Subregion-specific effects on striatal neurotransmission and dopamine-signaling by acute and repeated amphetamine exposure

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ABSTRACT

Repeated administration of psychostimulants, such as amphetamine, is associated with a progressive increased sensitivity to some of the drug’s effects, but tolerance towards others. We hypothesized that these adaptations in part could be linked to differential effects by amphetamine on dopaminergic signaling in striatal subregions. To test this theory, acute and long-lasting changes in dopaminergic neurotransmission were assessed in the nucleus accumbens (nAc) and the dorsomedial striatum (DMS) following amphetamine exposure in Wistar rats. By means of in vivo microdialysis, dopamine release induced by local administration of amphetamine was monitored in nAc and DMS of amphetamine naïve rats, and in rats subjected to five days of systemic amphetamine administration (2.0 mg/kg/day) followed by two weeks of withdrawal. In parallel, ex vivo electrophysiology was conducted to outline the effect of acute and repeated amphetamine exposure on striatal neurotransmission. The data shows that amphetamine increases dopamine in a concentration-dependent and subregion-specific manner. Furthermore, repeated administration of amphetamine followed by abstinence resulted in a selective decrease in baseline dopamine in the nAc, and a potentiation of the relative dopamine elevation after systemic amphetamine in the same area. Ex vivo electrophysiology demonstrated decreased excitatory neurotransmission in brain slices from amphetamine-treated animals, and a nAc selective shift in the responsiveness to the dopamine D2-receptor agonist quinpirole. These selective effects on dopamine signaling seen in striatal subregions after repeated drug exposure may partially explain why tolerance develops to the rewarding effects, but not towards the psychosis inducing properties of amphetamine.

1. Introduction

Psychostimulant addiction, such as amphetamine addiction, is a complex disorder that involves both behavioral and neurophysiological transformations. The striatum has been proposed as an important hub region involved in several different aspects of the disorder, with subregion-specific neuroadaptations contributing to hallmark symptoms and neurological processes associated with addiction (Koob and Volkow, 2010). The reinforcing properties of amphetamine are associated with increased release of dopamine in the striatum, especially in the ventral part, and facilitation of reward-driven behavior (Carboni et al., 1989; Koob and Volkow, 2010). Repeated administration furthermore elicits long-lasting transformations of dopaminergic neurotransmission in striatal circuits, which produce sensitization towards the stimulatory properties of amphetamine in both humans and laboratory animals (Gatica et al., 2020; Kalivas and Stewart, 1991; O’Daly et al., 2014; Robinson and Becker, 1986; Solis et al., 2019). The subjective experience of pleasure, however, may be followed by a negative affective state, which includes loss of motivation for natural rewards, anhedonia and anxiety (Koob and Le Moal, 2001, 2008). Indeed, repeated administration of psychostimulants appears to increase the reward threshold by attenuating dopaminergic signaling, which in turn may drive drug intake (Ahmed and Koob, 2004; Belujon et al., 2016; Kesby et al., 2018). Blunted dopaminergic signaling has also been reported in striatal subregions in cocaine or alcohol dependent individuals (Martínez et al., 2004, 2005). The putative hypofunction of dopaminergic transmission could be an important factor underlying anhedonia and decreased sensitivity to rewarding events (Der-Avakian and Markou, 2012), which

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is described both in addicted individuals and in animal models, even after protracted withdrawal (Der-Avakian and Markou, 2010; Leventhal et al., 2008; Volkow et al., 2007). Drug induced effects on dopaminergic neurotransmission in striatal subregions may further create a bias toward reward-driven behavior over cognitive control, which also has been reported in amphetamine-sensitized individuals (Kohn et al., 2014; O’Daly et al., 2014).

Apart from addiction, dysregulation of dopamine transmission has been implicated in a number of other psychiatric illnesses, including schizophrenia. This is especially interesting considering the high prevalence of amphetamine use among patients diagnosed with schizophrenia, even though amphetamine, being a psychosis-generating drug, may further aggravate positive symptoms (Chiang et al., 2019; Voce et al., 2018,2019). However, amphetamine could act to relieve negative symptoms, which is supported by clinical studies where patients with both schizophrenia and substance use disorder report fewer negative symptoms than those with schizophrenia only (Potvin et al., 2006). These findings indirectly support a role for dopaminergic neurotransmission in ventral striatum in negative symptomatology, which is also supported by imaging studies, showing that reduced activation of ventral striatum correlates with the severity of negative symptoms in medication-free schizophrenics (Juckel et al., 2006; Wolf et al., 2014).

