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Local blockade of NMDA receptors in the rat prefrontal cortex increases c-Fos expression in multiple subcortical regions

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Ketamine, phencyclidine and MK801 are uncompetitive NMDA receptor (NMDAR) antagonists which are used widely to model certain features of schizophrenia in rats. Systemic administration of NMDAR antagonists, in addition to provoking an increase in c-Fos expression, leads to important neurochemical and electrophysiological changes within the medial prefrontal cortex (mPFC). Since the mPFC is considered to exert a top-down regulatory control of subcortical brain regions, we examined the effects of local infusion of the NMDAR antagonist, MK801, into the mPFC on the expression of c-Fos protein (widely used marker of neuronal activation) in several subcortical structures. The experiment was performed on freely moving rats, bilaterally implanted with guide cannulae in the prelimbic mPFC, infused with MK801 or saline. Bilateral administration of MK801 to the mPFC produced changes in the behavior (increased stereotypy and decreased sleep-like behavior) and complex changes in c-Fos protein expression with significant increases observed in the nucleus accumbens (core and shell), amygdala (basolateral and central nuclei), the CA1 field of the hippocampus, and mediodorsal and paraventricular thalamic nuclei, as compared to the saline group. Together, we demonstrate that blockade of NMDA receptors in the mPFC is sufficient to lead to behavioral abnormalities and increased c-Fos expression in many, but not all, of the subcortical structures examined. Our findings suggest that some of the behavioral abnormalities produced by uncompetitive NMDAR antagonists may result from aberrant activity in cortico-subcortical pathways. These data support an increasing body of literature, suggesting that the mPFC is an important site mediating the effects of NMDAR antagonists.

Key words: c-Fos, MK801, medial prefrontal cortex, striatum, amygdala, hippocampus, thalamus, behavior

INTRODUCTION

Ketamine, phencyclidine and MK801 are uncompetitive NMDA receptor channel blockers that act by binding to a site located in the NMDA channel itself. In rodents, systemic administration of these antagonists produces behavioral hyperactivity, stereotypy, impairments in spatial memory, sensorimotor gating, as well as, altered social interaction considered to model some of the symptoms of schizophrenia (for review see Geyer and Moghaddam 2002, Mouri et al. 2007). The behavioral effects produced by NMDAR antagonists may result from altered activity of several structures, including the medial prefrontal cortex (mPFC), hippocampus, amygdala and nucleus accumbens (NAc) (O'Donnell and Grace 1998). Of these, the mPFC is an important target since its efferent pathways exert a top-down regulatory control of other structures either directly (by glutamatergic projection neurons) and/or indirectly through modulation of dopaminergic inputs to these regions (for review see Vertes 2006, Floresco et al. 2009).

Increasing evidence indicates that the mPFC is a critical site mediating the effects of NMDAR antagonists. For example, studies using freely moving rats have shown that systemic administration of NMDAR antagonists produce a sustained potentiation of the firing rate in the majority of pyramidal neurons and reduction in organized burst activity, which may attenuate signal transmission efficiency of the mPFC

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(Jackson et al. 2004, Homayoun and Moghaddam 2007). Also, systemic application has been shown to increase the release of glutamate, dopamine, and serotonin in the mPFC (Mathé et al. 1999, López-Gil et al. 2007). The importance of the mPFC in mediating some of the behavioral effects of NMDAR antagonists is highlighted further by studies demonstrating that local infusion to the mPFC can modify motor and cognitive performance (Jentsch et al. 1998, Stefani et al. 2003) and bilateral excitotoxic lesions of the mPFC abolish the disruptive action of systemically administered MK801 on pre-pulse inhibition (Schwabe and Koch 2004).

c-Fos protein is a product of the immediate early gene *c-fos,* which is rapidly transcribed after neuronal activation (Kovacs 1998). In previous studies, increases in c-Fos expression were found after electrical stimulation of the mPFC in several brain regions, including the NAc and amygdala (Arvanitogiannis et al. 2000). Also, pharmacological activation of the infralimbic cortex, induced by local infusion of the $GABA_A$ receptor antagonist picrotoxin, was found to increase the expression of c-Fos in several nuclei of the amygdala (Berretta et al. 2005). Although much is known about the effect of systemic administration of NMDAR antagonists on *c-fos* mRNA and c-Fos protein expression, where increases have been reported in several brain regions, including the mPFC itself and its projection regions including the NAc, amygdala and thalamus (Ahn et al. 2002, De Leonibus et al. 2002, Keilhoff et al. 2004, Väisänen et al. 2004, Imre et al. 2006, Kalinichev et al. 2008, Kargieman et al. 2008, Zuo et al. 2009), to date, few studies have attempted to examine changes in c-Fos following local injection of NMDAR antagonists. Considering the importance of the mPFC in mediating the effects of NMDAR antagonists, we hypothesized that local blockade of NMDAR within the mPFC may be sufficient to produce increases in c-Fos expression in subcortical regions. To this end, the uncompetitive NMDAR antagonist, MK801, or saline was bilaterally infused to the mPFC of awake rats and c-Fos measured in the NAc, amygdala, hippocampus and thalamus. Parts of this work have been published in the abstract form (Nowak et al. 2009, 2010).

