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Metabotropic glutamate receptors depress glutamate-mediated synaptic input to rat midbrain dopamine neurones *in vitro*

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1 Glutamate (AMPA receptor-mediated) excitatory postsynaptic potentials (e.p.s.ps.), evoked by electrical stimulation rostral to the recording site, were examined by intracellular microelectrode recording from dopamine neurones in parasagittal slices of rat ventral midbrain.

2 The e.p.s.p. was depressed by the group III metabotropic glutamate (mGlu) receptor agonist L-2-amino-4-phosphonobutyric acid (L-AP4; 0.01–30 μM) by up to 60% with an EC_{50} of 0.82 μM. The depression induced by L-AP4 (3 μM) was reversed by the group III preferring mGlu receptor antagonist, α-methyl-4-phosphonophenylglycine (MPPG; 250 μM).

3 The group I and II mGlu agonist, 1**S**,3**R**-aminocyclopentanedicarboxylic acid (ACPD; 3–30 μM) also depressed the e.p.s.p. in a concentration-dependent manner. The effect of ACPD (10 μM) was reversed by (+)-α-methyl-4-carboxyphenylglycine (MCPG; 1 mM; 4 cells). This effect of ACPD was also partially antagonized (by 50.3±15.7%, 4 cells) by MPPG (250 μM).

4 The selective agonist at group I mGlu receptors, dihydroxyphenylglycine (DHPG; 100 μM), decreased e.p.s.p. amplitude by 27.1±8.2% (7 cells), as did the group II mGlu receptor-selective agonist (1**S**,1′**R**,2′**R**,3′**R**)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV; 1 μM) by 26.7±4.3% (5 cells).

5 DHPG (10–100 μM) caused a depolarization of the recorded cell, as did ACPD (3–30 μM), whereas no such postsynaptic effect of either L-AP4 or DCG-IV was observed.

6 These results provide evidence for the presence of presynaptic inhibitory metabotropic glutamate autoreceptors from the mGlu receptor groups II and III on descending glutamatergic inputs to midbrain dopamine neurones. Group I mGlu receptors mediate a postsynaptic depolarization, and can also depress glutamatergic transmission, but may not necessarily be localized presynaptically. These sites represent novel drug targets for treatment of schizophrenia and movement disorders of basal ganglia origin.

Keywords: Dopamine neurones; basal ganglia; metabotropic glutamate receptors; glutamate receptor agonists; glutamate receptor antagonists; phenylglycine derivatives; synaptic potentials; e.p.s.p.; intracellular recording; brain slices

Introduction

Midbrain dopamine neurones from substantia nigra (SNc) and ventral tegmental area (VTA) and their various projections, principally to dorsal and ventral striatum, amygdala and prefrontal cortex, are critically involved in a variety of behaviours and pathological states. Their role in motor control (Dunnett & Robbins, 1992) arises from modulation of the circuitry of the basal ganglia (Gerfen, 1992; Chesselet & Delfs, 1996), while mesolimbic projections to the ventral striatum (nucleus accumbens) appear central to motivation and reward (Fibiger & Phillips, 1986; Robbins & Everitt, 1996), including the rewarding and habit-forming properties of drugs of abuse (Koob, 1992; Hyman, 1996). Central dopamine systems play a role in cognitive function (Dunnett & Robbins, 1992; Goldman-Rakic, 1995) and, as dopamine antagonists are widely-used antipsychotic agents, dopamine system dysfunction has been implicated additionally in psychosis.

With the cloning and sequencing of eight guanosine 5′-triphosphate (GTP) binding (G) protein-coupled receptors for glutamate, it has become clear in recent years that glutamate can also act as a neuromodulatory agent as well as a fast excitatory transmitter (see Pin & Duvoisin, 1995; Conn & Pin, 1997, for review). These metabotropic glutamate (mGlu) receptors may be grouped together by virtue of sequence homology and preferred second messenger coupling. Thus group I mGlu receptors activate phospholipase C and comprise $mGlu_1$ and $mGlu_5$. Group II couple negatively to adenylyl cyclase and comprise $mGlu_2$ and $mGlu_3$. $mGlu_{4,6,7}$ and $mGlu_8$ also decrease intracellular adenosine 3′:5′-cyclic monophosphate (cyclic AMP) levels, but their distinct pharmacology places them in group III (Pin & Duvoisin, 1995; Conn & Pin, 1997). Pharmacological discrimination of receptors between these three groups may now be made by using selective agonists, but development of selective antagonists, and of compounds capable of discriminating between members of a particular group is presently at an early stage (Conn & Pin, 1997). Electrophysiological studies in the central nervous system have demonstrated two principal consequences of mGlu receptor activation on neurones: direct excitation, and (presynaptic) modulation of neurotransmitter release, including that of glutamate itself (reviewed by Pin & Duvoisin, 1995; Conn & Pin, 1997).

The possibility that mGlu receptors might play a role in modulating dopamine system function was first suggested by the observation that intrastriatal injection of the non-selective mGlu receptor agonist, 1**S**,3**R**-1-amino-1, 3 cyclopentanedicarboxylate (ACPD), caused a contralateral rotational behaviour that was dependent on dopamine receptor activation (Sacaan *et al*., 1992). Indeed, messenger RNA encoding several mGlu receptor subtypes has been described both in the SNc/VTA, and also in many regions of basal ganglia known to project to the ventral midbrain (Testa *et al*., 1994). It has also been shown by intracellular recording in brain slices that ACPD exerts a direct excitatory action on midbrain neurones (Mercuri *et al*., 1993), and a slow synaptic current evoked by

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focal electrical stimulation, attributable to activation of metabotropic glutamate (mGlu) receptors on dopamine neurones, has also been described (Shen & Johnson, 1997). However, while fast evoked synaptic events in dopamine neurones resulting from ionotropic glutamate receptor activation have also similarly been obtained *in vitro* (Mereu *et al*., 1991; Johnson & North, 1992), the presence of presynaptic mGlu (auto-) receptors modulating these inputs has not been investigated. We have used a brain slice preparation to examine electrophysiologically the susceptibility of the descending glutamatergic input to single dopamine neurones to modulation by agonists at mGlu receptors. Our results implicate mGlu receptors from all three groups in decreasing electrically-evoked glutamate release onto dopamine neurones. Some of this work has been published previously in abstract form (Wigmore & Lacey, 1995; 1996).

Methods

Tissue preparation

Parasagittal slices (400 μm thick) of ventral midbrain containing the subthalamic nucleus and substantia nigra were freshly prepared from brains of male Wistar rats (100–180 g) killed by cervical dislocation following halothane anaesthesia, as previously described (Abbott *et al*., 1997). Slices were placed in a recording chamber (volume $\approx$0.3 ml) and superfused at 1–2 ml min^{-1} with artificial cerebrospinal fluid (aCSF) of composition (mM): NaCl 126, KCl 2.5, $CaCl_2$ 2.4, $MgCl_2$ 1.3, NaH_2PO_4 1.2, glucose 10 and $NaHCO_3$ 26, heated to 32–33°C and saturated with 95% O_2/5% CO_2. In order to study specifically the evoked excitatory postsynaptic potential (e.p.s.p.), the $GABA_A$ receptor antagonist picrotoxin (50 μM) was also routinely included in the superfusion medium.

