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Daily Monitoring of Dopamine Efflux Reveals a Short-Lasting Occlusion of the Dopamine Agonist Properties of *d*-Amphetamine by Dopamine Transporter Blockers GBR 12909 and Methylphenidate

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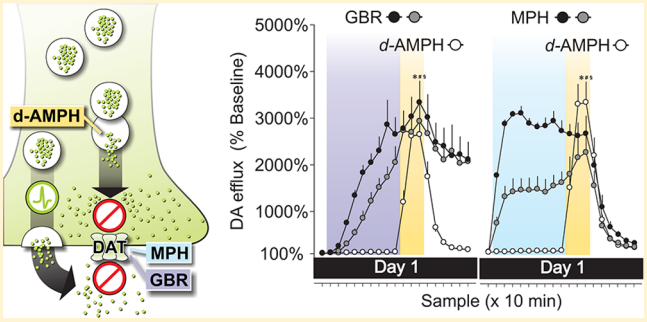
ABSTRACT: In vivo brain microdialysis was used in conjunction with "reverse-dialysis" of the dopamine-transporter (DAT) blockers GBR 12909 and methylphenidate (MPH) to observe the temporal course of their effects on *d*-amphetamine (*d*-AMPH)-induced increases in dopamine (DA) efflux in the rat nucleus accumbens (NAc). Reverse-dialysis of *d*-AMPH (10 μM) for 30 min resulted in a 2000−2500% increase in DA efflux. Pretreatment with GBR 12909 or MPH (20, 100 μM) for 90 min, which on their own elevated DA levels ∼2000−3000% above baseline values, dose-dependently occluded *d*-AMPH-evoked DA efflux. In GBR 12909-treated rats, basal levels of DA remained dramatically elevated at 24, 48, and 72 h following treatment, while levels in the MPH group returned back toward pretreatment values. Despite this contrast in basal DA efflux, the magnitudes of DA efflux evoked by a second exposure to *d*-AMPH were comparable in the two treatment groups. Together, these data support the development of DAT blockers as potential pharmacological interventions for the control of psychostimulant abuse. Furthermore, our data implicate DAT as a common site of action for both GBR 12909 and MPH, as well as *d*-AMPH.



KEYWORDS: *Addiction, d-amphetamine, dopamine transporter, GBR 12909, in vivo microdialysis, methylphenidate*

The abuse potential of psychostimulant drugs such as amphetamine (AMPH) and cocaine is attributed to their actions as indirect dopamine (DA) agonists at presynaptic sites on the terminal regions of the mesocorticolimbic DA system.[1,2] Compelling evidence for the links between the rewarding properties of psychostimulants and DA neurotransmission is provided by direct measurement of dramatic elevation in DA efflux into the extracellular compartment following intravenous (i.v.) self-administration of these drugs by animals as measured by the combined techniques of in vivo brain microdialysis and high-pressure liquid chromatography (HPLC) with electrochemical detection.[3−7]

Given the tremendous harm inflicted on personal health and the well-being of society, there is an urgent need to develop new medications that are effective in reducing addiction to psychostimulants. One approach to this challenge has focused on a specific group of compounds that serve as highly effective and selective DA uptake inhibitors. For a comprehensive review of this approach to the development of "agonist" therapy candidates, see Rothman et al.[8] Rothman and colleagues have championed the use of compounds within the aryl-1,4-dial(en)ylpiperazine class, specifically GBR 12909 (1-(2-[bis-(4-fluorophenyl)methoxy]ethyl)-4-(3-phenylpropyl)-piperazine), as potential medication for the treatment of psychostimulant drug addiction.[9−11] The neuropharmacological of properties of GBR 12909 are distinct from other DA reuptake inhibitors such as cocaine. Specifically, GBR 12909 has a much slower onset and markedly longer duration of action,[12−14] the latter characteristic being consistent with the indication that GBR 12909 and close analogues may bind irreversibly to the DAT.[15,16] In preclinical studies, GBR 12909 treatment attenuated cocaine self-administration in rats.[17,18] Pretreatment with a depot formulation of the drug, GBR-decanoate, selectively blocked operant responding for cocaine self-administration for up to a month, without affecting lever-pressing behavior for food reward.[19]

Initial in vivo microdialysis studies reported an elevation in DA efflux following systemic administration of GBR 12909,[12,20] sufficient to attenuate cocaine-induced increases in DA efflux.[21,22] Importantly, in rats treated with GBR-decanoate, elevation of DA efflux was observed up to 2 weeks later and furthermore, significantly antagonized the increase in DA efflux induced by i.v. doses (0.3 and 1.0 mg/kg) of methamphetamine.[23] A different in vivo measure of the DA agonist effects of AMPH involves monitoring changes in [^{11}C]raclopride binding as measured by PET. In a study with nonhuman primates, increased raclopride displacement of DA confirmed that GBR