At the same time, dorsal striatum may be more associated with positive symptoms (McCutcheon et al., 2019), which is strengthened by the fact that psychotic patients with schizophrenia show higher baseline levels of dopamine as well as a higher sensitivity to amphetamine-induced dopamine release in the dorsal striatum, especially in the associative part (Abi-Dargham et al., 1998, 2000; Laruelle et al., 1996, 1999).

An interesting difference between the rewarding and psychosis generating properties of amphetamine following repeated intake is that while increasingly higher doses are needed for the rewarding effects, indicating tolerance development, no tolerance or even sensitization appears to develop to the psychosis inducing properties. Thus, differential alterations of dopamine transmission in the ventral striatum (a “reward” region) and the associative striatum (a “psychosis” region) might be expected following repeated drug exposure. Therefore, to outline the effects displayed by acute and repeated amphetamine on striatal neurotransmission, in vivo microdialysis and ex vivo electrophysiology were performed in amphetamine naïve Wistar rats, and in rats subjected to five days of amphetamine-treatment followed by two weeks of abstinence. Recordings were conducted in the nucleus accumbens (nAc), which roughly corresponds to the ventral striatum, and the dorsomedial striatum (DMS), roughly corresponding to the human associative striatum.

2. Material and methods

2.1. Experimental outline

The overall aim of this study was to assess brain subregion-specific effects on dopaminergic neurotransmission elicited by acute and repeated exposure to amphetamine. In the first set of experiments, amphetamine was administered to drug-naïve animals (n = 57), and brain-region-specific responses were monitored. In the second set of experiments, animals (n = 86) received amphetamine or vehicle for five days and dopamine signaling and synaptic neurotransmission were assessed at baseline and during an amphetamine challenge after two weeks of abstinence.

2.2. Animals

Male Wistar rats (Envigo, Horst, Netherlands) were used in all experiments. Naïve animals used for in vivo microdialysis weighed 280–300 g at the time of the experiment, and animals subjected to amphetamine treatment weighed 260–280 g at the start of treatment, and 350–400 g at the time of microdialysis or electrophysiological experiments. Following one week of acclimatization to the animal facility, the animals were divided into groups of three and housed in standard rat cages (55 × 35 × 20 cm) at constant room temperature (22 °C) and humidity (65%) with regular light conditions (lights on 7 a.m. to 7 p.m.), with ad libitum access to food and water. The study was approved by the Ethics Committee for Animal Experiments, Gothenburg, Sweden.

2.3. Amphetamine treatment

Animals (n = 86) were divided into two groups and received either amphetamine (dextroamphetamine sulfate, Sigma-Aldrich, Stockholm, Sweden) (2.0 mg/kg, i. p., 2 ml/kg, n = 41) or vehicle (0.9% NaCl, i. p., 2 ml/kg, n = 41). Animals were treated for a total of five days (Thursday, Friday, Monday, Tuesday and Wednesday), and were allowed to recover for 2 weeks before in vivo microdialysis sampling or electrophysiological field recordings were conducted. To improve the robustness of presented findings, experiments were conducted in four different batches of animals.

2.4. Locomotion

In order to assess behavioral sensitization following amphetamine treatment, the first batch of animals was tested in a locomotor activity paradigm on the first and last day of treatment. Vertical and horizontal ambulatory movement were monitored in an arena (40 × 40 cm, Med Assoc., Fairfax, VT, USA) placed in a sound attenuated, ventilated box illuminated by a dim light. Movement was assessed by a two-layer grid of photocell beams, where consecutive beam breaks in the lower layer constituted horizontal ambulatory movement, and beam breaks in the upper layer registered as vertical movement (rearing). Data was tracked and compiled in 5-min blocks (Activity Monitor 7, Med Assoc., St. Albans, VT, USA). Animals were allowed to habituate to the box for 30 min, before they were picked up and administered amphetamine or vehicle (i.p.), after which they were placed back in the box for an additional 30 min.