Electrophysiological recording and stimulation

Intracellular recordings from dopamine neurones were made with sharp 2 M KCl-filled microelectrodes (resistance 50–130 MΩ) advanced into the dorso-rostral portion of the substantia nigra/VTA, which could be identified visually with a dissecting microscope (Wild-Leitz). Membrane potential and injected current were amplified and measured by an Axoclamp 2A microelectrode amplifier (Axon Instruments, Foster City, California, U.S.A.). Additional amplification to optimize analogue-to-digital signal conversion was achieved with a Brownlee 210A instrumentation amplifier (Brownlee Precision Co., Santa Clara, California, U.S.A.). Single synaptic events were evoked with electrical stimulation delivered with a bipolar stimulating electrode (twisted FORMVAR-coated 50 μm diameter nichrom wire; Advent Research Materials Ltd., U.K.) placed in or caudal to (within 500 μm of) the subthalamic nucleus, which could also be identified by visual inspection. Single-shock stimuli (10 mA maximum, 100 μs duration) were delivered every 10–30 s by a constant current stimulus isolation unit (AMPI Isoflex, Jerusalem, Israel). In order to record and measure accurately the amplitude of the resultant depolarizing synaptic potentials, cells were held at membrane potentials in the range $-$68 to $-$85 mV by passing a constant hyperpolarizing current across the recording electrode, thus preventing action potential firing during the synaptic event. Any deviation of more than $\pm$2 mV from the pre-set membrane potential during the experiment (including if due to drug application) was offset by manually-controlled current injection to maintain a constant driving force on the ions carrying the synaptic current. Stimulation current amplitude was routinely adjusted at the beginning of each experiment to obtain synaptic potentials of 3–10 mV in amplitude under control conditions, which did not elicit action potential firing, and held constant thereafter. Stimuli were triggered and stored with pCLAMP software (ver. 5.5; Axon Instruments) and delivered to the stimulus isolation unit by a Master-8 pulse generator (AMPI, Jerusalem, Israel). Membrane current and potential were monitored on a chart recorder (Gould Easygraph) and a digital storage oscilloscope, and individual events (both synaptic stimulation and current injection) triggered by pCLAMP, and written to the hard disk of an Elonex 450 PC.

Data analysis

Data analysis was carried out off-line by pCLAMP, Origin 4.0 (MicroCal) and Excel (Microsoft) software. E.p.s.p. depressions due to agonist action were calculated as $(1-X/Y)\times100\%$ where X = average of last 5 successive records from periods of application of agonist, and Y = average of last 5 successive records of control period. Similarly, the reversibility of agonists actions on washout, or by antagonist coapplication, was calculated by $(Z\text{-}X)/(Y\text{-}X)\times100\%$ where Z = average in presence of both agonist and antagonist, or following agonist washout. Concentration-effect curves were fitted to a logistic equation with unity slope by use of Origin 4.0. Statistical significance was calculated by means of one-way ANOVAs, and all data are expressed as mean $\pm$ s.e.mean unless otherwise stated.

Drugs

Drugs were applied in known concentrations in the superfusing solution by use of a three way tap system, with a dead time of 15–45 s before the drugs reached the recording chamber. This dead time was not accounted for in the Figures, thus the onset of drug application represents the onset of superfusion, rather than its entry into the recording chamber. D/L-2-amino-5-phosphonopentanoic acid (AP5), L-2-amino-4-phosphonobutyric acid (L-AP4), 1**S**,3**R**-1-amino-1, 3cyclopentanedicarboxylate (ACPD), **RS**-α-methyl-4-phosphonophenylglycine (MPPG), (+)-α-methyl-4-carboxyphenylglycine (MCPG), (1**S**,1′**R**,2′**R**,3′**R**)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV), (**S**,**R**)-dihydroxyphenylglycine (DHPG) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were all obtained from Tocris Cookson, U.K. Picrotoxin was obtained from Sigma, U.K.

Results

Identification of midbrain dopamine neurones

Of the 105 cells included in this study, 78 cells fired action potentials spontaneously at regular rates. In the 54 cells that fired at $\geqslant$1 Hz, the mean frequency was 2.2$\pm$0.13 Hz (maximum of 4.8 Hz), with the action potential usually followed by a pronounced afterhyperpolarization (Figure 1a). Twenty-seven cells did not fire action potentials spontaneously, but did so on injection of a depolarizing current. When membrane potential was increased to around $-$60 mV by passage of constant hyperpolarizing current (less than 200 pA), spontaneous firing was abolished (Figure 1b). A step hyperpolarization ($\geqslant$10 mV; 500 ms) resulted in a pronounced time-dependent inward rectification in all cells

