Sustained inhibitory transmission but dysfunctional dopamine D2 receptor signaling in dorsal striatal subregions following protracted abstinence from amphetamine

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ABSTRACT
Behavioral sensitization to amphetamine is a complex phenomenon that engages several neurotransmitter systems and brain regions. While dysregulated signaling in the mesolimbic dopamine system repeatedly has been linked to behavioral sensitization, later research has implicated dorsal striatal circuits and GABAergic neurotransmission in contributing to behavioral transformation elicited by amphetamine. The aim of this study was thus to determine if repeated amphetamine exposure followed by abstinence would alter inhibitory neurotransmission in dorsal striatal subregions. To this end, male Wistar rats received amphetamine (2.0 mg/kg) in an intermittent manner for a total of five days. Behavioral sensitization to amphetamine was measured in locomotor-activity boxes, while neuroadaptations were recorded in the dorsolateral (DLS) and dorsomedial striatum (DMS) using ex vivo electrophysiology at different timepoints of amphetamine abstinence (2 weeks, 4–5 weeks, 10–11 weeks). Data show that repeated drug-exposure produces behavioral sensitization to the locomotor-stimulatory properties of amphetamine, which sustains for at least ten weeks. Electrophysiological recordings demonstrated a long-lasting suppression of evoked population spikes in both striatal subregions. Furthermore, following ten weeks of abstinence, the responsiveness to a dopamine D2 receptor agonist was significantly impaired in brain slices from rats previously receiving amphetamine. However, neither the frequency nor the amplitude of spontaneous inhibitory currents was affected by treatment at any of the time points analyzed. In conclusion, passive administration of amphetamine initiates long-lasting neuroadaptations in brain regions associated with goal-directed behavior and habitual performance, but these transformations do not appear to be driven by changes in GABAergic neurotransmission.

1. Introduction
Repeated administration of psychostimulants, such as amphetamine, progressively transforms neuronal circuits and increases the sensitivity to the behavioral effects of the drug (Robinson and Becker, 1986; Segal and Mandell, 1974). Behavioral sensitization has been proposed to reflect neurochemical changes that are characteristic for drug addiction and is an established model for investigating drug-induced effects on the function of the nervous system (Robinson and Berridge, 1993; Steketee and Kalivas, 2011). Many of the neuroadaptations associated with behavioral sensitization have been linked to the basal ganglia and their connected nuclei, including the striatum (Adinoff, 2004; Gatica et al., 2020; Parikh et al., 2014; Vanderschuren and Kalivas, 2000). In rodents, the dorsal striatum can be subdivided into the dorsolateral (DLS) and dorsomedial (DMS) striatum, which dynamically encode the shift between goal-directed and habitual actions (Gremel and Costa, 2013). Acute and repeated exposure to amphetamine modulate neuronal firing in the DLS (Gatica et al., 2020; Ma et al., 2013), and dopamine D2 receptor expressing medium spiny neurons (MSNs) in the DMS are recruited during behavioral sensitization towards the locomotor-
stimulatory properties of amphetamine (Durieux et al., 2012). Neuronal ensembles in the DMS have also been implicated in amphetamine craving and drug-seeking behavior after extended abstinence (Caprioli et al., 2017; Li et al., 2018).

Behavioral sensitization to amphetamine is a complex phenomenon that engages several neurotransmitter systems, where the role of dopamine and glutamate neurotransmission has been especially acknowledged (Bamford et al., 2008; Huang et al., 2020; Jing et al., 2018; Kim and Vezina, 2002; Robinson and Becker, 1982; Vezina, 1996; Wang et al., 2013; Yoon et al., 2008). Recent research, however, implicate a role for the GABAergic system in sensitization to amphetamine and methamphetamine (Wearne and Cornish, 2019), and in the development of amphetamine use disorder (Jiao et al., 2015). Acute administration of amphetamine increases GABA levels in dorsal striatum (Bustamante et al., 2002; Del Arco et al., 1998), and sensitization to amphetamine is associated with an increase in mRNA levels of GABA receptors and transporters (Wearne et al., 2016). The GABA<sub>B</sub> receptor agonist baclofen prevents both the development and expression of amphetamine-induced locomotor sensitization (Cedillo and Miranda, 2013), while GABA<sub>A</sub> receptors appear to play a role in the acquisition of behavioral sensitization to methamphetamine (Ito et al., 2000). However, progressive and sustained effects on GABAergic signaling in dorsal striatum following behavioral sensitization to amphetamine have not been fully investigated.

