Journal of Neurochemistry

JOURNAL OF NEUROCHEMISTRY | 2011 | 118 | 24–33

doi: 10.1111/j.1471-4159.2011.07293.x

ORIGINAL ARTICLE

Microdialysis and mass spectrometric monitoring of dopamine and enkephalins in the globus pallidus reveal reciprocal interactions that regulate movement

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Abstract

Pallidal dopamine, GABA and the endogenous opioid peptides enkephalins have independently been shown to be important controllers of sensorimotor processes. Using *in vivo* microdialysis coupled to liquid chromatography–mass spectrometry and a behavioral assay, we explored the interaction between these three neurotransmitters in the rat globus pallidus. Amphetamine (3 mg/kg i.p.) evoked an increase in dopamine, GABA and methionine/leucine enkephalin. Local perfusion of the dopamine D_1 receptor antagonist SCH 23390 (100 μM) fully prevented amphetamine stimulated enkephalin and GABA release in the globus pallidus and greatly suppressed hyperlocomotion. In contrast, the dopamine D_2 receptor antagonist raclopride (100 μM) had only minimal effects suggesting a greater role for pallidal D_1 over D_2 receptors in the regulation of movement. Under basal conditions, opioid receptor blockade by naloxone perfusion (10 μM) in the globus pallidus stimulated GABA and inhibited dopamine release. Amphetamine-stimulated dopamine release and locomotor activation were attenuated by naloxone perfusion with no effect on GABA. These findings demonstrate a functional relationship between pallidal dopamine, GABA and enkephalin systems in the control of locomotor behavior under basal and stimulated conditions. Moreover, these findings demonstrate the usefulness of liquid chromatography–mass spectrometry as an analytical tool when coupled to *in vivo* microdialysis.

Keywords: amphetamine, dopamine, enkephalins, globus pallidus, mass spectrometry, microdialysis.

J. Neurochem. (2011) **118**, 24–33.

The globus pallidus (GP) is a critical nucleus along the indirect pathway of the basal ganglia (BG) and a known contributor to sensorimotor regulation (for a review, see Parent and Hazrati 1995a, b). The GP receives a massive innervation of striatopallidal medium spiny projection neurons (MSNs) which utilize both GABA and the endogenous opioid peptides, enkephalins (ENKs), as co-transmitters (Cuello and Paxinos 1978; Kita and Kitai 1988). The GP also receives dopamine (DA) input from the substantia nigra pars compacta (Lindvall and Björklund 1979; Hauber and Fuchs 2000). Although many studies have targeted opioidergic and DAergic systems independently, the interaction between ENKs and DA in the control of GP-mediated locmotor activity has yet to be uncovered.

Inhibition of the GP results in disinhibition (indirectly via a relay in the subthalamic nucleus) of BG output nuclei (Alexander *et al.* 1986; Parent and Hazrati 1995b). When disinhibited, these output nuclei, the substantia nigra pars reticulata and GP internus, overinhibit thalamic glutamatergic projections to motor cortex targets and inhibit movement (for review see Parent and Hazrati 1995a,b). Thus, inhibition of the GP corresponds to a loss of movement (akinesia) while disinhibition corresponds to hyperlocomotion. Indeed, inactivation of the GP by direct infusions of GABA receptor agonists or chemical lesions causes catalepsy (Ossowska *et al.* 1984; Avdelidis and Spyraki 1986; Hauber *et al.* 1998) while disinhibition by blocking GABA receptors activates

Received March 11, 2011; revised manuscript received April 08, 2011; accepted April 26, 2011.

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Abbreviations used: AMPH, amphetamine; BG, basal ganglia; DA, dopamine; ENKs, enkephalins; GP, globus pallidus; MSNs, medium spiny projection neurons; PENK, ENK precursor; RM, repeated measure.

locomotion (Ossowska *et al.* 1984; Maneuf *et al.* 1994). Moreover, the selective removal of striatopallidal MSNs, the main input of GABA to the GP, generates a hyperactive phenotype in mice (Durieux *et al*. 2009).

Opioid receptors also disinhibit the GP both *in vitro* and *in vivo* by lowering GABA concentrations (Maneuf *et al*. 1994; Mabrouk *et al.* 2008). Accordingly, μ and δ receptor (MOR and DOR, respectively) agonists stimulate locomotion when injected into the GP (Joyce *et al.* 1981; Dewar *et al.* 1985). Therefore, GABA and ENKs, both of which are released from the same subset of striatopallidal MSNs, appear to have opposite roles in maintaining pallidal function and locomotor output.

Although the striatum and nucleus accumbens are the primary targets of midbrain DA projection neurons, DA projections to the GP also play an important role in locomotion. DA receptor antagonists injected into the GP inhibit locomotor activity (Costall *et al.* 1972; Hauber and Lutz 1999; Galvan *et al.* 2001). In Parkinson's disease models, the GP undergoes a massive loss of DA terminals, and motor activity is restored by pallidal DA injections or DA reinnervation (Filion *et al.* 1991; Galvan *et al.* 2001; Fuchs and Hauber 2004; Bartlett and Mendez 2005; Bouali-Benazzouz *et al.* 2009). Taken together, these studies point to the GP as an important site for DA-mediated locomotion; although the mechanism has yet to be fully clarified.

Although opioids and DA both appear to sustain movement in the GP, it is unknown if they exert these effects independently or in concert with each other. Technical challenges have limited the number of studies investigating the role of DA on endogenous ENK release *in vivo* because of their low extracellular concentrations (in the pM level). Many investigators have therefore relied on ENK precursor (PENK) mRNA expression studies while manipulating DA to demonstrate a relationship between these two systems. However, these studies are inconclusive since PENK expression may not reflect ENK release. Thus, the precise effect of DA on ENK release remains unknown and conversely, the effect of tonic opioid receptor stimulation on pallidal DA release is also unknown.

The aim of the current study was to examine behaviorally relevant DA-ENK interactions in the GP. Using *in vivo* microdialysis coupled to capillary LC mass spectrometry (MS) for neurotransmitter measurements and a locomotor behavior assay, we tested whether DA and ENK mutually regulate one another and how this interaction contributes to GP inhibition (i.e. GABA levels) and movement. We stimulated DA release using systemic amphetamine (AMPH) and bilaterally administered both DA D_1- or D_2-like receptor (D_1R and D_2R) antagonists while simultaneously recording locomotor activation. Additionally, to reveal the influence of tonic ENK release over DA and GABA, we bilaterally perfused the non-specific opioid receptor antagonist naloxone into the GP under both basal and AMPH stimulated conditions. Overall, these studies uncover a substantial DA–GABA–ENK relationship in the GP for the control of locomotor functions.

Materials and methods

Microdialysis

Adult male Sprague–Dawley rats (Harlan, Indianapolis, IN, USA) weighing between 250 and 350 g were used for all experiments. Rats were housed in a temperature and humidity controlled room with 12 h light/dark cycles with food and water available *ad libitum*. Adequate measures were taken to prevent animal pain and discomfort. Moreover, bilateral microdialysis probe implantations were performed to uniformly deliver drugs into both hemispheres thus allowing for more robust pharmacological blockade of receptor targets and behavioral correlates. This technique also limits the numbers of animals needed per group because two sets of dialysate are collected per animal. All animals were treated as approved by the University of Michigan Unit for Laboratory Animal Medicine (ULAM) and in accordance with the National Institute of Health (NIH) Guidelines for the Care and Use of Laboratory Animals. These guidelines meet or exceed the issued ARRIVE guidelines.