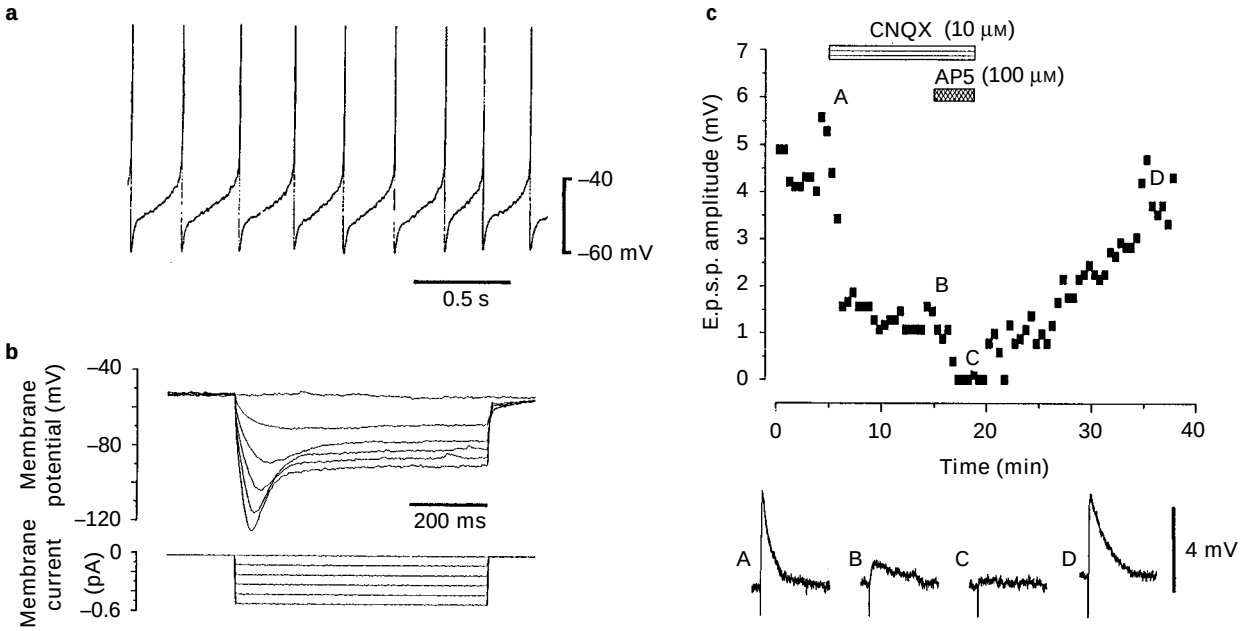

Figure 1 Characteristic membrane properties of midbrain dopamine neurones, and pharmacology of evoked glutamatergic e.p.s.ps. (a) Record of resting membrane potential from a dopamine neurone, which fired spontaneous action potentials at 4.0 Hz, with a threshold for action potential generation of ≈ -40 mV. (b) Superimposed records of membrane potential (above) illustrating the effect of injection of hyperpolarizing current pulses (500 ms duration) of increasing amplitude (below). The time-dependent inward rectification occurring with hyperpolarization is characteristic of dopamine neurones. Pulses superimposed on -30 pA constant injected current, required to prevent spontaneous action potential firing. (c) Upper panel: amplitude of e.p.s.ps evoked every 10 s plotted over the time course of an experiment where glutamate receptor antagonists CNQX (10 μM) and AP5 (100 μM) were applied in the superfusate for the periods shown by the striped and hatched bars, respectively. Lower records: representative e.p.s.ps (average of 3 successive events) from the same experiment taken at the four time points shown. The e.p.s.p. was completely abolished by the combined application of the AMPA and NMDA receptor antagonists. Membrane potential held at -68 mV with injected current throughout the experiment. Picrotoxin (50 μM) present throughout.

(Figure 1b). Input resistance, measured at steady-state by hyperpolarizing current injection steps of 0.1 nA for 500 ms from around -60 mV, was 124 ± 3.3 MΩ ($n = 105$). These electrophysiological properties are generally considered characteristic of the dopamine containing neurones of ventral midbrain (Lacey, 1993).

The fast glutamate receptor-mediated excitatory postsynaptic potential

Single shock electrical stimulation within 500 μm and rostral to the site of recording, in the presence of the $GABA_A$ receptor antagonist picrotoxin, elicited a fast depolarizing synaptic potential that attained peak amplitude within around 10 ms (Figure 1c). The picrotoxin-resistant e.p.s.p. was reversibly depressed by $78 \pm 4.7\%$ ($n = 4$) within 2–3 min of application of the non-N-methyl-D-aspartate (NMDA) type ionotropic glutamate receptor antagonist CNQX (10 μM; Figure 1c). This e.p.s.p. was therefore considered to be mediated primarily by (**RS**)-α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) type glutamate receptors. A small component sensitive to application of the NMDA antagonist AP5 (100 μM) was also observed, such that coapplication of CNQX (10 μM) and DL-AP5 (100 μM) led to a complete depression of the e.p.s.p. (Figure 1c). Thus under these conditions the e.p.s.p. is mediated by glutamate, acting principally on AMPA receptors.

The e.p.s.p. was depressed by group III mGlu receptor activation

The group III mGlu receptor selective agonist L-AP4 (0.3–30 μM; Pin & Duvoisin, 1995; Conn & Pin, 1997) caused a depression of the e.p.s.p. amplitude which reversed on washout (Figure 2a). Concentration-effect data pooled from 44 different experiments indicated an EC_{50} value for this effect of L-AP4 of 0.82 μM, with a maximal effect of around 60% depression occurring with 30 μM L-AP4 (Figure 2b). This effect of L-AP4 (3 μM) was completely reversed on coapplication of the mGlu receptor group III preferring antagonist (Jane *et al*., 1995) MPPG (250 μM; 3 cells; Figure 3), while MPPG (100 μM) reversed the L-AP4 (3 μM) depression of the e.p.s.p. by $68.6 \pm 16.2\%$ (3 cells). L-AP4 (0.3–30 μM) was without effect on membrane potential in all cells tested.

The e.p.s.p. was depressed by group I and group II mGlu receptor activation

The group I/II mGlu receptor selective agonist ACPD (3–30 μM; Pin & Duvoisin, 1995; Conn & Pin, 1997) also reversibly depressed the e.p.s.p. (Figure 4a) in a concentration-dependent manner (Figure 4b). In contrast to L-AP4, application of ACPD was often accompanied by a postsynaptic depolarization, as previously described (Mercuri *et al*., 1993), and this was offset by passing a hyperpolarizing current. The e.p.s.p. depression by ACPD (10 μM) was fully reversed ($n = 4$) by the group I/II - selective (Conn & Pin, 1997) mGlu receptor antagonist MCPG (1 mM; Figure 5a). Additionally, the e.p.s.p. depression caused by ACPD (30 μM) was also partially reversed (by $50.3 \pm 15.7\%$, 4 cells) by MPPG (250 μM; Figure 5b). This supports the suggestion that MPPG is not selective for group III receptors (Jane *et al*., 1995).

In order to establish better the relative contributions of group I and group II mGlu receptors to the action of the non-selective agonist ACPD, agonists selective for group I and for

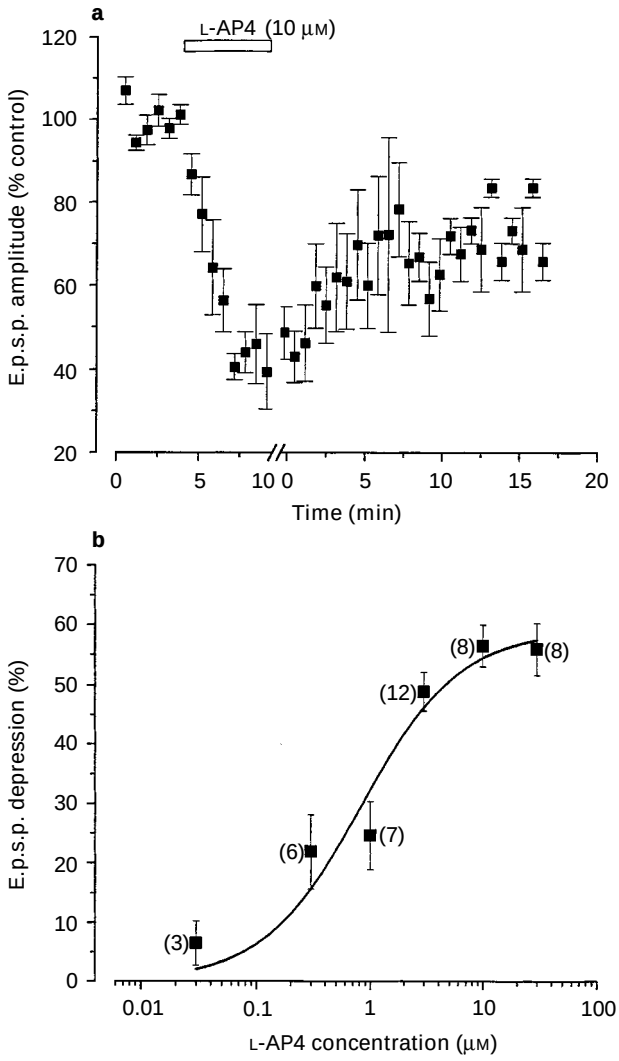

Figure 2 The selective group III mGlu receptor agonist L-AP4 reversibly depressed e.p.s.p. amplitude. (a) Plot of e.p.s.p. amplitude pooled for the duration of 4 experiments showing mean reversible depression of 56.5% caused by application by superfusion of group III agonist L-AP4 (10 μM; open bar). Each point represents mean e.p.s.p. amplitude for the 4 cells. The average e.p.s.p. amplitude of the control period was used as standard against which all other e.p.s.ps were compared. The abscissa scale shows the time after commencement of the pre-drug control period, and the time-course of each experiment was synchronized to the commencement of application of L-AP4. The data points during the wash period were time-locked to the commencement of washout of L-AP4, and the break in scale, representing 8 min, was due to the variability of agonist application time between experiments. (b) Concentration-effect data for the effect of L-AP4 in the range 0.03–30 μM. The maximum effect of around 60% depression occurred with a concentration of approximately 10 μM, with an EC_{50} of 0.82 μM. The number of experiments represented by each point are shown in parentheses. In (a) and (b), vertical lines show s.e.mean.

