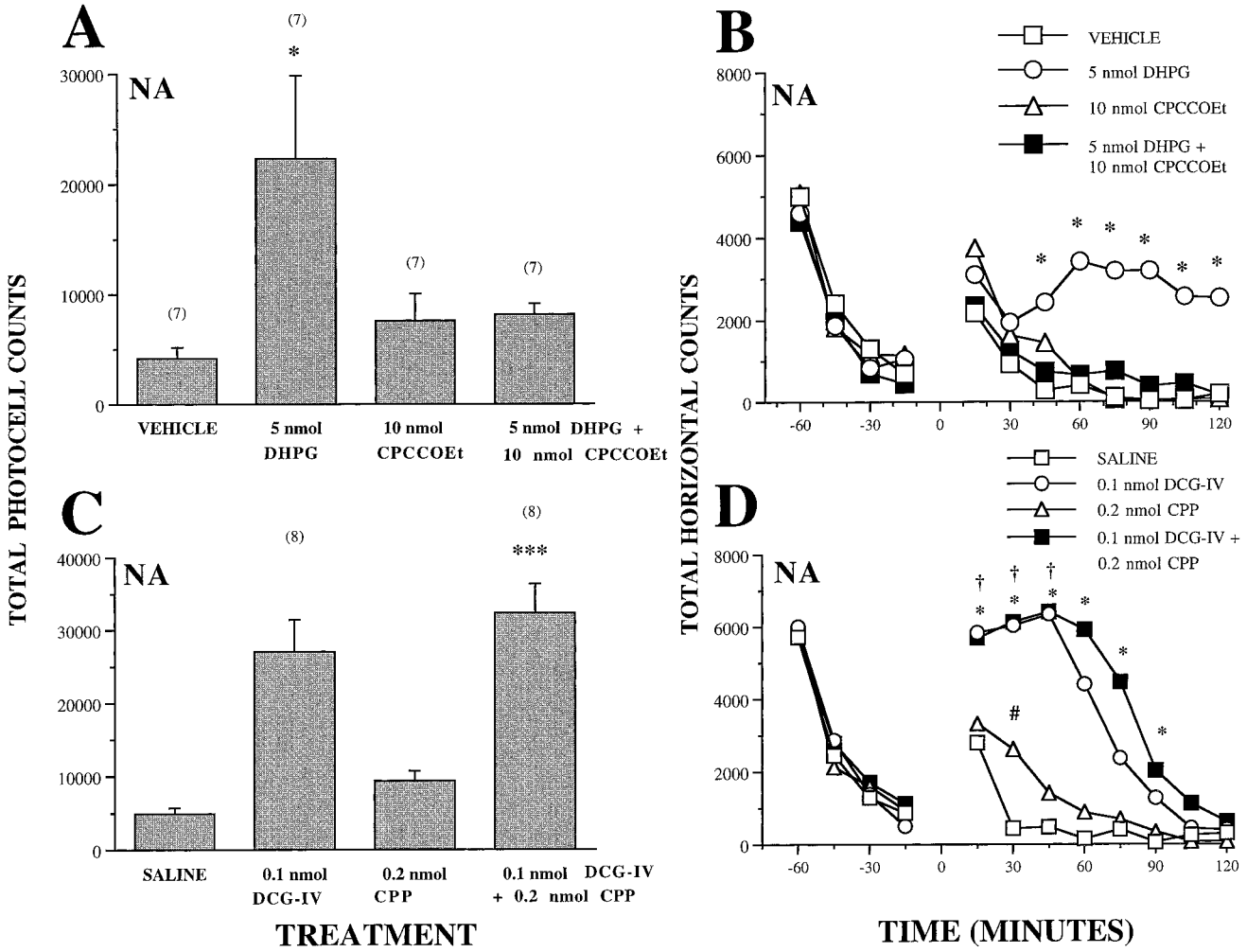

Fig. 4. Evaluation of the ability of distinct antagonists to block motor-stimulating effects of DHPG and DCG-IV in the NA. A and B, ability of group I antagonist CPCCOEt to block the motor response elicited by DHPG in the NA. The data are shown as mean ± S.E. of total horizontal photocell counts and horizontal time course and reveal that blockade of group I receptors by CPCCOEt (10 nmol) blocked the increase in photocell counts by DHPG (5 nmol). C and D, effects of concurrent administration of the NMDA antagonist CPP on the behavioral-activating ability of DCG-IV in the NA. CPP was unable to block motor activity produced by DCG-IV microinfusion in this region. A and B, $^{*}P < .05$ when comparing 5 nmol CPCCOEt with vehicle, C and D, $^{*}P < .05$ when comparing DCG-IV with saline; $^{\dagger}P < .05$ when comparing DCG-IV + CPP with saline; $^{\#}P < .05$ when comparing CPP with saline.

al., 1997). In this study, increased motor activity was abolished by an mGLuR1-selective receptor antagonist, suggesting that the motor response was mediated primarily by these receptors. Interestingly, there is evidence in other brain regions that the stimulation of group I mGluRs increases glutamate release via a presynaptic mechanism (Herrero et al., 1992; Moroni et al., 1998). It is then possible that DHPG microinfusion in the NA acts primarily on presynaptic mGluR 1 receptors and results in glutamate release. The potential release of glutamate within the NA could contribute to the observed motor stimulation. Alternatively, some studies indicate that spiny cells in the NA projecting to either the VTA or ventral pallidum express