2.5. In vivo microdialysis

Brain microdialysis experiments were performed in the nAc or DMS in two separate set of studies. First, dose-response relationship was evaluated following local administration of amphetamine (0, 0.1, 1, 10 or 100 μM) in the nAc and DMS of drug-naïve animals. In the second set of experiments, the acute response to local administration of amphetamine (10 μM) was monitored in animals following a two-week period of abstinence after five days of amphetamine treatment (see Fig. 3A). For implantation of dialysis probes, the animals were anesthetized with isoflurane (Baxter, Kista, Sweden) and mounted into a stereotaxic instrument (David Kopf Instruments, AngTho’s AB, Lidingö, Sweden). A custom-made dialysis probe was placed monolaterally in either the nAc: A/P: +1.85, M/L: −1.4 relative to bregma, and D/V: −7.8 mm relative to dura, or the DMS: A/P: +1.2, M/L: −2.0 relative to bregma, and D/V: −5.5 relative to dura. The probe was secured in place using two anchoring screws fastened into the skull and Harvard cement (DAB Dental AB, Gothenburg, Sweden). All rats received 2 ml 0.9% saline solution immediately after the surgery to prevent dehydration, and were single housed for approximately 48 h to recover until start of the microdialysis experiment.

On the day of the experiment, the dialysis probe was connected to a microperfusion pump (U-864 Syringe Pump, AngTho’s, Sweden) via a swivel, which allowed the animal to move freely in the cage. The probe was perfused with Ringer solution (consisting of mmol/l: 140 NaCl, 1.2 CaCl2, 3.0 KCl, and 1.0 MgCl2) at a rate of 2 μl/min. Dialysate samples were collected every 20 min and were immediately analyzed for dopamine content as previously described (Clarke et al., 2014). Once three stable baseline samples were obtained (less than ±10%
fluctuation), amphetamine (0.1, 1, 10 or 100 μM) diluted in Ringer’s solution was perfused locally via the microdialysis probe for 2 h after which an additional hour of Ringer’s solution concluded the experiment. The local administration was performed in order to more directly monitor the role of local circuits in amphetamine-induced dopamine elevation. Local administration of amphetamine in the nAc did not influence dopamine levels in the DMS (Dopamine levels 98.9 ± 3.0% in the DMS after 60 min perfusion of 10 μM amphetamine in the nAc, n = 4, data not shown).

The animals were euthanized immediately after the experiment, the brains removed and submerged in formalin-free fixative (Accustain, Sigma-Aldrich, Stockholm, Sweden) for future confirmation of probe placement. Animals with incorrect probe placement and/or substantial bleeding or excessive damage to the tissue were excluded from the statistical analysis. Correct probe placement is illustrated in Fig. 1A and C.

2.6. Electrophysiology

2.6.1. Brain slice preparation

Rats (n = 34) were anesthetized with isoflurane (Forene, Baxter, Sweden), decapitated, and brains rapidly removed and submerged in continuously oxygenated (95% O2, 5% CO2) modified artificial cerebrospinal fluid (aCSF) containing (in mM): 220 sucrose, 2 KCl, 1.3 NaH2PO4, 6 MgCl2, 0.2 CaCl2, 26 NaHCO3, 10 D-glucose. Coronal brain slices (250 μm) were sectioned using a Leica VT 1200 S Vibratome (Leica Microsystems AB, Bromma, Sweden), and were allowed to equilibrate at 31 °C for 30 min in oxygenated normal aCSF before maintained in room temperature for the remainder of the day. Slices were allowed to recover for at least 1 h before initiation of recordings, and no recordings were started later than 7 h after slicing.