We hypothesize that behavioral sensitization to amphetamine coincides with long-lasting changes in basal neurotransmission in dorsal striatal subregions. We also postulate that changes in local GABAergic neurotransmission play a key role in altering striatal output. To test this hypothesis, male Wistar rats received amphetamine for five days and behavioral sensitization and striatal neurotransmission were monitored for up to 11 weeks of abstinence from amphetamine.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (Janvier Labs, Le Genest-Saint-Ise, France, 260–290 g, approximately 7 weeks old) were housed in groups of three at constant room temperature (21 °C) and humidity (60 %) under regular light-dark conditions (lights on at 6:00 am and off at 6:00 pm) with ad libitum access to food and tap water. The animals were allowed to habituate to their housing environment at least one week before the start of the experiment. All injections and locomotor activity measurements were performed during the light cycle. Experimental protocols were approved by the Ethics Committee of Animal Experiments, Gothenburg, Sweden.

2.2. Amphetamine administration

Rats were randomly assigned to receive either vehicle (saline solution, 0.9 % NaCl) (n = 43) or amphetamine (n = 62). Dextroamphetamine sulphate (amphetamine) was dissolved in saline solution and administered intraperitoneally (i.p.) at 2.0 mg/kg or 0.5 mg/kg (at the volume of 1.0 ml/kg). The injection regimen consisted of two phases. During the induction phase animals received five discontinuous injections of amphetamine (2.0 mg/kg) over a period of one week (Thu, Fri, Mon, Tue, Wed). This treatment paradigm has previously been shown to be sufficient to produce long-lasting behavioral sensitization (Mendez et al., 2009). After the induction phase, the expression of behavioral sensitization was tested in different batches of animals for up to ten weeks of amphetamine abstinence. To avoid a ceiling effect, expression of behavioral sensitization was assessed using a moderate amphetamine dose of 0.5 mg/kg.

In a way to assess behavioral sensitization in relation to the onset of neurophysiological changes a subset of animals only received two injections of amphetamine (0.5 mg/kg), administered two weeks apart.

2.3. Locomotor activity measurements

Locomotor activity (ambulatory counts and rearing) was measured in subsets of rats during treatment and amphetamine abstinence. Locomotor activity was assessed on the first and last day of the induction phase (2.0 mg/kg amphetamine or vehicle) in the majority of animals, and subsequently at weekly intervals for the first five weeks, or after ten weeks of abstinence from amphetamine (expression phase, 0.5 mg/kg amphetamine) in a subset of rats. To minimize the number of animals used, some animals (n = 20) were exposed to an amphetamine challenge on two separate occasions. Behavioral responding in animals receiving one additional challenge dose did not differ significantly from animals receiving their first challenge dose. Locomotor activity was also measured in a subset of rats that only received two injections of amphetamine (0.5 mg/kg), administered two weeks apart.

Locomotor behavior was monitored in dimly lit testing box (40 × 40 cm, Med Associates Inc. Vermont, USA). Following 30 min of habituation the subjects received an injection of either vehicle or amphetamine, and the activity was recorded for additionally 30 min after injection. The activity was reported in 5-minute bins for ambulatory counts (consecutive horizontal beam breaks) or rearing activity (vertical beam breaks). Putative effects on locomotion produced by conditioning to the test box were also assessed by monitoring locomotion in response to a vehicle-injection, in animals previously receiving amphetamine. Animals receiving an additional challenge dose of amphetamine were not included in electrophysiological analysis. The experimenter handling the animals during locomotor behavior studies coded the animals for future electrophysiological recordings but was not blinded to treatment himself.

2.4. Slice preparation for electrophysiology

Rats (n = 54) were anesthetized with isoflurane (Forene, Kista, Sweden) and decapitated. Brains were rapidly extracted and submerged in ice-cold modified artificial cerebrospinal fluid (aCSF) consisting of (in mM): 220 sucrose, 2 KCl, 0.2 CaCl<sub>2</sub>, 6 MgCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub> and 10 d-glucose, continuously bubbling with a gas mixture of 95 % O<sub>2</sub>/5 % CO<sub>2</sub>. Coronal brain slices (250 μm) containing the striatum and encompassing cortex were prepared using a Leica VT 1200S vibratome (Leica Microsystems AB, Bromma, Sweden). Brain slices were transferred to 30 °C normal aCSF containing (in mM): 124 NaCl, 4.5 KCl, 2 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, 1.2 NaH<sub>2</sub>PO<sub>4</sub> and 10 d-glucose, continuously bubbled with a mixture of 95 % O<sub>2</sub>/5% CO<sub>2</sub> gas for 30 min and were then allowed to equilibrate for at least 1 h at room temperature. All electrophysiological recordings and data analysis were performed in blind.