Prior to surgery, animals were injected with general pain reliever carpofen (5 mg/kg s.c.) to minimize discomfort during and after surgery. Rats were then anesthetized with an intraperitoneal (i.p.) injection of a ketamine (65 mg/kg) and dexdormitor (0.25 mg/kg) mixture prepared in an isotonic salt solution. Concentrically designed microdialysis probes were then implanted bilaterally using a stereotaxic instrument (David Kopf Instruments, Tujunga, CA, USA), into the GP (2 mm dialyzing membrane, AN69, Hospal, Bologna, Italy) according to the following coordinates from bregma and dura: AP −1.3, ML ± 3.0, DV −7.0 (Paxinos and Watson 2007). Probes were secured to the skull by acrylic dental cement and metallic screws. Following surgery, rats were allowed to recover and experiments were run 24 h after probe implantation. Microdialysis probes were flushed at a flow rate of 1.5 μL/min with a modified Ringer solution (composition in mM: $CaCl_2$ 1.2; KCl 2.7, NaCl 148 and $MgCl_2$ 0.85) for 3 h using a Chemyx Fusion 400 syringe pump (Chemyx, Stafford, TX, USA). Perfusion flow rate was then reduced to 0.6 μL/min and samples were collected every 20 min. Most samples were analyzed the same day as experiment, however in the instances in which samples were frozen, 0.5 μL acetic acid was added to sample to preserve peptide content as previously described (Li *et al.* 2009). When experiments were completed, animals were killed and then brains were extracted and frozen at −80°C until histology. Probe position (Fig. 1) was verified by visual examination of 35 μm sections taken using a Leica cryostat (CM1900; Bannockburn, IL, USA).

Met-ENK and leu-ENK detection with capillary LC-MS^3

Met-ENK and leu-ENK were measured using a slightly modified version of a previously described method from our laboratory (Li *et al.* 2009). Chromatography columns and emitter tips were prepared in house using 28 and 10 cm lengths of 25 μm i.d. fused silica capillary, respectively. Columns were packed with 3 cm of 5 μm Alltima C18 reversed-phase particles (Alltech, Deerfield, IL, USA). Columns and tips were joined at the ion source using a Picoclear union (New

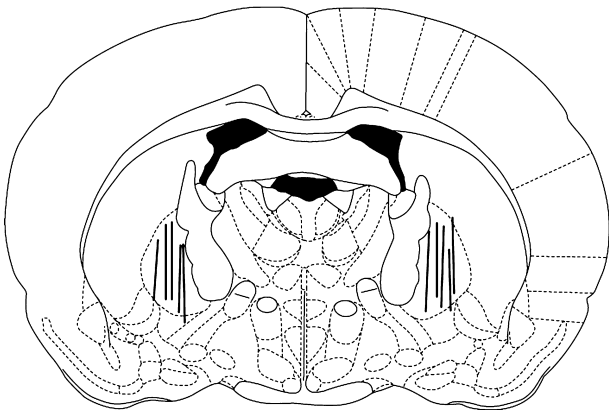

Fig. 1 Schematic showing representative probe placements of bilateral GP probe implantations as verified histologically.

Objective, Woburn, MA, USA). A high pressure (4000 psi) air-driven fluid pump (DHSF-151, Haskel Inc., Burbank, CA, USA) was used for sample loading and desalting, and a lower pressure (700 psi) micro HPLC pump (MicroPro, Eldex Laboratories, Napa, CA, USA) for gradient separation and eluting. An 8-min sample loading time and 3 min desalting time was used per 10 μL sample. Following loading and desalting, an injector valve switched to put the gradient pump online for elution. Mobile phase A consisted of LC-MS grade water with 0.2% acetic acid and mobile phase B consisted of LC-MS grade methanol with 0.2% acetic acid. The gradient program began with an isocratic step of 20% B for 1 min, then a linear increase to 95% over 2.5 min, followed by an isocratic step at 95% B for 0.5 min. The pump was programmed to then reset upon completion of gradient. The column and emitter tip were coupled to a PV-550 nanospray ESI source (New Objective, Woburn, MA, USA) interfaced to an LTQ XL linear ion trap MS (Thermo Fisher Scientific, Waltham, MA, USA). A +3.0 kV potential was applied to a liquid junction prior to the column for electrospray. Samples were injected using a WPS-3000TPL autosampler (Dionex, Sunnyvale, CA, USA), in full loop injection mode (5 μL loop). All valve switching and runs were controlled automatically with Xcalibur software (Thermo Fisher Scientific). The MS^3 pathways set on the linear ion trap for met-ENK and leu-ENK were: 574 → 397 → 278, 323, 380 and, 556 → 397 → 278, 323, 380, respectively.

Monoamine and amino acid analysis using QQQ MS

DA, AMPH and GABA were measured by a novel UPLC-MS assay developed in house. Briefly, 5 μL of dialysate was mixed with 2.5 μL of 100 mM borate buffer and 2.5 μL 2% (v/v in acetonitrile) benzoyl chloride solution. After 10 min reaction at 25°C, 2.5 μL internal standard containing $^{13}C_6$ benzoylated analytes was spiked into reach sample. Nine microliters of the total volume was injected for UPLC-MS/MS analysis. A 6 min separation was performed using Waters nanoAcquity (Waters, Milford, MA, USA) system and BEH C18 column (1 mm × 100 mm, 1.7 μm). Mobile phase A consisted of 10 mM ammonium formate and 0.15% formic acid. Mobile phase B was pure acetonitrile. Analytes were detected by Waters Quattro Ultima triple quadrupole mass spectrometer operating in multiple reaction monitoring mode.

Locomotor behavior analysis

While animals were undergoing microdialysis, Logitech (Apples, Switzerland) webcams were placed above cages. Webcams were connected via USB port to analysis PC running Matlab 2009 (Mathworks, Natick, MA, USA) software. Using the image acquisition toolbox in Matlab, data were collected via a custom designed motion monitoring program (Mark Dow, University of Oregon). Threshold of motion detection software was set at 150. This threshold limit was selected as not to detect small motions (such as breathing or whisker movement) but only larger motions such as walking around cage, running, rearing, etc. Data were collected every 60 s and then pooled into 5 min intervals. Data are expressed in terms of absolute locomotor activity as calculated by the detection software.

Data presentation and statistical analysis

Statistical analysis has been performed by two-way repeated measure (RM) analysis of variance (ANOVA). In case ANOVA yielded a significant F score, *post hoc* analysis has been performed by contrast analysis to determine group differences. In case a significant time × treatment interaction was found, the sequentially rejective Bonferroni test was used (implemented in Graphpad Prism 5) to determine specific differences (i.e. at the single time-point level) between groups. p values < 0.05 were considered to be statistically significant.

Materials

AMPH, benzoyl chloride, raclopride, SCH 23390, naloxone, met- and leu-ENK, DA, GABA, (including C^{13} labeled DA and GABA) were purchased from Sigma Chemical Co (St Louis, MO, USA). Ketamine, dexdormitor and carpofen were from MWI (Rochester Hills, MI, USA). While AMPH was dissolved in isotonic saline solution, raclopride, SCH 23390 and naloxone were dissolved in Ringer solution (described above). AMPH was administered with i.p. injections (1 mL/kg) whereas all other drugs were perfused through the microdialysis probe (reverse dialysis).