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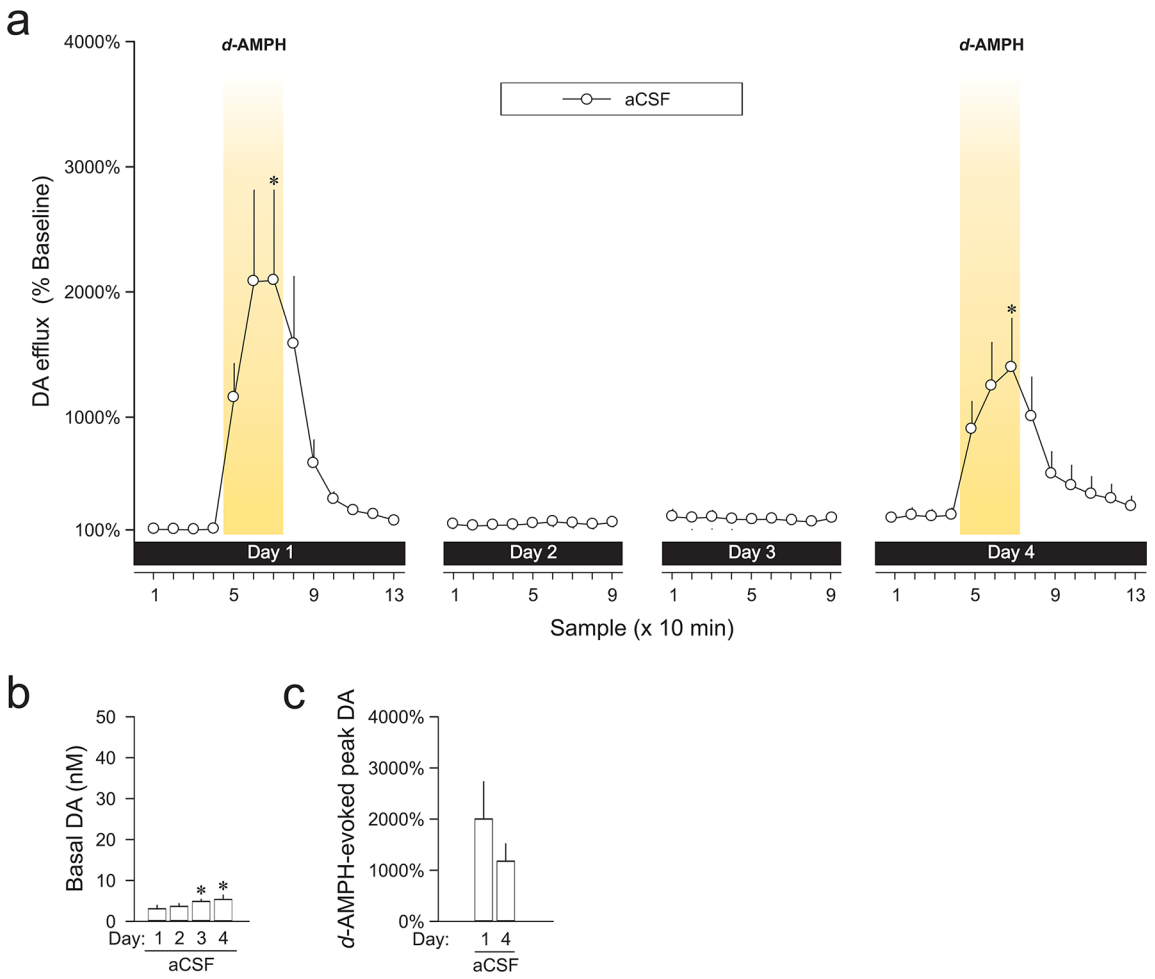

Figure 1. Effect of *d*-AMPH (10 μM; days 1 and 4) on DA efflux in the NAc. Shown are (a) change in DA efflux expressed as % change from baseline, (b) basal DA concentration, and (c) peak change in DA efflux evoked by *d*-AMPH. Yellow shading represents 30 min periods of *d*-AMPH reverse-dialysis. Data points represent mean + SEM (n = 4). *p < 0.05, (a) vs sample 4 on days 1 and 4; (b) and (c) vs Day 1.

12909 attenuated amphetamine-induced increase in striatal DA release.[24]

An alternate strategy to the development of medication for psychostimulant addiction based on highly selective DAT blockers as represented by GBR 12909 proposes the use of compounds that target multiple receptors or uptake sites.[8,25] Although methylphenidate (MPH) is an effective DAT blocker, its inhibitory effects extend to the serotonin and norepinephrine transporters.[26,27] Administration of MPH, both systemically and by reverse-dialysis, is associated with robust increases in extracellular levels of DA, serotonin and norepinephrine.[28−31]

The present study compared the effects of pretreatment with two DAT inhibitors, GBR 12909 and MPH, which differ in binding affinity and specificity for the DAT, on *d*-AMPH-induced DA efflux in the NAc. In light of the indication that GBR 12909 interaction with the DAT may be long-lasting,[15] which raises the possibility of prolonged elevation of extracellular DA, microdialysis experiments in the present study were extended past the typical 3−4 h monitoring to 4 days. This permitted the assessment of both the acute and delayed effects of pretreatment with either DAT inhibitor on the DA agonist properties of *d*-AMPH on the first and last day of the experiment. In addition, monitoring basal DA efflux at 24 h intervals allowed a comparison of the longer-term action of these two DAT blockers on basal DA efflux.

RESULTS AND DISCUSSION

Multiday Utilization of a Microdialysis Probe Does Not Compromise Basal or Evoked DA Efflux. The feasibility of in vivo brain microdialysis conducted across multiple days must consider possible implantation-induced injury, which reduces basal rates of neurotransmitter release, as well as gliosis on the semipermeable membrane, which interferes with diffusion of DA into the probe.[32−34] Thus, prior to investigating possible longer-term effects of exposure to either GBR 12909 or MPH on DA efflux, the initial experiment was designed to confirm that microdialysis could be conducted reliably over a 4-day interval. Rather than utilizing repeated insertion of probes and the consequent tissue trauma, we chose a single insertion protocol in which the microdialysis probe remained implanted in the NAc for the four days. Our results indicated that basal DA efflux, monitored for at least 90 min, was relatively stable (±10% fluctuation) on each of the four days (Figure 1a, n = 4). Surprisingly, in contrast to concerns that recovery of DA from the probe may be compromised, there was in fact a small but significant increase in DA concentration across days [$F(3,9)$ = 8.403, p = 0.006; Figure 1b]. A similar steady increase in basal levels of amino acid transmitters has been reported previously.[35] Changes in reuptake efficiency and/or tonic release of neurotransmitter