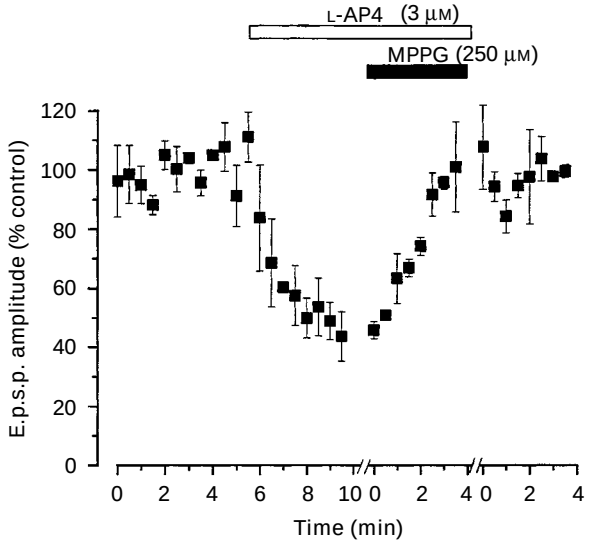

Figure 3 The effect of L-AP4 was reversed by the mGlu receptor antagonist MPPG. Pooled data from 3 different cells showing the effect of MPPG (250 μM; solid bar) on the depression of the e.p.s.p. caused by L-AP4 (3 μM; open bar). Data points represent averages from comparable time points in 3 different cells. Abscissa scale breaks represent up to 3 min in each case. Vertical lines show s.e.mean.

group II receptors were independently assessed. The group II mGlu receptor selective agonist DCG-IV (1 μM; Ishida *et al.*, 1993) reduced e.p.s.p. amplitude by $26.7 \pm 4.3\%$ ($n=5$; significant at $P<0.001$; Figure 6a), but was without effect on membrane potential. Washout of the effect of DCG-IV was incomplete after 8 min. The group I mGlu receptor selective agonist DHPG (100 μM; Schoepp *et al.*, 1994) also depressed the e.p.s.p. by $27.1 \pm 8.2\%$ ($n=7$; $P<0.05$; Figure 6b) at a concentration of 100 μM, and this effect persisted without recovery for washout periods of up to 7 min, although in 2 cells some recovery of e.p.s.p. amplitude was evident after washout of 10 min. DHPG (100 μM) additionally caused a membrane depolarization, which was offset by passing a hyperpolarizing current to permit study of e.p.s.p. amplitude. In a separate set of experiments, this effect of DHPG was not restricted in this way, resulting in depolarizations of 4.5 ± 1.4 mV ($n=8$; 10 μM DHPG). Taken together, these data indicate that the e.p.s.p. is susceptible to depression by activation of both group I and group II mGlu receptors, as well as by L-AP4-sensitive group III mGlu receptors. However, only group I mGlu receptor activation resulted in a direct effect on postsynaptic membrane excitability.

Discussion

We have shown that four different mGlu receptor agonists, three of which are selective for each of the three pharmacological groups of mGlu receptors, are capable of depressing fast glutamate-mediated e.p.s.ps in midbrain dopamine neurones. Only those acting at group I mGlu receptors also caused a direct postsynaptic effect, suggesting that at least group II and group III mGlu receptors are located presynaptically on the terminals of glutamate-releasing fibres.

Pharmacological identification of mGlu receptors regulating dopaminergic neurone activity: group III mGlu receptors

A clear role for group III mGlu receptors in depressing glutamatergic input to dopamine neurones is indicated by the ability of the group III-selective agonist L-AP4 to depress the e.p.s.p. L-AP4 has been shown to depress glutamatergic transmission at a number of central synapses (see Schoepp & Conn, 1993; Conn & Pin, 1997, for review), including the slow mGlu receptor-mediated e.p.s.c. in VTA dopaminergic neurones (Shen & Johnson, 1997). In many of these cases there is good electrophysiological evidence that a presynaptic site is involved, for example, in the hippocampal CA1 region (Gereau & Conn, 1995), medial nucleus of the trapezoid body

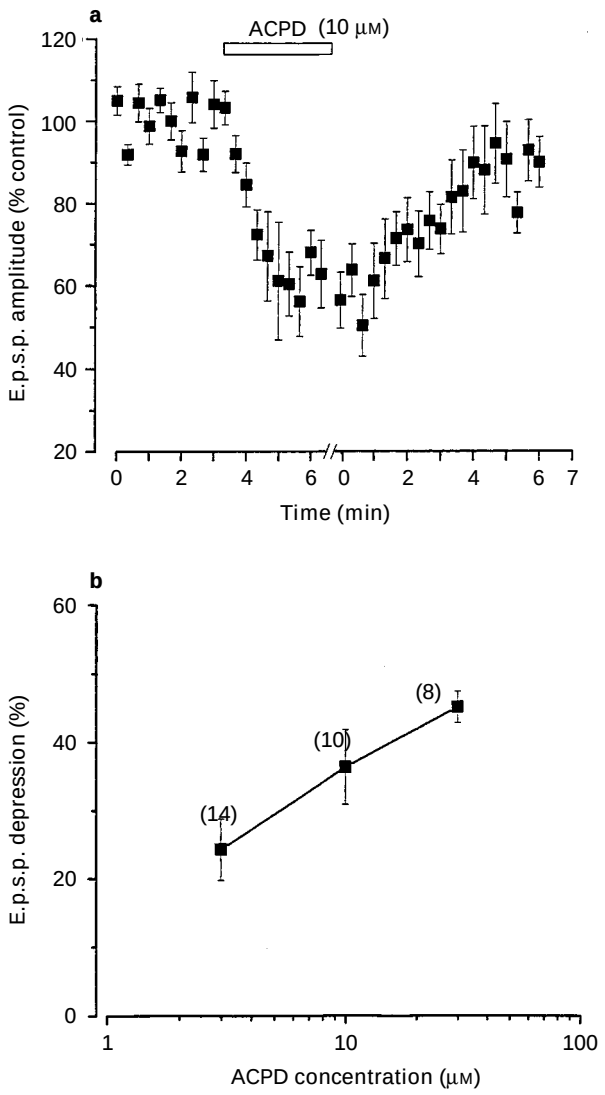

Figure 4 The group I/II - selective mGlu agonist ACPD depressed the e.p.s.p. in a concentration-dependent manner. (a) Plot of data points averaged from six cells illustrating the reversible depression of e.p.s.p. amplitude (by around 40%) by ACPD (10 μM; open bar). Abscissa scale break represents up to 11.5 min. (b) The action of ACPD was concentration-dependent over the range 3–30 μM. Data were pooled from the number of experiments shown in parentheses. In (a) and (b), vertical lines show s.e.mean.