Results

Effect of systemic AMPH on DA release and extracellular levels of AMPH in GP

We first wanted to demonstrate that midbrain DAergic projections to the GP were sensitive to the DA releasing effects of AMPH (3 mg/kg i.p.). Also, as our analytical technique allowed for the measurement of molecules including a primary amine in their structure, we measured extracellular AMPH (expressed as picograms per 5 μL of dialysate) levels in the GP. DA concentrations in GP dialysate were 1.4 ± 0.2 nM (n = 10) and remained stable over the time course of the experiment (Fig. 2a). RM ANOVA revealed a significant effect of treatment ($F_{1,5}$ = 10.25, p < 0.0001), time ($F_{5,32}$ = 146.6, p < 0.0001), and a time × treatment interaction ($F_{5,32}$ = 10.25, p < 0.0001). *Post hoc* analysis revealed that compared with saline-treated animals, AMPH (3 mg/kg i.p.) caused a large (~10-fold) increase in pallidal DA. This effect peaks at 40 min after injection and tapers off to become non-significant 2 h after injection.

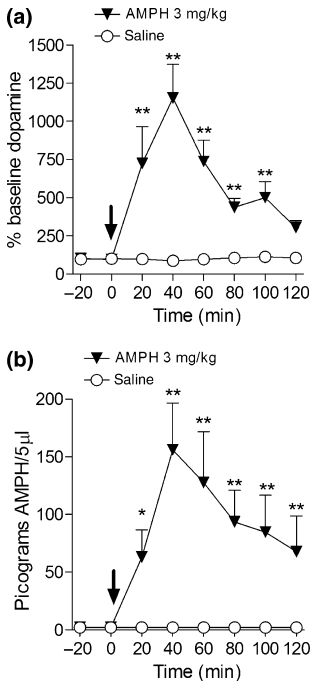

Fig. 2 Systemic AMPH (3 mg/kg i.p.) administration caused a ~10-fold increase in GP DA (a) compared with saline-treated animals ($n = 6$ per group). Data are expressed as means ± SEM of pre-treatment (basal) values. Extracellular AMPH was detected in the GP following systemic administration (b). Data are expressed as picograms of AMPH per 5 µL of dialysate injected onto LC-MS system. $^{*}p < 0.05$, significantly different from vehicle; $^{**}p < 0.01$, significantly different from vehicle.

The levels of AMPH in the GP closely mirror the amount of DA overflow observed following treatment (Fig. 2b). Compared with saline-treated animals, RM ANOVA revealed a significant effect of treatment ($F_{1,5} = 28.45$, $p < 0.0001$) and time ($F_{5,56} = 286.9$, $p < 0.0001$), and a time × treatment interaction ($F_{5,56} = 27.23$, $p < 0.0001$). No AMPH was observed in animals not treated with AMPH (saline-treated). Levels of AMPH reached ~150 pg/5 µL of dialysate at 40 min post-injection (Fig. 2b).

Effect of systemic administration of AMPH on ENK release in GP

Given that the GP is a major target for striatal ENKergic MSNs and that overall inhibitory tone of the GP is linked to normal and pathogenic movement, we sought to characterize the impact of DA release on ENK levels. We therefore used microdialysis to monitor the two main neuroactive ENKs (met-ENK and leu-ENK) following AMPH treatment. Dialysate concentrations of met-ENK and leu-ENK in the GP were 64 ± 29 pM ($n = 20$) and 15 ± 2 pM ($n = 20$), respectively, and remained stable over the time course of the experiment (Fig. 3a). RM ANOVA revealed a significant effect of treatment ($F_{3,15} = 17.47$, $p < 0.0001$), time ($F_{7,99} = 120.1$, $p < 0.0001$), and a time × treatment interaction ($F_{21,99} = 36.86$, $p < 0.0001$). *Post hoc* analysis revealed that AMPH caused an increase (starting 1 h after injection) in both met-ENK (120%) and leu-ENK (180%) levels compared with vehicle-treated animals (Fig. 3). This effect lasted until the end of the experiment (140 min post-injection). In pilot experiments, we found that low AMPH doses (2.5 mg/kg i.p.) caused delayed increases in ENK release whereas higher doses (5–12 mg/kg i.p.) caused immediate release in ENK in the GP/ventral pallidum (data not shown).

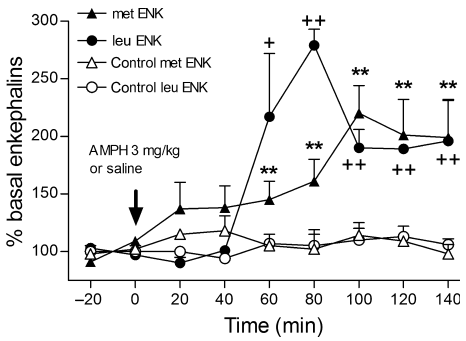

Fig. 3 Systemic AMPH (3 mg/kg i.p.) administration caused a ~2-fold increase in both met- and leu-ENK. Effects are only evident 60 min post-injection and continue until the end of the experiment. Data are expressed as mean ± SEM of pre-treatment (basal) values. $^{**}p < 0.01$, leu-ENK significantly different from vehicle; $^{++}p < 0.01$, met-ENK significantly different from vehicle.

Effect of local DA D_1 or D_2 antagonist perfusion in GP on AMPH-induced ENK release

To investigate whether AMPH stimulated ENK release in GP was caused by local DA receptor activation, we locally perfused via reverse dialysis the selective DA D_1 receptor antagonist, SCH 23390 (100 µM) and the selective DA D_2 receptor antagonist raclopride (100 µM) prior to and after systemic AMPH injections. RM ANOVA on met-ENK levels revealed a significant effect of treatment ($F_{2,10} = 10.87$, $p < 0.0001$), time ($F_{9,55} = 56.67$, $p < 0.0001$) and a time × treatment interaction ($F_{18,55} = 4.54$, $p < 0.0001$). RM ANOVA on leu-ENK levels also revealed significant effect of treatment ($F_{2,10} = 8.39$, $p < 0.0001$), time ($F_{9,55} = 38.98$, $p < 0.0001$) and a time × treatment interaction ($F_{18,55} = 10.80$, $p < 0.0001$). Neither DA receptor antagonist affected basal ENK release, however, SCH 23390 (100 µM) completely blocked AMPH-stimulated met-ENK and leu-ENK release (Fig. 4a). However, raclopride (100 µM) had no effect on AMPH-stimulated met-ENK release but appeared to partially attenuate leu-ENK release (Fig. 4b).