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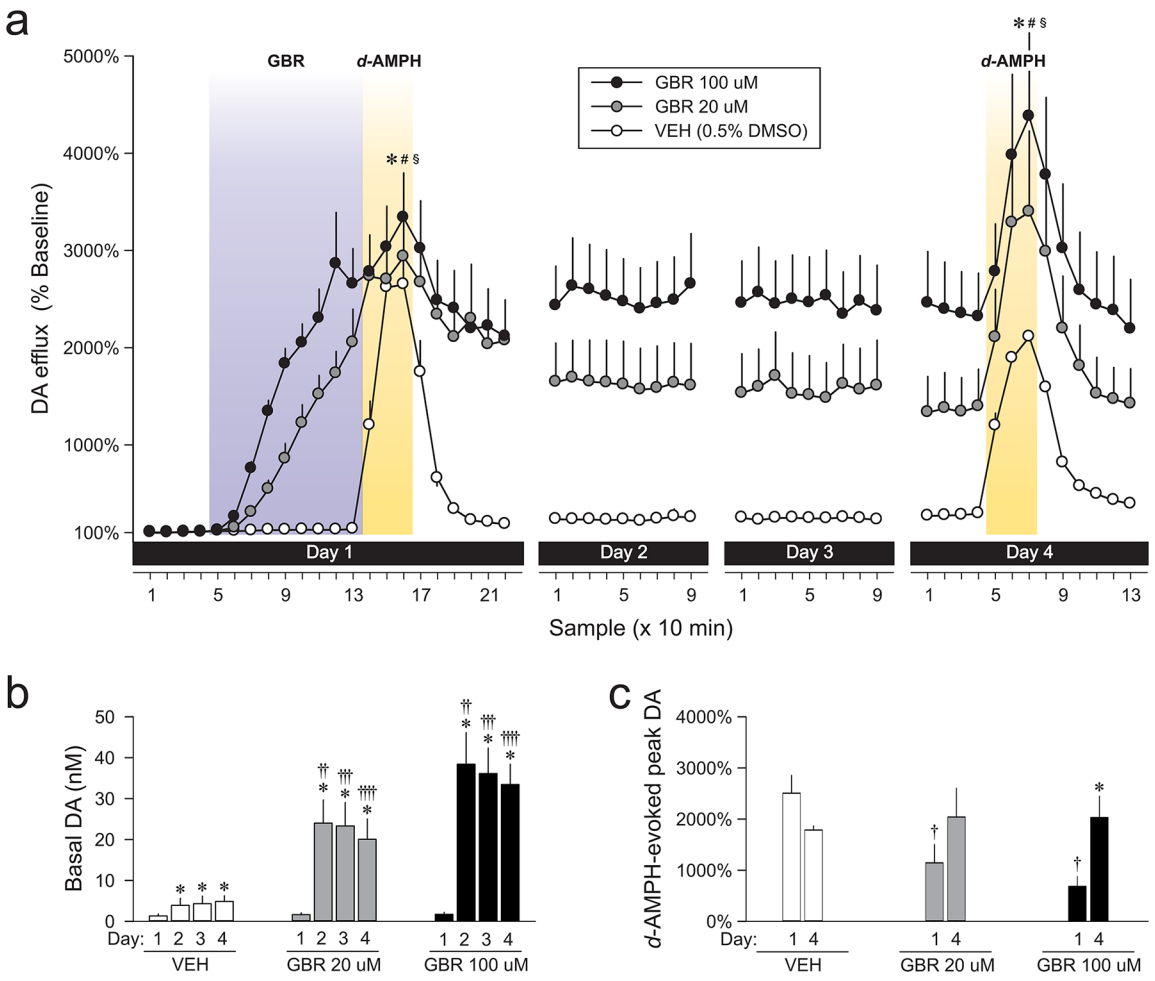

Figure 2. Effect of *d*-AMPH (10 μM; days 1 and 4) on DA efflux in the NAc following reverse-dialysis of GBR 12909 (GBR; 20, 100 μM). Shown are (a) change in DA efflux expressed as % change from baseline, (b) basal DA concentration, and (c) peak change in DA efflux evoked by *d*-AMPH. Purple and yellow shading represents the 90 min period of GBR or vehicle (VEH) and 30 min periods of *d*-AMPH reverse-dialysis, respectively. Data points represent mean + SEM (VEH, $n = 4$; GBR 20 μM, $n = 6$; GBR 100 μM, $n = 6$). $*^{(\mathrm{VEH})}, \#^{(20\mu\mathrm{M})}, \S^{(100\mu\mathrm{M})} p < 0.05$, (a) vs sample 4 on days 1 and 4; (b) and (c) vs day 1 within dose. $^{\dagger(\mathrm{Day1})}, ^{\dagger\dagger(\mathrm{Day2})}, ^{\dagger\dagger\dagger(\mathrm{Day3})}, ^{\dagger\dagger\dagger\dagger(\mathrm{Day4})} p < 0.05$ vs VEH within day.

may account in part for these observations, but this phenomenon has yet to be addressed experimentally.

DA release stimulated by the psychostimulant *d*-AMPH was also assessed on the first and last day of the experiment. Reverse-dialysis of *d*-AMPH (10 μM) for 30 min evoked a significant increase in DA efflux on days 1 and 4 [$F(9,27) = 7.126$, $p < 0.001$ and $F(9,27) = 13.327$, $p < 0.001$, respectively; Figure 1a]. Although the evoked increase on day 1 was greater in magnitude than that observed on day 4, the peak DA responses did not differ statistically (Figure 1c). Possible reduction in the efficacy of the probe to deliver the same amount of *d*-AMPH may explain in part the smaller DA response on day 4, but development of a small degree of tolerance to *d*-AMPH cannot be ruled out.

