Brain Structure and Function (2019) 224:171–190
https://doi.org/10.1007/s00429-018-1769-z

ORIGINAL ARTICLE

Disinhibition of the prefrontal cortex leads to brain-wide increases in neuronal activation that are modified by spatial learning

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Received: 11 April 2018 / Accepted: 3 October 2018 / Published online: 8 October 2018

Abstract

Deficient prefrontal cortex (PFC) GABA function is hypothesized to play a role in schizophrenia and other psychiatric disorders. In rodents, PFC $GABA_A$ receptor antagonism produces cognitive and behavioral changes relevant to these disorders, including impaired spatial memory assessed with the traditional working/reference memory radial maze task. This aspect of spatial memory does not depend on PFC, suggesting that deficient PFC GABAergic transmission may interfere with non-PFC-dependent cognitive functions via aberrant increases in PFC output. To test this, we assessed whether PFC $GABA_A$ antagonism (50 ng bicuculline methbromide) alters neuronal activation in PFC terminal regions, including the striatum, thalamus, hippocampus, amygdala, and cortical regions, of adult male rats using the immediate early gene, c-Fos, as an activity marker. A subset of these animals were also trained and/or tested on the working/reference memory radial maze task. These treatments caused widespread increases in neuronal activation in animals under baseline conditions, with notable exception of the hippocampus. Furthermore, PFC $GABA_A$ antagonism impaired task performance. In most instances, training and/or testing on the radial maze had no additional effects on neuronal activation. However, in both the hippocampus and rhomboid thalamic nucleus, PFC $GABA_A$ antagonism caused a selective increase in neuronal activation in animals trained on the maze. These results indicate that deficiencies in PFC GABAergic transmission may have widespread impacts on neuronal activity that may interfere with certain PFC-independent cognitive functions. Furthermore, these alterations in activity are modulated by plasticity induced by spatial learning in the hippocampus and rhomboid thalamic nucleus.

Keywords Prefrontal cortex · Hippocampus · Schizophrenia · GABA · cFos · Spatial memory · Experience-dependent plasticity

Introduction

GABAergic interneurons are a population of cells with immense diversity in their molecular, morphological and neurophysiological specializations (Tremblay et al. 2016). However, they share the common feature of regulating excitability and output of other neurons, in turn enabling co-ordination of activity at the microcircuit or network level. In the prefrontal cortex (PFC), interneuron activity and GABA release play a key role in establishing neuronal oscillations that are thought to be a mechanism that enables information processing necessary for many higher-order cognitive functions. For instance, fast-spiking interneurons that express the calcium-binding protein parvalbumin (PV) are necessary for initiating oscillatory activity within the gamma range (30–80 Hz) (Cardin et al. 2009; Sohal et al. 2009). Gamma oscillations occur when working memory and cognitive control mechanisms are engaged and abnormal gamma oscillations are associated with impairments in these aspects of cognition; as is observed in schizophrenia (Chen et al. 2014; Haenschel et al. 2009; Minzenberg et al. 2010).

Deficiencies in prefrontal GABAergic transmission have been observed in several pathological and non-pathological conditions, including schizophrenia (Benes 1995; Gonzalez-Burgos and Lewis 2012, Tse et al. 2015b), depression (Lener et al. 2017; Luscher et al. 2011) and aging (McQuail et al. 2015). In schizophrenia, one of the most reliable pathologies found in postmortem brain is a decrease in mRNA and protein expression of the GABA synthesis enzyme, GAD67 in prefrontal regions (Akbarian

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et al. 1995; Curley et al. 2011; Guidotti et al. 2000; Volk et al. 2000). Positron emission tomography (PET) imaging studies using a benzodiazepine site ligand revealed disrupted PFC GABAergic transmission in schizophrenia patients (Frankle et al. 2015), while the largest in vivo magnetic resonance spectroscopy study of schizophrenia to date found evidence for decreased PFC GABA levels in aged individuals (Rowland et al. 2016). Collectively, these findings suggest that certain symptoms of schizophrenia may be driven by decreased synthesis and/or release of GABA, especially within the PFC. Strikingly, many animal models of the disorder, whether neurodevelopmental (François et al. 2009; Tseng et al. 2008), pharmacological (Amitai et al. 2012; Behrens et al. 2007; Morshedi and Meredith 2007) or genetic (Ji et al. 2009; Lee et al. 2013; Shen et al. 2008), converge on disrupting PFC GABA function. Since changes in GABA function in schizophrenia are detected particularly within PFC regions that regulate higher-order cognition, they are well-placed to contribute to cognitive impairments associated with the disorder.

In recent years, our group and others have investigated how PFC GABAergic transmission regulates cognitive processes mediated by the frontal lobes by assessing how different domains of cognition are affected by pharmacological targeting of GABAergic transmission in the medial PFC of male rats. These studies have revealed that reducing PFC GABA signalling produces a myriad of cognitive deficits with relevance to schizophrenia, including impairments in attention (Auger et al. 2017; Paine et al. 2011; Pehrson et al. 2013; Pezze et al. 2014), working memory (Auger and Floresco 2016), visuospatial learning and memory (Auger and Floresco 2014) and cognitive flexibility (Enomoto et al. 2011). Intra-PFC administration of $GABA_A$ antagonists also increased phasic dopamine activity and locomotor response to stimulants (Enomoto et al. 2011), led to aberrant salience attribution (Piantadosi and Floresco 2014), and impaired social behavior and reward-related decision-making (Paine et al. 2015, 2017; Piantadosi et al. 2016). These latter findings suggest that deficiencies in PFC GABA signalling may also contribute to positive and negative symptoms of schizophrenia, respectively.

One of our previous studies revealed that PFC $GABA_A$ receptor antagonism produces a robust impairment in the classical reference/working memory (RM/WM) variant of the radial maze task (Auger and Floresco 2015). This was associated with increases in both RM and WM errors, mirroring the impairments observed in schizophrenia patients performing a virtual version of this task (Spieker et al. 2012). Another notable finding of this study that even though disinhibition of the PFC induces robust impairments on this task, PFC inactivation did not affect performance (Auger and Floresco 2015). Thus, it appears that manipulations that disinhibit PFC activity can interfere with certain cognitive or mnemonic functions that are not normally mediated by the PFC.

One possible explanation for these observations is that decreased PFC GABA function may lead to aberrant increases in activity of PFC projection neurons that consequently alters patterns of activation of neurons of downstream regions. This in turn may disrupt normal functioning of these downstream circuits that mediate PFC-independent forms of cognition. In this regard, PFC $GABA_A$ receptor antagonism elevates expression of the immediate early gene (IEG) c-Fos in medial PFC (Paine et al. 2011), but how local disinhibition of the PFC may impact neuronal activity in PFC efferent regions is not known. To address this question, we investigated how PFC $GABA_A$ antagonism impacts neuronal activation throughout the brain, using c-Fos expression as an index of neural activation. Given that PFC $GABA_A$ antagonism disrupts performance of the non-PFC-dependent RM/WM radial maze task, we were particularly interested in how PFC $GABA_A$ antagonism affected neuronal activation in animals that were trained and/or tested on this task. To this end, c-Fos expression following PFC $GABA_A$ antagonism was measured in behaviorally-naive animals, those that were trained on the RM/WM task, and rats that were trained and performed the behavior on the test day, enabling assessment of how PFC disinhibition alters terminal region neuronal activation following plasticity or behavioral activation associated with spatial memory processes. The PFC is one of the most densely connected structures within the brain, with many efferent projections that enable top-down control over circuits (Sesack et al. 1989; Vertes 2002, 2004). Therefore, we hypothesized local disinhibition of the PFC would alter neuronal activation in multiple regions receiving inputs from PFC. We targeted our analyses on regions known to be involved in spatial memory processes, including the striatum (Colombo et al. 1989; Floresco et al. 1997; Packard and White 1990; Schacter et al. 1989), thalamus (Aggleton and Nelson 2015; Harvey et al. 2017; Stokes and Best 1988, 1990), hippocampus (Becker et al. 1980; Duva et al. 1997; Floresco et al. 1997) and parahippocampal cortices (Otto et al. 1997; Pouzet et al. 1999). We also examined how this manipulation affected c-Fos expression in sensorimotor cortices adjacent to the PFC to gauge the extent of cortical activation induced by these treatments. In addition, we targeted the amygdala, because activity in this region may in some instances interfere with spatial memory functions of the hippocampus (White and McDonald 1993; McDonald and White 1993). Moreover, PFC disinhibition has been shown to perturb emotional and decision-making functions mediated in part by these nuclei (Ghods-Sharifi et al. 2009; Piantadosi and Floresco 2014; Piantadosi et al. 2016).

Materials and methods

Animals

Adult male Long Evans rats (Charles River; 275–300 g) were used in all experiments. Rats were acclimatized to the colony for ~ 1 week before undergoing surgery. After recovery, they were restricted to 85–90% of their free-feeding weight. Food restriction for cage controls commenced on the same day as animals that received surgery. Experiments were conducted in accordance with the Canadian Council on Animal Care and were approved by the University of British Columbia Animal Care Committee.

Surgical procedures

Subjects were implanted with bilateral guide cannulae targeting the prelimbic (PrL) PFC (flat skull co-ordinates AP: +3.2 mm, M/L: 0.8 mm and DV −2.8 mm), as in Auger and Floresco (2015). Anaesthesia was induced using ketamine/xylazine (50 and 10 mg/kg, respectively) and maintained using isoflurane vapor. Animals were given Anafen (5 mg/kg, s.c.) as a pre-surgery analgesic, and received the same dose of Anafen for at least 2 days following surgery. Animals were given ~ 10 days to recover from surgery before beginning maze training. They were given ad libitum access to food for 3–4 days following surgery, and then were food-restricted for a week prior to beginning maze training.

Experimental groups and timeline

The study consisted of seven experimental groups that differed based on drug treatment, maze training (i.e., behavioral history) and testing following infusion of drug or vehicle. Six of the groups received infusions of drug or saline prior to kill, while the 'Cage Control' group did not receive surgery, infusions, or behavioral training/testing. This last group was included to assess the impact of the surgical and infusion procedures on c-Fos expression. The 'Saline (SAL) Only' and 'Bicuculline (BIC) Only' groups received only infusions of vehicle or drug respectively (no behavioral training), and were included to assess the effects of intra-PFC $GABA_A$ antagonism under baseline conditions. Animals in the 'SAL Training' and 'BIC Training' groups underwent maze training until reaching criterion performance. On the following day, they received an infusion of vehicle or drug, but were not tested on the maze on the day of kill (i.e., no behavioral activation). These groups were included to assess whether plasticity associated with maze learning had an impact on c-Fos expression, particularly following prefrontal disinhibition. Finally, 'SAL Test' and 'BIC Test' groups were trained on the maze, received an infusion of vehicle or drug on test days, and performed the task prior to kiiling. The 'Test' groups were included to assess how performance of the maze task impacts c-Fos expression and how this may interact with PFC disinhibition. All animals that received infusions were killed 90 min after infusion or maze testing.