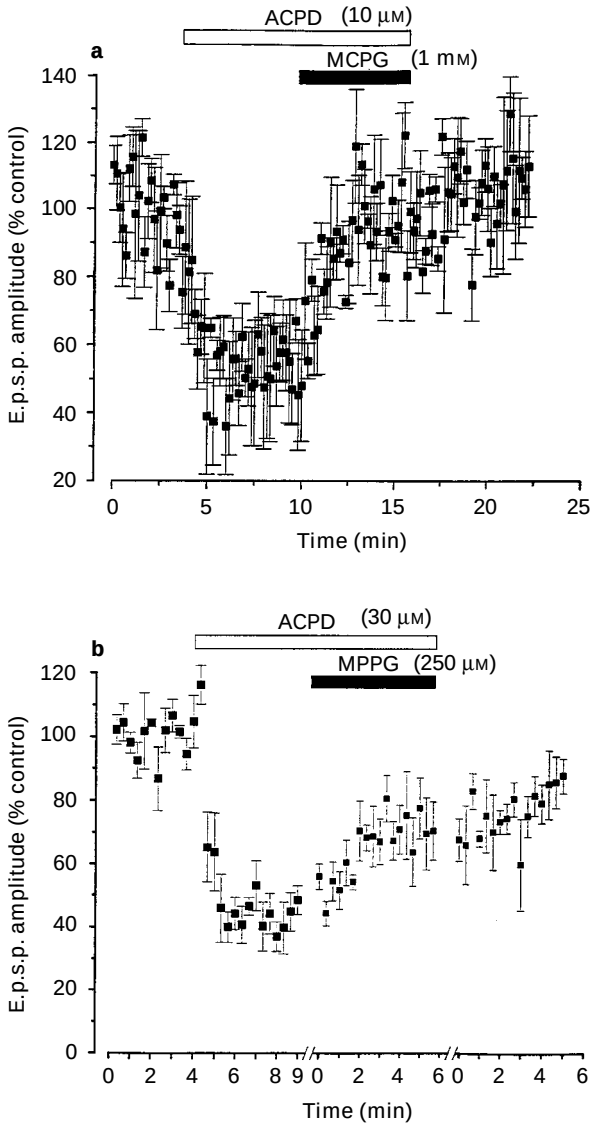

Figure 5 The effect of ACPD was antagonized by both MCPG and MPPG. (a) Plot of data pooled from 4 experiments showing reversal by MCPG (1 mM; solid bar) of the e.p.s.p. depression caused by ACPD (10 μM; open bar). (b) Plot of data pooled from 3 experiments showing reversal (by around 50%) by MPPG (250 μM; solid bar) of the e.p.s.p. depression caused by ACPD (30 μM; open bar). Abscissa scale breaks: before antagonist application, up to 2 min; before drug washout, up to 2.5 min. In (a) and (b), vertical lines show s.e.mean.

(Takahashi *et al*., 1996), cultured olfactory bulb neurones (Trombley & Westbrook, 1992), striatum (Calabresi *et al*., 1993) and nucleus accumbens (Manzoni *et al*., 1997). Indeed, antibodies to $mGlu_7$, one of the group III mGlu receptors, localize solely to presynaptic sites in the hippocampus (Shigemoto *et al*., 1996). Of the four group III mGlu receptors, $mGlu_6$ and $mGlu_8$ are thought to be localized primarily to optical pathways (Nakajima *et al*., 1993; Duvoisin *et al*., 1995) and may prove not to be expressed in ventral midbrain. The relative potency of L-AP4 in the present study (EC_{50} 1.6 μM) is consistent with an action on $mGlu_4$, rather than $mGlu_7$ (Conn & Pin, 1997). While the presence of mRNA encoding $mGlu_4$ in the neurones of the subthalamic nucleus (Testa *et al*., 1994), one possible source of the glutamatergic e.p.s.p., is consistent with this suggestion, the relatively small amounts might suggest that glutamatergic terminals arising from a different region would be more likely to be L-AP4-sensitive. The inhibition of the effect of L-AP4 by MPPG (250 μM) is also consistent with a role for group III mGlu receptors, and was obtained at concentrations comparable to those found effective in a similar paradigm in the lateral perforant path synapse in the hippocampus (Bushell *et al*., 1996). However, our observation that the same concentration of MPPG could also antagonize the effect of the group I/II agonist ACPD cautions against considering it selective for group III mGlu receptors (Jane *et al*., 1995).

Group II mGlu receptors

The non-selective group I and II mGlu receptor agonist ACPD (Pin & Duvoisin, 1995) also dose-dependently decreased the size of the evoked e.p.s.p. This effect was specific, as it could be reversed by the antagonist of group I and II mGlu receptors, MCPG. This action of ACPD was accompanied by a depolarization, as previously observed (Mercuri *et al*., 1993), which was offset with a 'manual voltage clamp' in order to study the e.p.s.p. The depression

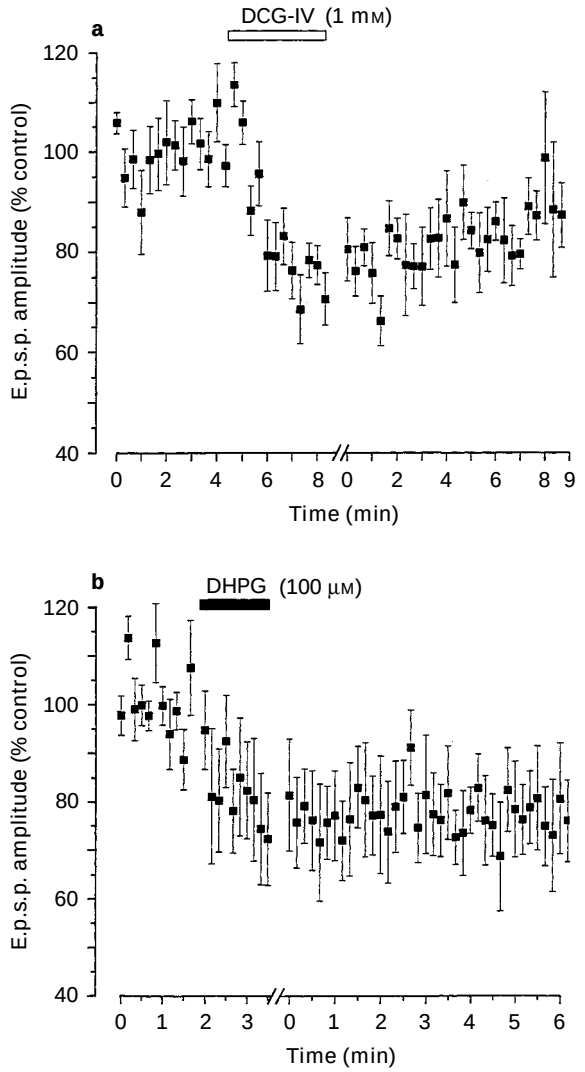

Figure 6 Selective agonists at group I and II mGlu receptors both depressed the e.p.s.p. (a) The group II agonist, DCG-IV (1 μM, open bar), depressed the e.p.s.p. (by 26.7±4.3%). This effect reversed slowly on washout of DCG-IV. Data pooled from 5 cells. Abscissa scale break, up to 2.5 min. (b) The group I agonist, DHPG (100 μM, solid bar), also depressed the e.p.s.p. by 27.1±8.2%). Data pooled from 7 cells. Abscissa scale break of up to 2.5 min. Reversibility on washout was not evident over this time course. In (a) and (b), vertical lines show s.e.mean.