DMSO-Containing Vehicle Does Not Alter Basal or Evoked DA. GBR 12909 is a compound with limited solubility in aCSF, but can be dissolved to necessary concentrations using small amounts of dimethyl sulfoxide (DMSO). In the present study, a 0.5% DMSO/aCSF solution was used to prepare all drugs administered by reverse-dialysis. A comparison of basal DA levels in animals that were perfused with the DMSO-containing vehicle (VEH) solution ($n = 4$) or aCSF only revealed no statistical differences between the two groups. As in the aCSF group described above, there was a small but gradual increase in basal DA levels across days that was significantly higher than that observed on day 1 [$F(3,9) = 4.424$, $p = 0.036$; Figure 2b].

The response of the VEH-treated control group to *d*-AMPH was also unaffected by the small amount of DMSO. On both days 1 and 4, *d*-AMPH evoked a significant increase in DA efflux from baseline [$F(9,27) = 41.597$, $p < 0.001$ and $F(9,27) = 160.044$, $p < 0.001$, respectively; Figure 2a] that was of comparable magnitude and time-course to that observed in the aCSF group. Furthermore, the *d*-AMPH-evoked increase in DA efflux on day 1 was again slightly higher than that on day 4 (Figure 2c), but did not reach statistical significance. These VEH data served as the control condition in both GBR 12909 and MPH reverse-dialysis experiments.

GBR 12909 but Not MPH Enhances Basal DA Efflux over 4 Days. The initial phase of the experiment compared the acute effects of the two DAT blockers, GBR 12909 and MPH, on DA efflux in the NAc. Reverse-dialysis of GBR 12909 was accompanied by a slow rise in DA levels, reaching peak increases of ~2000% in the 20 μM group ($n = 6$) and ~2700% in the 100 μM group ($n = 6$) by the end of the 90 min of treatment (Figure 2a). Surprisingly, when the perfusion medium was switched back to aCSF, elevated levels of DA were maintained for a further 90 min (i.e., until the termination

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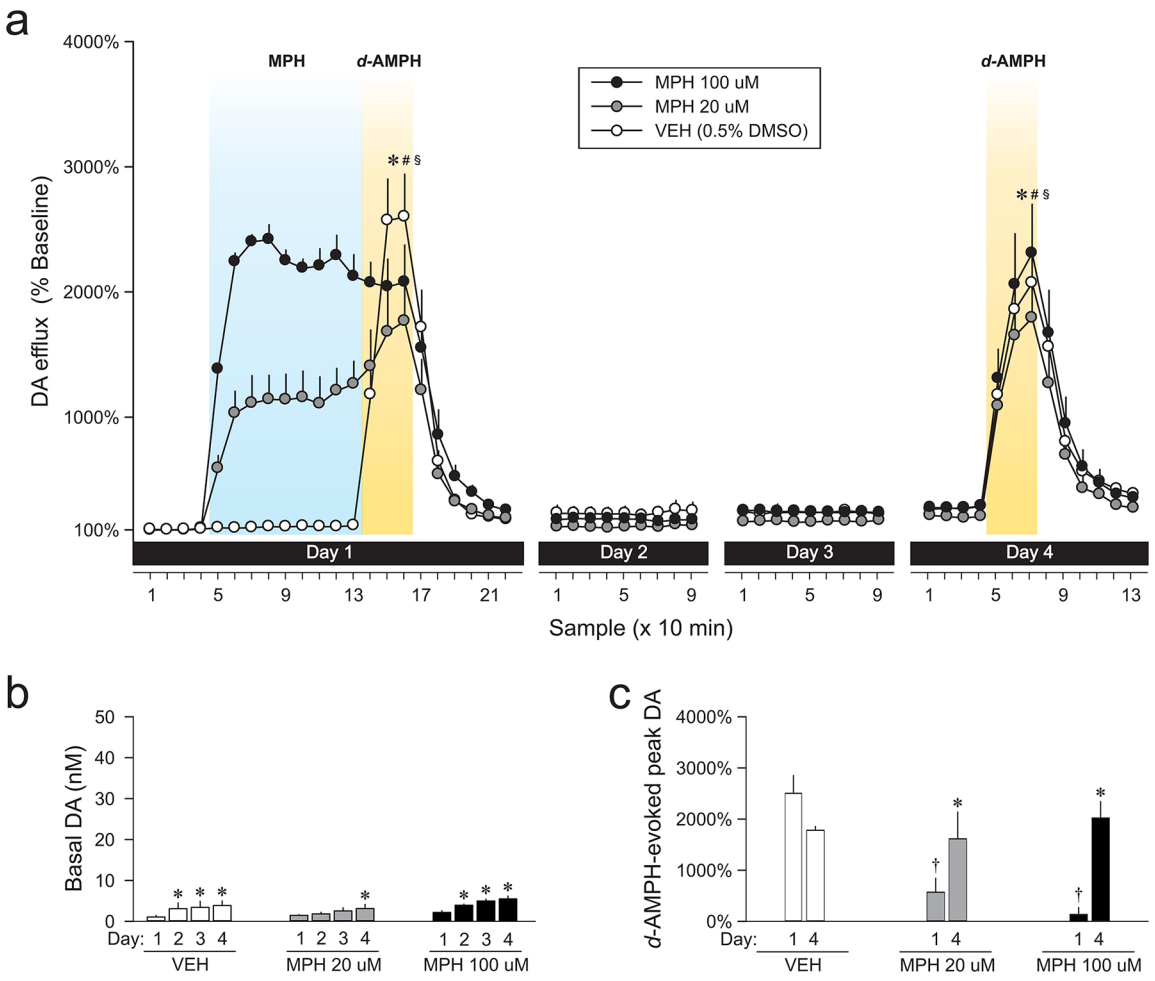