Following food restriction, animals in behavioral training groups underwent maze training until achieving criterion performance. Animals in cage control groups were treated identically, (i.e., they were food-restricted, handled and weighed daily) but they received no exposure to the maze. When animals in behavioral groups reached criterion performance, they received an infusion and were killed later that day. Animals in untrained conditions received mock infusions and were killed on the same days, so that animals in behavioral and non-behavioral groups underwent an equal duration of food restriction.

Behavioral procedures

All training and testing was conducted during the animals' light cycle. Testing was conducted on an 8-arm radial maze described previously (Auger and Floresco 2015). On the first day of training, rats were habituated to the maze for ten minutes with no food present. The next day, they underwent a second habituation session with sucrose-based food pellets (BioServ, Frenchtown NJ, USA) scattered across the maze. Animals were habituated once per day until they readily ate the pellets off of the maze. Once habituated, they commenced formal training with one trial per day. In each training session, the same four arms of the maze were baited for each animal, with different patterns of baiting across animals, with the caveat that no more than two consecutive arms were baited together. Errors were scored when a rat either (1) made an entry into an arm that was never baited, i.e., reference memory (RM) error or (2) re-entered an arm they entered earlier in the trial, i.e., short-term or working memory (WM) error. Rats were trained until they reached a criterion of approximately 1 or fewer total errors per day. At this point, they received a mock infusion. If the rat achieved criterion performance following the mock infusion, it received the test infusion the next day. If it did not, it was retrained until it displayed criterion performance following a mock infusion.

Drugs and microinfusions

Antagonism of PFC $GABA_A$ receptors was achieved using bicuculline methobromide (BIC). A dose of 50 ng BIC was chosen as it has been shown to produced robust deficits in performance of the RM/WM maze task (Auger and Floresco 2015) and other cognitive tasks (Auger et al. 2017; Enomoto et al. 2011; Piantadosi et al. 2016). Bilateral infusions were

made using 30-gauge injectors extending 0.8 mm below the guide cannulae. Drug or SAL vehicle were infused at a rate of 0.5 µl/75 s. Following infusions, the injectors were left in place for 1 min to allow for diffusion. Rats were then placed back in their home cages. Animals that were tested on the RM/WM maze task were placed on the maze 10 min after the infusion.

Tissue processing

Approximately 90 min after infusion and/or behavioral testing, animals were overdosed with chloral hydrate and transcardially perfused with 30 ml 0.9% saline following by 60 ml of 4% paraformaldehyde. Following extraction, brains were post-fixed in paraformaldehyde overnight and then transferred to 30% sucrose for cryoprotection and storage until slicing. Coronal 40 µm sections were collected in series of 10 along the rostral–caudal axis of the brain from the olfactory bulb to the cerebellum and stored at − 20 °C in antifreeze solution consisting of ethylene glycol, glycerol, and 0.1 M PBS.

Histology

To verify cannulae placements, sections containing PFC were mounted onto gelatin-coated slides and stained using Cresyl Violet. A representative cannula track is presented in Fig. 1a, and localization of approximate infusion placements are depicted in Fig. 1b.

c-Fos immunohistochemistry

The tissue was pre-washed at 4 °C overnight in 0.1 M PBS to remove antifreeze solution. Peroxidase activity was neutralized using a 30 min room temperature incubation in 0.3% hydrogen peroxide, followed by 3 × 10 min washes in 0.1 M PBS. Sections were incubated at 4 °C overnight in 1:1000 rabbit c-Fos antibody (SantaCruz Antibodies) diluted in 0.04% Triton-X and 3% normal goat serum in 0.1 M PBS. Following washing, tissue was incubated at 4 °C overnight in goat anti-rabbit biotinylated IgG (Vector Labs). The sections were then washed, incubated in ABC solution for 1 h at room temperature, and washed again before being developed using DAB solution (Vector Labs). Sections were then mounted onto Superfrost microscope slides, dehydrated and cleared in xylene, and coverslipped using Permount.

c-Fos quantification

Regions of interest and a list of abbreviations are depicted in Fig. 2. The average percent of immunoreactive (IR) area was quantified as an estimate of cell number as in Yagi et al. (2016), in light of the fact that BIC treatment led to high levels of c-Fos expression. Sections were examined and photographed at 40 × magnification using a Nikon E600 microscope. Digitized images were analyzed using ImageJ (National Institutes of Health, USA). The average optical density of 6 non-IR regions was used as a measure of background for each section, to account for differences in

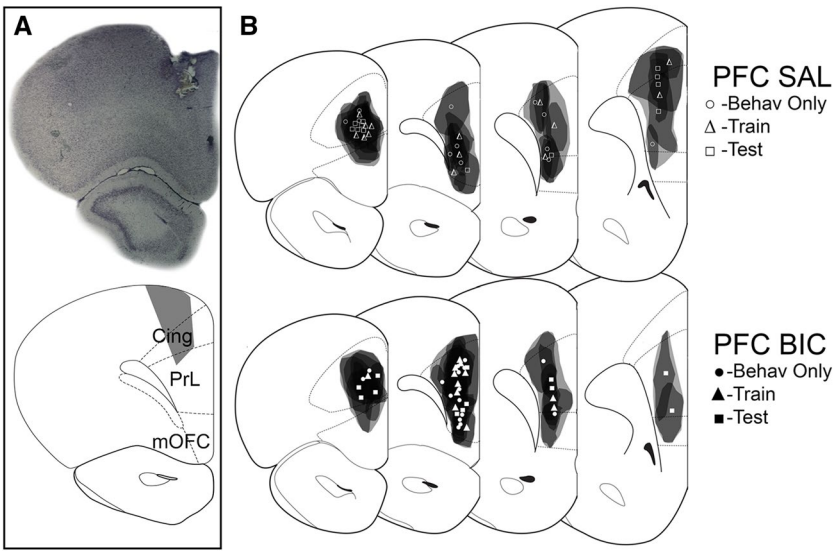

Fig. 1 Cannulae placements for animals receiving infusions of SAL or BIC. **a** Photomicrograph at × 10 magnification showing cannula placement. **b** Individual placement data. Shaded regions indicate the extent of the infusions, with darker regions reflecting areas with most infusion overlap and lighter regions indicating fewer infusions. Symbols are used to denote the group of individual subjects. Filled in symbols indicate BIC-treated animals, while open symbols indicated SAL-treated animals of Behav Only (filled circle/open circle), Trained (filled triangle/open triangle) and Tested (filled square/open square) groups

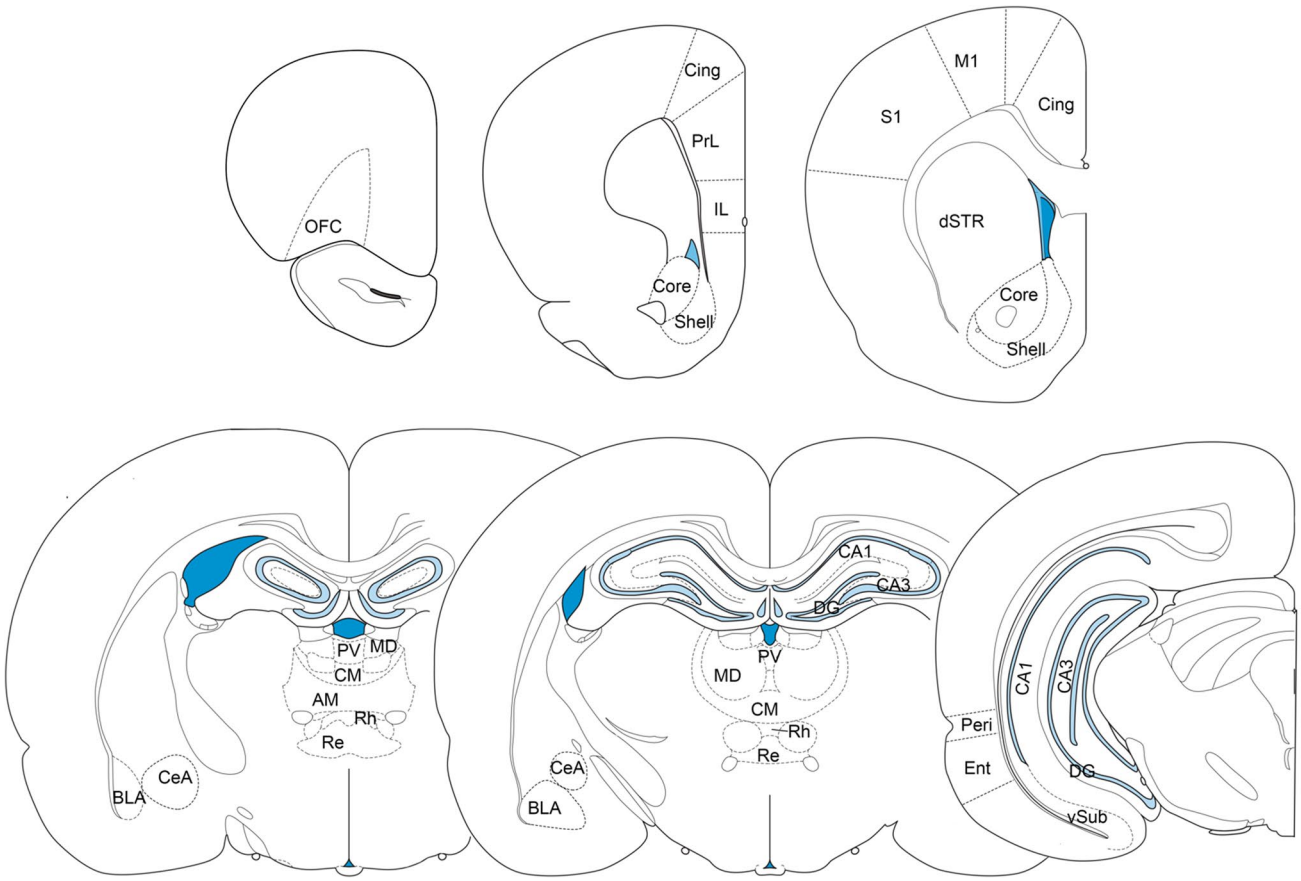

Fig. 2 Regions of interest for neuronal activation experiments. *OFC* lateral orbitofrontal cortex, *Cing* cingulate cortex, *PrL* prelimbic medial PFC, *IL* infralimbic medial PFC, *Core* nucleus accumbens core, *Shell* nucleus accumbens shell, *M1* primary motor cortex, *S1* primary somatosensory cortex, *BLA* basolateral amygdala, *CeA* central nucleus of amygdala, *PV* paraventricular nucleus of thalamus, *CM* centromedial nucleus of thalamus, *MD* mediodorsal nucleus of thalamus, *AM* anteromedial nucleus of thalamus, *Rh* rhomboid nucleus of thalamus, *Re* nucleus reuniens of thalamus, *DG* dentate gyrus of hippocampus, *CA3/1* cornu ammonis region 3/1 of hippocampus, *vSub* ventral subiculum, *Peri* perirhinal cortex, *Ent* entorhinal cortex

variations of background. IR area was then considered the area of the image with an optical density of 1.5 times the background. Brain regions of interest were traced, and the total area and IR area of the tracing recorded per section. Quantification of IR regions was typically carried out on 3–8 sections depending on the size of the region, and the reported percent IR area per region of interest reported is an average of these measurements. We confirmed that the % c-Fos IR area was a valid approximation of cell counts by comparing these measures for the PrL PFC of all animals in the experiment. Automated cell counting was performed using ImageJ on 4 images taken at 200× magnification from four separate sections of PrL PFC from each animal. A threshold of 1.5 times the background was applied and only cells darker than this were counted. The cells counted by the software were verified visually and any non-cellular items were excluded from the final count. As can be seen in Fig. 3, the % c-Fos IR area and c-Fos cell counts are highly correlated ($r = 0.88$, $p < 0.0001$). Further, there was