a high density of group I mRNA (Testa et al., 1995; Romano et al., 1995). However, because mGluR5 seems to be the dominant subtype on spiny cells and DHPG-induced motor activation was abolished by a selective mGluR1 antagonist, the present data argue against this postsynaptic mechanism.

Group II mGluR stimulation elicited a short duration increase in motor activity in the NA. The group II agonist DCG-IV has moderate affinity for NMDA receptors (Hayashi et al., 1993) and group III mGluRs (Brabet et al., 1998), posing the possibility that the motor effect by DCG-IV was not mediated by the group II mGluRs. Because NMDA agonists are known to elicit a motor stimulant effect after microinjection into the NA (Ohno and Watanabe, 1995; Pap and Bradberry, 1995), it is reasonable that agonism of NMDA receptors by DCG-IV may result in the observed motor increase. However, it is important to note that a very low dose (0.1 nmol) of DCG-IV was used to elicit motor behavior effectively minimizing its nonspecific action. Furthermore, the motor response to DCG-IV was partially blocked by coadministration with a group II/III-selective antagonist in either the NA or the VTA, indicating that the effect involves group II mGluRs. Perhaps most convincing is the fact that the potent NMDA antagonist CPP was unable to antagonize the motor response elicited by DCG-IV in the NA. It is unlikely that DCG-IV effects are mediated via group III receptor subtypes given that L-AP4 failed to produce a lasting motor activation when used in this study. In situ hybridization studies reveal the presence of mRNA encoding both mGluR2 and mGluR3 in the NA, supporting the possibility that DCG-IV may be

