Disruption of prefrontal cortical–hippocampal balance in a developmental model of schizophrenia: reversal by sulpiride

Pauline Belujon, Mary H. Patton and Anthony A. Grace
Departments of Neuroscience, Psychiatry and Psychology, University of Pittsburgh, Pittsburgh, USA

Abstract
The nucleus accumbens (NAc) receives converging inputs from the medial prefrontal cortex (mPFC) and the hippocampus which have competitive interactions in the NAc to influence motivational drive. We have previously shown altered synaptic plasticity in the hippocampal–NAc pathway in the methylazoxymethanol acetate (MAM) developmental model of schizophrenia in rodents that is dependent on cortical inputs. Thus, because mPFC–hippocampal balance is known to be partially altered in this model, we investigated potential pathological changes in the hippocampal influence over cortex-driven NAc spike activity. Here we show that the reciprocal interaction between the hippocampus and mPFC is absent in MAM animals but is able to be reinstated with administration of the antipsychotic drug, sulpiride. The lack of interaction between these structures in this model could explain the attentional deficits in schizophrenia patients and shed light onto their inability to focus on a single task.

Introduction
The dorsolateral prefrontal cortex in primates, which is homologous to the medial PFC (mPFC) in rodents (Ongur & Price, 2000) plays a major role in higher cognitive functions, such as working memory (for review see Goldman-Rakic, 1990), or set-shifting (Floresco et al. 2006), and deficits in prefrontal cortical activity are believed to be a central component in numerous psychiatric disorders, such as schizophrenia (Meyer-Lindenberg et al. 2001). Moreover, numerous functional studies highlight a major role of the hippocampus, a region involved in context-dependent processing (Jarrard, 1995), in schizophrenia (Bogerts et al. 1993; Tamminga et al. 1992). Cortical and hippocampal inputs are integrated within the nucleus accumbens (NAc), and disruption of the balance between those two inputs may play a major role in schizophrenia. We have previously shown that the ventral hippocampus and the mPFC exhibit dopamine-dependent reciprocal interactions in the NAc. Thus, high-frequency stimulation (HFS) of the hippocampus induces a long-lasting depression in mPFC-evoked activity in the NAc and vice versa (Goto & Grace, 2005), indicating a competition between the two structures. Moreover, this competition is dopamine-dependent, regulating the balance of information processing within the NAc (Goto & Grace, 2005).

In rodents, prenatal administration of methylazoxymethanol acetate (MAM), an antimitotic agent, leads to anatomical and behavioural disruptions in adults that are comparable to some of the deficits that have been described in schizophrenia patients (Flagstad et al. 2004; Gourevitch et al. 2004; Moore et al. 2006). We have previously shown that hippocampal–accumbens synaptic plasticity is disrupted in the MAM model, and that the deficits observed were dependent on cortical activity (Belujon et al. 2011). Moreover, we demonstrated that those alterations were normalized when the antipsychotic drug sulpiride was administrated systemically (Belujon et al. 2011). In the present study, we used the developmental MAM rodent model of schizophrenia to investigate the influence of the hippocampus on the cortico-accumbens synaptic plasticity and the effect of the antipsychotic drug sulpiride.

Methods

Animals
All experiments were performed in accordance with the guidelines outlined in the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh.
MAM

MAM- and saline-treated rats were prepared as described previously (Lodge & Grace, 2007; Moore et al. 2006). Briefly, timed pregnant female Sprague–Dawley rats (Hilltop, USA) arrived at gestational day (GD) 15 and were housed individually under a 12-h light/dark cycle (lights on 07:00 hours). On GD 17, dams were injected with the mitotoxin MAM (diluted in saline, 22 mg/kg i.p.) or with a vehicle, saline (1 ml/kg i.p.). Male pups were weaned on postnatal day (PD) 21 and housed in groups of 2–3 with littersmates.

Extracellular recordings

In vivo single-unit recordings were done in male adult offspring of PD 90 or older (MAM: 33 neurons in 33 rats derived from 13 dams; saline: 20 neurons in 20 rats derived from nine dams). Rats were anaesthetized with chloral hydrate (400 mg/kg), placed in a stereotaxic apparatus (Kopf, USA) and implanted with a catheter in the lateral tail vein to allow for intravenous (i.v.) injections. Extracellular electrodes were pulled from glass micro-pipettes (WPI, USA) and filled with a 2% Chicago Sky Blue dye solution in 2 M NaCl. Microelectrodes were lowered through the NAc (A/P +1.5 mm from bregma; M/L +1.1 mm from midline; D/V −5 to −7.5 mm from dura). Single-unit signals were amplified and filtered using a Fintronics amplifier (500–5000 Hz). Recording were displayed on an oscilloscope (B&K Precision, USA) and transferred via an interface to a computer equipped with LabChart v. 7 software. Neuronal activity with a signal-to-noise ratio of 3:1 or greater was recorded and used for analyses. Only one set of recordings was obtained from each animal.