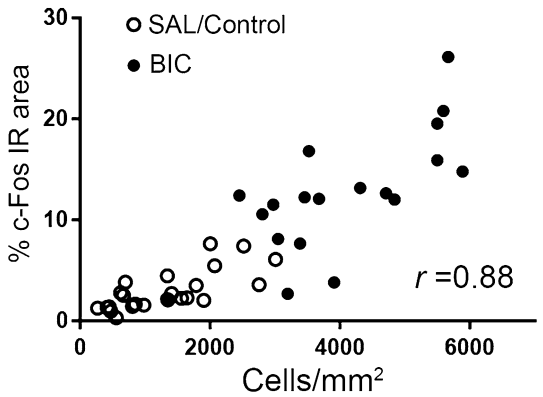

Fig. 3 The % c-Fos IR area is highly correlated with numbers of c-Fos positive cells counted in the PrL mPFC

a high correlation between these measures within both SAL ($r = 0.74$, $p < 0.0001$) and BIC treatment groups ($r = 0.72$, $p < 0.0005$).

Data analyses

Behavioral data from animals that performed the task on infusion test days (SAL Test and BIC Test) were analyzed with a mixed ANOVA with group as a between-subjects factor and error type (RM or WM) or latency type (time to initiate, TTI; or average time per choice, ATC) as repeated measures.

The c-Fos data were subjected to a multi-tier analysis. The initial analysis compared cFos expression in 23 different brain regions from animals with no behavioral training (baseline—Cage Controls, SAL only and BIC only groups), to clarify how PFC disinhibition may increase cFos expression in the absence of any behavioral manipulation. These data were analyzed with a mixed ANOVA with treatment group as a between-subjects factor and brain region as a repeated measure, and multiple comparisons were conducted using Dunnett's test with the SAL Only group as a control.

The more comprehensive analyses compared baseline groups with those that were tested and/or trained on the task. These analyses consisted of a series of factorial ANOVAs, comparing differences in c-Fos expression within related groups of brain regions, to reduce the number of comparisons made. These regions included frontal cortical, striatal, thalamic, amygdalar, hippocampal and parahippocampal regions. Each of these analyses were comprised of three-way ANOVAs, with drug treatment (SAL/Control or BIC) and behavioral history (No training, training, training + testing) as between-subjects factors and brain region as a within-subjects factors. The results of these ANOVAs were further decomposed with additional ANOVAs and Tukey's tests as necessary. In these analyses, the primary comparisons of interests were main effects of drug treatment, drug treatment x behavioral history, drug treatment × region and the three-way interaction. For the hippocampal regions, dorsal versus ventral position was included as an additional within-subjects factor, with the four-way interaction also being of interest.

Data from 1 to 2 brain regions were missing in a small number of animals ($n = 6$, total data points missing = 9/920 total points); for instance, because of a lost or damaged section during staining. These missing values were imputed using expectation maximization algorithm in Systat (version 13.00.05, Systat Software Inc), a method of imputation which does not alter the means of the groups. Inclusion of the imputed data points did not alter the outcome of any analyses when compared to those conducted with the data removed, except for analyses of thalamic nuclei, where a main effect of behavioral history and region × drug treatment interaction that were at trend levels of significance before inclusion of imputed data, achieved statistical significance with imputed data included. Imputation did not change the results of the ANOVA within the Rh or AM, where the missing points occurred. Rather, because imputation enabled inclusion of data from other nuclei where data were available, trend level effects achieved significance. Importantly, both the analyses from the dataset with values imputed or missing contained a significant three-way interaction within the thalamus. Thus, we chose to report the analyses containing values imputed using expectation maximization to keep a consistent subject number through all analyses, and to be able to include the completed data from other sub-regions in each analysis.

Results

In the present study, we assessed neuronal activation in a total of 46 animals, 25 of which were trained on the RM/WM task. On test days, trained animals were divided into groups that received only an infusion but were not exposed to the maze on that day ('SAL Train'; $n = 7$, or 'BIC Train'; $n = 6$), or those that both received a drug or vehicle infusion and performed the maze task on the test day ('SAL Test'; $n = 6$; and 'BIC Test' $n = 6$). The untrained groups consisted of animals receiving no infusion ('Cage Controls', $n = 7$), or infusions of vehicle ('SAL Only'; $n = 6$) or drug ('BIC Only'; $n = 8$).

PFC $GABA_A$ antagonism increases neuronal activation throughout brain under baseline conditions

We first analyzed the effects of intra-PFC SAL or BIC infusion on neuronal activation in untrained animals (baseline-Fig. 4). This analysis compared c-Fos expression in 23 brain regions in animals receiving no infusion ('Cage Controls', $n = 7$), or infusions of vehicle ('SAL Only'; $n = 6$) or drug ('BIC Only'; $n = 8$). PFC administration of BIC led to increased c-Fos expression throughout the brain (main effect of drug treatment: $F_{2,18} = 25.89$, $p < 0.001$). Overall, the levels of c-Fos expression varied significantly across regions (main effect of region: $F_{22,396} = 7.01$, $p < 0.001$), and the extent to which BIC treatment enhanced c-Fos expression was different across regions (significant interaction of drug treatment and region: $F_{44,396} = 2.93$, $p < 0.001$). Subsequent partitioning of the interaction term with one-way ANOVAs and Dunnett's tests with treatment group as a between-subjects factor and 'SAL Only' as the control group revealed that c-Fos expression was greater in BIC Only in comparison to SAL Only in most studied regions, as displayed in Fig. 4 and Table 1. One notable exception to this was within the hippocampus proper, where no differences between groups were found in any sub-region (DG, CA3 or CA1). We chose to combine the data of dorsal and ventral HPC to simplify the analysis, because, though a preliminary analysis revealed

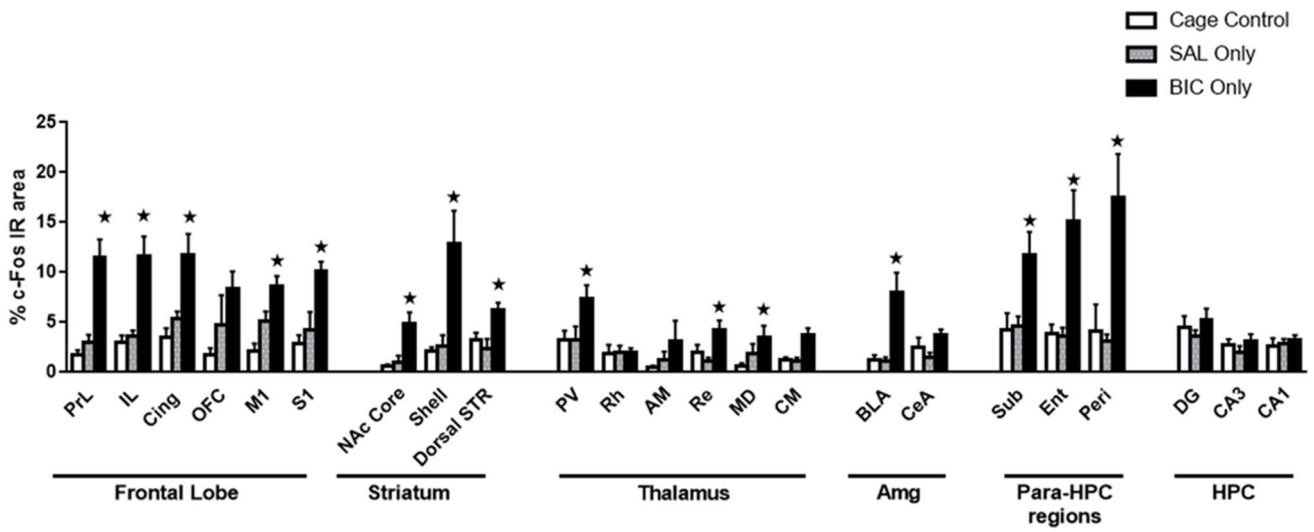

Fig. 4 Neuronal activation data for untrained animals (baseline). Animals receiving intra-PFC BIC infusions had increased c-Fos expression in most structures, in comparison to cage controls or intra-PFC SAL. One notable exception was in HPC sub-regions, which did not display an increase in c-Fos expression following PFC disinhibition under baseline conditions. $^{*}p < 0.05$ versus SAL

Table 1 Results of ANOVAs conducted on neuronal activation data in baseline animals in individual brain regions with treatment as between-group factor

Region	*F* ratio	Region, Cont'd	*F* ratio, Cont'd
PrL	20.44***	Rh	0.02 n.s
IL	10.01**	MD	9.56**
OFC	3.12 n.s	CM	2.85 n.s
Cing	9.05**	DG	0.54 n.s
M1	12.78***	CA3	0.78 n.s
S1	12.22***	CA1	0.23 n.s
Core	6.67*	Ent	9.96**
Shell	6.58**	Peri	6.49**
STR	7.20**	Sub	5.56*
PV	4.31*	BLA	8.05**
AM	0.95 n.s	CeA	2.37 n.s
Re	3.80*		

$^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

that c-Fos expression was slightly increased in ventral relative to dorsal sub-regions ($F_{1,18} = 5.42$, $p < 0.05$), dorsal–ventral position did not interact with treatment (both Fs < 0.35) and the lack of increased activation following BIC treatment was observed through all HPC sub-regions.