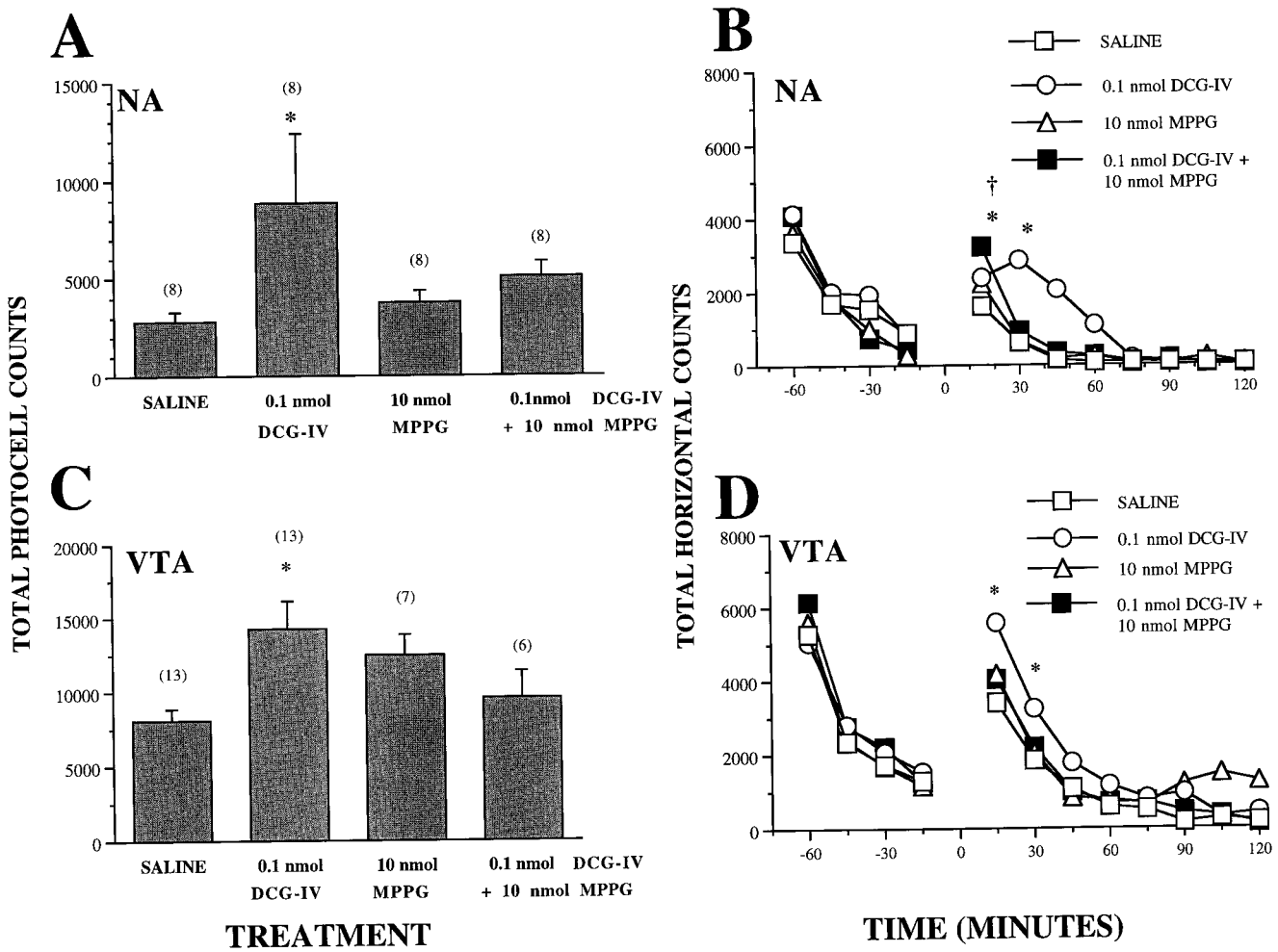

Fig. 5. Determination of the ability of MPPG to block the motor-stimulating effects of DCG-IV in the NA and VTA. A and B, ability of group II/III antagonist MPPG to block the motor stimulant response to DCG-IV microinjection into the NA. The data are shown as mean ± S.E. of total horizontal photocell counts and horizontal time course and reveal that blockade of group II/III receptors by MPPG (10 nmol) partially blocked the increase in photocell counts by DCG-IV (0.1 nmol). The number of determinations are shown in parentheses. C and D, ability of group II/III-specific antagonist MPPG to block the increased motor activity elicited by DCG-IV in the VTA. The data are shown as mean ± S.E. of total horizontal photocell counts and horizontal time course. The number of determinations is shown in parentheses. $^{*}P < .05$ when comparing DCG-IV with saline; $^{\dagger}P < .05$ when comparing DCG-IV + MPPG with saline.