2.5. Electrophysiological field potential recordings

Field potential recordings were performed as previously described (Adermark et al., 2011). Briefly, one hemisphere of a brain slice containing the striatum and the overlying cortex was placed in the recording chamber under a continuous flow (2.0 ml/min) of pre-heated aCSF (30 °C). A stimulating electrode (monopolar tungsten electrode, World Precision Instruments, FL, USA, type TM33B) was placed at the border of the subcortical white matter and the DLS or intrastriatal in DMS. Field populations spikes (PS) were evoked using a paired-pulse stimulation protocol with a 50 ms interpulse interval evoked at a frequency of 0.05 Hz. Evoked field populations spikes primarily reflect current flow associated with excitatory neurotransmission and is rapidly blocked by the AMPA receptor antagonist CNQX (Danielson et al., 2021a; Lagstrom et al., 2019). Sustained changes in striatal neurotransmission elicited by repeated amphetamine exposure were estimated by stepwise increasing afferent stimulation strength (input/output function). In a subset of experiments, the response to the dopamine D2 receptor agonist quinpirole (5 μM) or 5-hydroxytryptamine (5-
3. Results

3.1. Repeated administration of amphetamine induces long-standing behavioral sensitization

Animals received amphetamine (2.0 mg/kg) or saline in a discontinuous manner for a total of five days and were then monitored over ten weeks in drug abstinence (Fig. 1A). Five days of amphetamine administration (2.0 mg/kg) enhanced the locomotor-stimulatory effect of the

Fig. 1. Long-lasting changes in locomotion and rearing activity following repeated exposure to amphetamine. A) Rats received five injections of amphetamine (2.0 mg/kg) and were monitored over ten weeks of abstinence from the drug. The locomotor-stimulatory properties of a challenge dose of amphetamine (0.5 mg/kg) were tested once weekly over the first five weeks, and then once more after ten weeks of abstinence. B) Time course graph showing ambulatory counts in response to challenge dose of amphetamine (0.5 mg/kg). C-D) Repeated administration of amphetamine (2.0 mg/kg) enhanced drug-induced locomotion and rearing when comparing the first administration with the fifth. E-F) The behavioral response to a challenge dose of amphetamine (0.5 mg/kg) was augmented in animals previously receiving amphetamine (2.0 mg/kg) in a long-standing manner. A conditioned response to the locomotor box was not observed following a vehicle-injection in animals previously receiving amphetamine (week 1, week 3 and week 5). Bar graphs show accumulated beam breaks over 30 min following amphetamine injection. Data are presented as mean values ± SEM. Numbers underneath bars correspond to number of rats. **p < 0.01.
drug (paired t-test: 1st vs. 5th amphetamine exposure: ambulatory locomotion (consecutive beam breaks): \( t_{(34)} = 6.95, p < 0.001 \); vertical activity (rearing): \( t_{(34)} = 6.19, p < 0.001 \) (Fig. 1B-D). Repeated administration of saline did not affect locomotor behavior significantly (ambulatory locomotion: \( t_{(36)} = 0.25, p = 0.80 \); vertical activity (rearing): \( t_{(36)} = 1.14, p = 0.26 \) (Fig. 1C-D).