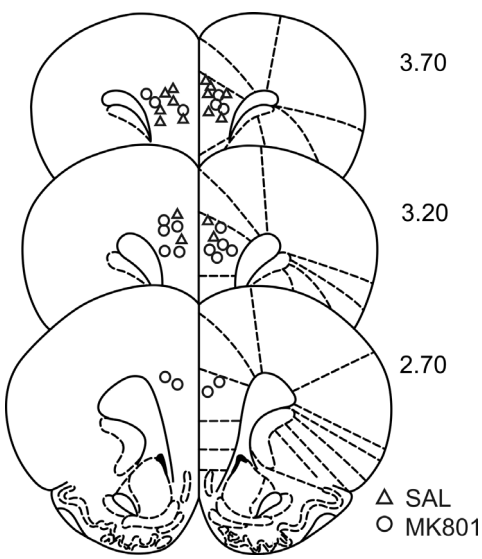

Fig. 1. Schematic representation of placements of cannulae tips targeted to the prelimbic area of mPFC. Localization of prefrontal guide cannulae ranged between 2.70–3.70 mm anterior to bregma. Modified from Paxinos G, Watson C, The Rat Brain in Stereotaxic Coordinates (4th edition). Figures 7, 8, 9, Copyright (C) 1998, with permission from Elsevier.

METHODS

Animals

Twenty male Wistar rats weighing 280–320 g were individually housed in standard Plexiglas cages (type IV, 27 × 42 × 20 cm, Tecniplast) with unlimited access to water and standard laboratory rat chow, in L:D 12:12 h conditions. All efforts were made to minimize animal suffering, and to reduce the number of animals used. The study was approved by a local ethics committee and conducted in accordance with the Polish Law on Animal Experimentation and the Directive 86/609/EEC.

Implantation of guide cannulae

Rats were anaesthetized with isoflurane and positioned in a stereotaxic frame with blunt ear bars. Twenty-eight gauge stainless steel guide cannulae (Bilaney, Germany) were bilaterally implanted in the prelimbic mPFC according to coordinates (in mm) of the stereotaxic atlas (Paxinos and Watson 1998) AP: +3.2, ML: ± 0.6, DV: 2.6. Guides were attached firmly to skull with small stainless steel screws and dental cement. Obturators were inserted to prevent obstruction by debris.

Experimental procedure

The experiment was preceded by six days of habituation. All rats were habituated both to immobilization (5 min, in preparation for infusion) and to the open

field arena (30 min). On the day of the experiment, rats were randomly assigned to saline or MK801 groups. The animals were gently restrained and an infusion cannula which extended 1 mm below the tip of the guide was locked in place inside the guide enabling infusion at a constant depth. The infusion cannula was left in place for 60 seconds. This was followed by infusion of saline (0.9% NaCl) or MK801 (4 μg, Sigma) in a volume of 0.5 μl for 60 s. This dose was chosen since pilot studies showed that 4 μg of MK801 is sufficient to evoke marked changes in oscillatory activity when infused into mPFC. The cannula was left in place for the next 60 seconds and then removed. Left and right sides were infused separately. Rats were immediately placed in the open field arena (95 × 95 × 40 cm) and the behavior was video-recorded for 90 minutes after infusion. Before each trial the arena was cleaned with water containing a detergent and dried with a towel.

c-Fos immunohistochemistry

Rats were killed with an overdose of pentobarbital 90 min after infusion and perfused transcardially with ice-cold phosphate buffered saline (PBS, pH 7.4 Sigma) followed by 4% paraformaldehyde solution. Brains were dissected and postfixed in 4% paraformaldehyde solution overnight and thereafter in 20% and 30% sucrose (Sigma) solutions. The brains were deep frozen and stored at −72°C until the day of sectioning in the cryostat (−21°C). Forty-micrometer thick coronal sections were taken, stored for up to 10 days in PBS with sodium azide (0.01%) and then subjected to standard c-Fos immunohistochemistry according to Savonenko and coauthors (1999). The sections were washed three times in PBS and thereafter incubated for 10 min in 0.3% H_2O_2 in PBS, washed twice in PBS, then incubated with a polyclonal antibody (anti-c-Fos, 1:1 000; Santa Cruz