Lastly, throughout the brain, there were no differences in c-Fos expression between SAL Only and cage controls in any of the regions assessed, as indicated by a separate ANOVA that compared only these two treatment groups (no effect of treatment ($F_{1,11} = 0.44$, n.s.) or treatment × brain region interaction ($F_{22,242} = 0.76$, n.s.). In light of the fact the SAL Only and cage control groups did not differ, data from these two groups were combined into a single control group designated 'Control-No Train' that was used for subsequent analyses that included data from those trained on the radial maze. Taken together, these analyses indicate that PFC $GABA_A$ antagonism induces broad increases in neuronal activation in numerous downstream brain regions.

PFC GABA antagonism impairs RM/WM radial maze performance

Previous studies from our laboratory have shown that PFC $GABA_A$ antagonism disrupts performance of the RM/WM radial maze task using a within-subjects design and a task variant that required animals to perform 5 back-to-back trials of the RM/WM task. In that study, animals made significantly more RM and WM errors on both the first and subsequent trials of the test session (Auger and Floresco 2015). In the present study, a subset of the animals were trained on a standard, single trial variant of the RM/WM task and on test days, some of these rats that received either SAL or BIC infusions prior to being tested on the maze and killed. Analysis of the behavioral data revealed that intra-PFC BIC significantly impaired performance (Fig. 5a; $F_{1,10}$=9.98, $p = 0.01$). No significant treatment × error type interaction was observed ($F_{1,10} = 0.78$, n.s.), indicating that intra-PFC BIC induced a comparable increase of both RM and WM errors. Taken together with the findings from our previous study, the present results indicate that PFC $GABA_A$ antagonism disrupts both RM and WM, and that this impairment is observed using both within- and between-subjects designs and using single or multiple daily trials.

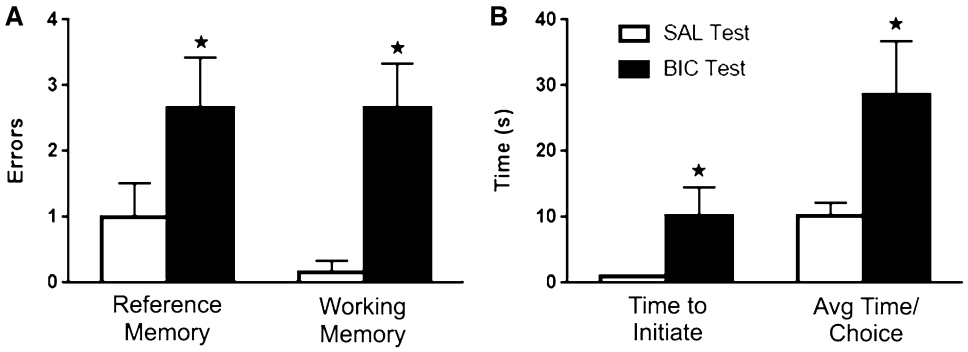

Fig. 5 Behavioral data for animals tested in the RM/WM radial maze. **a** Subjects receiving intra-PFC BIC infusions made significantly more RM and WM errors in compared to SAL-treated animals. **b** Subjects receiving intra-PFC BIC infusions were both slower to initiate the trial and to make subsequent choices. *$p < 0.05$, main effect of BIC treatment

Latency data were analyzed with a similar mixed ANOVA, with treatment as between-subjects factors and task phase (time to initiate the trial, TTI and average time per subsequent choice, ATC) as within-subjects factors (Fig. 5b). As was observed in our previous study (Auger and Floresco 2015), PFC $GABA_A$ antagonism significantly increased both types of choice latencies. Analysis of the data yielded a significant main effect of drug treatment ($F_{1,10} = 6.56$, $p < 0.05$) but no drug treatment × phase interaction ($F_{1,10} = 1.27$, n.s.).

PFC GABA antagonism increases neuronal activation following training and testing on the RM/WM task

Reducing PFC GABA transmission disrupted performance of the RM/WM task. In marked contrast, inactivation of the PFC does not affect RM/WM radial maze performance, indicating that normal patterns of activity in this region is not essential for efficient search on this task (Auger and Floresco 2015). In light of this consideration, one of the main objectives of this study was to assess how PFC disinhibition influenced neuronal activation in animals that were trained and tested on the RM/WM task. Thus, we compared c-Fos expression in groups of animals receiving intra-PFC infusions of BIC or vehicle/control groups that were (1) untrained ('BIC Only' and 'Control-No Training'), (2) trained on the RM/WM task ('BIC Train' and 'SAL Train') and (3) trained on the RM/WM task had performed the task on test day ('BIC Test' and 'SAL Test'). The data were analyzed using factorial ANOVAs with intra-PFC BIC treatment and behavioral history as between-subjects factors, and brain region as a within-subjects factor. To simplify the analysis, rather than analyzing all brain regions together, data were groups into related brain regions and analyzed with separate ANOVAs.

a. *Frontal regions* Neuronal activation data from PFC regions that included the prelimbic (PrL), infralimbic (Il), lateral orbitofrontal cortex (OFC), cingulate (Cing), primary motor (M1) and primary somatosensory (S1) were analyzed together using a factorial ANOVA (Fig. 6a). A representative tracing of the PrL is depicted in Fig. 6b. PFC $GABA_A$ antagonism significantly increased c-Fos expression in all areas assessed, as indicated by a main effect of treatment ($F_{1,40} = 70.70$, $p < 0.001$). The levels of c-Fos expression varied between regions ($F_{5,200} = 7.65$, $p < 0.001$), and more importantly, the analysis revealed that the extent to which intra-PFC BIC increased c-Fos expression also differed depending on region (treatment x region interaction; $F_{5,200} = 2.67$, $p < 0.05$). Partitioning this interaction revealed that BIC treatment increased c-Fos expression in all brain regions relative to the control groups (Control-No Train, Sal Train and Sal Test; all ps < 0.01). Moreover, BIC-induced activation was higher in medial PFC regions near the infusion site (PrL, IL, Cing), relative to the other frontal regions ($p < 0.01$). On the other hand, c-Fos expression in the control groups did not differ across regions.

Notably, there were no significant treatment × behavioral history or treatment × region × behavioral history interactions (all Fs < 1.1, n.s.). Behavioral training had no significant effects in control animals (Fig. 6c–e). Further, the magnitude of neuronal activation induced by BIC treatment did not differ between rats that had no maze exposure and those that were either trained and/or tested on the maze prior to BIC treatment (Fig. 6f–i).

b. *Striatum* Analysis of the neuronal activation data from the core and shell of the nucleus accumbens (NAc) and dorsal striatum (dSTR) (Fig. 7a) revealed c-Fos expression was significantly increased throughout these regions following PFC $GABA_A$ antagonism (main effect of treatment; $F_{1,40} = 27.14$, $p < 0.001$). The analysis also yielded a significant main effect of region ($F_{2,80} = 28.46$, $p < 0.001$), and treatment × region interaction ($F_{2,80} = 16.14$, $p < 0.001$). Simple main effects analyses revealed that intra-PFC BIC significantly increased c-Fos expression in all regions relative to the control groups ($p < 0.001$), but this effect was significantly greater in the NAc shell ($p < 0.01$) compared to

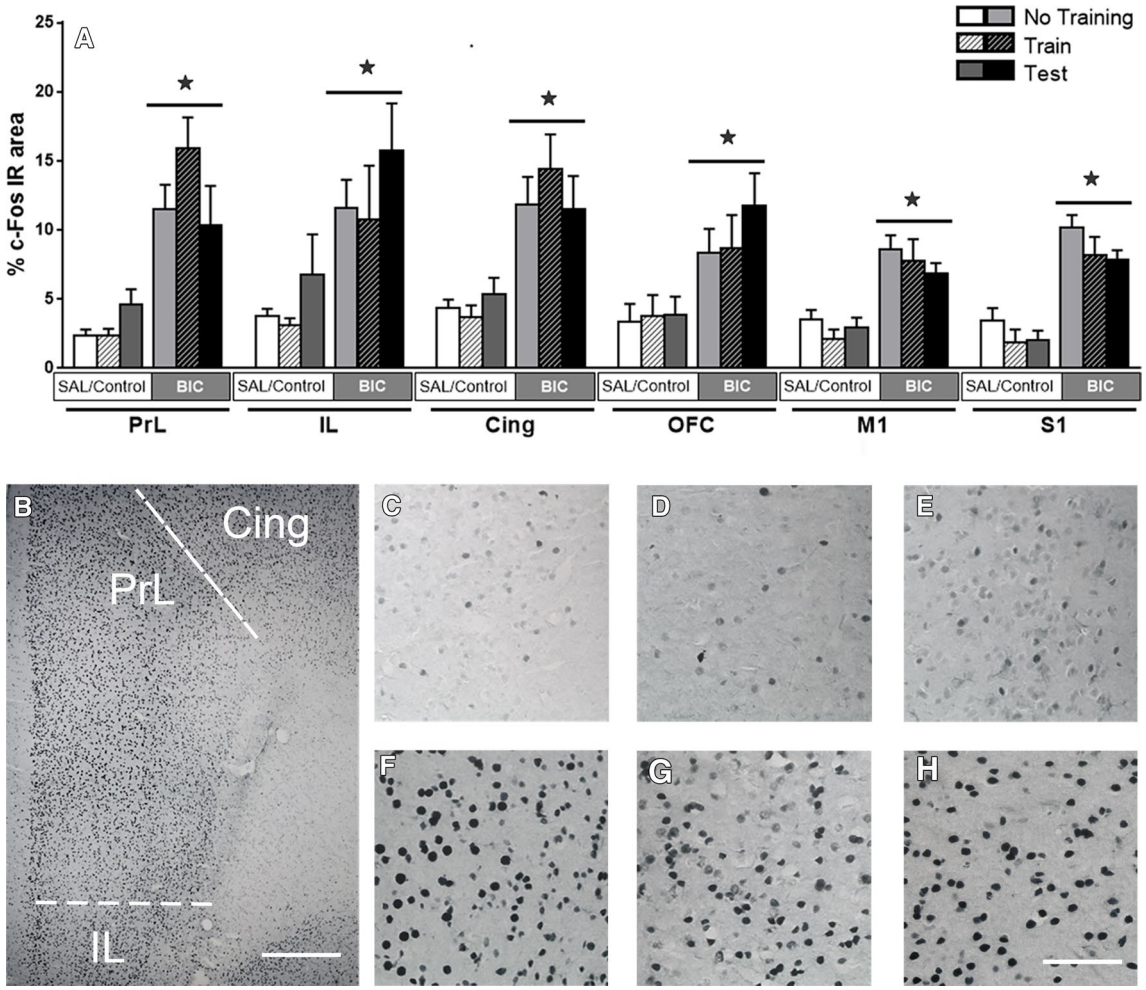

Fig. 6 Frontal cortical neuronal activation data in animals trained and tested on the RM/WM radial maze. **a** Intra-PFC BIC treatment increased c-Fos expression throughout frontal regions, irrespective of behavioral history. B-I, representative micrographs of the PrL in all groups. **b** Photomicrograph of PrL PFC of BIC Only at 40× magnification. Scale bar corresponds to 500 µm. **c–e** SAL treatment groups, **f–i** BIC treatment groups, 200× magnification. Scale bar corresponds to 100 µm. **c, f** Behav Only groups. **d, g** Trained groups. **e, i** Tested groups. *$p < 0.05$, main effect of BIC treatment

the other two regions (see Fig. 7b, c). Moreover, there were no main effects or interactions with the behavioral history factor (all Fs < 1.72, n.s.). Taken together, this analysis indicated that PFC disinhibition increases neuronal activation throughout the STR, with this effect being most pronounced in the NAc shell. Furthermore, the magnitude of neuronal activation induced by effects of PFC disinhibition was not modulated by previous behavioral history.