of the e.p.s.p. by ACPD could not be attributed to an action upon either solely group I or group II mGlu receptors. The selective group II agonist DCG-IV, used at a concentration (1 μM) that might be expected to be near-maximally effective (Bushell *et al*., 1996), depressed the e.p.s.p. by 27%, without any attendant postsynaptic effect. Depression of glutamatergic transmission attributable to group II mGlu receptor activation has been observed at several synapses including in the spinal cord (Ishida *et al*., 1993), the hippocampal mossy fibre-CA3 synapse (Kamiya *et al*., 1996), the corticostriatal (Calabresi *et al*., 1993) and cortico-accumbens pathway (Manzoni *et al*., 1997), and the medial (Macek *et al*., 1996) and lateral (Bushell *et al*., 1996; but see Macek *et al*., 1996) perforant paths in the dentate gyrus, but notably not in hippocampal Schaffer collateral-CA1 pathway (Gereau & Conn, 1995; Manzoni & Bockaert, 1995). In several of these studies, strong electrophysiological evidence for a presynaptic site of drug action has been obtained, and it would appear that presynaptic group II mGlu autoreceptors, often operating in concert with group III mGlu receptors at the same synapse, are widely distributed in the brain (Conn & Pin, 1997). The lack of postsynaptic action of DCG-IV in the present study indicates that this situation also pertains to the glutamatergic input to midbrain dopamine neurones. While the present study does not permit discrimination between the two members of group II, the subthalamic nucleus expresses both $mGlu_3$ and, unusually for neurones in basal ganglia, $mGlu_2$ as well (Testa *et al*., 1994), and thus both possibilities remain open at this time. Additionally, the possibility of group II mGlu receptors expressed on glial cells contributing indirectly to presynaptic inhibition, as demonstrated in the hippocampal CA1 region (Conn & Pin, 1997), cannot be discounted.

Group I mGlu receptors

A high concentration (100 μM) of the selective group I mGlu receptor agonist DHPG depressed the e.p.s.p. by 27%. Given that the group II agonist DCG-IV caused a depression of 27%, it is tempting to conclude that, if the effects of these two agonists are indeed maximal, the fact that their arithmetical sum is roughly equivalent to the depression (45%) caused by ACPD (30 μM), which was itself a near-maximal effect, then the effect of ACPD on the e.p.s.p. derives from it acting on group I and group II mGlu receptors to a roughly equal extent.

However, the ACPD depolarization was mimicked by DHPG, but not DCG-IV, indicating that the postsynaptic mGlu receptors that can increase dopamine neurone excitability belong solely to group I. A similar conclusion has been reached for neurones elsewhere, including those in the hippocampal CA1 pyramidal layer (Davies *et al*., 1995) and basolateral amygdala (Keele *et al*., 1997). Of the two group I mGlu receptors types, only mRNA encoding $mGlu_1$ (but not $mGlu_5$) has been observed in midbrain dopamine neurones (Testa *et al*., 1994), suggesting that the depolarization caused by ACPD and DHPG is mediated by postsynaptic $mGlu_1$ receptors. Because of this postsynaptic effect, it is more difficult to conclude from the present experiments that the e.p.s.p. depression caused by DHPG (and the portion of the effect of ACPD attributable to group I mGlu receptor activation) is also a result of presynaptic inhibition, and that the relevant group I mGlu receptor is located presynaptically. Indeed, evidence of depression of glutamatergic transmission by selective group I mGlu receptor activation is scarce, and there are many descriptions of negative findings. Nonetheless, e.p.s.c. depression in hippocampal CA1 pyramidal neurones by DHPG (and ACPD) has been described (Gereau & Conn, 1995; Manzoni & Bockaert, 1995) and attributed to a presynaptic mechanism, by virtue of altered paired-pulse ratios and a failure to alter the amplitude of mini e.p.s.cs. However, both $mGlu_1$ and $mGlu_5$ have been localized immunohistochemically in the hippocampus at only postsynaptic sites (Luján *et al*., 1996). NMDA receptor activation may be critical for the depressant effect of DHPG on transmission in the hippocampal CA1 region (Harvey *et al*., 1996), and activation of group I mGlu receptors can modulate NMDA receptor function (Harvey & Collingridge, 1993; Pisani *et al*., 1997; Yu *et al*., 1997). It is therefore conceivable that postsynaptic group I mGlu receptors may cause presynaptic inhibition by promoting the NMDA receptor-dependent release of an inhibitory retrograde messenger. However, whether this accounts for the present observations on midbrain dopamine neurones remains to be established.

Functional and therapeutic implications

The orientation of the slice preparation and the rostral location of the stimulating electrodes makes it likely that the glutamatergic synaptic inputs studied here arose from descending fibres from the prefrontal cortex (Phillipson, 1979; Naito & Kita, 1994), amygdala (Gonzales & Chesselet, 1990) or subthalamic nucleus (Kita & Kitai, 1987; Chergui *et al*., 1994). As selective stimulation of just one of these pathways could not be performed, the e.p.s.p. probably results from glutamate released from fibres of mixed anatomical origin. It cannot be assumed that fibres from these sources all express the same complement of mGlu receptors on their midbrain terminals, or even that terminals from the same source are homogeneous in this respect. This would account for the failure to produce much more than around 60% depression of e.p.s.p. amplitude with any of the agonists tested, suggesting that selective targeting of these inputs might be possible with specific mGlu autoreceptor ligands. Contrasting results were obtained in the spinal cord, where, as ACPD and L-AP4 separately caused maximal depressions of the monosynaptic e.p.s.c. of up to at least 80% of control, it was concluded that the majority of fibres express both ACPD- and L-AP4-sensitive mGlu receptors presynaptically (Cao *et al*., 1995).

A simplistic conclusion arising from these findings is that agonists of group II or group III mGlu receptors would reduce glutamatergic synaptic drive on midbrain dopamine neurones, and thence reduce the activity of ascending mesencephalic dopamine systems. This would be expected to be of use in treating psychosis. Alternatively, if it is assumed that these presynaptic glutamate autoreceptors indeed play a physiological role in regulating ongoing glutamate release (as has been demonstrated at hippocampal mossy fibre synapses by Scanziani *et al*., 1997), then antagonists at these receptor subtypes conversely might increase excitation of dopamine neurones. This could be of benefit in Parkinson's disease, although it may also promote excitotoxic cell death of dopamine neurones (Blandini *et al*., 1996). Ligands at group I receptors would have less predictable effects in these terms, as they would influence both inhibition of glutamate release and postsynaptic excitability.