Effect of GP perfusion of DA D_1 or D_2 antagonists on AMPH-induced GABA release and locomotor activity

Previous studies have shown that locomotor states are dependent on GP inhibition (Maneuf *et al.* 1994). As

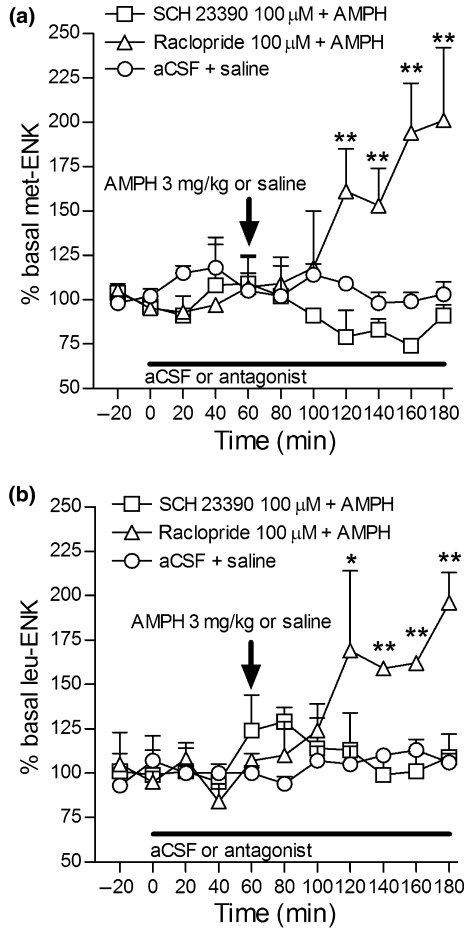

Fig. 4 SCH 23390 (100 μM) perfusion in the GP, but not raclopride (100 μM) reversed AMPH stimulated met-ENK (a) and leu-ENK (b) release. DA receptor antagonists were perfused for 1 h prior to systemic AMPH injections and continued until the end of the experiments (black bar). Data are expressed as mean ± SEM of pre-treatment (basal) values. *$p < 0.05$, leu-ENK significantly different from vehicle. **$p < 0.01$, met- or leu-ENK significantly different from vehicle.

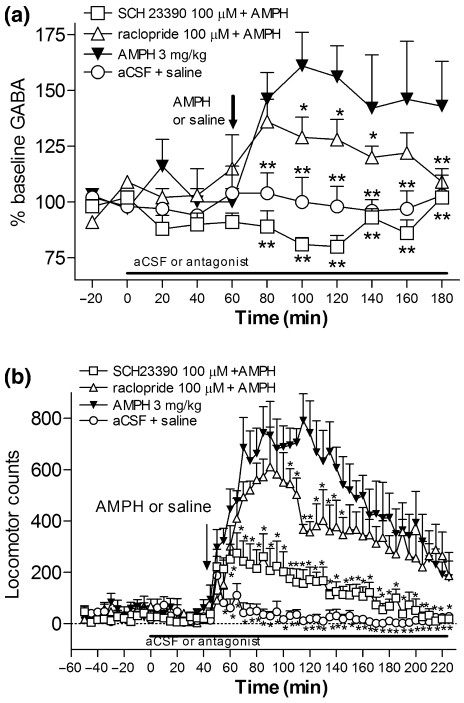

Fig. 5 Systemic AMPH (3 mg/kg i.p.) administration caused a ~50% increase in GP GABA (a) compared with saline-treated animals ($n = 5$ per group). This effect was attenuated by the GP perfusion of raclopride (100 μM) and reversed by perfusion of SCH 23390 (100 μM). Raclopride or SCH 23390 perfusion had no effect on basal GABA levels. Data are expressed as mean ± SEM of pre-treatment (basal) values. AMPH (3 mg/kg i.p.) stimulated locomtor activity (b). This effect was greatly attenuated by local SCH 23390 (100 μM) perfusion and partially attenuated by raclopride (100 μM). Antagonists were perfused for 1 h prior to systemic AMPH injections and continued until the end of the experiments (black bar). Data are expressed as locomotor counts ± SEM as calculated by MATLAB software. *$p < 0.05$, significantly different from AMPH-treated group; **$p < 0.01$, significantly different from AMPH-treated group.

AMPH-induced ENK release was dependent on pallidal DA receptors, we sought to determine if these effects correlated with GABAergic processes and locomotion. Dialysate GABA concentration in the GP was 5.2 ± 1.2 nM ($n = 23$) and remained stable over the time course of the experiment. RM ANOVA on GABA concentration revealed a significant effect of treatment ($F_{3,15} = 4.82$, $p < 0.0001$), time ($F_{9,143} = 49.54$, $p < 0.0001$) and a time × treatment interaction ($F_{27,143} = 4.78$, $p < 0.0001$). Local perfusion of either DA antagonist alone had no effect on GABA levels by themselves, whereas AMPH (3 mg/kg) increased it (maximal ~50%; Fig. 5a). Perfusion of the DA D_1 receptor antagonist SCH 23390 (100 μM) completely blocked GABA release caused by AMPH (3 mg/kg). SCH 23390 (100 μM) not only blocked the AMPH-induced increase but also caused a small, but significant reduction (~20%) in GABA 40 and 60 min post-injection (Fig. 5a). Raclopride (100 μM) only partially blocked the effects GABA releasing effects of AMPH (Fig. 5a).

While performing microdialysis we also monitored locomotor behavior during DA receptor antagonist perfusion. Basal locomotor levels were 35 ± 4 counts ($n = 76$; Fig. 5b). RM ANOVA on locomotor activity revealed a significant effect of treatment ($F_{3,12} = 19.72$, $p < 0.0001$), time ($F_{55,840} = 1401$, $p < 0.0001$) and a time × treatment interaction ($F_{165,840} = 85.30$, $p < 0.0001$). Local perfusion of either SCH 23390 (100 μM) or raclopride (100 μM) had no effect on basal levels of locomotor activity. AMPH (3 mg/kg) caused a large and reliable increase in locomotor activity (maximal ~800 locomotor counts 70 min post-injection). Compared with animals receiving AMPH only, the SCH 23390 + AMPH group significantly differed from 10 min post-injection, until the end of the experiment (Fig. 5b). The

raclopride + AMPH only group differed from the AMPH group between 60 and 105 min post-injection.

Effect of naloxone on basal GABA and DA levels in the GP

Our data showed that AMPH-stimulated, but not tonic DA release strongly affected pallidal ENK concentration (Fig. 4). As ENK analogues stimulate locomotor activity in the GP presumably through the regulation of classical neurotransmitters, we wanted to determine if tonic ENK release was sufficient to cause changes in DA or GABA levels. We therefore perfused the non-specific opioid receptor antagonist naloxone (10 μM) into the GP while monitoring GABA and DA. Dialysate GABA concentration in the GP was 7.7 ± 0.9 nM (n = 12) and remained stable over the time course of the experiment. RM ANOVA on GABA concentration showed a significant effect of treatment ($F_{1,4}$ = 10.73, p < 0.0001), time ($F_{9,60}$ = 266.80, p < 0.0001) and a time × treatment interaction ($F_{9,60}$ = 8.60, p < 0.0001). *Post hoc* analysis revealed that naloxone perfusion elevated GABA levels in the GP (maximal ~60%), starting 20 min after perfusion onset and lasting until the end of the experiment (Fig. 6a).

Dialysate DA levels in the GP were 5.6 ± 0.6 nM (n = 12) and remained stable over the time course of the experiment (Fig. 6b). RM ANOVA on DA levels showed a significant effect of treatment ($F_{1,4}$ = 5.39, p < 0.0001), time ($F_{9,50}$ = 97.66, p < 0.0001) and a time × treatment interaction ($F_{9,50}$ = 8.93, p < 0.0001). *Post hoc* analysis revealed that starting 20 min after onset of naloxone perfusion, DA levels were reduced (maximal ~40%). This effect was transient as it was nearly completely reversed by 160 min post-naloxone perfusion onset (Fig. 6b).