2.6.2. Electrophysiological field potential recordings

Electrophysiological field potential recordings were performed as previously described (Adermark et al., 2011; Licheri et al., 2019). In brief, one hemisphere of the brain slice was constantly perfused with pre-warmed aCSF (30 °C, 2 ml/min) containing (in mM); 124 NaCl, 4.5

Fig. 1. Amphetamine increases the microdialysate concentration of dopamine in a concentration- and brain region-dependent manner. A) Schematic illustration of probe-placement in the nAc. B) Time course graph showing changes in extracellular dopamine levels as assessed by in vivo microdialysis. Locally administered amphetamine increased extracellular dopamine levels in the nAc in a concentration-dependent manner. C) Schematic illustration of probe-placement in the DMS. D) Locally administered amphetamine increased extracellular dopamine levels in the DMS in a concentration-dependent manner compared to vehicle treated controls. E) The relative increase in dopamine induced by local administration of amphetamine (10 and 100 μM) was significantly higher in the nAc, while the absolute dopamine elevation was not significantly different. G) Extracellular levels of dopamine did not differ between the nAc and the DMS (measured at t = -20-0 min). All values are presented as means ± SEM. n = number of rats, ns = non-significant. **p < 0.01, ***p < 0.001.
KCl, 2 CaCl₂, 1 MgCl₂, 26 NaHCO₃, 1.2 NaH₂PO₄ and 10 g-glucose. Field population spikes (PSs) were evoked with a stimulating electrode (TM33B, World Precision Instruments, FL, USA, 20 s stimulation interval), and registered with a recording electrode (outer diameter 1.5 μm, resistance ranging from 2.5 to 5.5 MΩ, World Precision Instruments, FL, USA) in striatal subregions, and PS amplitudes were measured. Stimulating electrodes were positioned locally in nAc shell or DMS (Fig. 2A), around 0.3 mm from recording electrode. To define the role of AMPA receptors in mediating evoked PS amplitudes, the AMPA receptor antagonist CNQX (10 μM) was bath-perfused. To assess changes in neurotransmission produced by amphetamine-administration PSs were evoked with increasing stimulation strength (18–72 μA) creating a stimulation/response curve, while changes in release probability were estimated by comparing the paired pulse ratio (PPR, PS two/PS one, 50 ms interpulse interval). In a subset of experiments, excitatory neurotransmission was isolated by bath perfusion of the GABAₐ receptor antagonist bicuculline (bicuculline methiodide, 20 μM). To monitor changes in dopamine-receptor signaling, a stable baseline was recorded for 10 min before amphetamine (10 μM), or the dopamine D₂ receptor agonist quinpirole (quinpirole hydrochloride, 5 μM), were administered via bath perfusion. In a subset of experiments, slices were pre-treated with the dopamine D₂ receptor antagonist sulpiride (10 μM) prior to amphetamine. In these experiments, sulpiride-treated slices were compared to aCSF-perfused slices, recorded in parallel. Bicuculline was dissolved in H₂O to 20 mM, and amphetamine and quinpirole to 80 mM, and further diluted in aCSF shortly before use. Drugs were purchased from Tocris (Abingdon, UK). In all experiments, an amphetamine- and vehicle-treated rat were run in parallel, using the same sets of buffers. Data are presented as one value per animal, and the value presented is a mean of three to four recordings from the same animal.

2.7. Statistics

Electrophysiological recordings were analyzed using Clampfit 10.2 (Molecular devices, Axon CNS, CA, USA), Microsoft Excel and GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). For analysis of dopamine content in dialysis samples, behavioral and electrophysiological data, repeated measure 2-way ANOVA was used for comparisons over time, and input/output function, while t-tests were used when applicable. All parameters are given as mean ± SEM, and the level of significance was set to p < 0.05.