However, such conclusions ignore several potential complications: (1) differential effects of mGlu receptor ligands on glutamatergic inputs arising from different sources might impact differentially upon circuits involved in different behaviours. For example, prefrontal cortical afferents innervate selectively VTA neurones (Phillipson, 1979); targeting these might have relatively little influence on control of voluntary movement, but be more important for mesolimbo-cortical (motivational, rewarding and cognitive) function. (2) Glutamatergic inputs to dopamine neurones are likely also to innervate the GABAergic neurones of the adjacent substantia nigra pars reticulata (Kita & Kitai, 1987). As these are output relays for the basal ganglia, and their excitation will promote akinesia (see Gerfen, 1992, for example), this would offset any beneficial effect in Parkinson's disease of mGlu autoreceptor antagonists on dopamine neurones (above). (3) Inhibitory presynaptic mGlu (hetero-) receptors (Conn & Pin, 1997) may well also be present upon the GABA-mediated inputs to midbrain neurones, and their activation would functionally oppose modulation of dopamine systems through mGlu autoreceptors. (4) Any mGlu receptor ligand, even if receptor-specific, is likely to have effects elsewhere in the brain besides just the ventral midbrain, including the circuits that ascending dopamine systems themselves would influence in, for example, striatum (Sacaan *et al*., 1992; Calabresi *et al*., 1993), nucleus accumbens (Manzoni *et al*., 1997), and subthalamic nucleus (Kaatz & Albin, 1995; Abbott *et al*., 1997). This would also complicate predictions of behavioural outcome of such approaches and will require careful future evaluation.

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References

ABBOTT, A., WIGMORE, M.A. & LACEY, M.G. (1997). Excitation of rat subthalamic nucleus neurones in vitro by activation of a group I metabotropic glutamate receptor. *Brain Res.,* **766,** 162–167.

BLANDINI, F., PORTER, R.H.P. & GREENAMYRE, J.T. (1996). Glutamate and Parkinson's disease. *Mol. Neurobiol.,* **12,** 73–94.

BUSHELL, T.J., JANE, D.E., TSE, H.-W., WATKINS, J.C., GARTHWAITE, J., COLLINGRIDGE, G.L. & TSE, H.W. (1996). Pharmacological antagonism of the actions of group II and III mGluR agonists in the lateral perforant path of rat hippocampal slices. *Br. J. Pharmacol.,* **117,** 1457–1462.

CALABRESI, P., PISANI, A., MERCURI, N.B. & BERNARDI, G. (1993). Heterogeneity of metabotropic glutamate receptors in the striatum - electrophysiological evidence. *Eur. J. Neurosci.,* **5,** 1370–1377.

CAO, C.Q., EVANS, R.H., HEADLEY, P.M. & UDVARHELYI, P.M. (1995). A comparison of the effects of selective metabotropic glutamate receptor agonists on synaptically evoked whole cell currents of rat spinal ventral horn neurones *in vitro*. *Br. J. Pharmacol.,* **115,** 1469–1474.

CHERGUI, K., AKAOKA, H., CHARLÉTY, P.J., SAUNIER, C.F., BUDA, M. & CHOUVET, G. (1994). Subthalamic nucleus modulates burst firing of nigral dopamine neurones via NMDA receptors. *NeuroReport,* **5,** 1185–1188.

CHESSELET, M.F. & DELFS, J.M. (1996). Basal ganglia and movement disorders: An update. *Trends Neurosci.,* **19,** 417–422.

CONN, P.J. & PIN, J.P. (1997). Pharmacology and functions of metabotropic glutamate receptors. *Annu. Rev. Pharmacol. Toxicol.,* **37,** 205–237.

DAVIES, C.H., CLARKE, V.R.J., JANE, D.E. & COLLINGRIDGE, G.L. (1995). Pharmacology of postsynaptic metabotropic glutamate receptors in rat hippocampal CA1 pyramidal neurones. *Br. J. Pharmacol.,* **116,** 1859–1869.

DUNNETT, S.B. & ROBBINS, T.W. (1992). The functional role of mesotelencephalic dopamine systems. *Biol. Rev.,* **67,** 491–518.

DUVOISIN, R., ZHANG, C. & RAMONELL, K. (1995). A novel metabotropic glutamate receptor expressed in the retina and olfactory bulb. *J. Neurosci.,* **15,** 3075–3083.

FIBIGER, H.C. & PHILLIPS, A.G. (1986). Reward, motivation, cognition: psychobiology of mesentelencephalic dopamine systems. In *Handbook of Physiology, Section 1: The Nervous System. Volume IV, Intrinsic Regulatory Systems of the Brain*. ed. Mountcastle, V.B. & Bloom, F.E. pp.647–675. Bethesda, Maryland: American Physiological Society.

GEREAU, R.W. & CONN, P.J. (1995). Multiple presynaptic metabotropic glutamate receptors modulate excitatory and inhibitory synaptic transmission in hippocampal area CA1. *J. Neurosci.,* **15,** 6879–6889.

GERFEN, C.R. (1992). The neostriatal mosaic: multiple levels of compartmental organization in the basal ganglia. *Annu. Rev. Neurosci.,* **15,** 285–320.

GOLDMAN-RAKIC, P.S. (1995). Cellular basis of working memory. *Neuron,* **14,** 477–485.

GONZALES, C. & CHESSELET, M.F. (1990). Amygdalo-nigral pathway: an anterograde study in the rat with *Phaseolus vulgaris* leucoagglutinin (PHA-L). *J. Comp. Neurol.,* **297,** 182–200.

HARVEY, J. & COLLINGRIDGE, G.L. (1993). Signal transduction pathways involved in the acute potentiation of NMDA responses by 1S,3R-ACPD in rat hippocampal slices. *Br. J. Pharmacol.,* **109,** 1085–1090.

HARVEY, J., PALMER, M.J., IRVING, A.J., CLARKE, V.R.J. & COLLINGRIDGE, G.L. (1996). NMDA receptor dependence of mGlu-mediated depression of synaptic transmission in the CA1 region of the rat hippocampus. *Br. J. Pharmacol.,* **119,** 1239–1247.

HYMAN, S.E. (1996). Addiction to cocaine and amphetamine. *Neuron,* **16,** 901–904.

ISHIDA, M., SAITOH, T., SHIMAMOTO, K., OHFUNE, Y. & SHINOZAKI, H. (1993). A novel metabotropic glutamate receptor agonist: marked depression of monosynaptic excitation in the newborn rat isolated spinal cord. *Br. J. Pharmacol.,* **109,** 1160–1177.

JANE, D.E., PITTAWAY, K., SUNTER, D.C., THOMAS, N.K. & WATKINS, J.C. (1995). New phenylglycine derivatives with potent and selective antagonist activity at presynaptic glutamate receptors in neonatal rat spinal cord. *Neuropharmacology,* **34,** 851–856.

JOHNSON, S.W. & NORTH, R.A. (1992). Two types of neurone in the rat ventral tegmental area and their synaptic inputs. *J. Physiol.,* **450,** 455–468.