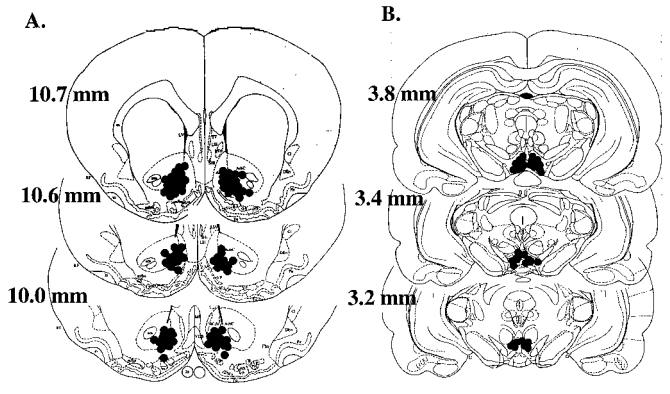

Fig. 6. Location of cannula tips in the NA and VTA. Coordinates are representative of stereotaxic location with respect to interaural zero according to the atlas of Paxinos and Watson (1986), The majority of NA microinjections fell on the medial border of the core and shell. VTA placements tended to be located throughout the rostrocaudal VTA.

acting at postsynaptic binding sites (Ohishi et al., 1993, 1995; Testa et al., 1995). In contrast, an electrophysiological study reveals that group II agonists presynaptically inhibit glutamate synaptic potentials in the NA (Manzoni et al., 1997). Similarly, Hu et al. (1999) found DCG-IV to inhibit dopamine release in the NA. Given that the enhanced release of both glutamate and dopamine has been shown to produce motor activity (Burns et al., 1994), it is unlikely that presynaptic inhibition of their release by DCG-IV contributes to the motor stimulant effect observed in this study.

It is important to note that studies using group II mGluR agonists other than DCG-IV were unsuccessful at evoking spontaneous motor activation. However, in these cases the compounds used were either administered systemically (Helton et al., 1998; Moghaddam and Adams, 1998) or by i.c.v. injection. The latter study also used high doses of agonist, and the involvement of NMDA receptors was revealed (Kronthaler and Schmidt, 1998). Regardless, the compounds used in the studies mentioned above had access to whole brain circuitry, and effects outside the NA or VTA may mask the behavioral activation associated with the group II mGluR agonist used in the present study.

mRNA encoding the group III receptor subtypes mGluR4 and mGluR7 is found in moderate abundance in the NA

(Ohishi et al., 1995; Testa et al., 1995). However, stimulation of these receptors over the dose range examined produced a minimal effect on activity. Moreover, Hu et al. (1998) found that L-AP4 markedly reduced basal levels of extracellular dopamine, indicating a presynaptic effect on dopamine terminals in the NA to inhibit dopamine release. This action would not be expected to mediate a motor stimulant effect because reduced dopamine transmission inhibits motor activity.

Motor Activity Elicited by mGluR Receptor Stimulation in VTA. Similar to the NA, both group I and group II, but not group III, mGluR agonists stimulated motor activity when microinjected into the VTA. Interestingly, the opposite profile exists for the expression of mRNA encoding the mGluR subtypes. Thus, mGluR7 mRNA and protein are relatively abundant in the VTA (Ohishi et al., 1995; Kinoshita et al., 1998), whereas mRNA encoding the group I and group II mRNAs is absent or minimal (Ohishi et al., 1993a,b). However, mGluR1 and mGluR2/3 protein content is moderate in the ventral mesencephalon, indicating a presynaptic localization (Romano et al., 1995; Petralia et al., 1996). This implies that the behavioral effects of the group I and group II agonists are mediated by presynaptic modulation of transmitter release. Because group I agonists presynaptically augment glutamate release (Herrero et al., 1992; Macek et al., 1998; Moroni et al., 1998), a reasonable mechanism for DHPG-induced motor activity in the VTA is enhanced glutamate release, resulting in the stimulation of ionotropic glutamate receptors on dopamine (Suaud-Chagny et al., 1992). Moreover, the ventral portion of the prefrontal cortex is a primary source of glutamatergic innervation of the VTA (Sesack et al., 1989), and this cortical region is relatively enriched in mRNA encoding mGluR1 (Testa et al., 1995). Alternatively, it was recently reported that mGluR receptor stimulation of dopamine cells produces a decrease followed by an increase in cell membrane potential or firing frequency (Meltzer et al., 1997; Fiorillo and Williams, 1998). This appears to be a postsynaptic effect mediated by group I mGluRs (Fiorillo and Williams, 1998). Thus, despite the low abundance of group I mRNA in the VTA, the latter studies support a direct postsynaptic action to stimulate dopamine cells and elicit motor activation.