3. Results

3.1. Amphetamine increases extracellular dopamine in a concentration- and partially brain region-dependent manner

In the first set of experiments, changes in extracellular dopamine levels were studied using in vivo microdialysis. To monitor concentration-dependent and region-specific responses to amphetamine, the drug was locally administered using reversed microdialysis. Local perfusion of 10 and 100 μM, but not 1 μM, of amphetamine significantly increased extracellular dopamine over time in a concentration-dependent manner, both in the nAc (vehicle vs. 1, 10 or 100 μM amphetamine: treatment effect: F(3, 26) = 94.2, p < 0.001; Dunnett’s post hoc: vehicle vs 1 μM, q(26) = 1.45, p = 0.35; vehicle vs 10 μM, q(26) = 5.77, p < 0.001; vehicle vs 100 μM, q(26) = 15.1, p < 0.001) (Fig. 1B), and in the DMS (Vehicle vs. 1, 10 or 100 μM amphetamine: Treatment effect: F(3, 26) = 92.0, p < 0.001; Dunnett’s post hoc: vehicle vs 1 μM, q(23) = 2.43, p = 0.06; vehicle vs 10 μM, q(23) = 6.69, p < 0.001; vehicle vs 100 μM, q(23) = 15.6, p < 0.001) (Fig. 1D). The relative increase in dopamine elicited by 10 and 100 μM amphetamine was...
significantly higher in the nAc as compared to the DMS (10 μM: nAc vs DMS, t(13) = 4.31, p < 0.001; 100 μM: nAc vs DMS, t(14) = 4.79, p < 0.001) (Fig. 1E), while absolute levels were not significantly different (10 μM: nAc vs DMS, t(13) = 1.94, p = 0.074; 100 μM: nAc vs DMS, t(14) = 0.712, p = 0.49) (Fig. 1F). Baseline extracellular levels of dopamine were not significantly different when comparing nAc with the DMS (t(52) = 0.30, p = 0.76) (Fig. 1G).

3.2. Baseline neurotransmission and dopamine-signaling is subregion-specific

To further assess subregion-specific effects on dopaminergic signaling and striatal neurotransmission, field potential recordings were conducted in striatal subregions. The amplitude of evoked potentials were significantly larger in the DMS as compared to the nAc (F(1, 22)= 36, p < 0.001) (Fig. 2B), which coincided with a reduced probability of transmitter release, as assessed by PPR (t(28)= 2.10, p = 0.045) (Fig. 2D). Evoked potentials in both subregions were rapidly suppressed by perfusion of the AMPA receptor antagonist CNQX (10 μM) (nAc: t(10)= 59, p < 0.001; DMS: t(11)= 43, p < 0.001), suggesting that evoked PS amplitudes primarily corresponds to glutamatergic activation of post-synaptic receptors (Fig. 2E). The responsiveness to dopamine-regulating substances also depended on the subregion analyzed. Administration of amphetamine (10 μM, 10 min) depressed PS amplitude to a greater extent in the DMS (F(1, 21) = 6.84, p = 0.016), as did the dopamine D2 receptor agonist quinpirole (5 μM, 20 min) (F(1, 15) = 5.57, p = 0.032) (Fig. 2). PS amplitude was not fully restored upon drug wash-off, resulting in a slightly sustained depression of evoked potentials in both subregions (nAc: 92 ± 2.0%, t(14) = 4.12, p = 0.001; DMS: 86 ± 4.4%, t(10) = 3.13, p = 0.011) (Fig. 2F). Amphetamine induced depression was not inhibited by the dopamine D2 receptor antagonist sulpiride (10 μM) (t(1, 18) = 1.69, p = 0.21) (Fig. 2G). Sulpiride did not modulate PS amplitude by itself (F(1, 10) = 1.13, p = 0.31) (data not shown).

3.3. Repeated administration of amphetamine produces behavioral sensitization

In the next set of experiments, neurophysiological and behavioral transformations elicited by repeated administration of amphetamine were assessed. Five days of systemic administration of amphetamine produced behavioral sensitization with regards to ambulatory activity, with significantly higher ambulatory counts in the last session as compared to the first (amphetamine 1st vs 5th, t(30) = 3.05, p = 0.005),
without a significant change in horizontal activity in the vehicle treated group (vehicle 1st vs. 5th, t_{26} = 1.99, p = 0.057) (Fig. 3B). The amphetamine treatment also sensitized rearing activity, as shown by an increase in vertical counts (amphetamine 1st vs. 5th, t_{29} = 2.10, p = 0.045), with no change in the vehicle treated group (t_{20} = 0.08, p = 0.94) (Fig. 3C).