Figure 3. Effect of *d*-AMPH (10 μM; days 1 and 4) on DA efflux in the NAc following reverse-dialysis of methylphenidate (MPH; 20, 100 μM). Shown are (a) change in DA efflux expressed as % change from baseline, (b) basal DA concentration, and (c) peak change in DA efflux evoked by *d*-AMPH. Blue and yellow shading represents the 90 min period of MPH or vehicle (VEH) and 30 min periods of *d*-AMPH reverse-dialysis, respectively. Data points represent mean + SEM (VEH, n = 4; MPH 20 μM, n = 4; MPH 100 μM, n = 6). $*^{(\mathrm{VEH}),\ \#(20\mu\mathrm{M}),\ \S(100\mu\mathrm{M})}p < 0.05$, (a) vs sample 4 on days 1 and 4; (b) and (c) vs day 1 within dose. $^{\dagger(\mathrm{Day1})}p < 0.05$ vs VEH within day.

of the experiment) on day 1, and on subsequent days. A comparison of rats treated with VEH and GBR 12909 (20, 100 μM) indicated that a dose-dependent elevation of basal DA concentration was present on days 2−4 [$F(6,39) = 6.909$, $p < 0.001$; Figure 2b). In contrast, reverse-dialysis of MPH was accompanied by a rapid rise in DA efflux that reached a plateau within 30 min and sustained only for the duration of the treatment (Figure 3a). The magnitude of increased DA efflux in the presence of MPH was 2-fold higher in the 100 μM group (~2400%, n = 6) than in the 20 μM group (~1200%, n = 4) on day 1, but this dose-dependent pattern was not present on subsequent days (Figure 2b). However, as with the control group, DA levels in MPH-treated rats showed small stepped increases of 0.5−1 nM across subsequent days (Figure 3b). The slow rate of increase in DA efflux and the maintenance of the elevated levels for several days following exposure to GBR 12909 is reminiscent of the "slow onset/offset kinetics" ascribed to DAT blockers (including GBR 12909),[36] which has been suggested to have lower abuse liability than those with faster and shorter time-course of effects (including MPH and cocaine).

Earlier studies have reported that GBR 12909 resulted in elevated levels of DA efflux that persist for several hours in the striatum;[12,20,31] however, the present data are the first demonstration that increased DA efflux evoked by GBR 12909 (in a nondepot formulation), administered both locally or systemically, lasts several days past the period of exposure (Figures 2 and 4). The longer-term effects of GBR 12909 treatment on DA efflux are consistent with the molecular characterization of GBR-like molecules. In particular, the piperazine family of DAT-specific inhibitors may bind with very strong affinity, possibly irreversibly, to maintain long-term blockade of the DAT over several days.[15,16] Another factor that may influence the duration of prolonged elevation of DA is the rate of turnover of the DAT protein. According to Kimmel and colleagues, the half-life of DAT is estimated to be in the order of 2−3 days.[37] This suggests that as the DAT complex, to which GBR 12909 is bound, is replaced by new DAT protein, there would be normalization of function (i.e., reuptake and reverse-transport) over time. In support of this idea, in a preliminary experiment comparing changes in basal levels on days 2−4 with those on day 8, we observed that basal DA values had returned to normal one week following treatment with GBR 12909. Alternatively, Andersen has proposed a secondary binding site for GBR 12935, a close analog of GBR 12909.[38] In detailed characterization of rat striatal DAT binding, Andersen reported that GBR 12935 bindings strongly to a DA uptake complex as well as a piperazine acceptor site.

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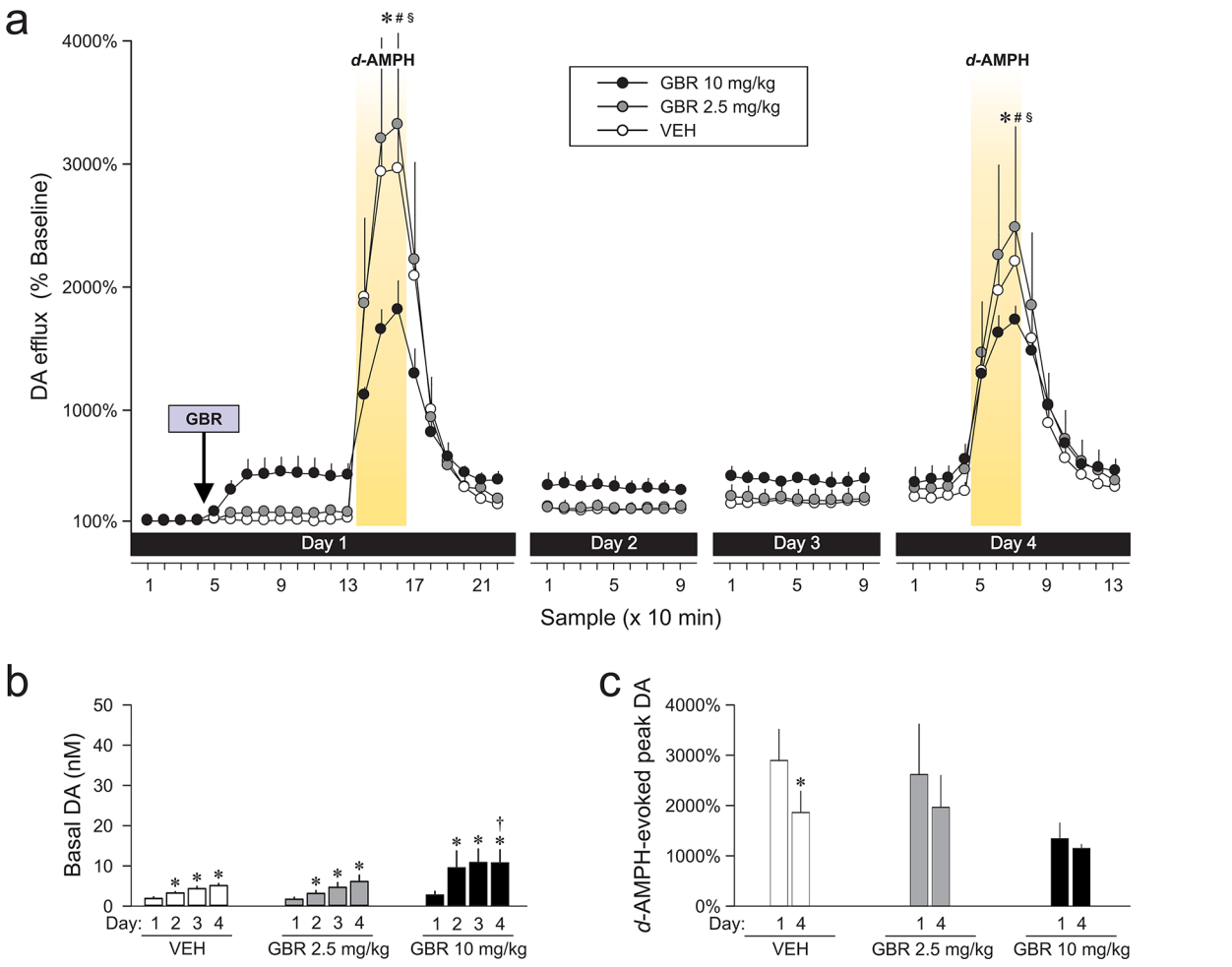