c. *Thalamus* Neuronal activation data from the paraventricular (PV), anteromedial (AM), nucleus reuniens (Re), rhomboid (Rh), mediodorsal (MD) and centromedial (CM) nuclei of the thalamus were analyzed (Fig. 8a) and this revealed PFC $GABA_A$ antagonism significantly increased c-Fos expression across these thalamic regions (main effect of drug treatment: $F_{1,40} = 12.31$, $p = 0.001$). Similar to PFC and STR, the levels of c-Fos expression varied in different thalamic regions (main effect of region: $F_{5,200} = 27.45$, $p < 0.001$), with this effect accompanied by a drug treatment × region interaction ($F_{5,200} = 2.32$, $p < 0.05$, see Fig. 8b–e). Of particular interest, the analysis also produced a three-way interaction of drug treatment, region and behavioral history ($F_{10,200} = 3.34$, $p < 0.001$). This was subsequently partitioned with a series of two-way ANOVAs, analysing data from each thalamic region separately, with treatment and history as between-subjects factors.

For the PV, the ANOVA revealed that intra-PFC BIC treatment increased c-Fos expression in all behavioral groups (main effect of drug treatment: $F_{1,40} = 10.83$, $p < 0.01$). However, there was also a main effect of behavioral history

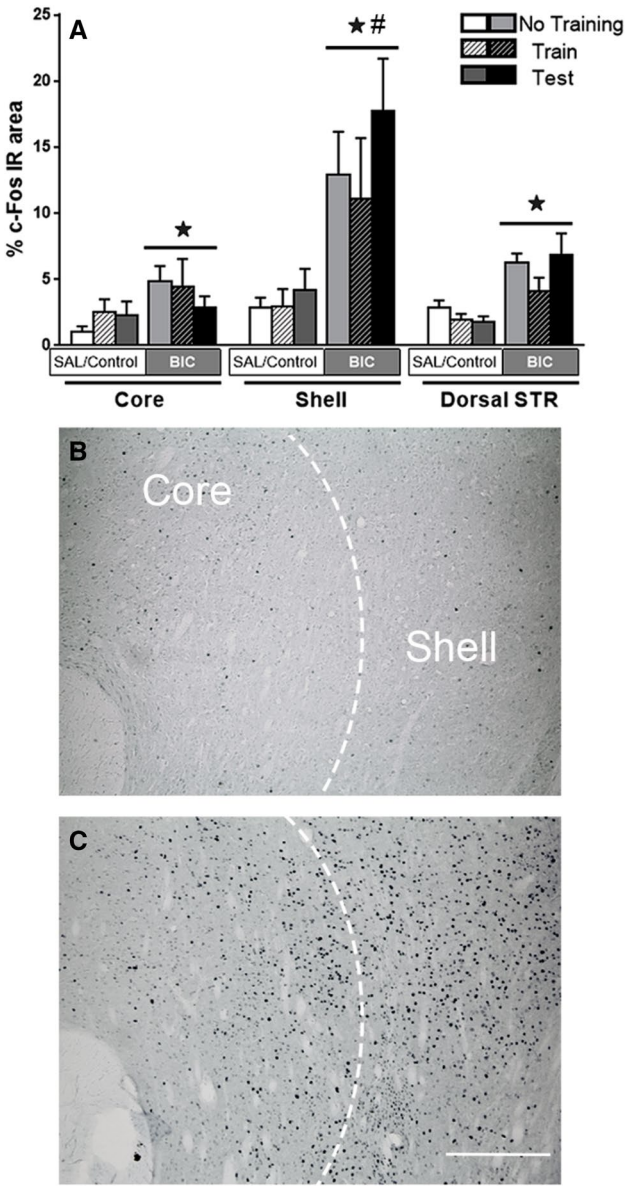

Fig. 7 Striatal neuronal activation data in animals trained and tested on the RM/WM radial maze. **a** Intra-PFC BIC treatment significantly increased neuronal activation throughout the STR irrespective of behavioral history, although the increase was most prominent in the NAc Shell. **b, c** Representative micrographs of the NAc of **b** SAL and **c** BIC-treated animals with no behavioral training or testing at ×100 magnification. Scale bar denotes 250 µm. $^{*}p<0.05$, main effect of BIC treatment, $^{\#}p<0.05$ NAc shell versus other striatal regions

($F_{2,40}=4.35$, $p<0.05$) and in particular, the effects of behavioral training/testing were different in SAL versus BIC treatment groups (drug treatment × behavioral history interaction: $F_{2,40}=3.48$, $p<0.05$). Subsequent analyses further revealed that for the control groups, higher expression of c-Fos was observed in the PV of rats that had performed the task on test day relative to the other groups ($F_{2,23}=13.05$, $p<0.001$, and Tukey's, $p<0.05$). In comparison, for the BIC-treated groups, behavioral history had no significant effect on c-Fos expression within the PV nucleus ($F_{2,17}=1.91$, n.s.), although the levels of expression was numerically greater in rats with maze experience compared to the BIC-No Train group. Furthermore, even though BIC treatment significantly increased c-Fos expression within the PV of all behavioral groups, the level of c-Fos expression in the PV was not different between SAL- and BIC-treated animals who performed the task on the test day ($F_{1,9}=0.37$, n.s.). Thus, testing on the maze increased neuronal activation in the PV, as did PFC $GABA_A$ antagonism. However, there did not appear to be an additive effect of these two treatments.

In the Rh nucleus, both PFC $GABA_A$ antagonism ($F_{1,40}=6.54$, $p<0.05$) and behavioral history ($F_{2,40}=4.80$, $p<0.05$) affected c-Fos expression, although no significant interaction between the two factors was observed ($F_{2,40}=2.03$, n.s.). The main effect of behavioral history reflected the fact that across both treatment groups, animals with maze experience displayed higher levels of c-Fos expression compared to the No Train groups (Tukey's, $p<0.05$). Although no significant effect of drug was found when each of the behavioral groups were analyzed separately (all Fs <3.02, n.s.), both the trained and tested groups showing more c-Fos expression relative to the untrained groups following PFC $GABA_A$ antagonism. From these results, we conclude (i) maze exposure alone increased activity in the Rh and (ii) PFC $GABA_A$ antagonism specifically increased neuronal activation in the Rh of animals that had been trained on the RM/WM task.

For the remaining thalamic nuclei (AM, Re, MD and CM), individual factorial ANOVAs revealed that only PFC $GABA_A$ antagonism impacted levels of c-Fos expression (main effect of treatment; all Fs >5.35, $p<0.01$), with no effects of behavior or interactions of drug treatment and behavioral history (all Fs <2.2, n.s.). Thus, PFC $GABA_A$ antagonism increased neuronal activation in these regions to a similar degree in all groups, irrespective of behavioral history.

d. *Temporal lobe structures* Separate factorial ANOVAs were performed for amygdalar (Amg, Fig. 9), parahippocampal structures and hippocampal (HPC) regions (Fig. 10). The Amg regions included the basolateral Amg (BLA) and central Amg (CeA) (Fig. 9a). The analysis revealed a main effect of drug treatment ($F_{1,40}=23.91$, $p<0.01$), as well as three-way interaction of drug, behavioral history and region ($F_{2,40}=15.16$, $p<0.05$, see also Fig. 9b, c). PFC disinhibition increased c-Fos expression within the BLA ($F_{1,40}=23.60$, $p<0.001$) in a manner that was independent of behavioral history (treatment × behavioral history interaction; $F_{1,40}=1.31$, n.s.). Analysis of c-Fos expression in the CeA revealed only a main effect of drug ($F_{1,40}=10.41$,