3.4. Brain region selective changes in dopamine levels after repeated amphetamine exposure

To assess sustained transformations in dopaminergic neurotransmission, amphetamine-induced dopamine release was monitored in the nAc and DMS of animals two weeks after receiving repeated amphetamine-injections for five days. Repeated administration of amphetamine selectively suppressed extracellular dopamine levels in the nAc (amphetamine vs vehicle, t_{17} = 2.26, p = 0.037) (Fig. 3D), with no effect in the DMS (amphetamine vs vehicle, t_{10} = 0.19, p = 0.86) (Fig. 3G). In addition, even though the absolute increase in extracellular dopamine levels elicited by 10 μM amphetamine was similar to vehicle-treated control (F(1, 17) = 0.05, p = 0.82) (Fig. 3E), the relative change was significantly larger in amphetamine-treated animals (F(1, 17) = 6.38, p = 0.022) (Fig. 3F). Repeated administration of amphetamine did not significantly alter the dopamine-elevating property of amphetamine when locally administered into the DMS, neither with regard to the absolute (F(1, 10) = 0.085, p = 0.78) (Fig. 3H), nor the relative elevation of dopamine levels (F(1, 10) = 0.03, p = 0.87) (Fig. 3I).

3.5. Repeated administration of amphetamine produces selective effects on striatal neurotransmission

In a way to measure downstream effects produced by acute and repeated amphetamine exposure, electrophysiological recordings were performed ex vivo. Electrophysiological field potential recordings were conducted two weeks after the last amphetamine exposure. In the nAc shell, repeated amphetamine exposure did not produce significant effects on stimulus/response curves or PPR (stimulus/response: F(1, 26) = 1.40, p = 0.24; PPR: t_{26} = 0.94, p = 0.35) (Fig. 4A). However, isolation of excitatory currents by inhibition of GABAergic neurotransmission revealed a robust suppression of evoked potentials (F(1, 10) = 10.2, p = 0.010), although PPR remained unaffected (t_{10} = 0.06, p = 0.95) (Fig. 4). Acute exposure to amphetamine (10 μM) suppressed PS amplitude, but the effect was not modulated in brain slices from animals receiving previous amphetamine injections (F(1, 31)<0.001, p = 0.98) (Fig. 4E). The responsiveness to the dopamine D2 receptor agonist quinpirole (5 μM), however, was qualitatively altered from suppressive to slightly stimulative in brain slices from animals previously receiving amphetamine (amphetamine: F(1, 16) = 8.72, p = 0.0094) (Fig. 4F).

In the DMS, repeated administration of amphetamine did not affect evoked potentials in either aCSF-, nor bicuculline-perfused slices (aCSF: stimulus/response: F(1, 26) = 1.65, p = 0.21; PPR: t_{26} = 1.36, p = 0.19; bicuculline: stimulus/response: F(1, 17) = 0.81, p = 0.38; PPR: t_{17} = 0.047, p = 0.96) (Fig. 5A–D). The responsiveness to amphetamine or quinpirole was not significantly modulated in brain slices from animals previously receiving amphetamine (amphetamine: F(1, 16) = 0.015, p = 0.90; quinpirole: F(1, 20) = 0.39, p = 0.54) (Fig. 5).

4. Discussion

The data presented here shows that amphetamine modulates striatal neurotransmission and dopamine release in a region-specific manner. While the relative dopamine release induced by local amphetamine administration in vivo was more pronounced in the nAc, acute effects on evoked PS amplitudes by amphetamine or the dopamine D2 receptor agonist quinpirole were more pronounced in the DMS. We also show...
that five days of amphetamine treatment not only was sufficient to produce behavioral sensitization, but also enough to suppress baseline dopamine levels and to produce a relative potentiation of the dopamine-elevating properties of amphetamine, selectively in the nAc. Importantly, isolation of excitatory transmission revealed a significant effect by amphetamine on synaptic output, suggesting that amphetamine may produce long-lasting transformations not only in dopamine signaling, but also in excitatory neurotransmission in the nAc.

Amphetamine promotes dopamine elevations through multiple mechanisms of action, by inhibiting uptake and degradation, by enhancing synthesis, by depleting vesicular stores, by promoting mechanisms of action, by inhibiting uptake and degradation, by inhibiting dopamine-transporter reversal and by augmenting vesicular release (Avelar et al., 2013). In this study we chose to administer amphetamine on synaptic output, suggesting that amphetamine may produce long-lasting transformations not only in dopamine signaling, but also in excitatory neurotransmission in the nAc.