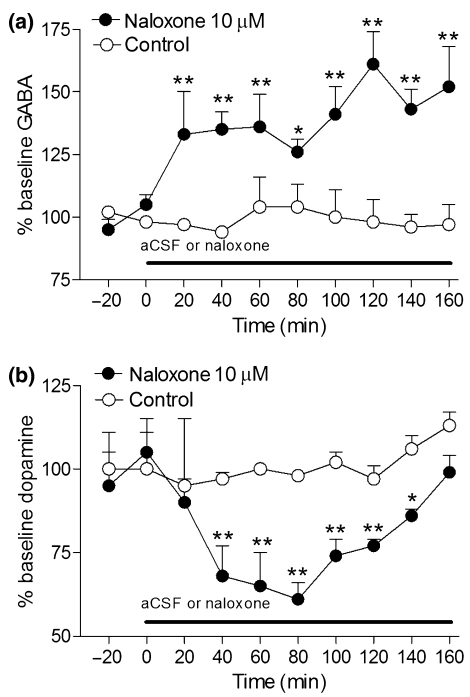

Fig. 6 Naloxone perfusion (10 μM) in the GP increased GABA (a) and decreased DA (b) compared with control group (n = 5 per group). Data are expressed as mean ± SEM of pre-treatment (basal) values. *p < 0.05, significantly different from vehicle; **p < 0.01, significantly different from vehicle.

Effect of naloxone on AMPH-induced GABA and DA levels in the GP and locomotor activity

Based on the finding that naloxone stimulated GABA and reduced DA release and since AMPH stimulated ENK release and hyperlocomotion, we wanted to determine how naloxone affected AMPH-stimulated GABA, DA and movement. Therefore, while performing microdialysis we monitored locomotor behavior while locally perfusing naloxone in the GP, prior to, and after a systemic AMPH (3 mg/kg i.p.) injection.

Dialysate GABA concentration in the GP was 9.1 ± 1.0 nM (n = 18) and remained stable over the time course of the experiment (Fig. 7a). RM ANOVA on GABA showed a significant effect of treatment ($F_{2,5}$ = 7.48, p < 0.0001), time ($F_{9,121}$ = 113.0, p < 0.0001) and a time × treatment interaction ($F_{18,121}$ = 10.38, p < 0.0001). Naloxone perfusion alone elevated GABA levels in the GP (at 40 min after onset of perfusion) and did not affect AMPH-stimulated GABA release (Fig. 7a).

Dialysate DA concentration in the GP was 5.2 ± 0.6 nM (n = 18) and remained stable over the course of the experiment (Fig. 7b). RM ANOVA on DA concentration revealed a significant effect of treatment ($F_{2,5}$ = 23.94, p < 0.0001), time ($F_{9,123}$ = 188.3, p < 0.0001) and a time × treatment interaction ($F_{18,123}$ = 56.18, p < 0.0001). Systemic AMPH (3 mg/kg i.p.) stimulated DA release in the GP and this effect was attenuated by local naloxone (10 μM) perfusion by about ~50% (Fig. 7b).

Basal locomotor levels were 43 ± 3 counts (n = 20; Fig. 5). RM ANOVA on locomotor activity revealed a significant effect of treatment ($F_{2,8}$ = 17.02, p < 0.0001), time ($F_{55,672}$ = 1108, p < 0.0001) and a time × treatment interaction ($F_{110,672}$ = 43.91, p < 0.0001). Local perfusion of naloxone (10 μM) had no effect on basal levels of locomotor activity. AMPH (3 mg/kg) caused a very large and reliable increase in locomotor activity (maximal ~800 locomotor counts 70 min post-injection). Compared with animals receiving AMPH the naloxone + AMPH groups differed from the AMPH starting 50 min after injection until the end of the experiment.

Discussion

Rationale and overview of findings

As the GP is a known regulator of movement and a major target of GABA, ENKs and DA, the current study explored the interactions between these neurotransmitter systems and

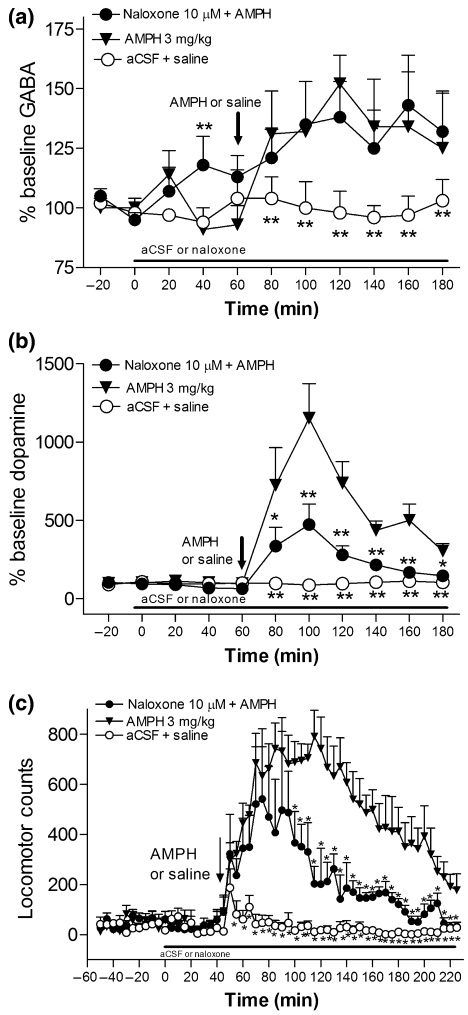

Fig. 7 Systemic AMPH (3 mg/kg i.p.) administration caused a ~50% increase in GP GABA (a) compared with saline-treated animals ($n = 5$ per group). Naloxone (10 μM) also stimulated GABA release starting 40 min after perfusion onset. Naloxone perfusion did not affect GABA release following systemic AMPH administration (a). Naloxone (10 μM) did attenuate AMPH stimulated DA release in the GP (b). Data are expressed as mean ± SEM of pre-treatment (basal) values. Naloxone (10 μM) attenuated AMPH-induced hyperactivity (c), Naloxone was perfused for 1 h prior to systemic AMPH injections and continued until the end of the experiments (black bar). Data are expressed as locomotor counts ± SEM as calculated by MATLAB software. *$p < 0.05$, significantly different from AMPH-treated group; **$p < 0.01$, significantly different from AMPH-treated group.

their role in movement at this site. Systemic AMPH stimulated DA, GABA and ENK release in the GP which correlated with an expected rise in locomotor activity. Bilateral perfusion of the D_1R antagonist SCH 23390 fully prevented AMPH-stimulated ENK and GABA release in the GP while greatly suppressing AMPH-induced hyperactivity. Perfusion of the D_2R antagonist raclopride had little effect on AMPH-stimulated ENK release and only partially attenuated AMPH-stimulated GABA release and hyperlocomotion. These results suggest a primary role for pallidal D_1Rs over D_2Rs in striatopallidal MSN activation and DA-mediated locomotion in the GP. We also showed that under basal conditions, opioid receptor blockade in the GP stimulated GABA while suppressing DA release, suggesting tonic ENKergic regulation of these neurotransmitters. Finally, under AMPH stimulated conditions, naloxone attenuated DA release and locomotor activation without further enhancing GABA release.