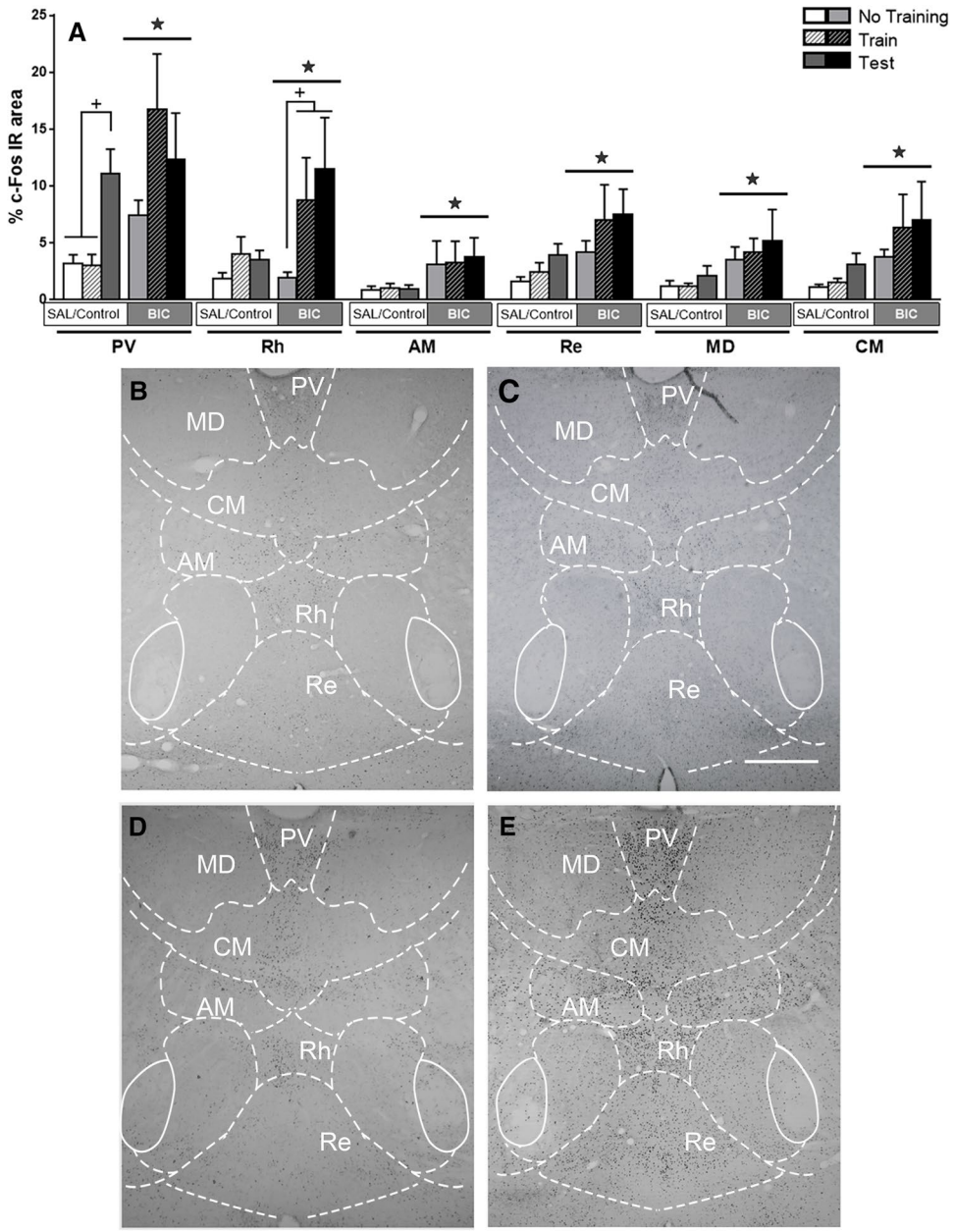

Fig. 8 Thalamic neuronal activation data in animals trained and tested on the RM/WM radial maze. **a** Intra-PFC BIC treatment significantly increased c-Fos expression throughout thalamic regions. In the PV, testing on the maze increased c-Fos expression in SAL-treated animals. No increase in c-Fos expression was observed in the Rh of BIC-treated animals that were untrained. **b**–**e** Representative micrographs of the thalamus of **b** Behav Only, **c** SAL Test, **d** BIC Only and **e** BIC Test groups. Scale bar denotes 500 µm. *$p < 0.05$, main effect of BIC treatment. $^{+}p < 0.05$ interaction of treatment and behavioral group

$p < 0.01$, all other Fs < 2.52, n.s.). Moreover, BIC treatment increased levels of neuronal activation to a larger degree in the BLA when compared to the CeA ($F_{1,40} = 9.02$, $p < 0.01$), whereas in SAL-treated animals, no observable differences in neuronal activation relative to behavioral history were observed ($F_{2,23} = 0.3$, n.s.). When taken together, these analyses reveal that BIC treatment increased neuronal activation in both Amg sub-regions, but to a greater degree in the BLA versus CeA.

For the parahippocampal cortical structures, which included the entorhinal (Ent) and perirhinal (Peri) cortices, and the ventral subiculum (vSub), only a main effect of BIC treatment was found ($F_{1,40} = 35.40$, $p < 0.001$, all other Fs < 1.35, n.s.; Fig. 10a). Thus, PFC $GABA_A$ antagonism increased neuronal activation throughout these structures, regardless of behavioral history (see Fig. 10d, e). Furthermore, all these structures tended to have similar levels of c-Fos expression and did not vary in the extent to which PFC $GABA_A$ antagonism increased their activation.

In contrast to the relatively straightforward effects in the Amg and parahippocampal regions, the effects of PFC disinhibition on neuronal activation in the HPC (including DG, CA3 and CA1 subfields) were more complex. Note that in our initial experiment, reducing PFC $GABA_A$

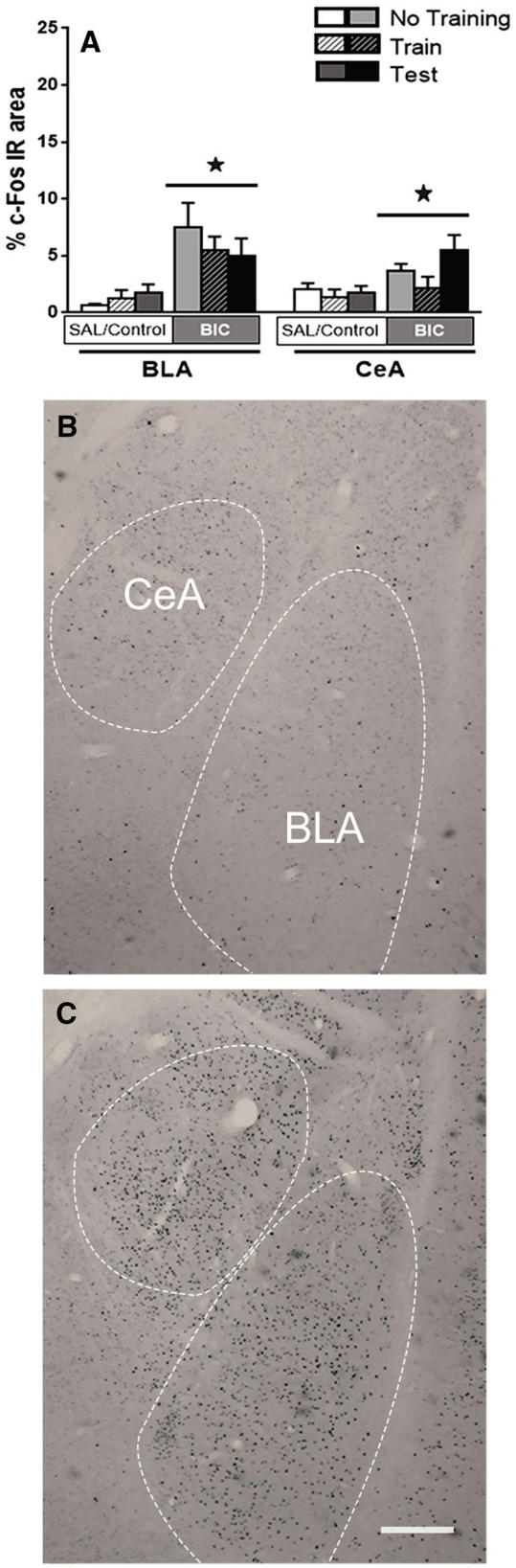

Fig. 9 Amygdala neuronal activation data in animals trained and tested on the RM/WM radial maze. **a** Intra-PFC BIC treatment increased c-Fos expression in BLA and CeA. **b, c** Representative photomicrographs taken at ×40 magnification of SAL Only (**b**) and BIC Only groups (**c**). Scale bar denotes 500 µm. *$p < 0.05$, main effect of BIC treatment

transmission did not increase c-Fos expression in the HPC of rats that remained in their home cages for the duration of the experiment (Fig. 5). However, when the analyses incorporated data from rats with maze experience (Fig. 10b), this produced significant main effects of drug treatment ($F_{1,40} = 24.72$, $p < 0.001$), behavioral history ($F_{2,40} = 8.84$, $p = 0.001$) and region ($F_{2.80} = 31.71$, $p < 0.001$). There was also a drug treatment × region interaction ($F_{2,80} = 3.25$, $p < 0.05$), which reflected higher levels of c-Fos expression in the DG of BIC-treated rats relative to the CA3 or CA1. Of particular interest, this analysis also yielded a significant drug treatment × behavioral history interaction ($F_{2,40} = 5.20$, $p = 0.01$). The three-way interaction was not significant ($F_{4,80} = 0.70$, n.s.). However, there was a significant four-way interaction of drug, behavior, sub-region and dorsal versus ventral position ($F_{4,80} = 3.50$, $p < 0.05$).

The drug treatment × behavioral history interaction was partitioned using separate one-way ANOVAs with behavioral history as a between-subjects factor for data obtained from SAL/Control and BIC-treated animals. For SAL/Control rats, the results of the ANOVA only approached trend levels of significance ($F_{2,34} = 2.98$, $p = 0.07$). Notably, c-Fos expression in these animals was highest in the DG and CA3 in rats that performed the task on test day, compared to the other two groups. Thus, in the absence of drug treatment, engagement of a spatial task may have led to a subtle increase in HPC activation.

In contrast, behavioral history had a major influence on how BIC treatment affected HPC neuronal activation ($F_{2,34} = 13.64$, $p < 0.001$). Specifically, c-Fos levels were higher in BIC-treated rats that were trained and/or tested on the maze compared those that did not receive any training ($p < 0.01$, see Fig. 10f–i). Additional partitioning of the treatment × history interaction confirmed that BIC treatment did not increase c-Fos expression in rats that did not receive training relative to controls ($F_{1,19} = 1.38$, n.s.). However, for rats in the train and test history conditions, BIC treatment did increase hippocampal neuronal activation relative to SAL (both Fs > 7.97, both ps < 0.05). Taken together, these analyses indicate that reducing PFC GABA activity by itself is insufficient to increase neuronal activation in the HPC. In contrast, it appears that neural plasticity associated with learning a spatial memory task alters PFC output pathways so that disinhibitory increases in frontal lobe activity can cause aberrant increases in HPC activation.