Expression of behavioral sensitization was tested with a challenge dose of amphetamine (0.5 mg/kg). In drug-naive animals, administration of the challenge dose of amphetamine did not significantly increase locomotion or rearing, as compared to a vehicle-injection (Fig. 1E-F). However, rats that were pre-treated with amphetamine during the induction phase showed an enhanced locomotor response to the challenge dose of amphetamine (0.5 mg/kg) compared to rats receiving their first amphetamine-administration. Animals previously receiving amphetamine did not show enhanced locomotion in response to a vehicle injection, suggesting that conditioning to the testing environment did not contribute to the behavior (One-way ANOVA: ambulatory counts: \( F_{(3,38)} = 22.92, p < 0.001 \); Veh-Veh vs. Veh-Amph: \( q_{(38)} = 2.10, p = 0.46 \); Veh-Amph vs. Amp-Amph: \( q_{(38)} = 10.58, p < 0.001 \); Amph-Veh vs. Amp-Veh: \( q_{(38)} = 9.58, p < 0.001 \); rearing activity: \( F_{(3,38)} = 19.9, p < 0.001 \); Veh-Veh vs. Veh-Amph: \( q_{(38)} = 2.05, p = 0.30 \); Veh-Amph vs. Amp-Amph: \( q_{(38)} = 9.92, p < 0.001 \); Amph-Amph vs. Amp-Veh: \( q_{(38)} = 8.90, p < 0.001 \) (Fig. 1E-F)). The sensitized response to the locomotor stimulatory properties of amphetamine sustained for at least ten weeks after the last amphetamine exposure (Amph vs. vehicle: ambulatory counts: week 2: \( t_{(20)} = 7.30, p < 0.001 \); week 3: \( F_{(2,22)} = 83.4, p < 0.001 \); week 4: \( t_{(10)} = 7.09, p < 0.001 \); week 5: \( F_{(2,28)} = 70.7, p < 0.001 \); week 10: \( t_{(16)} = 6.65, p < 0.001 \); rearing activity: week 2: \( t_{(20)} = 6.20, p < 0.001 \); week 3: \( F_{(2,22)} = 46.7, p < 0.001 \); week 4: \( t_{(20)} = 6.31, p < 0.001 \); week 5: \( F_{(2,28)} = 51.2, p < 0.001 \); week 10: \( t_{(16)} = 6.63, p < 0.001 \) (Fig. 1E, F). Ambulatory counts did not differ between weeks (\( F_{(5,56)} = 1.94, p = 0.10 \)).

There was, however, a significant variability between weeks when assessing rearing activity (\( F_{(5,58)} = 2.73, p = 0.028 \), even though post hoc analysis did not show a significant separation between any specific abstinence week (\( p > 0.05 \)).

### 3.2. One injection of amphetamine is sufficient to produce behavioral sensitization

In a way to monitor the onset of behavioral sensitization in parallel to neurophysiological transformations, one batch of animals (\( n = 14 \)) received a single amphetamine injection (0.5 mg/kg). Subsets of these animals were then assessed with regards to behavioral sensitization (two weeks later) or neuroadaptations (acute or two weeks later). Amphetamine-induced locomotion was significantly enhanced when monitored in the same set of rats two weeks after the initial exposure (paired t-test: \( t_{(6)} = 3.94, p = 0.017 \) (Fig. 2B), indicating that one injection followed by a period of abstinence is sufficient to produce behavioral sensitization. While one single amphetamine exposure was insufficient to produce acute (24 h abstinence) neurophysiological adaptations, but a robust depression of evoked potentials was seen after two weeks abstinence in both striatal subregions (DLS: \( F_{(2,66)} = 20.7, p < 0.001 \); post hoc: Veh vs. Veh + 0.5Amph (acute): \( q_{(66)} = 1.96, p = 0.25 \); Veh vs. Veh + 0.5Amph (2 weeks): \( q_{(66)} = 7.15, p < 0.001 \); Veh + 0.5Amph (acute) vs. Veh + 0.5Amph (2 weeks): \( q_{(66)} = 8.34, p < 0.001 \); DMS: \( F_{(2,71)} = 10.9, p < 0.001 \), post hoc: Veh vs. Veh + 0.5Amph (acute): \( q_{(71)} = 1.20, p = 0.68 \); Veh vs. Veh + 0.5Amph (2 weeks): \( q_{(71)} = 5.28, p = 0.0011 \); Veh + 0.5Amph (acute) vs. Veh + 0.5Amph (2 weeks): \( q_{(71)} = 5.89, p < 0.001 \) (Fig. 2D-E).

### 3.3. Long-lasting depression of striatal excitability during amphetamine abstinence

To further outline the long-term effects on neurotransmission elicited by repeated amphetamine exposure (2.0 mg/kg, five injections), field potential recordings were conducted during acute (24 h) or protracted abstinence. In the DLS, input/output function was not significantly modulated during the acute abstinence phase, but a sustained depression was observed after longer periods of abstinence (Two-way ANOVA:
acute: $F_{(1,72)} = 1.09$, $p = 0.30$; 4–5 weeks $F_{(1,56)} = 5.02$, $p = 0.029$; 10–11 weeks: $F_{(1,86)} = 14.4$, $p = 0.0003$ (Fig. 3A–C). In the DMS, input/ output function was significantly depressed at all time-points (Two-way ANOVA: acute: $F_{(1,86)} = 5.19$, $p = 0.025$; 4–5 weeks: $F_{(1,51)} = 6.70$, $p = 0.013$; 10–11 weeks: $F_{(1,67)} = 4.97$, $p = 0.029$) (Fig. 3E–F).