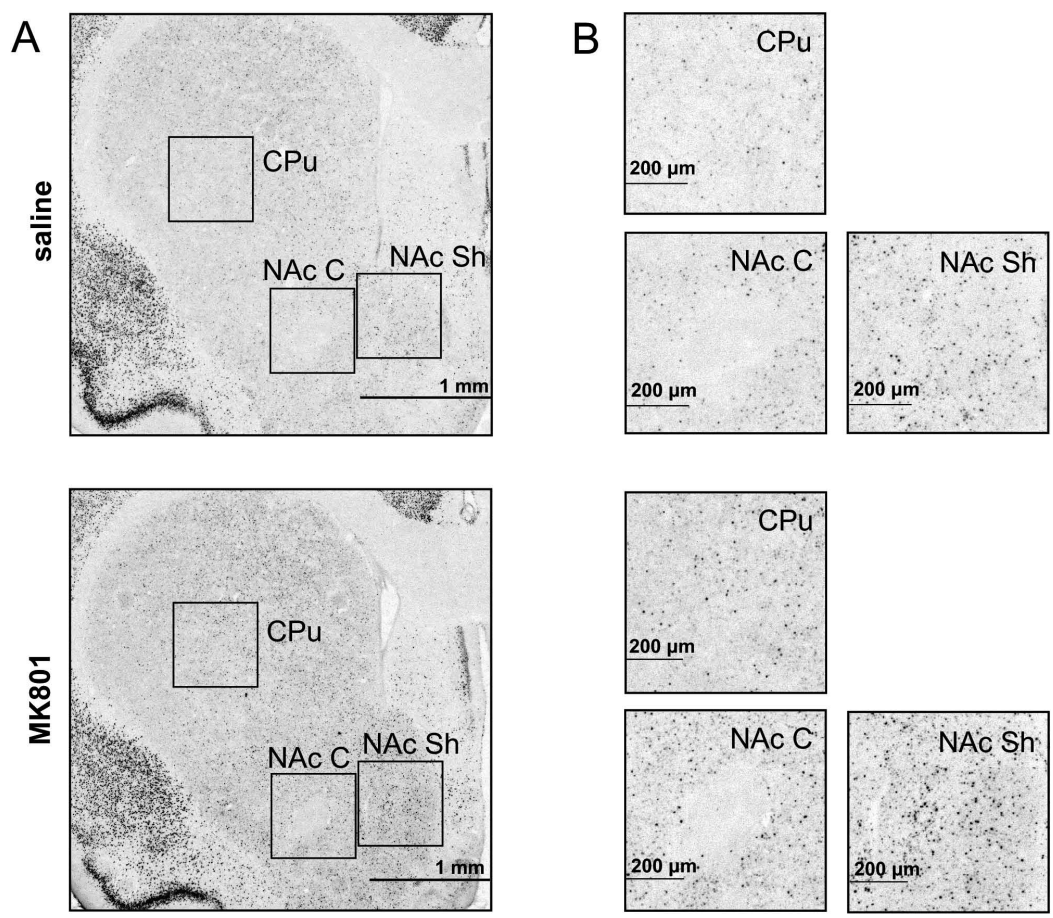

Fig. 2. c-Fos protein expression in the striatum. (A) Examples of c-Fos-stained brain sections illustrating the difference in the striatal subregions after infusion of saline or MK801 into mPFC. (B) The example frames from A are shown in greater magnification (×5). (CPu) caudate putamen, (NAc C) nucleus accumbens core, (NAc Sh) nucleus accumbens shell.

no. sc-52) for 48 h at 4ºC in PBS and normal goat serum (3%). The sections were subsequently washed three times in PBS with Triton X-100 (0.3%; Sigma), incubated with goat anti-rabbit biotinylated secondary antibody (1:1 000; Vector) in PBS/Triton and normal goat serum (3%) for 2 h, washed three times in PBS/Triton, incubated with avidin–biotin complex (1:1 000, in PBS/Triton; Vector) for 1 h and washed three times in PBS. The immunostaining reaction was developed using the glucose oxidase–diaminobenzidine–nickel method. The sections were incubated in PBS with diaminobenzidine (0.05%), glucose (0.2%), ammonium chloride (0.04%) and ammonium nickel sulfate (0.1%) (all from Sigma) for 5 min, then 10% (w/v) glucose oxidase (Sigma; 10 U/ml in water) was added. The staining reaction was stopped by three washes with PBS. The sections were mounted on gelatin-covered slides, air dried, dehydrated in ethanol solutions and xylene, and embedded in Entellan (Merck). The immunostaining was performed in two batches, each containing representative slices of all investigated brain structures from all animals. c-Fos stained brain slices were microphotographed with Nikon ECLIPSE 80i microscope using 2× objective (0.06 N.A; with high resolution, 1 pixel representing 5.43 μm^2) and bilaterally assessed for c-Fos activation using ImageJ software (WCIF, Toronto, Canada). The expression of c-Fos protein was analyzed in the striatum (nucleus accumbens, shell and core and caudate putamen), hippocampus (CA1, CA2, CA3 and dentate gyrus), amygdala (basolateral, central, medial and cortical nuclei) and thalamus (nucleus reuniens, paraventricular nucleus – anterior and posterior part and mediodorsal nucleus). For each structure and its subdivision, c-Fos counts were bilaterally assessed on the basis of measures from two to four brain slices (in most cases three). For each brain structure, the number of c-Fos immunopositive nuclei was counted and divided by the area occupied by this structure