Dopamine terminals in the GP respond to AMPH

We showed that AMPH stimulated GP DA release in a manner similar to the striatum and nucleus accumbens (Di Chiara and Imperato 1998). This novel result is consistent with local AMPH perfusion studies which stimulated DA release in the GP (Fuchs *et al.* 2005). We further extend these observations by showing the behavioral effects of AMPH-stimulated DA overflow and the roles of DA receptor subtypes in the GP.

Evidence for pre-syanptic D_1Rs in GP

Although, many studies emphasize the role of pallidal D_2Rs (Floran *et al.* 1997; Marshall *et al.* 2001), others have consistently shown higher D_1R binding compared with D_2R in the GP (Dubois *et al.* 1986; Mansour *et al.* 1990; Yung *et al.* 1995). D_1Rs are present on striatopallidal terminals because striatal lesions reduced D_1R binding in the GP by ~65% (Barone *et al.* 1987). In support of this evidence, D_1Rs mediate GABA release in GP slices (Floran *et al.* 1990) presumably via striatopallidal terminals, although the contribution of other GABAergic elements such as GABAergic interneurons cannot be excluded in those preparations.

Presynaptic D_1 controls the GABA/ENK release

D_1Rs are known to enhance cellular activity and neurotransmitter release through coupling to Gαs, the formation of adenylyl cyclase, and the influx of Ca^{2+} and (Kebabian and Calne 1979; Cameron and Williams 1993; Bergson *et al.* 1995). Like the release of classical neurotransmitters, peptide release is also Ca^{2+} dependent (for review, see Ludwig and Leng 2006) and therefore prolonged or intense D_1R stimulation might also prompt ENK release. We found that D_1Rs in the GP were the primary mediators of AMPH-induced GABA and ENK release since these effects were blocked by local perfusion of D_1R antagonist SCH 23390. This result is consistent with DA actions on stimulatory D_1Rs on striatopallidal terminals since striatopallidal MSNs are a major source of GABA and the primary source of ENKs in the GP (Cuello and Paxinos 1978; Kita and Kitai 1988). Thus, DA acts at pre-synaptic D_1Rs on striatopallidal terminals to enhance GABA and ENK release and may be required to fine tune pallidal inhibition. These results are

consistent with a previous study showing acute AMPH-induced increases in PENK mRNA expression in striatal MSNs (Wang and McGinty 1996). Therefore, D_1R stimulation facilitates ENK release and subsequent increases in PENK mRNA expression (Wang and McGinty 1996) possibly to maintain ENK stores.

Post-synaptic control of locomotor activity

Post-synaptic D_1Rs and D_2Rs have also been described in the GP (Carlson *et al.* 1986; Walters *et al.* 1987; Hurley *et al.* 2001; Shin *et al.* 2003). Compared with pre-synaptic D_1Rs, post-synaptic D_1Rs and D_2Rs on GP output neurons are better positioned to act as final effectors on locomotor output. Therefore, when DA is greatly elevated (in our case ~10-fold by AMPH), direct post-synaptic DA receptor stimulation (mainly D_1Rs) would enhance locomotor activity despite increases in GABA (Fig. 5) and GP inhibition. Post-synaptic D_1Rs located on pallidonigral or pallidosubthalamic projections would both inhibit the substantia nigra pars reticulata and lead to disinhibition of the nigrothalamocortical loop and stimulate locomotion. Although this hypothesis remains speculative, the fact that D_1R blockade in the GP greatly attenuated AMPH-induced hyperlocmotion and GABA and ENK suggests that DA activates locomotion downstream of GP disinhibition. Supporting this idea, SCH 23390 perfusion in the ventral pallidum blocked hyperlocomotion induced by local MOR stimulation (Alesdatter and Kalivas 1993). Thus, D_1R blockade appeared to over-ride opioid-mediated pallidal disinhibition.

Role of D_2Rs in locomotor activity and GABA release

Compared with D_1Rs, the current study showed that D_2R blockade was less effective at attenuating AMPH-stimulated hyperactivity and GABA release while only mildly attenuating leu-ENK release (with no effect on met-ENK release). This suggests a different localization and function of D_2Rs compared with D_1Rs in the GP. As D_2Rs are generally considered inhibitory, their post-synaptic localization to GABAergic pallidothalamic neurons would allow DA to stimulate locomotion by disinhibitng the thalamus. This type of localization can explain our finding that raclopride only attenuated AMPH-induced hyperactivity. This suggestion is supported by a recent study demonstrating that DA inhibits pallidothalamic GABAergic transmission via DA D4 receptor (a D_2R; Gasca-Martinez *et al.* 2010). Thus based on their lower expression level, they perhaps serve a secondary role for stimulating locomotor activity compared to D_1Rs.

Opioid receptors in GP are also involved in locomotor activity

Like DA, pallidal ENK release likely contributes to locomotor activity. ENK knockout mice have significantly reduced locomotor activity (König *et al*. 1996) whereas ENKase inhibitors stimulate locomotion (Michael-Titus *et al.* 1987; Baamonde *et al.* 1992; Jutkiewicz *et al.* 2006). Because the GP is a primary target of ENKergic fibers (Cuello and Paxinos 1978), it likely mediates many of these effects on movement based on its position in the BG. Supporting this view, exogenous MOR or DOR selective agonists and ENK analogues injected directly into the GP stimulate locomotor activity (Joyce *et al.* 1981; Dewar *et al.*1985; Anagnostakis *et al.* 1992). *In vitro* studies and *in vivo* studies have both shown opioid receptor mediated inhibition of GABA in the GP (Maneuf *et al.* 1994; Mabrouk *et al.* 2008). Therefore, much evidence points towards ENKs as locomotor promoting neuromodulators through GP disinhibtion. Despite this evidence, no studies have directly measured endogenous ENK release while simultaneously monitoring locomotor behavior. Furthermore, the role of DA in these effects has yet to be described. One study did show that in the DA depleted state (i.e. 6-hydroxydopamine-lesioned animals), selective DOR stimulation in the GP failed to enhance locomotor activity (Mabrouk *et al.* 2008). This suggests the possibility that DA signaling, and not solely pallidal disinhibtion, is responsible for opioid-mediated hyperlocomotion in the GP.

In the GP, MORs have been primarily localized pre-synaptically on striatopallidal terminals (Olive *et al.* 1997; Stanford and Cooper 1999). This is consistent with our finding that naloxone perfusion increased GABA release presumably through the inhibition of striatopallidal ENK tone. A novel finding is that this disinhibition coincided with elevated DA levels suggesting that under basal conditions, ENKs are tonically released in the GP to disinhibit DA.