Fig. 5. No significant effect on dorsal striatal neurotransmission or dopamine D2 receptor signaling in the DMS following repeated amphetamine administration. A-B) Stimulus/response curve and PPR was not significantly modulated in brain slices from rats previously exposed to amphetamine. C-D) There was no effect by treatment on stimulus/response curve or PPR after isolation of excitatory neurotransmission using GABA receptor antagonist. E-F) Synaptic depression induced by bath perfusion of amphetamine or quinpirole was not significantly modulated by repeated amphetamine exposure. G) Example traces showing evoked potentials in the DMS in slices from a vehicle- or amphetamine-treated rat. Calibration: 2 m s, 0.2 mV. All values are presented as mean ± SEM. n = number of rats, ns = non-significant.

Although absolute levels showed no significant elevation of dopamine, it should however be noted that neither reward related behavior nor behaviors associated with psychosis were assessed in this study. Future studies correlating neuroadaptations with behavioral effects are thus needed to further address causal relationships.

Dopamine release elicited by cocaine administration has also been shown to result in a temporally and regionally specific increase in phasic dopamine release, and these effects appear to be regulated by dopaminergic autoreceptors (Aragona et al., 2008). There could thus be an inverse relationship between the extracellular dopamine levels and dopamine release following an amphetamine challenge, which has previously been suggested for cocaine (Weiss et al., 1992). However, here extracellular dopamine levels were not significantly different when comparing the two brain regions, suggesting that other mechanisms might contribute. Importantly, the dopamine D2 receptor agonist quinpirole produced a more pronounced depression of synaptic output when assessed in the DMS. Dopamine D2 receptors are expressed on both striatal neurons and dopaminergic terminals, and both populations are involved in feedback inhibition. The increased responsiveness in the DMS could thus suggest that feedback inhibition though the D2 receptor is more pronounced in the dorsal striatum as compared to nAc. These ideas are also supported by a previous study showing that the D2 heteroreceptor-mediated control of dopamine levels is more efficient in the dorsal striatum than in the nAc (Anzalone et al., 2012). Brain subregion-specific control of dopamine release by dopamine D2 receptors may thus partially explain the concentration-dependent disparity in dopamine release detected following local amphetamine-exposure.

Microdialysis, performed two weeks after the five day-long amphetamine treatment period, revealed a selective suppression of baseline dopamine levels in the nAc, and a potentiation of amphetamine-induced dopamine when assessing the relative effect. Although absolute levels showed no significant elevation of dopamine, the potentiation of the relative increase in combination with an altered
Exposure to psychostimulants has repeatedly been shown to result in persistent changes in dopamine receptor availability within the striatum (Ashok et al., 2017). Even though this may not always be the case when assessing dopamine D2 receptor expression after shorter exposure periods in rat (Ilbonhomme et al., 1995; Sun et al., 2015), we found that repeated amphetamine exposure significantly changed the responsiveness to the dopamine D2 receptor agonist quinpirole in the nAc. Not only was the depressant effect of the drug completely blocked but the response appeared converted to a slight stimulatory effect. Paradoxical excitation via D2 receptor activation has previously been reported during hypercholinergic states, and may result from a change in coupling from the preferred G-i/o-arrestin pathway to non-canonical β-arrestin signaling (Eskow Jaunarajs et al., 2015, 2019; Scarduzio et al., 2017). Interestingly, a similar shift in the responsiveness to quinpirole was recently shown in brain slices from nicotine-exposed rats (Morud et al., 2016), and human studies suggest that both stimulant users and smokers show lower striatal D2/D3 receptor availability (Proebstl et al., 2019; Volkow et al., 2001; Wiers et al., 2017). Both amphetamine and nicotine produce chronic behavioral sensitization (Kolta et al., 1985; Morud et al., 2016), and generate significantly higher dopamine elevations in the nAc as compared to the DMS (Adermark et al., 2016). These psychostimulants thus activate dopamine signaling and induce transformations in the dopaminergic system in a comparable manner.