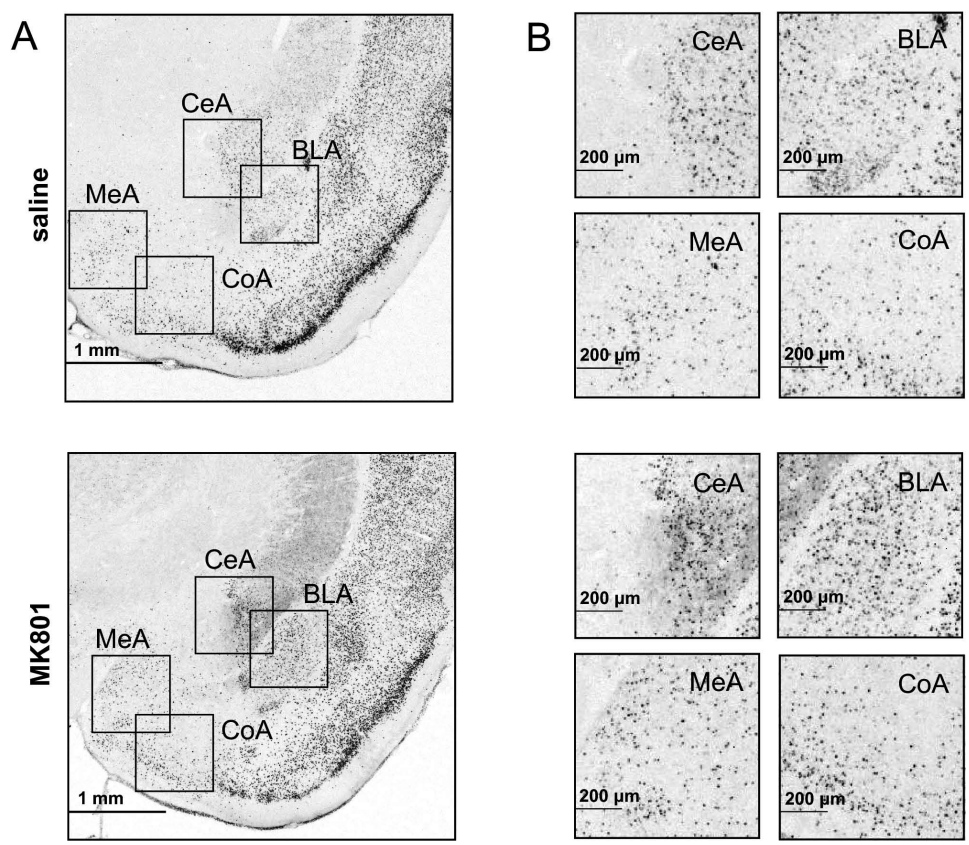

Fig. 3. c-Fos expression in the amygdala. (A) Examples of c-Fos-stained brain sections illustrating the distributions of c-Fos-immunopositive nuclei in the amygdala after infusion of MK801 or saline into the mPFC. (B) The example frames from A are shown in greater magnification (×5). (BLA) basolateral nucleus of amygdala; (CeA) central nucleus of amygdala; (CoA) cortical nucleus of amygdala; (MeA) medial nucleus of amygdala.

Table I

c-Fos protein expression in the investigated brain areas

Brain region	Saline (n/0.1 mm^2)	MK801 (n/0.1 mm^2)	*P*	Significance
STRIATUM				
NAc C, nucleus accumbens core	7.3 ± 0.6	11.2 ± 0.6	0.0003	**
NAc Sh, nucleus accumbens shell	12.3 ± 0.8	19.6 ± 1.4	0.0007	*
CPu, caudate putamen	14.0 ± 2.5	17.7 ± 2.6	0.321	NS
AMYGDALA				
BLA, basolateral nucleus of amygdala	16.6 ± 1.1	27.1 ± 1.6	<0.0001	**
Ce, central nucleus of amygdala	19.4 ± 2.3	34.8 ± 2.3	0.0002	**
Co, cortical nucleus of amygdala	22.5 ± 1.9	30.9 ± 3.9	0.0914	NS
Me, medial nucleus of amygdala	21.1 ± 1.1	21.1 ± 1.6	0.996	NS
THALAMUS				
Re, nucleus reuniens	18.2 ± 2.4	31.4 ± 3.3	0.0056	NS
MD, mediodorsal thalamic nucleus	10.1 ± 2.3	26.6 ± 2.8	0.0005	**
PVA, anterior part of paraventricular nucleus	37.1 ± 2.7	49.9 ± 3.1	0.009	NS
PVP, posterior part of paraventricular nucleus	32.9 ± 2.5	49.0 ± 3.2	0.0013	*
HIPPOCAMPUS				
CA1 field	5.1 ± 0.3	9.7 ± 0.6	<0.0001	**
CA2 field	7.5 ± 0.8	8.3 ± 0.8	0.4850	NS
CA3 field	6.1 ± 0.3	6.6 ± 0.5	0.4138	NS
DG, Dentate gyrus	7.2 ± 0.3	9.3 ± 0.5	0.005	NS

Results are presented as an average number of c-Fos immunopositive nuclei counted per 0.1 mm².
Data are presented as mean ± SEM (unpaired *t*-test Bonferroni corrected *P* values: * $P<0.0033$, ** $P<0.00067$).

(expressed in 0.1 mm^2). The anatomical border of each structure was marked manually by researcher using a built-in function from the ImageJ menu and was identified using adjacent Nissl-stained sections. The number of c-Fos positive nuclei and structure area were calculated automatically by the software. Data collection was done by researcher blind to the group division.

Verification of placement of guide cannulae was done using Nissl-stained brain slices. Only data from rats with guides correctly implanted in the prelimbic part of mPFC were taken for further analysis.