Contrary to the neurochemical findings, naloxone (or DA receptor antagonists) perfusion alone had no discernible effect on basal locomotor activity. However, based on low basal locomotor activity of animal subjects, our assay is limited in detecting motor inhibition from a basal state. Nonetheless, AMPH-stimulated hyperlocomotion and DA were attenuated by naloxone perfusion but did not further facilitate GABA release. This suggests that AMPH-stimulated DA and hyperlocomotion are facilitated by phasic ENK release from striatopallidal neurons possibly through some other mechanism apart from GABAergic inhibition. For instance, post-synaptic DORs on pallidothalamic neurons could account for direct locomotor stimulating effects without indirect disinhibition. Interestingly, past studies showed that systemic naloxone pre-treatment attenuated AMPH-induced hyperactivity and striatal DA release (Hooks *et al.* 1992; Schad *et al.* 1995). The authors of those studies suggested, but did not demonstrate, an indirect mechanism whereby AMPH would stimulate endogenous opioids which would then in turn stimulate DA release through the inhibition of midbrain GABAergic interneurons (Schad *et al*. 1995). Our studies reveal that AMPH does indeed stimulate ENK release, although the effect of naloxone occurs at terminal sites and not necessarily through actions at

midbrain DA cell bodies. Whatever the mechanism, the fact that naloxone perfusion stimulated GABA release and reduced DA while also reducing AMPH-stimulated DA strongly suggests that the endogenous ENKs act to facilitate DA release and locomotor activity at terminal sites.

Interaction model and conclusions

Our data are consistent with a model in which DA release in the GP can evoke both GABA and ENK release primarily through D_1Rs, presumably on pre-synaptic terminals of striatopallidal neurons. DA activation of D_1Rs is also primarily responsible for AMPH-induced hyperlocomotion presumably by post-synaptic activation of pallidonigral or pallidosubthalamic neurons while D_2Rs are involved to a lesser extent; perhaps through inhibition of pallidothalamic projections. As GABA release from striatopallidal neurons is important in providing inhibitory tone, release of ENK from the same neurons may be a signal to suppress excessive GABA release thus accounting for the opposite effects on behavior of the two neurotransmitters released from striatopallidal neurons. Thus, ENK participates in signaling by inhibiting GABA and disinhibiting DA in the GP to sustain locomotion. These results further characterize the role of pallidal DA, GABA and ENKs and may aid in the development of future therapeutics where DA dysregulation causes abnormal movement such as Parkinson's disease, dyskinesia or psychostimulant abuse.

Acknowledgements

We thank Mr Mark Dow for his help with video tracking software. This work was supported by National Institute on Drug Abuse T32 training grant (DA07268) to the Biology of Drug Abuse program at the University of Michigan and R37 EB003320 to R.T.K.

References

Alesdatter J. E. and Kalivas P. W. (1993) Inhibition of mu opioid-induced motor activity in the ventral pallidum by D_1 receptor blockade. *Behav. Pharmacol.* **4**, 645–651.

Alexander G. E., DeLong M. R. and Strick P. L. (1986) Parallel organization of functionally segregated circuits linking basal ganglia and cortex. *Annu. Rev. Neurosci.* **9**, 357–381.

Anagnostakis Y., Krikos Y. and Spyraki C. (1992) Pallidal substrate of morphine-induced locomotion. *Eur. Neuropsychopharmacol.* **2**, 65–72.

Avdelidis D. and Spyraki C. (1986) Dopamine dependent behaviours in rats with bilateral ibotenic acid-induced lesions of the globus pallidus. *Brain Res. Bull.* **16**, 25–32.

Baamonde A., Daugé V., Ruiz-Gayo M., Fulga I. G., Turcaud S., Fournié-Zaluski M. C. and Roques B. P. (1992) Antidepressant-type effects of endogenous enkephalins protected by systemic RB 101 are mediated by opioid delta and dopamine D_1 receptor stimulation. *Eur. J. Pharmacol.* **216**, 157–166.

Barone P., Tucci I., Parashos S. A. and Chase T. N. (1987) D-1 dopamine receptor changes after striatal quinolinic acid lesion. *Eur. J. Pharmacol.* **138**, 141–145.

Bartlett L. E. and Mendez I. (2005) Dopaminergic reinnervation of the globus pallidus by fetal nigral grafts in the rodent model of Parkinson's disease. *Cell Transplant.* **14**, 119–127.

Bergson C., Mrzljak L., Smiley J. F., Pappy M., Levenson R. and Goldman-Rakic P. S. (1995) Regional, cellular, and subcellular variations in the distribution of D_1 and D_5 dopamine receptors in primate brain. *J. Neurosci.* **15**, 7821–7836.

Bouali-Benazzouz R., Tai C. H., Chetrit J. and Benazzouz A. (2009) Intrapallidal injection of 6-hydroxydopamine induced changes in dopamine innervation and neuronal activity of globus pallidus. *Neuroscience* **164**, 588–596.

Cameron D. L. and Williams J. T. (1993) Dopamine D_1 receptors facilitate transmitter release. *Nature* **366**, 344–347.

Carlson J. H., Bergstrom D. A. and Walters J. R. (1986) Neurophysiological evidence that D-1 dopamine receptor blockade attenuates postsynaptic but not autoreceptor-mediated effects of dopamine agonists. *Eur. J. Pharmacol.* **123**, 237–251.

Costall B., Naylor R. J. and Olley J. E. (1972) Catalepsy and circling behaviour after intracerebral injections of neuroleptic, cholinergic and anticholinergic agents into the caudate-putamen, globus pallidus and substantia nigra of rat brain. *Neuropharmacology* **11**, 645–663.

Cuello A. C. and Paxinos G. (1978) Evidence for a long Leu-enkephalin striopallidal pathway in rat brain. *Nature* **271**, 178–180.

Dewar D., Jenner P. and Marsden C. D. (1985) Behavioural effects in rats of unilateral and bilateral injections of opiate receptor agonists into the globus pallidus. *Neuroscience* **15**, 41–46.

Di Chiara G. and Imperato A. (1998) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc. Natl Acad. Sci. USA* **85**, 5274–5278.

Dubois A., Savasta M., Curet O. and Scatton B. (1986) Autoradiographic distribution of the D_1 agonist [3H]SKF 38393, in the rat brain and spinal cord. Comparison with the distribution of D_2 dopamine receptors. *Neuroscience* **19**, 125–137.

Durieux P. F., Bearzatto B., Guiducci S., Buch T., Waisman A., Zoli M., Schiffmann S. N. and de Kerchove d'Exaerde A. (2009) D_2R striatopallidal neurons inhibit both locomotor and drug reward processes. *Nat. Neurosci.* **12**, 393–395.

Filion M., Tremblay L. and Bédard P. J. (1991) Effects of dopamine agonists on the spontaneous activity of globus pallidus neurons in monkeys with MPTP-induced Parkinsonism. *Brain Res.* **547**, 152–161.

Floran B., Aceves J., Sierra A. and Martinez-Fong D. (1990) Activation of D_1 dopamine receptors stimulates the release of GABA in the basal ganglia of the rat. *Neurosci. Lett.* **116**, 136–140.

Floran B., Floran L., Sierra A. and Aceves J. (1997) D_2 receptor-mediated inhibition of GABA release by endogenous dopamine in the rat globus pallidus. *Neurosci. Lett.* **237**, 1–4.

Fuchs H. and Hauber W. (2004) Dopaminergic innervation of the rat globus pallidus characterized by microdialysis and immunohistochemistry. *Exp. Brain Res.* **154**, 66–75.

Fuchs H., Nagel J. and Hauber W. (2005) Effects of physiological and pharmacological stimuli on dopamine release in the rat globus pallidus. *Neurochem. Int.* **47**, 474–481.