The biphasic dose-response effect of group II receptor stimulation in the VTA poses the possibility that multiple receptors may be activated by DCG-IV. As outlined, although DCG-IV has high affinity for group II mGluRs, it also has moderate affinity for NMDA receptors (Hayashi et al., 1993). The motor stimulant effect elicited by the moderate dose of DCG-IV was reduced by a group II/II mGluR antagonist, indicating the involvement of group II receptors. However, it is possible that the motor inhibition observed after the highest dose of DCG-IV may arise from stimulation of NMDA receptors. In accordance with this, a recent study by Kronthaler and Schmidt (1998) reported that another potent group II agonist, (2*S*,3*S*,4*S*)-α-carboxycyclopropyl-glycine, induced catalepsy in animals when used i.c.v. at extremely high doses (62.5–500 nmol), and this effect was greatly reduced by the NMDA antagonist dizocilpine. Analysis of the time course of the behavioral response after the higher dose reveals that early motor stimulation is followed by motor inhibition. Given that excessive stimulation of NMDA receptors produces depolarization block of dopamine neuronal activity (Johnson et al., 1992), it is possible that the later inhibition of motor activity may arise from the induction of depolarization block by NMDA receptor stimulation.

Summary. The present report demonstrates that group I and group II mGluR stimulation in the VTA and NA elicits motor activation. To a lesser extent, group III receptor stimulation in the NA was also behaviorally active. Based on the present findings and the literature reviewed, it is proposed that the primary action of DHPG is on mGluR1 located presynaptically on glutamatergic afferents, where the stimulation of these receptors results in the release of glutamate. In contrast, the present data and current literature are most consistent with DCG-IV action on the spiny cells of the NA, whereas a preferential presynaptic-versus-postsynaptic action of DCG-IV in the VTA is not implied by extant data.

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References

Attarain S and Amalric M (1997) Microinfusion of the metabotropic glutamate receptor agonist 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid into the nucleus accumbens induces dopamine-dependent locomotor activation in the rat. *Eur J Neurosci* **9:**809–816.

Brabet I, Parmentier M-L, De Colle C, Bockaert J, Acher F and Pin J-P (1998) Comparative effect of L-CCG-I, DCG-IV and gamma-carboxy-L-glutamate on all cloned metabotropic glutamate receptor subtypes. *Neuropharmacology* **37:**1043–1051.

Burns LH, Everitt BJ, Kelley AE and Robbins TW (1994) Glutamate-dopamine interactions in the ventral striatum: Role in locomotor activity and responding with conditioned reinforcement. *Psychopharmacology* **115:**516–528.

Conn PJ and Pin J-P (1997) Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol* **37:**205–237.

Donzati BA and Uretsky NJ (1983) Effects of excitatory amino acids in locomotor activity after bilateral microinjection into the rat nucleus accumbens: Possible dependence on dopaminergic mechanisms. *Neuropharmacology* **22:**971–981.

Fallon JH and Moore RY (1978) Catecholamine innervation of basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and striatum. *J Comp Neurol* **180:**545–580.