Behavioural measures

The behavior of animals was video recorded (MPEG-2 format) and an observer-based program (BehaView, Boguszewski, http://www.pmbogusz.net/software/behaview) was used to measure the duration of several behaviors. Locomotor activity was quantified by counting the total number of crossovers an animal made from one square to another (the open field arena was virtually divided into 1 central and 8 peripheral zones). Additionally, we measured the number of entries into the central zone and total time

spent in it. We also measured the duration of other behaviors: inactive state (i.e. when animal was staying immobile, with almost no postural changes), rearing (when animal raised on hind limbs, supporting on the arena wall or not, and very often sniffing), grooming (head or back grooming or scratching with a hind paw) and sleeping-like (when an animal laid immobile with the body in a curled-up position, eyes closed, regular breathing).

Systemic administration of MK801 to rats produces some changes in behavior, such as increases in locomotor activity and stereotyped sniffing (Koek et al. 1988, Hoffman 1992, Andine et al. 1999). In animals infused with MK801 we observed persistent, unusual sniffing behavior which we classified as a stereotypy. While the animal was standing on four limbs, it sniffed with the head raised up and occasionally slowly waved it, we will refer to this behavior as "a stereotyped sniffing". The beginning and end of every type of behavior was determined and set manually by an experimenter blind to the group.

Statistics

For c-Fos expression, averaged across at least four measurements (bilateral assessment from two to four brain slices), a two-way repeated measures ANOVA with structures as repeated-measures was used. A Student's unpaired *t*-test was used as a *post hoc* for pair-wise comparisons between saline and MK801-infused rats [with *P* values Bonferroni-corrected according to the number of tested structures (=15): * $P<0.0033$ (0.05/15), ** $P<0.00067$ (0.01/15)]. For analysis of behavior a two-way repeated measure ANOVA with time as the repeated measure was performed and the Bonferroni *post-hoc* test used to find individual points of difference between MK801 and saline-infused groups. Values were considered significant if $P<0.05$.

RESULTS

Verification of cannulae placement

Localization of the guide cannulae tips are shown in Figure 1 for saline ($n=8$) and MK801 ($n=10$) rats. Two rats were excluded from the study, one due to incorrect placement of guide cannulae and the other due to technical reasons during the infusion.

The effect of local infusion to the mPFC on c-Fos expression

The expression of c-Fos protein was analyzed in the striatum, hippocampus, thalamus, and amygdala. Results are presented as the average number of c-Fos immunopositive nuclei counted within a given structure divided by the area it occupied (expressed as a number per 0.1 mm^2). A two-way ANOVA revealed a significant effect of drug infusion ($F_{1,16}=3.46$, $P<0.001$), structure ($F_{14,224}=75.07$, $P<0.0010$) and structure × drug interaction ($F_{14,224}= 4.99$, $P<0.0001$),

Table I shows the effect of local infusion of MK801 and saline to the mPFC on c-Fos expression. In the ventral striatal region injection of MK801 produced a significant increase in the number of immunopositive c-Fos stained nuclei compared to saline in both the core (NAc C, $P=0.003$) and shell (NAc Sh, $P=0.007$) of the NAc, while no significant difference was found for the caudate-putamen (CPu) (Fig. 2). Comparison of c-Fos expression in the amygdalar complex showed a significant difference for the basolateral (BLA, $P<0.0001$) and central (CeA, $P=0.0002$) nuclei, while the two other investigated nuclei (cortical, CoA and medial, MeA) did not show a statistically significant difference in c-Fos expression (Fig. 3). Similarly, two out of the four investigated thalamic nuclei showed a significant increase in the number of c-Fos positive cells: the mediodorsal (MD, $P=0.0005$) and paraventricular nuclei (posterior part, PVP, $P=0.0013$) (Fig. 4). Within the hippocampus c-Fos counts were significantly higher only in the CA1 field ($P<0.0001$) for the MK801-infused rats. Differences for CA2, CA3 and dentate gyrus were non-significant (Fig. 5).

The effect of local infusion to the mPFC on animal behavior

Behaviour during the whole experimental session was analyzed in eighteen consecutive time intervals each lasting 5 min (see Fig. 6). Locomotor activity measured as the number of crossovers gradually decreased with time and was not different for both experimental groups (MK801- and saline-infused animals). Two-way ANOVA revealed a significant effect of time ($F_{17,272}=17.40$, $P<0.0001$) with no significant effect of treatment or drug × time interaction. A two-way ANOVA did not reveal significant differences in time spent in the central zone (no significant effect of

treatment, time or drug × time interaction between experimental groups, not shown in Fig. 6). Saline-treated rats showed a gradual reduction in overall activity over time and were typically lying still [inactive state; significant effect of time ($F_{17,272}$=2.93, P=0.0001)] or sleeping in the corner of the cage by the end of the experiment. Consistent with this, rats infused with saline slept significantly longer than MK801-infused rats [significant effect of drug ($F_{1,272}$=13.95, P=0.002), time ($F_{17,272}$=3.14, P<0.0001) and drug × time interaction ($F_{17,272}$=3.01, P<0.0001) between experimental groups)]. Bonferroni *post hoc* analysis revealed a significant difference between sleep-like state duration in MK801 and saline infused rats for the following time periods: 45–50 min (P<0.01), 50–55 min (P<0.05), 70–75 min (P<0.001) and 85–90 min after infusion (P<0.001). Duration of rearing (one of the exploratory behaviors analyzed) did not differ between saline- and MK801-treated groups (no significant effect of treatment, time or drug × time interaction between experimental groups). Duration of grooming, a non-locomotor behavior, was found to be not different for both MK801 and saline-infused rats. Two-way ANOVA did not show a significant effect of treatment, time or drug × time interaction.