Stimulation

Single-pulse, low-frequency stimulation (LFS) and HFS were applied via a concentric bipolar stimulating electrode (NEX-100X; Rhodes Medical Instruments, USA) to the fimbria (saline rats: A/P −1.6 mm from bregma; M/L −1.3 mm from midline; D/V −4.5 mm from dura; MAM rats: A/P −2 mm from bregma; M/L +1.3 mm from midline; D/V 4.2 mm from dura). A chemotrode (combination of a stimulating electrode and guide cannula, allowing for drug infusion at the stimulation site if necessary) was placed in the mPFC at a 15° angle (saline rats: A/P +2.9 mm from bregma; M/L +1.9 mm from midline; D/V −3 mm from dura; Belujon & Grace, 2008; MAM: A/P +3.2 mm from bregma; M/L +1.3 mm from midline; D/V −3.6 mm from dura). Coordinates used in MAM-treated rats were adjusted to compensate for the 10% reduction in brain size (Flagstad et al. 2004).

Only neurons that responded monosynaptically to both mPFC and fimbria stimulations were recorded. The fimbria and the mPFC received alternating single-pulse stimuli delivered using a dual output stimulator (S8800; Grass Technologies, USA) (frequency 0.5 Hz, intensity 1 mA; pulsewidth 0.25 ms). Once a cell was isolated, the current administered to the mPFC was adjusted to evoke an action potential ~50% of the time and baseline responses were recorded to have at least 5 min of stable recordings. Spike probabilities were measured by dividing the number of spikes observed by the number of stimuli in 2-min intervals for the baseline period and 5-min intervals after treatment. It should be noted that only a few neurons (5/53) showed more than one spike per mPFC stimulation; the majority responding to the stimulation with a single spike. After recording a stable baseline HFS (20 Hz, 200 pulses, suprathreshold) or LFS (5 Hz, 500 pulses, suprathreshold) was administered to the fimbria and mPFC-evoked activity in the NAc was recorded for at least 30 min after the stimulation (frequency 0.5 Hz, baseline intensity, 0.25 ms pulsewidth). For the HFS experiments, seven saline-treated and six MAM-treated dams were used, whereas six saline-treated and five MAM-treated dams were used for LFS experiments. In experiments in which sulpiride (5 mg/kg i.v.) was injected intravenously, a subsequent evoked activity (10 min) was recorded before tetanization of the fimbria. For those experiments, eight dams were used for the pre-injection of supiride study and three for the pre-injection of saline.

It should be noted that although the majority of the cells (46/53 neurons) were recorded in the shell part of the NAc, some (3/20 neurons from saline-treated animals, and 4/33 neurons for MAM-treated rats) were in the core part of the NAc (Fig. 1a). Since no difference was observed between these two parts, the data have been combined.

Drugs and drug infusions

Following a baseline period, sulpiride (Sigma Aldrich, USA, 5 mg/kg) or vehicle (saline, 1 ml/kg) were injected intravenously.

Histology

After each experiment, electrode placement was verified by ejection of Chicago Sky Blue dye into the recording site. To verify the stimulation electrode placement, a 10 s pulse at 200 μA was administered. Rats were euthanized with a lethal dose of chloral hydrate and brains were removed following decapitation. The tissue was fixed in 8% paraformaldehyde for at least 48 h and then transferred to a 25% sucrose solution for cryoprotection. Once saturated, brains were frozen and sliced coronally at 60 μm thickness using a cryostat and mounted onto gelatin-chromalum-coated slides. Tissue was stained with combination of Neutral Red and Cresyl Violet.

Analysis

Data were analysed using a one-way ANOVA with repeated measures followed by the Holm–Sidak test, with...
Fig. 1. Disrupted medial prefrontal cortex (mPFC)-evoked heterosynaptic plasticity induced by high-frequency stimulation (HFS) of the fimbria, but not by low-frequency stimulation (LFS) in methylazoxymethanol acetate (MAM)-treated rats. (a) Schematic showing the representative placements of stimulating electrodes in the mPFC (left) and the fimbria (right) and location of recording electrodes in the nucleus accumbens (NAc) (middle), shown as coronal sections of the rat brain, taken from the atlas of Paxinos & Watson (1996). Numbers to the right indicate millimeters A/P from bregma; approximately 50% of placements are shown. (b) Representative example
time as the within-subject factor. Multiple comparisons were analysed using a two-way ANOVA followed by the Holm–Sidak test, with treatment as the between-subject factor and time as the within-subject factor.