Figure 4. Effect of *d*-AMPH (10 μM; days 1 and 4) on DA efflux in the NAc following systemic injection of GBR 12909 (GBR; 2.5, 10 mg/kg, i.p.). Shown are (a) change in DA efflux expressed as % change from baseline, (b) basal DA concentration, and (c) peak change in DA efflux evoked by *d*-AMPH. Arrow indicates time of GBR or vehicle (VEH) treatment. Yellow shading represents 30 min periods of *d*-AMPH reverse-dialysis. Data points represent mean + SEM (VEH, $n = 4$; GBR 2.5 mg/kg, $n = 4$; GBR 10 mg/kg, $n = 4$). $*^{(\mathrm{VEH})}$, $\#^{(20\mu\mathrm{M})}$, $\S^{(100\mu\mathrm{M})}p < 0.05$, (a) vs sample 4 on days 1 and 4; (b) and (c) vs day 1 within dose. $\dagger^{(\mathrm{Day1})}p < 0.05$ vs VEH within day.

Andersen also emphasized a distinction between the binding [^{3}H]GBR 12935 to the DAT as being separate from substrates for DA uptake and release.

Pretreatment with Either GBR 12909 or MPH Occludes *d*-AMPH-Evoked DA on Day 1 but Not on Day 4. The second phase of the experiment assessed the effect of pretreatment with either GBR 12909 or MPH on DA agonist properties of *d*-AMPH. Following a 90 min exposure to either GBR 12909 or MPH (20, 100 μM), *d*-AMPH (10 μM) was applied directly into the NAc by reverse-dialysis for 30 min on the first day as well as 72 h later. GBR 12909 pretreatment resulted in a near complete occlusion of DA efflux induced by subsequent administration of *d*-AMPH on day 1. The DA response elicited by *d*-AMPH in control subjects [$F(9,27) = 41.597$, $p < 0.001$; Figure 2a] was dose-dependently attenuated by GBR 12909 [$F(2,13) = 5.451$, $p = 0.019$; Figure 2c]. Pretreatment with MPH also resulted in a robust inhibitory effect on DA efflux evoked by *d*-AMPH on day 1 [$F(2,11) = 22.877$, $p < 0.001$; Figure 3c]. However, a repeated d-AMPH challenge revealed that GBR 12909- or MPH-mediated inhibition of the DA releasing properties of d-AMPH was short-lasting. As described above, basal levels in GBR 12909 animals were elevated significantly in a dose-dependent manner over several days. On day 4, although DA efflux was significantly higher than pretreatment values (~1400% in the 20 μM group, 2300% in the 100 μM group), *d*-AMPH evoked a further enhancement of DA efflux (~2000%) above the already elevated levels (Figure 2a). The peak evoked DA responses were of comparable magnitude in the VEH and GBR 12909 (20, 100 μM) groups (Figure 2c). In the MPH-treated group, pretreatment with MPH 72 h earlier, similarly failed to attenuate a *d*-AMPH-evoked increase from baseline (Figure 3c).

The mechanism by which AMPH elevates extracellular levels of DA is believed to involve a depletion of DA from vesicles into the cytosolic space and the transport of this pool of DA down the concentration gradient through the DAT in the reverse direction.[39] The entry of *d*-AMPH molecules into the DA terminal may also involve the DAT in a carrier-mediated exchange/diffusion process.[40] However, as suggested by the results of Day 1, GBR 12909 blockade of DAT may prevent the reuptake of DA as well as the entry of *d*-AMPH into the nerve terminal. It is important to note that the DAT may not be the exclusive point of entry for AMPH. Indeed, at higher concentrations, AMPH may utilize an alternate route, wherein the highly lipophilic nature of AMPH may permit its entry into

the cell by diffusion through the phospholipid bilayer.[41] While a consideration of these mechanisms may explain the blunting of *d*-AMPH-evoked DA efflux observed on day 1, the restitution of *d*-AMPH's effects on day 4 remains a puzzle. Earlier in the discussion, we suggested that the sustained elevation of basal DA levels across three days may be related to the strong affinity of GBR 12909 for the DAT.[15,16] An alternative to this account is suggested by the findings of Melikian and colleagues which confirm that DAT endocytosis is accelerated following exposure to psychostimulants.[42] If GBR 12909 shared this property of psychostimulants to alter DAT trafficking away from the plasma membrane, then the consequent reduction in reuptake sites may provide an alternative explanation for the elevated basal levels of DA observed in the present findings. Whichever account may best explain the sustained elevation of basal DA levels, an importance consequence of both would be the removal of GBR 12909-bound DAT and its replacement by new DAT protein (within 2−3 days),[37] which would serve as an effective substrate for *d*-AMPH uptake and facilitated efflux of DA.