Bilateral infusion of MK801 to the mPFC was associated with the presence of episodes of stereotyped side-to-side head movements (in the majority of rats – 7 out of 10 rats) and sustained sniffing with the head raised up [significant effect of drug ($F_{1,272}$=10.82, P=0.005) and drug × time interaction ($F_{17,272}$=1.80, P=0.028)]. Bonferroni *post hoc* analysis revealed a significant difference between MK801 and saline infused rats for 2 time periods: 40–45 min (P<0.05) and 45–50 min (P<0.01) after infusion.

DISCUSSION

In this study, we found that local injection of MK801, at a behaviorally relevant dose, produced a significant increase in c-Fos protein expression in the NAc (shell and core), amygdala (basolateral and central nucleus), hippocampus (CA1 field) and thalamus (mediodorsal and paraventricular nucleus). Since c-Fos can be used as a marker of neuronal activation this suggests that local blockade of NMDA receptors in the mPFC can lead to the activation of diverse downstream structures. To date, most investigators examining the effect of NMDAR antagonists on c-Fos levels have used the systemic route of drug administration. The

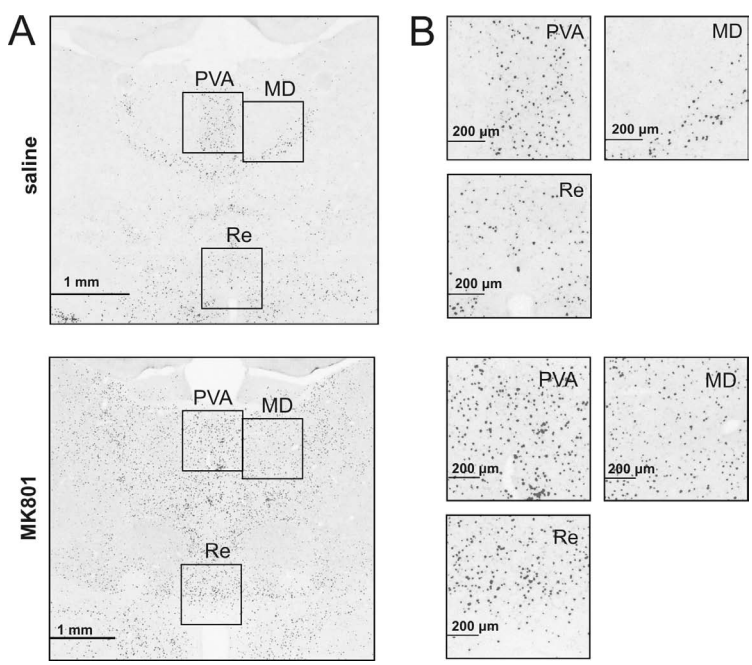

Fig. 4. c-Fos protein expression in the thalamus. (A) Examples of c-Fos-stained brain sections illustrating distributions of c-Fos-immunoreactive nuclei in the thalamus after infusion of MK801 or saline into mPFC. (B) The example frames from A are shown in greater magnification (×5). (Re) nucleus reuniens; (MD) mediodorsal thalamic nucleus; (PVA) anterior part of paraventricular nucleus.

vast majority have demonstrated that NMDAR antagonists increase the levels of c-Fos protein or mRNA in the NAc (Hussain et al. 2001, Habara et al. 2001, Gotoh et al. 2002, Imre et al. 2006), hippocampus (Näkki et al. 1996, Väisänen et al. 2004, Imre et al. 2006), and thalamic nuclei (Dragunow and Faull 1990, Näkki et al. 1996, De Leonibus et al. 2002, Imre et al. 2006, Kargieman et al. 2008). There is also some evidence that systemic NMDAR blockade can increase c-Fos expression in the amygdala (Imre et al. 2006), however in a separate study *c-fos* mRNA levels were unchanged (Väisänen et al. 2004). The novelty of the present finding is that we show that local blockade of NMDAR in the mPFC alone is sufficient to induce c-Fos expression in these subcortical structures – a finding which supports a growing body of literature suggesting that the mPFC is an important target mediating the effects of NMDAR antagonists.