Results

Locations of stimulating and recording electrodes are presented in Fig. 1a. The mean baseline spike probability, the latency of fimbria-evoked responses and the current used to obtain 50% spike probability at baseline were not statistically different between MAM- and saline-treated animals (t test: \( t \) = 0.1604, \( p = 0.4904 \) and \( p = 0.0961 \), respectively; \( n = 20 \) saline- and \( n = 33 \) MAM-treated animals).

Reciprocal interaction between hippocampal and PFC are absent in MAM rats

We have previously shown that the hippocampus and the mPFC exert a functionally reciprocal interaction within the NAc (Goto & Grace, 2005). In this study, we confirmed these results in saline animals (Fig. 1a). Indeed, tetanization of the fimbria induced a significant and persistent depression (\( \approx 50\% \), >30 min) of the mPFC-evoked spike probability of accumbens neurons in saline-treated animals (baseline: 0.39 ± 0.08; post-HFS: 0.16 ± 0.09; \( F = 7.989 \), \( p < 0.05 \), \( n = 12 \)). However, in MAM animals, tetanization of the fimbria induced an mPFC-evoked spike probability that was highly variable but overall yielded no significant effect. Indeed, the mPFC-evoked spike probability was within a 95% confidence interval (CI) of the baseline (baseline: 0.48 ± 0.05; post-tetanus: 0.49 ± 0.08; 95% CI 0.37–0.59, \( n = 12 \)). The difference between the post-HFS mPFC-evoked spike probability was significantly different between MAM- and saline-treated rats (two-way ANOVA, Holm Sidak post-hoc, \( F = 18.841 \), \( p < 0.05 \)). Therefore, the fimbria tetanization-evoked synaptic plasticity induced in the mPFC-NAc pathway (herein referred to as heterosynaptic plasticity) is absent in MAM-treated animals.

LFS-induced synaptic plasticity is not disrupted in MAM-treated rats

While HFS usually induces long-lasting enhancement of synaptic efficacy (long-term potentiation, LTP), LFS generally mediates long-term depression (LTD). We have previously shown that LTD of the fimbria induced depression in the hippocampus–accumbens pathway, which was not different between MAM- and saline-treated rats (Belujon et al. 2011). Here, we studied the heterosynaptic plasticity of mPFC afferents to the NAc induced by LFS of the fimbria. As shown in the hippocampus–accumbens pathway (Belujon et al. 2011), LTD of the fimbria induced a LTD (\( \approx 30\% \), >30 min) in the MFC-NAc pathway in both MAM- and saline-treated rats. Thus, in MAM-treated rats, the spike probability decreased from 0.53 ± 0.10 (baseline) to 0.19 ± 0.04, 30 min after LTD (\( F = 3.151 \), \( p < 0.05 \), \( n = 8 \)). In saline-treated rats, the spike probability

of extracellular recordings from accumbens neurons evoked by mPFC stimulation before and after tetanization of the fimbria in saline-treated animals (left) and MAM-treated animals (right). (c) Mean percent change (±S.E.M.) in mPFC-evoked spike probability, normalized to the baseline, after HFS to the fimbria in saline-treated (●) and MAM-treated (■) animals (\( p < 0.05 \); arrow indicates time of stimulation). (d) Mean of the percent change in mPFC-evoked responses (±S.E.M.) following HFS in saline-treated (●) and MAM-treated (■) animals. (e) Mean percent change (±S.E.M.) in mPFC-evoked spike probability, normalized to the baseline, after LFS to the fimbria in saline-treated (●) and MAM-treated (■) animals (\( p < 0.05 \); arrow indicates time of stimulation). (f) Mean of the percent change in mPFC-evoked responses (±S.E.M.) following LFS in saline-treated (●) and MAM-treated (■) animals.
increased from 0.54 ± 0.03 (baseline) to 0.29 ± 0.10, 30 min after LFS to the fimbria \( (F = 5.48, p < 0.05, n = 8) \). Moreover, there was no significant difference between the depression of the mPFC–NAc pathway in MAM-treated animals compared to saline-treated animals (two-way ANOVA, Holm–Sidak post-hoc, \( F = 0.19, p = 0.669 \)).

Therefore, with LFS of the fimbria, mPFC-evoked spiking in the NAc exhibits a similar type of depression in both MAM- and saline-treated rats.