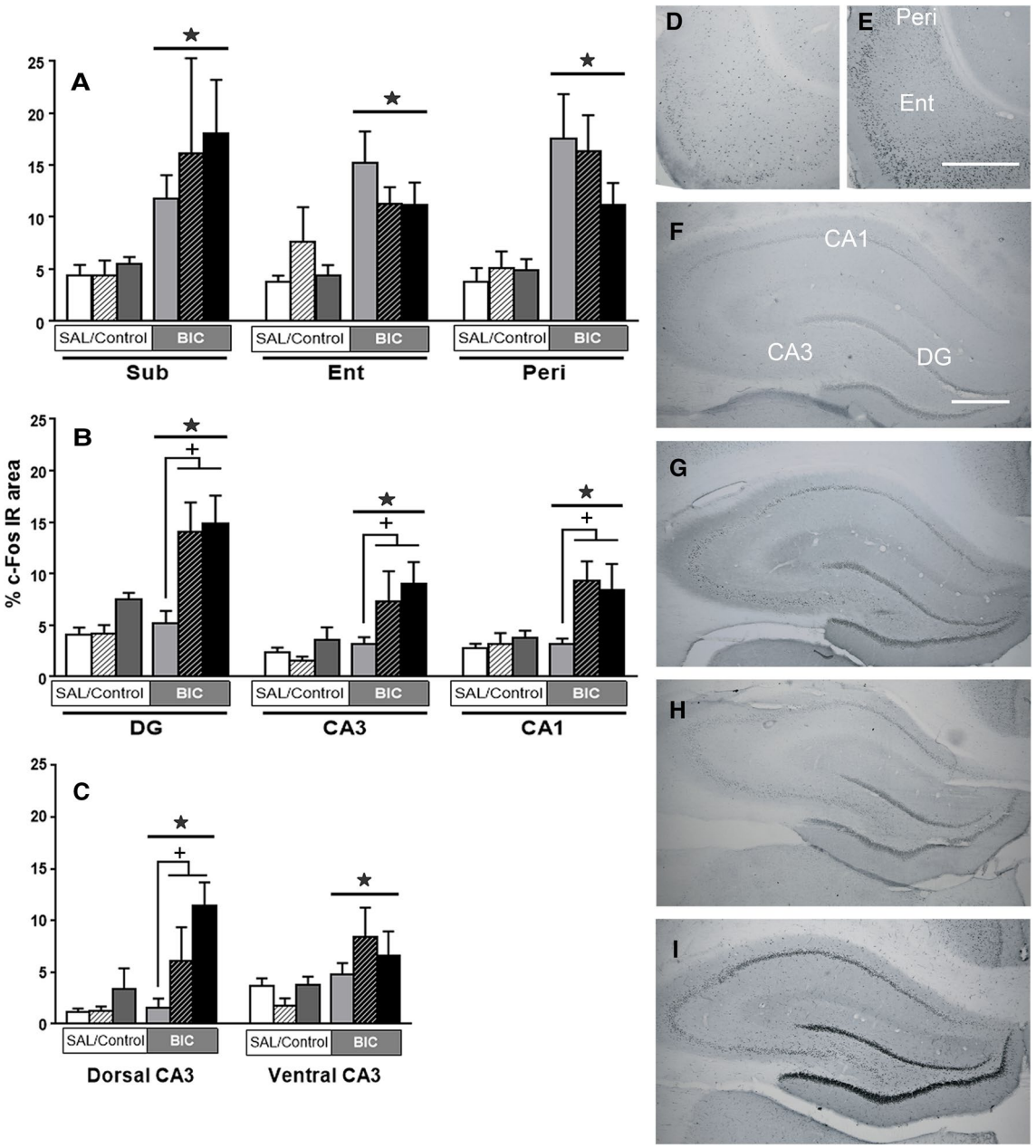

Fig. 10 Temporal lobe neuronal activation data in animals trained and tested on the RM/WM radial maze. Intra-PFC BIC treatment increased c-Fos expression in parahippocampal regions, including Ent and Peri cortices and the subiculum (**a**) and hippocampus proper (**b**). **c** In CA3 of HPC, a significant increase in activation in trained versus untrained animals following BIC treatment was observed only in the dorsal, but not ventral, aspect. No differences were observed between dorsal and ventral DG or CA1 (not shown). **d, e** Representative micrographs of Peri and Ent cortices taken at ×40 magnification. Scale bar represents 1000 µm. **d** SAL Only, **e** BIC Only. **f–i** the dorsal HPC of **f** No Training, **g** SAL Test, **h** BIC Only and **i** BIC Test groups. Scale bar denotes 500 µm.*$p < 0.05$, main effect of BIC treatment; $^{+}p < 0.05$ interaction of treatment and behavioral group, with trained animals receiving BIC infusions showing higher levels of activation than untrained animals in all HPC sub-regions

Across all treatment and behavioral history groups, there tended to be higher activation in ventral HPC in comparison to dorsal HPC, though, this effect did not achieve statistical significance ($F_{1,40} = 3.2$, $p > 0.08$). However, there was a significant four-way interaction of dorsal versus ventral position, drug, behavioral history, and HPC sub-region ($F_{4,80} = 3.50$, $p < 0.05$). Partitioning of this interaction revealed no differences between the patterns of effects in dorsal versus ventral DG or CA1 (both Fs < 1.88, $p > 0.05$), with higher activation observed in trained groups that received BIC. In CA3 of BIC-treated animals, the selective increase in activation in trained animals was observed

only in dorsal (Fig. 10d; $F_{2,40} = 3.61$, $p < 0.05$), not ventral CA3 ($F_{2,40} = 1.964$, $p > 0.15$). However, visual inspection of the ventral CA3 data suggests a similar pattern of effects in comparison to other HPC sub-regions. Furthermore, in the analyses that included the whole HPC, there was a significant drug × behavior history interaction, but no interaction of drug, behavioral history, and sub-region suggesting that neuronal activation following BIC treatment was higher in trained versus untrained animals throughout the HPC.

Discussion

The main finding of the present study is that prefrontal cortex (PFC) disinhibition increases neuronal activation in PFC efferent regions, including throughout the striatum, thalamus, amygdalar and other cortical regions. Remarkably, increases in neuronal activation in the hippocampus, a region that does not receive direct PFC input (Sesack et al. 1989; Vertes 2004), were only observed in animals that had been trained on the RM/WM maze task. A similar pattern of activation was observed in the rhomboid thalamic nucleus, suggesting remodelling within this PFC-thalamic-hippocampal pathway may have potentiated the effects of PFC disinhibition, together with either enhanced entorhinal cortical input or local hippocampal plasticity. Thus, our findings raise the possibility that experience-dependent plasticity within circuits, in this case induced by spatial learning, may affect the extent to which local imbalances in excitation and inhibition within the PFC affect the activity and function of other brain regions.

Baseline effects of PFC disinhibition

In the present study, infusions of $GABA_A$ antagonist were predominately localized within the anterior PrL PFC (Fig. 1), with these treatments causing a marked increase in neuronal activation near the infusion area, as has been reported by another group (Paine et al. 2011). Comparable levels of activation were observed through adjacent IL and Cing cortical regions. Likewise, increased activation was also observed in more distant cortical regions, including the Ent and Peri cortices. Furthermore, cortical regions that do not receive direct inputs from the PrL, including M1 and S1 (Sesack et al. 1989; Vertes 2004), also showed increases in activation, perhaps via inputs from Cing (Sesack et al. 1989) or relay thalamic nuclei (Haque et al. 2010). However, the magnitude of increase in sensorimotor regions tended to be less than cortical regions that were directly connected to the PrL PFC. Thus, local PFC disinhibition appears to have widespread effects on cortical activity patterns, in some cases even having polysynaptic effects at baseline, though monosynaptic effects tended to be strongest.

PFC $GABA_A$ antagonism also increased neuronal activation in many subcortical regions receiving PFC innervation, including throughout the STR, Amg, and thalamus. These findings were similar to a recent study employing [^{18}F] flurodeoxyglucose PET imaging that showed increased neural activity throughout the brain following unilateral PFC administration of the same dose of BIC in anaesthetized rats (Parthoens et al. 2015). However, we notably observed no increase in c-Fos expression in the HPC, which does not receive direct PFC innervation, in BIC-treated animals under baseline conditions (no behavioral training). In STR regions, the effect of BIC treatment was greater in the NAc shell in comparison to the core or dSTR. The NAc shell receives direct inputs from more ventral aspects of the medial PFC, including the PrL and IL cortices (Sesack et al. 1989) where the majority of BIC infusions occurred. On the other hand, the NAc core and dSTR tend to receive inputs from more dorsomedial portions of the PFC, i.e., the Cing (Sesack et al. 1989), or perhaps disynaptic input via the CM thalamic nucleus (Vertes 2004; Vertes et al. 2012). Thus, similar to cortical regions, regions that received direct PrL inputs tended to have a larger induction of c-Fos expression. However, some thalamic nuclei, including the AM and CM, which receive substantial PrL input (Vertes 2002, 2004), did not show significant changes in activation when only baseline data was analyzed. Therefore, additional factors including numbers of PFC projection neurons targeting the area, degree of axonal arborisation of PFC efferents, number of polysynaptic PFC inputs into the region or local inhibitory tone may also regulate changes in activation in response to altered PFC inputs.

Effects of PFC disinhibition following spatial learning or performance

In most of the brain regions examined, the effects of PFC disinhibition were the same in untrained versus trained or tested groups. This was true even in regions known to be involved in spatial learning and memory or goal-directed behavior, such as anterior and MD thalamus (Stokes and Best 1988, 1990; Floresco et al. 1999), striatum (Floresco et al. 1997) and parahippocampal structures (Otto et al. 1997) that may have been recruited during performance of the maze behavior. In some instances, regions that did not have significant effects of BIC treatment in baseline conditions (i.e., CeA, AM, CM, and OFC) showed main effects of drug, but no interactions of drug and behavior, suggesting PFC BIC treatment increased neuronal activation regardless of behavioral history in these regions as well. The different results of the more restricted baseline analyses and those that included all rats may reflect that the effect of BIC treatment was of lesser magnitude or reliability in these regions in comparison to others that displayed significant increases

in c-Fos expression after BIC treatment when only baseline animals were analyzed. For instance, the level of neuronal activation induced by BIC treatment in these regions may have been more variable because of subtle differences in cannulae placement or individual differences in strength of connectivity between PFC and these regions. It is likely that the significant effects observed in these regions after inclusion of data from trained animals are attributable to the additional statistical power afforded by these analyses, i.e., that more animals received PFC infusions of BIC versus SAL in the analysis that included different behavioral groups. Specifically, the initial baseline analyses consisted of 8 rats in the drug group and 6–7 in the control groups, whereas the full analyses included ns of 26/20 in the drug/control groups. Nevertheless, the data indicate that previous experience with the maze task or engaging in search behavior on test day did not affect the extent to which PFC disinhibition caused aberrant increases in activity in most regions.

The most notable exception to the observations described above was in the HPC, which does not receive direct input from PFC regions, and only receives disynaptic inputs via the midline thalamus and Ent (Prasad and Chudasama 2013). In this region, neuronal activation was not altered by PFC $GABA_A$ antagonism in animals that did not have any experience with the spatial task. However, PFC disinhibition did increase HPC activation in rats trained on the RM/WM task, regardless of whether they performed the behavior on test day. A parsimonious explanation for this effect is that spatial learning may have strengthened inputs to the HPC that potentiated the effects of PFC disinhibition, leading to enhanced HPC activation. The dorsal HPC receives PFC input via lateral Ent and AM and Rh nuclei of thalamus, while ventral CA1 receives this input via nuclei of the midline thalamus, including the Re, PV, and Rh (Cassel et al. 2013; Prasad and Chudasama 2013). Intriguingly, the Rh thalamic nucleus, which projects to CA1 of HPC as well as NAc, BLA and several frontal cortical regions (Vertes et al. 2006), displayed a similar pattern of activation to the HPC, with increased neuronal activation occurring selectively in Rh of trained animals. Likewise, activation in the PV following BIC treatment tended to be higher in trained animals, though the interaction was not significant. Therefore, it appears that spatial learning that leads to plasticity within corticothalamic-HPC pathways may potentiate the effects of PFC disinhibition. However, given that thalamic regions that project to the HPC only target CA1, and potentiation of the effects of PFC BIC following training occurred throughout the HPC, our findings indicate that enhancement of Ent input and/or local plasticity within the HPC also play a role in the enhancement of neuronal activation following behavioral training.