Possible mechanisms leading to the induction of c-Fos in subcortical structures

Conclusions drawn on the basis of local infusion have one weakness that some of the changes observed after the intra-cortical infusion may result from the diffusion of drug to other structures. In order to avoid that, we infused a very small amount of drug (4 μg/0.5 μl) into the prelimbic part of prefrontal cortex. Moreover, in awake rats, local injection of NMDAR antagonists to the mPFC has been shown to provoke marked changes in the local neurochemistry. For example, local injection of ketamine (Lorrain et al. 2003), MK801 and PCP (Yonezawa et al. 1998) and the competitive NMDAR antagonist 2-amino-5-phosphonopentanoate (AP5) (Feenstra et al. 2002) all increase dopamine release in the mPFC. Local infusion of CPP (3-(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid, competitive NMDAR antagonist), has also been shown to increase extracellular concentration of glutamate in the mPFC (Ceglia et al. 2004, Abekawa et al. 2008), however, others have shown no effect of ketamine (Lorrain et al. 2003). In addition, a decrease in the concentration of GABA in the mPFC has been reported after local injection of PCP and MK801 (Yonezawa et al. 1998). The concentration of MK801 used in our study would be expected to provoke substantial changes in the local neurochemistry of the mPFC which would almost certainly disrupt the firing of projection neurons and leading to complex changes in downstream structures. Indeed, systemic administration of MK801 is known to increase the firing of pyramidal neurons of the mPFC, in awake rats (Jackson et al. 2004). However, in anaesthetized rats local application of NMDAR antagonists (PCP and MK801) to

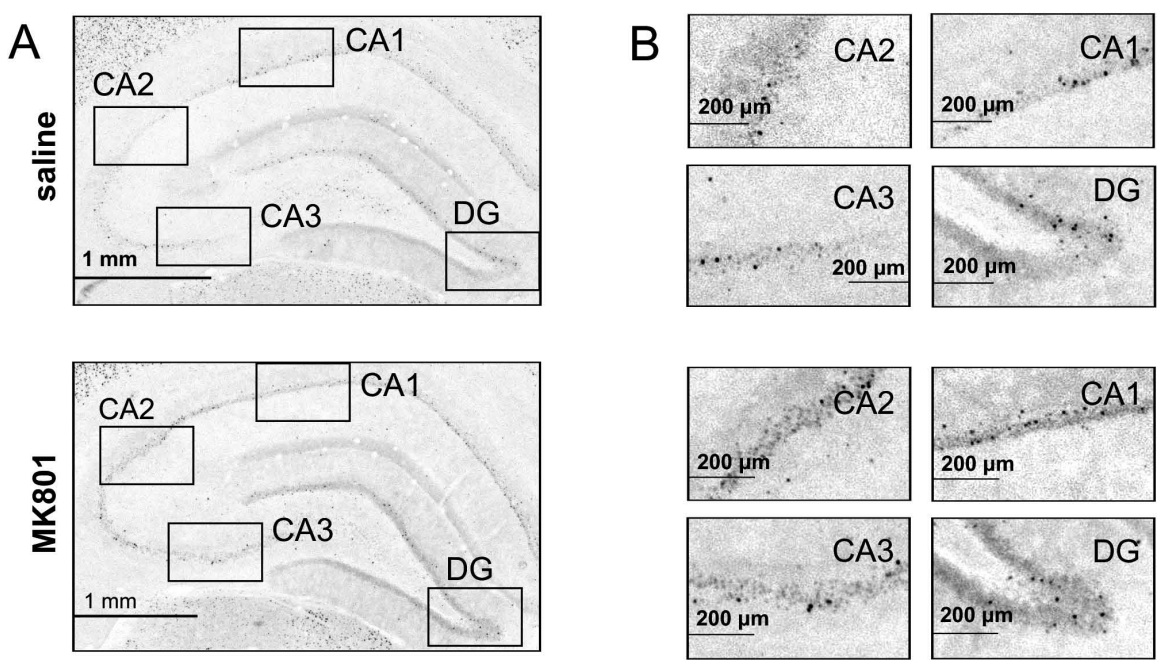

Fig. 5. c-Fos protein expression in the hippocampus. (A) Examples of c-Fos-stained brain sections illustrating the difference in the hippocampus after infusion of saline or MK801 into mPFC. (B) The example frames from A are shown in greater magnification (×5). (CA1) CA1 field; (CA2) CA2 field; (CA3) CA3 field; (DG) dentate gyrus.

the mPFC was not found to increase spontaneous firing of projection neurons (Suzuki et al. 2002, Jodo et al. 2005). Whether this is an effect of anaesthesia, when cortical activity is widely depressed, or due to a drug-drug interaction, or the doses used remains to be demonstrated. Notwithstanding, the observation that local injection of NMDAR antagonists can interfere healthy neuronal processing is corroborated by numerous findings that intra-PFC injection can produce working memory deficits (Akirav and Maroun 2006, Baviera et al. 2008, Gilmartin and Helmstetter 2010).

The mPFC sends excitatory glutamatergic projections to both shell and core of the NAc and several nuclei of the amygdala, as well as to midline/medial structures of the thalamus (Sah et al. 2003, Vertes 2004). It is likely that enhanced c-Fos protein expression in the NAc, some nuclei of amygdala and thalamus reflects enhanced excitatory input. Increased c-Fos expression in the amygdala has been reported following activation of the mPFC by local picrotoxin infusion (Berretta et al. 2005) and electrical stimulation of the mPFC (Arvanitogiannis et al. 2000).