**The antipsychotic drug, sulpiride, restores HFS-induced LTD in MAM-treated rats**

We have previously shown that systemic injection of sulpiride reversed the altered vSub-NAc synaptic plasticity in MAM-treated animals (Belujon et al. 2011). In the present study, we injected sulpiride systemically 10 min before HFS of the fimbria and examined whether it affected mPFC-driven spiking in the NAc (Fig. 2). In MAM-treated rats, blocking D2 receptors with sulpiride did not affect the mPFC-evoked spike probability. The mPFC-evoked spike probability after sulpiride injection was within the 95% CI of the baseline spike probability (baseline: 0.44 ± 0.04; post-sulpiride: 0.41 ± 0.02; 95% CI 0.34 – 0.54). Injection of saline also had no effect on the spike probability (baseline: 0.51 ± 0.02; post-saline: 0.53 ± 0.05; 95% CI 0.45 – 0.57). Tetanization of the fimbria in MAM-treated rats induced a LTD (≥ 50%, > 25 min) with a pre-injection of sulpiride. The baseline spike probability was 0.44 ± 0.04 and decreased to 0.25 ± 0.11, 20 min after HFS to the fimbria \( (F = 4.951, p < 0.05, n = 8) \); Fig. 2a). Pre-injection of saline did not induce any change in the mPFC-evoked spiking of the NAc after HFS to the fimbria (baseline: 0.51 ± 0.02, post-saline: 0.53 ± 0.05, post-HFS: 0.55 ± 0.03; \( F = 0.56, p = 0.781, n = 5 \); Fig. 2a). Moreover, the mPFC-evoked spike probability after sulpiride-HFS was significantly different compared to vehicle injection (two-way ANOVA, Holm–Sidak post-hoc, \( F = 6.974, p < 0.05 \)). The HFS-induced depression observed after injection of sulpiride in MAM-treated rats is comparable to the HFS-induced LTD in saline animals (Fig. 2a). Therefore, injection of antipsychotic drug in MAM-treated rats restores the altered mPFC-evoked heterosynaptic plasticity.

**Discussion**

The present study demonstrates that in the MAM model, the mPFC-evoked heterosynaptic plasticity induced by tetanus of the fimbria is absent in the NAc and that the LTD observed in control rats is restored after injection of the antipsychotic drug sulpiride in MAM rats.

We found no clear effect of fimbria HFS on the mPFC–NAc pathway of MAM animals. One explanation of this result is that the mPFC of MAM animals is already in a hyperactive state, and thus HFS of the hippocampus cannot induce LTD in the mPFC–NAc pathway. Thus, mPFC neurons were found to fire faster in MAM-treated rats (Lavin et al. 2005) and showed more robust tetanization-induced LTP (Goto & Grace, 2006). Indeed, a loss of parvalbumin-containing interneurons is shown in the PFC (among other structures) in schizophrenia patients (Lewis & Moghaddam, 2006), as well as in the developmental rodent MAM model of schizophrenia (Lodge et al. 2009). This loss could disrupt the effects exerted by multiple inputs to the mPFC, which in the normal rat may in part facilitate the reciprocal interactions between this region and the hippocampus. The site of action of sulpiride in restoring reciprocal interactions between the mPFC and hippocampus is unclear. However, it has been shown that increased dopamine transmission in the NAc tends to shift the mPFC–hippocampal balance in favour of the mPFC (Goto & Grace, 2005). Perhaps in the MAM-treated rat, antipsychotic drug-induced attenuation of the impact of increased NAc dopamine transmission may serve to restore the balance in the system to allow the interaction to emerge.

Therefore, in the MAM-exposed rat, the balance between the mPFC and the hippocampus is disrupted. We have previously postulated that the interaction between the mPFC and the hippocampus is important for gating of attentional states (Grace, 2012; Sesack & Grace, 2010). Thus, the hippocampal subiculum, being a source of context-dependent gating, is proposed to act by keeping an individual focused on a task. Furthermore, the hippocampal input to the NAc is potentiated by D2 stimulation. In contrast, the mPFC is proposed to drive behavioural flexibility, and is down-modulated by D1 stimulation. Therefore, in an individual performing a task resulting in a reward, the increase in dopamine transmission would increase hippocampal inputs to increase attention and performance of a given task, while inhibiting the mPFC from shifting to an alternate task. However, if the action is not rewarded, there is a decrease in the dopamine system (Schultz, 2010), enabling the mPFC to now shift focus. However, if activation of the hippocampus can no longer attenuate the mPFC, a condition could arise in which the subject is caused to increase focused attention on multiple contingencies, due to activation of both the hippocampal-driven task focus and the mPFC-driven flexibility in response. An antipsychotic drug could then serve to normalize this relationship. While admittedly hypothetical, such a relationship could account for disrupted focus and an overwhelming bombardment of stimuli competing for attention (Saks, 2008).

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