Fiorillo CD and William JT (1998) Glutamate produces inhibitory postsynaptic potentials in dopamine neurons. *Nature (Lond)* **394:**78–82.

Hayashi Y, Momiyama A, Takahashi T, Ohishi H, Ogawa-Meguro R, Shemoto R, Mizuno N and Nakanishi S (1993) Role of metabotropic glutamate receptor in synaptic modulation in the accessory olfactory bulb. *Nature (Lond)* **366:**687–690.

Helton DR, Tizzano JP, Monn JA, Schoepp DD and Kallman MJ (1998) Anxiolytic and side-effect profile of LY354740: A potent, highly selective, orally active agonist for group II metabotropic glutamate receptors. *J Pharmacol Exp Ther* **284:**651–660.

Herrero I, Miras-Portugal MT and Sanchez-Prieto J (1992) Positive feedback of glutamate exocytosis by metabotropic presynaptic receptor stimulation. *Nature (Lond)* **360:**163–166.

Hu G, Duffy P, Swanson C, Ghasemzadeh MB and Kalivas PW (1998) The regulation of dopamine transmission by metabotropic glutamate receptors. *J Pharmacol Exp Ther*, in press.

Imperato A, Honore T and Jensen LH (1990) Dopamine release in the nucleus caudatus and in the nucleus accumbens is under glutamatergic control through non-NMDA receptors: A study in freely-moving rats. *Brain Res* **530:**223–228.

Johnson SW, Seutin V and North RA (1992) Burst firing in dopamine neurons induced by N-methyl-D-aspartate: Role of electrogenic sodium pump. *Science (Wash DC)* **258:**665–667.

Kalivas PW and Alesdatter JE (1993) Involvement of N-methyl-D-aspartame receptor stimulation in the ventral tegmental area and amygdala in behavioral sensitization to cocaine. *J Pharmacol Exp Ther* **267:**486–495.

Kalivas PW, Churchill L and Klitenick MA (1993) The circuitry mediating the translation of motivational stimuli into adaptive motor responses, in *Limbic Motor Circuits and Neuropsychiatry* (Kalivas PW and Barnes CD eds) pp 237–287, CRC Press, Boca Raton. FL.

Kinoshita A, Shigemoto R, Ohishi H, van der Putten H and Mizuno N (1998) Immunohistochemical localization of metabotropic glutamate receptors, mGluR7a and MGluR7b, in the central nervous system of the adult rat and mouse: A light and electron microscopic study. *J Comp Neurol* **393:**332–352.

Kronthaler UO and Schmidt WJ (1998) The mGluRs group II agonist (2S,3S,4S)-alpha-carboxycyclopropyl-glycine induces catalepsy in the rat, which is pronouncedly antagonised by dizocilpine and D, L-amphetamine. *Neurosci Lett* **253:**25–28.

Kuczenski R and Segal D (1989) Concomitant characterization of behavioral and striatal neurotransmitter response to amphetamine using in vivo microdialysis. *J Neurosci* **9:**2051–2065.

Litschig S, Gasparini F, Rueegg D, Stoehr N, Flor PJ, Vranesic I, Prezeau L, Pin JP, Thomsen C and Kuhn R (1999) CPCCOEt, a noncompetitive metabotropic glutamate receptor 1 antagonist, inhibits receptor signaling without affecting glutamate binding. *Mol Pharmacol* **55:**453–461.