Galvan A., Floran B., Erlij D. and Aceves J. (2001) Intrapallidal dopamine restores motor deficits induced by 6-hydroxydopamine in the rat. *J. Neural. Transm.* **108**, 153–166.

Gasca-Martinez D., Hernandez A., Sierra A., Valdiosera R., Anaya-Martinez V., Floran B., Erlij D. and Aceves J. (2010) Dopamine inhibits GABA transmission from the globus pallidus to the thalamic reticular nucleus via presynaptic D4 receptors. *Neuroscience* **169**, 1672–1681.

Hauber W., Lutz S. and Münkle M. (1998) The effects of globus pallidus lesions on dopamine-dependent motor behaviour in rats. *Neuroscience* **86**, 147–157.

Hauber W. and Lutz S. (1999) Dopamine D_1 or D_2 receptor blockade in the globus pallidus produces akinesia in the rat. *Behav. Brain Res.* **106**, 143–150.

Hauber W. and Fuchs H. (2000) Dopamine release in the rat globus pallidus characterised by in vivo microdialysis. *Behav. Brain Res.* **111**, 39–44.

Hooks M. S., Jones D. N., Justice J. B., Jr and Holtzman S. G. (1992) Naloxone reduces amphetamine-induced stimulation of locomotor activity and in vivo dopamine release in the striatum and nucleus accumbens. *Pharmacol. Biochem. Behav.* **42**, 765–770.

Hurley M. J., Mash D. C. and Jenner P. (2001) Dopamine D(1) receptor expression in human basal ganglia and changes in Parkinson's disease. *Brain Res. Mol. Brain Res.* **87**, 271–279.

Joyce E. M., Koob G. F., Strecker R., Iversen S. D. and Bloom F. E. (1981) The behavioural effects of enkephalin analogues injected into the ventral tegmental area and globus pallidus. *Brain Res.* **221**, 359–370.

Jutkiewicz E. M., Torregrossa M. M., Sobczyk-Kojiro K., Mosberg H. I., Folk J. E., Rice K. C., Watson S. J. and Woods J. H. (2006) Behavioral and neurobiological effects of the enkephalinase inhibitor RB101 relative to its antidepressant effects. *Eur. J. Pharmacol.* **531**, 151–159.

Kebabian J. W. and Calne D. B. (1979) Multiple receptors for dopamine. *Nature* **277**, 93–96 (review).

Kita H. and Kitai S. T. (1988) Glutamate decarboxylase immunoreactive neurons in rat neostriatum: their morphological types and populations. *Brain Res.* **447**, 346–352.

König M., Zimmer A. M., Steiner H., Holmes P. V., Crawley J. N., Brownstein M. J. and Zimmer A. (1996) Pain responses, anxiety and aggression in mice deficient in pre-proenkephalin. *Nature* **383**, 535–538.

Li Q., Zubieta J. K. and Kennedy R. T. (2009) Practical aspects of in vivo detection of neuropeptides by microdialysis coupled off-line to capillary LC with multistage MS. *Anal. Chem.* **81**, 2242–2250.

Lindvall O. and Björklund A. (1979) Dopaminergic innervation of the globus pallidus by collaterals from the nigrostriatal pathway. *Brain Res.* **172**, 169–173.

Ludwig M. and Leng G. (2006) Dendritic peptide release and peptide-dependent behaviours. *Nat. Rev. Neurosci.* **7**, 126–136 (review).

Mabrouk O. S., Volta M., Marti M. and Morari M. (2008) Stimulation of delta opioid receptors located in substantia nigra reticulata but not globus pallidus or striatum restores motor activity in 6-hydroxydopamine lesioned rats: new insights into the role of delta receptors in Parkinsonism. *J. Neurochem.* **107**, 1647–1659.

Maneuf Y. P., Mitchell I. J., Crossman A. R. and Brotchie J. M. (1994) On the role of enkephalin cotransmission in the GABAergic striatal efferents to the globus pallidus. *Exp. Neurol.* **125**, 65–71.

Mansour A., Meador-Woodruff J. H., Bunzow J. R., Civelli O., Akil H. and Watson S. J. (1990) Localization of dopamine D_2 receptor mRNA and D_1 and D_2 receptor binding in the rat brain and pituitary: an in situ hybridization-receptor autoradiographic analysis. *J. Neurosci.* **10**, 2587–2600.

Marshall J. F., Henry B. L., Billings L. M. and Hoover B. R. (2001) The role of the globus pallidus D_2 subfamily of dopamine receptors in pallidal immediate early gene expression. *Neuroscience* **105**, 365–378.

Michael-Titus A., Preterre P., Giros B and Costentin J. (1987) Role of endogenous enkephalins in locomotion evidenced by acetorphan, an "enkephalinase" inhibitor. *J. Pharmacol. Exp. Ther.* **243**, 1062–1066.

Olive M. F., Anton B., Micevych P., Evans C. J. and Maidment N. T. (1997) Presynaptic versus postsynaptic localization of mu and delta opioid receptors in dorsal and ventral striatopallidal pathways. *J. Neurosci.* **17**, 7471–7479.

Ossowska K., Wedzony K. and Wolfarth S. (1984) The role of the GABA mechanisms of the globus pallidus in mediating catalepsy, stereotypy and locomotor activity. *Pharmacol. Biochem. Behav.* **21**, 825–831.

Parent A. and Hazrati L. N. (1995a) Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. *Brain Res. Brain Res. Rev.* **20**, 91–127.

Parent A. and Hazrati L. N. (1995b) Functional anatomy of the basal ganglia. II. The place of subthalamic nucleus and external pallidum in basal ganglia circuitry. *Brain Res. Brain Res. Rev.* **20**, 128–154.

Paxinos G. and Watson C. (2007) *The Rat Brain in Stereotaxic Coordinates*, 6th edn. Academic Press, Amsterdam.

Schad C. A., Justice J. B., Jr and Holtzman S. G. (1995) Naloxone reduces the neurochemical and behavioral effects of amphetamine but not those of cocaine. *Eur. J. Pharmacol.* **275**, 9–16.

Shin R. M., Masuda M., Miura M., Sano H., Shirasawa T., Song W. J., Kobayashi K. and Aosaki T. (2003) Dopamine D_4 receptor-induced postsynaptic inhibition of GABAergic currents in mouse globus pallidus neurons. *J. Neurosci.* **23**, 11662–11672.

Stanford I. M. and Cooper A. J. (1999) Presynaptic mu and delta opioid receptor modulation of GABAA IPSCs in the rat globus pallidus in vitro. *J. Neurosci.* **19**, 4796–4803.

Walters J. R., Bergstrom D. A., Carlson J. H., Chase T. N. and Braun A. R. (1987) D_1 dopamine receptor activation required for postsynaptic expression of D_2 agonist effects. *Science* **236**, 719–722.

Wang J. Q. and McGinty J. F. (1996) D_1 and D_2 receptor regulation of preproenkephalin and preprodynorphin mRNA in rat striatum following acute injection of amphetamine or methamphetamine. *Synapse* **22**, 114–122.

Yung K. K., Bolam J. P., Smith A. D., Hersch S. M., Ciliax B. J. and Levey A. I. (1995) Immunocytochemical localization of D_1 and D_2 dopamine receptors in the basal ganglia of the rat: light and electron microscopy. *Neuroscience* **65**, 709–730.