The data presented here show that evoked PSs primarily are mediated through activation of AMPA receptors located on MSNs. Since striatal MSNs express either dopamine D1 or dopamine D2 receptors, this may putatively affect the response to amphetamine. However, amphetamine-induced depression was insensitive to dopamine D2 receptor antagonist. This is in line with previous studies, suggesting that dopamine acts directly on presynaptic D1-like receptors to depress excitatory transmission (Harvey and Lacey, 1996; Nicola et al., 1996), or activate astrocytes which in turn depress neurotransmission via adenosine signaling (Corkrum et al., 2020). Following amphetamine administration, evoked PS amplitudes were not fully restored during drug wash-off. This finding is partially in line with previous studies showing that longer wash-off periods are required to normalize neurotransmission after amphetamine perfusion ex vivo (Nicola et al., 1996). That would also be in line with our microdialysis recordings, showing that 60 min of drug wash-off was required in order for dopamine levels to return to baseline. It is thus not fully clear that amphetamine produces a sustained depression of evoked PS amplitudes.

The data presented here suggest that neurophysiological transformations are more pronounced in the nAc as compared to the DMS. This finding could in part be connected to the more pronounced elevation of dopamine in the nAc following systemic administration of amphetamine (Di Chiara and Imperato, 1988; Drevets et al., 1999; Sharp et al., 1987). Dopamine is a key neurotransmitter involved in learning and memory (Stuber et al., 2010; Wolf, 2010), and may promote long-lasting transformations in several neurotransmitter systems (Clarke and Adermark, 2015). Furthermore, while D2 receptor-mediated synaptic currents in medium spiny neurons are slower in the nAc as compared to dorsal striatum, higher concentrations of dopamine are required to activate D2-receptors in dorsal striatum (Marcott et al., 2018). These findings collectively suggest that the amphetamine-induced elevation of dopamine leads to more pronounced neuroadaptations in the nAc (Chen et al., 2010; Collo et al., 2014).

Indeed, blocked or even inverted dopamine D2 receptor signaling in the nAc in combination with lower baseline dopamine levels, as observed here, may be a major driving force for drug intake in an attempt to achieve the sought-after but now elusive drug-induced state (Ballard et al., 2015; Mizoguchi et al., 2004). In psychosis prone amphetamine abusers or in schizophrenic patients using amphetamine (Ringen et al., 2008) this could be detrimental, since dopamine baseline levels and dopamine D2 receptor responsiveness were largely intact in the DMS. Hence increased dosing in order to obtain the desired effect may result in psychosis generated via excessive activation of intact dopamine D2 receptors in the DMS.

In our electrophysiological recordings, inhibition of GABA receptors was required in order to reveal drug-induced transformations in baseline neurotransmission. This finding suggests that both excitatory and inhibitory neurotransmission are suppressed following amphetamine treatment, which is partially in line with previous studies, showing decreased inhibitory currents in response to acute amphetamine (Gentonze et al., 2002), and a long-lasting suppression of dorsal striatal glutamatergic excitability following repeated administration of amphetamine (Bamford et al., 2008). At the same time, repeated amphetamine is also associated with increased mRNA levels of GABA receptors and GABA transporters in cortical areas (Wearne et al., 2016), which in turn may contribute to reduced excitatory neurotransmission in downstream targets such as DMS and nAc (Hart et al., 2018; Voorn et al., 2004).

Overall, the data presented here show that amphetamine modulates striatal neurotransmission and dopaminergic signaling in a brain region-specific manner. We also show that repeated exposure leads to sustained transformations not only in the dopaminergic system but also with respect to excitation and inhibition in neuronal circuits of the striatum. These subregion selective effects seen in the nAc and DMS after repeated amphetamine exposure may partially explain why increasingly higher doses are needed for the rewarding effects, while no tolerance develops to the psychosis inducing properties of amphetamine.

Author contributions

LA and BS designed the study. KD performed in vivo microdialysis and analyzed the corresponding data with assistance from ME. OL and LA performed electrophysiological recordings and analyzed the corresponding data. LA assembled the data and wrote the main draft of the manuscript. All authors contributed to the final draft of the manuscript and revised it critically.

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