KAATZ, K.W. & ALBIN, R.L. (1995). Intrastriatal and intrasubthalamic stimulation of metabotropic glutamate receptors: A behavioural and Fos immunohistochemical study. *Neuroscience,* **66,** 55–65.

KAMIYA, H., SHINOZAKI, H. & YAMAMOTO, C. (1996). Activation of metabotropic glutamate receptor type 2/3 suppresses transmission at rat hippocampal mossy fibre synapses. *J. Physiol.,* **493,** 447–455.

KEELE, N.B., ARVANOV, V.L. & SHINNICK-GALLAGHER, P. (1997). Quisqualate-preferring metabotropic glutamate receptor activates Na^+-Ca^{2+} exchange in rat basolateral amygdala neurones, *J. Physiol.,* **499,** 87–104.

KITA, H. & KITAI, S.T. (1987). Efferent projections of the subthalamic nucleus in the rat: light and electron microscopic analysis with the PHA-L method. *J. Comp. Neurol.,* **260,** 435–452.

KOOB, G.F. (1992). Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol. Sci.,* **13,** 177–184.

LACEY, M.G. (1993). Neurotransmitter receptors and ionic conductances regulating the activity of neurones in substantia nigra pars compacta and ventral tegmental area. *Prog. Brain Res.,* **99,** 251–276.

LUJAN, R., NUSSER, Z., ROBERTS, J.D.B., SHIGEMOTO, R. & SOMOGYI, P. (1996). Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. *Eur. J. Neurosci.,* **8,** 1488–1500.

MACEK, T.A., WINDER, D.G., GEREAU, R.W., LADD, C.O. & CONN, P.J. (1996). Differential involvement of group II and group III mGluRs as autoreceptors at lateral and medial perforant path synapses. *J. Neurophysiol.,* **76,** 3798–3806.

MANZONI, O., MICHEL, J.-M. & BOCKAERT, J. (1997). Metabotropic glutamate receptors in the rat nucleus accumbens. *Eur. J. Neurosci.,* **9,** 1514–1523.

MANZONI, O. & BOCKAERT, J. (1995). Metabotropic glutamate receptors inhibiting excitatory synapses in the CA1 area of rat hippocampus. *Eur. J. Neurosci.,* **7,** 2518–2523.

MERCURI, N.B., STRATTA, F., CALABRESI, P., BONCI, A. & BERNARDI, G. (1993). Activation of metabotropic glutamate receptors induces an inward current in rat dopamine mesencephalic neurons. *Neuroscience,* **56,** 399–407.

MEREU, G., COSTA, E., ARMSTRONG, D.M. & VICINI, S. (1991). Glutamate receptor subtypes mediate excitatory synaptic currents of dopamine neurons in midbrain slices. *J. Neurosci.,* **11,** 1359–1366.

NAITO, A. & KITA, H. (1994). The cortico-nigral projection in the rat: An anterograde tracing study with biotinylated dextran amine. *Brain Res.,* **637,** 317–322.

NAKAJIMA, Y., IWAKABE, H., AKAZAWA, C., NAWA, H., SHIGEMOTO, R., MIZUNO, N. & NAKANISHI, S. (1993). Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate. *J. Biol. Chem.,* **268,** 11868–11873.

PHILLIPSON, O.T. (1979). Afferent projections to the ventral tegmental area of Tsai and iterfascicular nucleus: a horseradish peroxidase study in the rat. *J. Comp. Neurol.,* **187,** 117–144.

PIN, J.-P. & DUVOISIN, R. (1995). The metabotropic glutamate receptors: Structure and functions. *Neuropharmacology,* **34,** 1–26.

PISANI, A., CALABRESI, P., CENTONZE, D. & BERNARDI, G. (1997). Enhancement of NMDA responses by group I metabotropic glutamate receptor activation in striatal neurones. *Br. J. Pharmacol.,* **120,** 1007–1014.

ROBBINS, T.W. & EVERITT, B.J. (1996). Neurobehavioural mechanisms of reward and motivation. *Curr. Opin. Neurobiol.,* **6,** 228–236.

SACAAN, A.I., BYMASTER, F.P. & SCHOEPP, D.D. (1992). Metabotropic glutamate receptor activation produces extrapyramidal motor system activation that is mediated by striatal dopamine. *J. Neurochem.,* **59,** 245–251.

SCANZIANI, M., SALIN, P.A., VOGT, K.E., MALENKA, R.C. & NICOLL, R.A. (1997). Use-dependent increases in glutamate concentration activate presynaptic metabotropic glutamate receptors. *Nature,* **385,** 630–634.

SCHOEPP, D.D. & CONN, P.J. (1993). Metabotropic glutamate receptors in brain-related function and pathology. *Trends Pharmacol. Sci.,* **14,** 13–20.

SCHOEPP, D.D., GOLDSWORTHY, J., JOHNSON, B.G., SALHOFF, C.R. & BAKER, S.R. (1994). 3,5-Dihydroxyphenylglycine is a highly selective agonist for phosphoinositide-linked metabotropic receptors in the rat hippocampus. *J. Neurochem.,* **63,** 769–772.

SHEN, K.Z. & JOHNSON, S.W. (1997). A slow excitatory postsynaptic current mediated by G-protein-coupled metabotropic glutamate receptors in rat ventral tegmental dopamine neurons. *Eur. J. Neurosci.,* **9,** 48–54.

SHIGEMOTO, R., KULIK, A., ROBERTS, J.D.B., OHISHI, H., NUSSER, Z., KANEKO, T. & SOMOGYI, P. (1996). Target-cell-specific concentration of a metabotropic glutamate receptor in the presynaptic active zone. *Nature,* **381,** 523–525.

TAKAHASHI, T., FORSYTHE, I.D., TSUJIMOTO, T., BARNES-DAVIES, M. & ONODERA, K. (1996). Presynaptic calcium current modulation by a metabotropic glutamate receptor. *Science,* **274,** 594–597.

TESTA, C.M., STANDAERT, D.G., YOUNG, A.B. & PENNEY, J.B., JR (1994). Metabotropic glutamate receptor mRNA expression in the basal ganglia of the rat. *J. Neurosci,* **14,** 3005–3018.

TROMBLEY, P.G. & WESTBROOK, G.L. (1992). L-AP4 inhibits calcium currents and synaptic transmission via a G-protein coupled glutamate receptor. *J. Neurosci,* **12,** 2043–2050.

WIGMORE, M.A. & LACEY, M.G. (1995). Inhibition by metabotropic glutamate receptors of excitatory synaptic input to rat midbrain dopamine neurons *in vitro*. *Soc. Neurosci. Abstr.* **21,** 1660.

WIGMORE, M.A. & LACEY, M.G. (1996). Depression of glutamate input to midbrain dopamine neurones in rat midbrain slices by metabotropic glutamate receptor agonists. *Br. J. Pharmacol.,* **118,** 43P.

YU, S.P., SENSI, S.L., CANZONIERO, L.M.T., BUISSON, A. & CHOI, D.W. (1997). Membrane-delimited modulation of NMDA currents by metabotropic glutamate receptor subtypes 1/5 in cultured mouse cortical neurons. *J. Physiol.,* **499,** 721–732.

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