3.4. Membrane properties are not altered by amphetamine exposure

To determine if repeated amphetamine affects striatal neurotransmission by altering baseline neuronal properties, membrane capacitance and resistance were analyzed in striatal MSNs from rats receiving five days of amphetamine or vehicle injections followed by abstinence. In the DLS, membrane capacitance was significantly depressed after extended abstinence (Cm: acute (19–40 h): $t_{(20)} = 1.49$, $p = 0.15$; 4–5 weeks: $t_{(50)} = 0.94$, $p = 0.35$; 10–11 weeks: $t_{(56)} = 2.24$, $p = 0.029$), but there was no effect by treatment on membrane resistance (Rm: acute: $t_{(20)} = 0.22$, $p = 0.98$; 4–5 weeks: $t_{(49)} = 0.78$, $p = 0.49$; 10–11 weeks: $t_{(54)} = 0.46$, $p = 0.65$) (Fig. 4). Membrane properties of MSNs in the DMS were not affected at any time-point analyzed (Cm: acute: $t_{(28)} = 0.486$, $p = 0.63$; 4–5 weeks: $t_{(38)} = 1.27$, $p = 0.20$; 10–11 weeks: $t_{(70)} = 1.11$, $p = 0.27$; Rm: acute: $t_{(27)} = 1.59$, $p = 0.12$; 4–5 weeks: $t_{(37)} = 1.44$, $p = 0.16$; 10–11 weeks: $t_{(70)} = 0.48$, $p = 0.63$) (Fig. 4).

3.5. Inhibitory neurotransmission during amphetamine abstinence

To determine if changes in GABAergic neurotransmission could underlie the long-lasting transformations observed in field potential recordings, sIPSCs were recorded in MSNs in the DLS and DMS. Neither the frequency nor the amplitude of sIPSCs were significantly affected during the acute stage of abstinence in either brain subregion (DLS: frequency: $t_{(16)} = 0.86$, $p = 0.40$; amplitude: $t_{(16)} = 1.14$, $p = 0.20$; DMS: frequency: $t_{(23)} = 1.01$, $p = 0.32$; amplitude: $t_{(23)} = 0.77$, $p = 0.44$) (Fig. 5). However, a decrease in sIPSC rise-time was apparent selectively in the DLS (DLS: rise-time: $t_{(17)} = 3.71$, $p = 0.0017$; decay-time: $t_{(17)} = 0.59$, $p = 0.56$; DMS: rise-time: $t_{(23)} = 1.74$, $p = 0.095$; decay-time: $t_{(22)} = 1.67$, $p = 0.11$), and this change was still present after 4–5 weeks abstinence (DLS: frequency: $t_{(41)} = 1.44$, $p = 0.16$; amplitude: $t_{(41)} = 0.28$, $p = 0.78$; rise-time: $t_{(42)} = 2.20$, $p = 0.034$; decay-time: $t_{(42)} = 1.73$, $p = 0.091$; DMS: frequency: $t_{(42)} = 0.19$, $p = 0.85$; amplitude: $t_{(43)} = 0.830$, $p = 0.41$; rise-time: $t_{(43)} = 0.85$, $p = 0.40$; decay-time: $t_{(43)} = 0.72$, $p = 0.48$) (Figs. 5, 6). No effects on sIPSC parameters were detected in either brain subregion after 10–11 weeks of abstinence (DLS: frequency: $t_{(29)} = 0.21$, $p = 0.84$; amplitude: $t_{(29)} = 0.66$, $p = 0.51$; rise-time: $t_{(29)} = 0.69$, $p = 0.50$; decay-time: $t_{(29)} = 0.94$, $p = 0.35$; DMS: frequency: $t_{(47)} = 1.11$, $p = 0.27$; amplitude: $t_{(46)} = 0.53$, $p = 0.60$; rise-time: $t_{(47)} = 0.024$, $p = 0.98$; decay-time: $t_{(46)} = 0.67$, $p = 0.51$) (Fig. 6).