With respect to local HPC plasticity, previous studies have often defined neural assemblies that represent a given memory as the set of neurons showing an induction of c-Fos or other IEGs following recall of the memory (Reijmers et al. 2007; Trouche et al. 2016; Zhou et al. 2009). Under basal conditions, it is possible that only a portion of the assembly expresses c-Fos, but increased input, as can occur following PFC disinhibition, may induce expression throughout the assembly via increased number or strength of synapses that are established over the course of learning. It is also possible that c-Fos expression spread to connected cells outside the assembly. This aberrant HPC activation may underlie disruptions in radial maze performance induced by PFC disinhibition, given the critical role this region plays in mediating search guided by spatial RM and WM (Becker et al. 1980; Olton and Papas 1979). Since both ventral and dorsal HPC showed similar increases in activation in trained animals following PFC disinhibition, and similar changes were observe across HPC subfields, the present experiments cannot identify to what extent PFC disinhibition caused aberrant HPC activation via increased activity in thalamic, Ent and/or local HPC circuitry. However, each of these options would represent novel forms of plasticity following spatial learning that will be of considerable interest to investigate in the future.

Effects of training and testing on the RM/WM radial maze on c-Fos expression in control animals

In most brain regions observed, training and testing on the RM/WM maze task had no effect on c-Fos expression in animals receiving control infusion treatments. This is somewhat surprising, in light of the fact that several of the regions examined, including STR and HPC, have been implicated in performance of identical or similar spatial tasks (Colombo et al. 1989; Floresco et al. 1997; Packard and White 1990; Schacter et al. 1989). Furthermore, inductions of c-Fos or other IEGs have been observed in the thalamus, HPC and prefrontal regions following performance of RM/WM (He et al. 2002b) and WM-only (Vann et al. 2000a, b) variants of the radial maze task. However, past work has shown hippocampal IEG expression often reaches peak levels during learning of similar spatial tasks, and returns to levels near baseline in well-trained animals (He et al. 2002a, b; Vann et al. 2000b). On the other hand, tasks requiring more complex hippocampal operations, such as pattern separation or completion, continue to induce high levels of neuronal activation in hippocampal subfields in well-trained animals (Yagi et al. 2016). Given the relatively simple nature of the task employed in the present study, the extended training rats received may have refined the ensemble of cells recruited to execute the task efficiently, leading to a subtle induction of c-Fos beyond the limit of detection of our methods of quantification. Peak c-Fos expression induced by radial maze performance may also have occurred before or after the 90 min time point of the present study. Indeed, trends

towards increased IEG expression in some HPC subfields were observed in trained or tested control animals, suggesting that the lack of a significant effect may merely be attributable to insufficient statistical power and/or different time course of c-Fos expression. However, PFC BIC treatment increased neuronal activation only in the HPC of trained rats, making it likely that learning of the maze task induced some experience-dependent modification of the HPC circuitry.

Neuronal activation in limbic structures implicated in anxiety and fear

The primary focus of this study was to explore how PFC disinhibition affects neuronal activation in brain regions known to facilitate performance of the RM/WM task. However, we also examined neuronal activation in structures implicated in anxiety and conditioned fear, in light of the fact that PFC $GABA_A$ antagonism can produce increased anxiety-like behavior (Bi et al. 2013) and impairs the ability to discriminate between aversive and neutral stimuli in a fear-conditioning paradigm (Piantadosi and Floresco 2014). Surprisingly, the PV nucleus of the thalamus, which has been implicated in maintenance and retrieval of fear memory (Do Monte et al. 2016) showed a selective induction of neuronal activation in groups that were tested on the RM/WM maze task, an effect that was observed in both SAL and BIC-treated animals. The PV has also been implicated in feeding, indicating PV neuronal activation may have reflected consumption of the food reward, which only occurred for tested animals (Matzeu et al. 2014).

Neuronal activation was increased in Amg regions following PFC $GABA_A$ antagonism in all behavioral groups. In the Amg, these increases were more prominent in the BLA when compared to the CeA. Similarly, Jones et al. (2011) showed that a combination of stress and intra-PFC BIC infusion increased neuronal activation to a larger degree in the BLA in comparison to the CeA, although no data from behaviorally-naive animals were included in that study. Taken together with these previous findings, the present data suggest that alterations in anxiety or fear-conditioning that result from deficient PFC GABA signalling may result in part from altered activity within the Amg and PV nucleus, in addition to local disinhibition of activity within the PFC.

PFC imbalances in excitation and inhibition impact neuronal activation throughout the brain

Maintenance of an appropriate balance between excitatory and inhibitory neural transmission is critical for their efficient neural functioning and execution of the behaviors they mediate. While transient deviations from this balance are known to occur during development or when learning and plasticity occur (Letzkus et al. 2015), prolonged disturbances of excitation–inhibition (E–I) balance are associated with pathological states, including schizophrenia and autism. In the present study, antagonism of PFC $GABA_A$ receptors was employed to diminish PFC GABAergic transmission, leading to increased pyramidal cell activity (Lodge 2011), and thus transiently disrupting the PFC E–I balance. Disturbances in E–I induced by reduced PFC GABA function might be expected to disturb local oscillations and tuning of individual neurons (Rao et al. 2000) or affect gating of irrelevant activity/noise within PFC, leading to deficits in PFC-mediated cognition. However, the observation that PFC $GABA_A$ antagonism also affects non-PFC-dependent functions, such as spatial reference memory (Auger and Floresco 2015; present study) raises the possibility that increased output from PFC projection neurons could be altering activity and function of regions that receive PFC input. Indeed, the present findings indicate that local disinhibition of the PFC, leading to increased glutamatergic PFC projection neuron signal outflow, has profound effects on patterns of activity within many structures that receive either monosynaptic or disynaptic PFC innervation. Importantly, the extent of the change in activation was also modulated by plasticity induced by learning in the relevant circuitry.

Notably, increases in neuronal activation following PFC disinhibition were observed both in task-related regions, which in this case include HPC, STR and thalamus, and task-unrelated regions, such as the Amg and sensorimotor cortices (Vann et al. 2000a). Therefore, PFC disinhibition may also disrupt cognition by inducing aberrant activations in task-unrelated networks. As noted previously, the medial PFC is not required for performance of this variant of the radial maze task (Auger and Floresco 2015), raising the possibility that PFC activity that was elevated above normal in the maze context could have also contributed to RM/WM impairments. Importantly, it is not yet clear how PFC disinhibition would affect E–I balance within individual PFC terminal regions, as the identity of the neurons expressing c-Fos was not assessed in this study. If PFC disinhibition leads to preferential activation of inhibitory interneurons in a terminal region, a net inhibition could result, while other structures where the main targets are glutamatergic may be disinhibited. Finally, reduced inhibitory transmission in the frontal lobes may disrupt PFC-dependent behaviors via perturbations of co-ordinated activity of PFC-cortical–subcortical networks.

Implications for schizophrenia and other psychiatric disorders

One of the pathophysiological mechanisms posited to underlie schizophrenia and other psychiatric disorders is a disruption in cortical E–I balance, with deficits in PFC GABA function being one of the key alterations leading to this

imbalance. Here, and in previous work, we and others have employed pharmacological reduction of PFC $GABA_A$ activity to provide insight into how PFC GABAergic transmission, and subsequent disruption of the E–I balance, affects behavior and underlying neural activity (Enomoto et al. 2011; Paine et al. 2011, Tse et al. 2015a). Although altered E–I balance induced by PFC $GABA_A$ antagonism is acute, it is sufficient to reproduce many features of schizophrenia including qualitatively similar cognitive deficits in attention (Auger et al. 2017; Paine et al. 2011), working memory (Auger and Floresco 2016), decision-making (Paine et al. 2015; Piantadosi et al. 2016) and cognitive flexibility, and behaviors reflective of positive (Enomoto et al. 2011; Piantadosi and Floresco 2014) and negative symptoms (Paine et al. 2017; Piantadosi et al. 2016). The present findings add to this literature to show that the cortical disinhibition induced by a deficient PFC GABA signalling also has the potential to alter the activity and function of circuits throughout the brain in a manner that may be relevant to schizophrenia. A substantial body of recent work has examined circuit-level alterations in schizophrenia (Hunt et al. 2017; Li et al. 2017; Yoon et al. 2013) or models of the disorder (Floresco et al. 2009; Hartung et al. 2016), and the present work suggests that deficiencies in inhibitory transmission within PFC could be an important contributor to many of these effects. While some of this literature has focussed on circuits that mediate cognitive functions known to be impaired in schizophrenia, heightened activity in off-task networks that may disrupt cognitive performance has also been observed (Anticevic et al. 2013), similar to aberrant activations in regions not associated with RM/WM performance observed here. Decreased PFC GABA function in schizophrenia is thought to take place alongside deficiencies in GABA within other structures, including other cortical regions (Hashimoto et al. 2008), STR and thalamus (Thompson et al. 2009). Importantly, the present findings suggest that even deficiencies in GABAergic transmission that are restricted to the PFC may interfere with function of other circuits implicated in cognitive and behavioral abnormalities present in schizophrenia, providing insight into how decreased PFC GABAergic transmission may contribute to different symptoms of the disorder.

Conclusion

The present study has revealed that deficiencies in PFC GABAergic transmission can have substantial consequences on neuronal activation in both near and distant PFC projection neuron targets. Intriguingly, learning of a spatial RM/WM radial maze task strengthened these effects in HPC and thalamic regions, revealing that increased inputs from thalamic and entorhinal cortical pathways or local remodelling of circuitry may represent novel forms of plasticity associated with learning of these types of spatial tasks. In turn, these forms of experience-dependent plasticity appear to modulate how PFC terminal regions respond to increase in PFC output. Given that dysfunctional PFC GABA signalling is observed in schizophrenia and other psychiatric disorders, these findings indicate that deficiencies in PFC GABA also have the potential to contribute to the circuit-level alterations that have been observed in these disorders. Future studies that investigate how disinhibiting distinct PFC circuits impacts behavior will provide further insight into how diminished PFC GABA function plays a role in these psychiatric conditions.

Acknowledgements This work was supported by a grant from the Canadian Institutes of Health Research (MOP 130393) to SBF. We thank Stephanie Lieblich and Paula Duarte-Guterman for their technical guidance in conducting these experiments.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

Ethical standards All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

Research involving human and/or animal participants All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted. This article does not contain any studies with human participants performed by any of the authors.

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