Meltzer LT, Serpa KA and Christoffersen CL (1997) Metabotropic glutamate receptor-mediated inhibition and excitation of substantia nigra dopamine neurons. *Synapse* **26:**184–193.
Meredith GE, Pennartz CMA and Groenewegen HJ (1993) The cellular framework for chemical signalling in the nucleus accumbens. *Prog Brain Res* **99:**3–24.
Mogenson GJ, Jones DJ and Yim CY (1980) From motivation to action: Functional interface between the limbic system and the motor system. *Prog Neurobiol* **14:**69–97.
Moghaddam B and Adams BW (1998) Reversal of phencyclidine effects by a group II metabotropic receptor agonist in rats. *Science (Wash DC)* **281:**1349–1352.
Moroni F, Cozzi A, Lonbardi G, Sourtcheva S, Leonardi P, Carfi M and Pellicciari R (1998) Presynaptic $mGlu_1$ type receptors potentiate transmitter output in the rat cortex. *Eur J Pharmacol* **347:**189–195.
Ohishi H, Akazawa C, Shigemoto R, Nakanishi S and Mizuno N (1995) Distributions of the mRNAs for L-2-amino-4-phosphonobutyrate-sensitive metabotropic glutamate receptors, mGluR4 and mGluR7, in the rat brain. *J Comp Neurol* **360:**555–570.
Ohishi H, Shigemoto R, Nakanishi S and Mizuno N (1993a) Distribution of the mRNA for a metabotropic glutamate receptor (mGluR3) in the rat brain: An in situ hybridization study. *J Comp Neurol* **335:**252–266.
Ohishi H, Shigemoto R, Nakanishi S and Mizuno N (1993b) Distribution of the mRNA for a metabotropic glutamate receptor, mGluR2, in the central nervous system of the rat. *Neuroscience* **53:**1009–1018.
Ohno M and Watanabe S (1995) Persistent increase in dopamine release after activation of metabotropic glutamate receptors in the rat nucleus accumbens. *Neurosci Lett* **200:**113–116.
Pap A and Bradberry CW (1995) Excitatory amino acid antagonists attenuate the effects of cocaine on extracellular dopamine in the nucleus accumbens. *J Pharmacol Exp Ther* **274:**127–132.
Paxinos G and Watson C (1986) *The Rat Brain in Stereotaxic Coordinates*, Academic Press, New York.
Pellegrino LK and Pellegrino AS and Cushman AJ (1979) *A Stereotaxic Atlas of the Rat Brain*, Plenum Press, New York.
Petralia RS, Wang Y-X, Niedzielski AS and Wenthold RJ (1996) The metabotropic glutamate receptors, mGLUR2 and mGLUR3, show unique postsynaptic, presynaptic and glial localizations. *Neuroscience* **71:**949–976.
Pulvirenti L, Berrier R, Kreifeldt M and Koob GF (1994) Modulation of locomotor activity by NMDA receptors in the nucleus accumbens core and shell regions of the rat. *Brain Res* **664:**231–236.
Romano C, Sesma MA, McDondald CT, O'Malley K, Van Den Pol AN and Olney JW (1995) Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. *J Comp Neurol* **355:**455–469.
Sesack SR and Pickel VM (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminal in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. *J Comp Neurol* **320:**145–160.
Suaud-Chagny MF, Chergui K, Chouvet G and Gonon F (1992) Relationship between dopamine release in the rat nucleus accumbens and the discharge activity of dopaminergic neurons during local in vivo application of amino acids in the ventral tegmental area. *Neuroscience* **49:**63–72.
Swanson CJ, Heath S and Kelley AE (1997) Differential behavioral responses after dopaminergic stimulation of the core and shell subregions of nucleus accumbens. *Pharmacol Biochem Behav* **58:**933–945.
Testa CM, Standaert DG, Landwehrmeyer GB, Penney JB and Young AB (1995) Differential expression of mGluR5 metabotropic glutamate receptor mRNA by rat striatal neurons. *J Neurosci* **354:**241–252.
Testa CM, Standaert DG, Young AB and Penney JBJ (1994) Metabotropic glutamate receptor mRNA expression in the basal ganglia of the rat. *J Neurosci* **14:**3005–3018.
Vezina P and Kim J-H (1999) Metabotropic glutamate receptors and the generation of locomotor activity: interactions with midbrain dopamine. *Neurosci Biobehav Rev* **23:**577–589.

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