3.6. Protracted abstinence transforms dopamine D2 receptor signaling

In the last set of experiments, the effect by amphetamine on other signaling pathways that could contribute to the depressed synaptic output was outlined. Considering the role of amphetamine in affecting dopaminergic and serotonergic neurotransmission, the dopamine D2 receptor agonist quinpirole (5 μM) or 5-HT (5 μM) were bath perfused. Field potential recordings revealed a significant suppression of evoked PS amplitudes in response to bath perfusion of the dopamine D2 receptor agonist quinpirole (5 μM) in slices from both vehicle-treated and amphetamine-treated rats after up to one month of abstinence (DLS: acute: $F_{(1,16)} = 2.55$, $p = 0.13$; 4–5 weeks: $F_{(1,21)} = 0.55$, $p = 0.47$; DMS: acute: $F_{(1,16)} = 0.28$, $p = 0.60$; 4–5 weeks: $F_{(1,12)} = 0.09$, $p = 0.78$). Following 10–11 weeks of abstinence, however, a significantly impaired response to dopamine D2 receptor agonist was observed in both DLS ($F_{(1,23)} = 16.3$, $p < 0.001$) and DMS ($F_{(1,21)} = 19.2$, $p < 0.001$) (Fig. 7). The depressant effect by 5-HT was not affected by treatment at any time-point analyzed (DLS: acute: $F_{(1,22)} = 0.0043$, $p = 0.95$; 10–11 weeks: $F_{(1,20)} = 0.034$, $p = 0.86$; DMS: acute: $F_{(1,20)} = 0.13$, $p = 0.72$; 10–11 weeks: $F_{(1,20)} = 0.034$, $p = 0.86$).

![Fig. 3. Long-lasting depression of synaptic output from dorsal striatum after repeated amphetamine exposure. A–C) Evoked synaptic potentials in the DLS were not affected during acute amphetamine abstinence, but significantly depressed after extended abstinence. D) Example traces show evoked populations spikes in the DLS in brain slices from rats treated with either vehicle (upper trace) or amphetamine (lower trace) after 11 weeks of abstinence. Calibration is 0.2 mV and 2 ms. E–G) Repeated amphetamine exposure followed by abstinence resulted in a depression of evoked potentials in the DMS, which lasted throughout the study. H) Example traces show evoked populations spikes in the DMS in brain slices from rats treated with either vehicle (upper trace) or amphetamine (lower trace) vehicle (upper trace) or amphetamine (lower trace) after 11 weeks of abstinence. Calibration is 0.2 mV and 2 ms. Data are presented as mean values ± SEM. Field potentials were recorded in parallel to whole cell recordings, using brain slices from the same rats. n = number of recordings, retrieved from at least 6 rats/group. *p < 0.05, ***p < 0.001.](image-url)
weeks: $F_{(1,20)} = 0.98$, $p = 0.34$) (Fig. 7).

4. Discussion

The aim of this study was to test the postulate that repeated exposure to amphetamine produces a long-lasting behavioral sensitization that coincides with altered synaptic output from dorsal striatal subregions, and that these effects are driven by changes in GABAergic neurotransmission. However, even though repeated amphetamine-exposure generated a long-standing suppression of synaptic output from both the DLS and DMS, amphetamine did not produce robust or long-lasting changes in neither membrane properties of medium spiny neurons, nor of spontaneous inhibitory neurotransmission. While the responsiveness to 5-HT was not affected by treatment, the synaptic depression induced by dopamine D2 receptor activation was significantly blunted after 10 weeks of amphetamine abstinence. These progressive neuroadaptations are partially in line with previous studies showing drug-induced changes in explicit brain regions arising after three months abstinence (Li et al., 2013; Licheri et al., 2020; Morud et al., 2018). While the role of these progressive neuroadaptations remains to be determined, blunted dopamine D2 receptor signaling in the striatum may increase the risk of developing compulsive drug-seeking behavior (Nelson and Killcross, 2013).

Repeated administration of amphetamine produced a long-lasting behavioral sensitization, which is in line with previous studies (Paulson et al., 1991; Robinson et al., 1988; Vestin et al., 2022). In fact, one single injection of a low dose of amphetamine (0.5 mg/kg) followed by two weeks abstinence was sufficient to produce both behavioral sensitization and striatal neuroadaptations. This finding is supported by a previous study, showing that a single exposure to amphetamine (5 mg/kg) induces behavioral, neurochemical, and neuroendocrine sensitization that intensifies over three weeks abstinence (Vanderschuren et al., 1999). Behavioral sensitization has been proposed to reflect many of the neurochemical changes that are characteristic for drug addiction (Robinson and Berridge, 1993; Steketee and Kalivas, 2011), and while a causal relationship cannot be established the data presented here further supports a role for striatal neuroadaptations in contributing to behavioral sensitization (Durieux et al., 2012; Vanderschuren et al., 1999). It should be noted that behavioral studies and electrophysiological recordings were performed during the light phase of the cycle. While this treatment paradigm has been shown to produce robust behavioral sensitization (Mendez et al., 2009; Vestin et al., 2022), this might have influenced the experimental outcome (Nelson et al., 2021).