Note on Reverse-Dialysis and Behavior. It is important to note that while intra-NAc reverse-dialysis of GBR 12909 or MPH led to highly significant increases in DA efflux, no changes in behavior were observed. This is not surprising, since a drug applied by this technique is expected to remain in the immediate vicinity of the dialyzing membrane.[43,44] Accordingly, all animals in the study typically lay quietly in a corner of the dialysis chamber, sleeping most of the time. Only on a limited number of occasions was the 30 min period of *d*-AMPH reverse-dialysis associated with slight increases in exploratory and sniffing behavior.

Systemic Administration of GBR 12909 Occludes *d*-AMPH-Evoked Elevation in DA efflux. In a final extension of the study, GBR 12909 at 2.5 or 10 mg/kg or its vehicle solution (n = 4 for all groups) was administered systemically. Once again, GBR 12909 treatment led to a dose-dependent increase in basal DA efflux (∼200% and ∼500% in the 2.5 and 10 mg/kg groups, respectively; Figure 4a), albeit of smaller magnitude than that observed in reverse-dialysis experiments. The basal DA concentrations on the days following treatment were significantly higher than baseline levels on day 1 [$F(6,27) = 6.063$, $p < 0.001$; Figure 4b]. On day 1, challenge with *d*-AMPH evoked significant increases in DA efflux in control rats (Figure 4c), which was attenuated by the higher dose of GBR 12909 (10 mg/kg; $p = 0.056$). On day 4, *d*-AMPH-evoked DA response in the GBR 12909-pretreated group was not statistically different than that observed in control rats. In contrast to results observed following reverse-dialysis of GBR 12909 (Figure 2), even following pretreatment with the highest systemic dose (10 mg/kg, i.p.), there remained substantial *d*-AMPH-evoked DA efflux in the NAc on day 1. This result is most likely related to the reduced effectiveness of a systemic treatment to antagonize the effects of a high dose of *d*-AMPH (10 μM for 30 min) administered via reverse-dialysis. In future studies, we may examine the effects of systemically administered GBR 12909 on intravenous injections of *d*-AMPH at doses used routinely in self-administration studies (0.1−0.25 mg/kg).

CONCLUDING REMARKS

In summary, the present data confirm previous reports that pretreatment with GBR 12909 elevates basal DA efflux and antagonizes the DA-releasing property of *d*-AMPH. The findings here additionally demonstrate that the latter effect is not present 72 h after initial treatment with this DAT blocker. The present findings also have important implications for the claim that GBR-like compounds may provide effective pharmacotherapy for psychostimulant addiction. Specifically, our finding that a single injection of GBR 12909 elevates basal DA concentration up to 3 days raises the possibility that intermittent treatment with GBR 12909, related to the rate of DAT protein turnover, may be effective in attenuating the rewarding effect of DA. Although the highest dose of systemic GBR 12909 employed here (10 mg/kg, i.p.) only partially occluded the increase in DA efflux evoked by reverse-dialysis of *d*-AMPH, the possibility still remains that potential therapeutic effects may be mediated by a sustained elevation of basal DA levels in the NAc. This in turn could modulate rapid phasic increases in DA efflux thought to mediate the rewarding effects of psychostimulant drugs.

METHODS

Subjects. Male Long Evans rats (250−275 g) were obtained from Charles River (St. Constant, Quebec, Canada) and housed in pairs upon arrival and then individually following surgery. The colony room was maintained at ∼21 °C with a 12 h light/dark cycle (lights on at 7 p.m.). Rats had free access to rat chow (Purina Rat Chow) and water in home cages and testing chambers unless otherwise noted. All experimental procedures were conducted in accordance with the ethical standards set by the Canadian Council on Animal Care and approved by the University of British Columbia Animal Care Committee.

Surgery. On the day of surgery, rats were anesthetized using 4% isoflurane (AErrane, Baxter Co., Toronto, Canada) mixed with oxygen, and then maintained with 2.0−2.5% isoflurane for the remainder of the surgery. Rats received a subcutaneous injection of an analgesic (ketoprofen 0.05 mL) and were placed in a stereotaxic apparatus in flat skull position (mouth bar at −3.2 mm). A heating pad placed underneath the stereotaxic to counter the hypothermic effects of isoflurane anesthesia. All coordinates were determined using the atlas of Paxinos and Watson (1997). Each rat was implanted with nitric acid-passivated stainless steel guide cannulae (19 gauge × 15 mm) directly above the NAc (from bregma, +1.7 mm anterio-posterior, ± 1.1 mm medio-lateral; from dura, −1.0 mm dorso-ventral). Guides were secured using skull screws and dental acrylic. Stainless steel obdurators maintained patency of the guides until probe implantation. Rats were allowed to recover from surgery for a minimum of one week prior to serving as subjects in microdialysis experiments.

In Vivo Microdialysis. Microdialysis probes were assembled 1−2 days prior to implantation. Probes were concentric in design, constructed from Filtral 12 AN69HF semipermeable hollow fibers (340 um OD × 2 mm, 65 kDa molecular weight cutoff; Hospal, Neurnberg, Germany) and silica inlet−outlet lines (75/150 um ID/OD). Typical in vitro probe recovery of an external DA standard solution at room temperature (21 °C) was 12%. One day prior to experiments, probes were connected to an Instech liquid swivel (Plymouth Meeting, PA), thoroughly flushed with artificial cerebrospinal fluid (aCSF; in mM: 10 sodium phosphate buffer, 1.2 $CaCl_2$, 3.0 KCl, 1.0 $MgCl_2$, 147 NaCl, pH 7.4) and then inserted into the NAc via the guide cannulae such that the 2 mm membrane spanned −4.8 to −6.8 mm from dura. Flow rate through the probe was maintained at 1 μL/min until the termination of the experiment. Following implantation, animals were placed in a Plexiglas chamber (40 × 40 × 40 cm^3) with food and water and remained there overnight for ∼16 h. In the morning, samples were collected at 10 min intervals.

High Pressure Liquid Chromatography/Electrochemical Detection. DA was isolated from the dialysate using high performance liquid chromatography and quantified via electrochemical detection. The systems consisted of an ESA 582 pump (Bedford, MA), a pulse damper (Scientific Systems Inc., State College, PA), a Rheodyne Inert manual injector (model 9125i, 20 μL injection loop;

Rohnert Park, CA), a Tosoh Bioscience Super ODS TSK column (2 um particle, 2 mm × 10 mm; Montgomeryville, PA), and an Antec Leyden Intro Electrochemical detector with VT-03 flow cell with a Ag/AgCl reference electrode ($V_{applied}$ = +650 mV; Leyden, The Netherlands). The mobile phase (70 mM sodium acetate buffer, 40 mg/L EDTA and 6 mg/L sodium dodecyl sulfate (variable), pH 4.0, 10% methanol] flowed through the system at 0.17 mL/min. EZChrome Elite software (Scientific Software, Pleasanton, CA) was used to acquire and analyze chromatographic data.

Drugs and Preparation. GBR 12909 dihydrochloride was obtained from Sigma-Aldrich (Oakville, Ontario, Canada), and MPH hydrochloride was synthesized at the TRIUMPH chemistry lab at UBC (Vancouver, Canada). For reverse-dialysis experiments, GBR 12909 (20, 100 μM) and MPH (20, 100 μM) were prepared daily in a vehicle composed of 0.5% DMSO in aCSF (pH 6.5). For systemic administration, GBR 12909 (2.5, 10, and 20 mg/kg) was prepared daily in a vehicle composed of propylene glycol (15%), ethanol (15%), and sterile H_2O (70%) with sonication and vortexing.

Experimental Design. Microdialysis experiments were conducted over 4 consecutive days. On day 1, once a stable baseline was established (with four consecutive samples showing less than 10% fluctuation in DA concentration), the treatment phase of the experiment was initiated. In reverse-dialysis experiments, treatment consisted of intra-NAc application of GBR 12909 (20 or 100 μM), MPH (20 or 100 μM), or vehicle. In systemic studies, GBR 12909 (2.5 and 10 mg/kg) or vehicle was administered i.p.). Ninety minutes following treatment, *d*-AMPH (10 μM) was applied by reverse-dialysis for 30 min. On days 2 and 3, samples were collected and analyzed for 90−120 min without treatment. On day 4, after establishing a stable baseline (as in day 1), *d*-AMPH (10 μM) was administered again by reverse-dialysis into the NAc for 30 min.

Histology. Following microdialysis experiments, rats were deeply anesthetized with isoflurane. Brains were promptly removed and stored in 20% w/v sucrose and 4% v/v paraformaldehyde solution for 5−7 days. Brains were then sliced into 50 um coronal sections, stained with cresyl violet, and examined for verification of probe placement. As shown in Figure 5, membrane tracts were localized to the shell/core border of the NAc, spanning 1.2 to 2.2 mm anterior to bregma.

Data Analyses. Microdialysis data are presented as percent change from the mean value of the last four samples of the baseline period. Basal DA levels were calculated as the mean concentration of the last four baseline samples prior to treatment on days 1 and 4 or the last four samples collected on days 2 and 3. Statistical analyses involved analysis of variance, with sample time or day of microdialysis as within-subjects factors and treatment dose as a between-group factor, followed by the Holm-Sidak method of planned comparisons. All statistical analyses were performed using SigmaPlot (12.3).

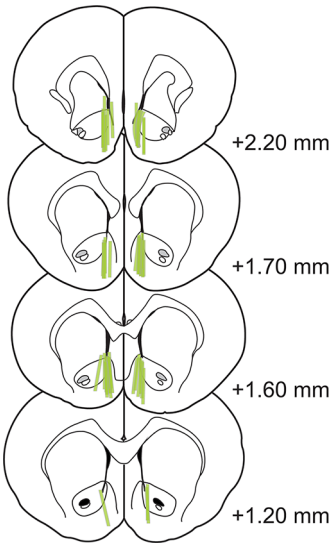

Figure 5. Location of microdialysis probes. Vertical lines represent the semipermeable membrane portion of probes (2 mm length and 300 μm outer diameter). Drawings of coronal sections were adapted from Paxinos and Watson (1997). Distance from bregma is indicated.

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Author Contributions

S.Y.A. and A.G.P. contributed equally to the experimental design, data evaluation, and preparation of the manuscript. S.Y.A. oversaw all aspects of the experiments.

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ABBREVIATIONS

aCSF, artificial cerebrospinal fluid; *d*-AMPH, *d*-amphetamine; DA, dopamine; i.p., intraperitoneally; i.v., intravenous; NAc, nucleus accumbens; MPH, methylphenidate

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