Amphetamine exposure followed by abstinence resulted in a depressed amplitude of evoked field potentials in both the DLS and DMS that was not explained by an increased inhibitory tone. It could also not be linked to changes in membrane properties, or impaired dopamine D2 receptor signaling or serotonergic signaling. Rather, the decrease in evoked potential amplitudes could be a sign of a hypoglutamatergic state, connected to changes in AMPA receptor availability or excitatory inputs. Striatal field potential amplitudes are primarily mediated through AMPA receptor signaling (Lagstrom et al., 2019), and repeated
amphetamine exposure have been reported to progressively depress AMPA subunit mRNA expression in subregions of the striatum (Furlong et al., 2018; Lu and Wolf, 1999). In addition, repeated exposure to methamphetamine produces a long-lasting presynaptic corticostriatal depression (Bamford et al., 2008), which could further contribute to a hypoglutamatergic state. Lastly, while GLAST and GLT-1 are not affected during acute abstinence (Armstrong et al., 2004; Sidiropoulou et al., 2001), glial glutamate transporters progressively decline with extended abstinence from psychostimulants (Fischer et al., 2021), which may further act to reduce excitatory neurotransmission in dorsal striatum (Adermark et al., 2021). Importantly, neuroadaptations in dorsal striatal subregions presented here were elicited by passive

Fig. 5. Minor changes in spontaneous inhibitory postsynaptic currents during acute amphetamine abstinence. A–D) In the DLS, only rise-time was significantly altered after repeated amphetamine exposure. E) Example traces showing recorded sIPSCs in DLS from a vehicle-treated rat. Calibration to the left is 50 pA and 1 s, and to the right 50 pA and 20 ms. F) Example traces showing recorded sIPSCs in DLS from an amphetamine-treated rat. Left calibration, 50 pA and 1 s, right calibration, 50 pA and 20 ms. G–J) There were no significant effects on recorded sIPSCs in the DMS. K) Example traces showing recorded sIPSCs in DMS from a vehicle-treated rat. Calibration to the left is 50 pA and 1 s, and right calibration to 50 pA and 20 ms. Data are presented as mean values ± SEM. Number in parenthesis corresponds to the number of neurons recorded, taken from at least 5 rats/group. Recordings from both treatment groups were conducted in parallel. **p < 0.01.

Fig. 6. Selective effects by amphetamine on postsynaptic properties in MSNs in the DLS. A) In the DLS, neither frequency nor amplitude were significantly modulated by treatment at follow up, but the decrease in rise-time sustained after one month of abstinence from amphetamine. B) No effects by previous amphetamine exposure on recorded sIPSCs were seen in the DMS after one month abstinence. C–D) GABAergic neurotransmission onto dorsal striatal MSNs was not altered in any brain subregion after three months of abstinence from amphetamine. Data are presented as mean values ± SEM. Number in parenthesis corresponds to the number of neurons recorded, taken from at least 6 rats/group. Recordings from both treatment groups were conducted in parallel. *p < 0.05.
administration, suggesting that amphetamine produces neuroplasticity that is independent of action learning.

While a robust depression of evoked potentials was apparent in the DMS during acute abstinence, activity in the DLS progressively declined with extended abstinence. Progressive neuroadaptations are in line with previous studies, showing that psychostimulants such as nicotine and cocaine recruit corticostriatal networks in a spatially and temporally distinct sequence (Adermark et al., 2016; Belin and Everitt, 2008; Belin et al., 2009; Belin-Rauscent et al., 2012; Licheri et al., 2020). A progressive decrease in membrane capacitance was also observed in DLS MSNs from animals previously receiving amphetamine. The reduced capacitance may be associated with a decrease in cell size (Gertler et al., 2008), and could be linked to drug-induced rearrangement of actin filaments (Matsumoto and Tasaki, 1977).

Since membrane capacitance influences synaptic efficacy and the speed with which electrical signals propagate (Matsumoto and Tasaki, 1977), decreased capacitance could have contributed to further depress evoked potentials in DLS or to suppress dopamine D2 receptor responses.

While the DMS is associated with the initial drug seeking behavior and development of behavioral sensitization (Conversi et al., 2008; Durieux et al., 2012), the DLS is implicated in late-stage habitual drug intake and compulsive drug seeking (Giuliano et al., 2019; Murray et al., 2012). The data presented here, however, demonstrated sustained neuroadaptations in the DMS, even after protracted abstinence. Sustained recruitment of the DMS during amphetamine abstinence is partially supported by previous studies showing that inhibition of D1 receptors in either the DLS or the DMS, or inhibition of neuronal ensembles in the DMS, decreases cue-induced methamphetamine seeking after prolonged but not early withdrawal (Caprioli et al., 2017; Li et al., 2015). Neurotransmission in the DMS thus appears to be especially susceptible to amphetamine exposure. This is especially interesting considering the postulated role of the DMS (roughly corresponding to associative striatum) in psychosis (Danielsson et al., 2021b; Herga et al., 2016; Mitchell et al., 1998), a disorder repeatedly associated with amphetamine abuse (Bramness and Rognli, 2016).

Amphetamine has been suggested to change GABAergic networks (Wearne and Cornish, 2019), and we hypothesized that repeated exposure to amphetamine would produce long-lasting effects on GABAergic neurotransmission in striatal subregions that coincided with behavioral sensitization. However, during extended amphetamine abstinence, whole cell recordings did not show any effects on sIPSC frequency or amplitude, which is partially in line with a previous study conducted in the prefrontal cortex (Paul et al., 2016). Repeated exposure to amphetamine, however, was associated with a significant decline in rise-time, selectively in the DLS. Since all recordings were performed in parallel, and the effect on rise-time was selective in the DLS and sustained during longer abstinence periods, this change is most likely a specific effect mediated by repeated amphetamine exposure. The change in rise time may be partially connected to a change in the expression of GABA_A receptor subunits (Jiao et al., 2016), which could affect the kinetics of channel openings rather than amplitude (Dixon et al., 2014). Still, it is not clear how these changes may impinge on synaptic transmission, especially since we found no effect by treatment on evoked field potentials in the DLS during the acute abstinence phase.

Systemic administration of amphetamine increases monoamines, and striatal release of dopamine and 5-HT may interact to influence behavioral responses to amphetamine (Hernandez et al., 1987; Kuczenski and Segal, 1989). Treatment-effect on striatal output could thus be driven by changes in monoamine signaling. Acute administration of 5-HT significantly depressed evoked field potentials, which is in line with a previous study performed in the DLS (Mathur et al., 2011). The responsiveness to 5-HT was however not sensitive to treatment, suggesting that serotoninergic signaling in the striatum is not compromised by repeated amphetamine exposure followed by abstinence (El-Sherbeni et al., 2020). A depression of evoked potentials was also apparent in response to bath perfused quinpirole. While there was no effect by treatment during early abstinence, dopamine D2 receptor signaling was significantly impaired in both DLS and DMS following protracted amphetamine abstinence. These findings are partially in line with clinical observations (Ashok et al., 2017), and supported by rodent studies showing dysfunctional dopamine D2 receptor signaling following amphetamine sensitization (Chen et al., 1999; Danielsson et al., 2021a;
Blunted dopamine D2 receptor signaling in the ventral striatum has been linked to diminished D2 receptor expression (Chen et al., 1999), but amphetamine may also affect striatal dopamine signaling by increasing dopamine D2 receptor dimerization (Wang et al., 2010), or by changing the dopaminergic tone (Ashok et al., 2017; Danielsson et al., 2021a; Gatica et al., 2020). Activity at distinct dopamine receptor subtypes have been linked to amphetamine induced-disruption of goal-directed behavior (Nelson and Killcross, 2013), but it remains to be determined if impaired dopamine D2 receptor signaling following extended amphetamine abstinence leads to decreased voluntary control over behavior.

In conclusion, the data presented here suggest that five days of amphetamine exposure is sufficient to induce a long-standing behavioral sensitization towards the locomotor-stimulatory properties of amphetamine, and to produce neuroadaptations in dorsal striatal subregions that continue to develop during drug abstinence. Changes in synaptic output from the striatum appeared to be associated with selective changes in excitatory neurotransmission and did not coincide with adaptations in GABAergic signaling. Striatal neuroadaptations could be a neurobiological underpinning of behavioral sensitization, but may also be important for amphetamine seeking behaviors (Caprioli et al., 2017; Li et al., 2015; Vanderschuren et al., 2005), and contribute to the high risk of relapse, which is common among amphetamine users even after prolonged abstinence (Brecht and Herbeck, 2014).

CRediT authorship contribution statement
LA, ME and AL designed the study. AL performed behavioral studies and wrote the main draft of the manuscript together with LA. VI and LA performed electrophysiological recordings and data analysis with assistance from JA. ME and BS assisted during interpretation of main findings. All co-authors critically reviewed the manuscript for critical content. Authors declare no conflict of interest.

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