The explanation of increased c-Fos protein expression in the CA1 is not straightforward as the mPFC is not known to send a direct excitatory projection to the hippocampus. Induction of c-Fos expression in the CA1 may reflect enhanced activity of some hippocampal afferent structures directly connected with the mPFC. The primary route from the prefrontal cortex to the hippocampus has been hypothesized to be *via* the nucleus reuniens (Re) (Vertes 2006). Our results did not show significant changes in the induction of c-Fos expression in the Re, while they were noticeable in other thalamic nuclei (MD and PVP). However, the increase of c-Fos expression in the Re was close to significance level. We also cannot exclude a possibility that changed activity in mPFC is transmitted to the CA1 field by another possible pathway.

Local blockade of NMDA receptors in the mPFC modifies behavior

Local administration of the competitive NMDAR antagonist, CPP, to the mPFC is known to produce a whole range of behavioral alterations including increase in locomotor activity (Del Arco et al. 2008), rearing and the unique hyperactivity syndrome called darting (Savelli et al. 1995). In our study, using the uncompetitive NMDAR antagonist, MK801, we observed increased side-to-side head movement (in 7 out of 10 rats) and sustained atypical sniffing with a raised head. At the same time we did not notice significant differences in locomotor activity and rearing. Also grooming behavior was unaffected by MK801 treatment which is in accordance with results obtained by Savelli and colleagues (1995) after CPP infusion to mPFC.

Whether there is a causative link between the degree of alertness and amount of stereotypies displayed by MK801 treated animals and the c-Fos protein expression in distinct subcortical nuclei remains uncertain. The differences between various types of behavior were significant in our study around 40 min after MK801 infusion. The brains were taken at 90

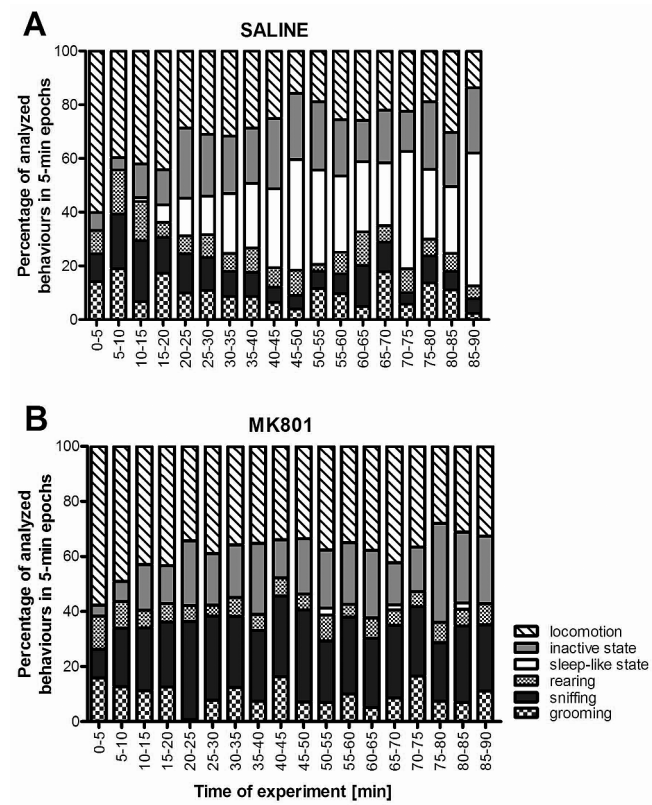

Fig. 6. The effect of local infusion of saline (A) and MK801 (B) into the mPFC on animal behavior (i.e. locomotion, inactive state, sleep-like state, rearing, stereotyped sniffing and grooming) in an open field arena during experiment evaluated for 5-min periods. The significant differences between the duration of behaviors occurred for: sleep-like state (45–55, 70–75 and 85–90 min) and stereotyped side-to-side head movements and sniffing (40–50 min). For detailed information see text. Duration of each behavior presented as percentage of 5 min time segment in 18 consecutive periods.

minutes after the injection. Taking into account that c-Fos protein expression is known to reach maximum level at 60–120 min after the onset of stimulus and then gradually decline, reaching baseline levels after around 4 h (e.g., Morgan and Curran 1991, Meyza et al. 2007), we cannot completely exclude the possibility that observed neuronal activation is a result of both direct action of MK801 and secondary behavioral changes. Nonetheless, since we did not see changes in locomotor activity throughout the course of the experiment (both hand scored and in automated analysis performed with EthoVision, Noldus NL – not shown here) it is difficult to explain the significant increase in the number of c-Fos positive cells in the CA1 field of the hippocampus, which has been previously linked with responsiveness to spatial challenges (Moser et al. 2008, Meyza et al. 2009). Also increases in neuronal activation in basolateral and central amygdala cannot be explained by response to novel environment, as all animals were habituated both to short restraint required for infusion and to the testing chamber. Therefore, although we cannot exclude the possibility that some of the observed changes in c-Fos expression were secondary to MK801 induced behavioral activation, the enhanced c-Fos expression seems to be primarily an effect of MK801 infusion.

CONCLUSIONS

In summary, we show that local blockade of NMDA receptors in the mPFC, at a dose sufficient to produce behavioral changes was associated with an induction of c-Fos protein in the NAc, amygdala (BLA and CeA), CA1 field of the hippocampus, and MD and PVP nuclei of thalamus. Considering that local blockade of NMDA receptors in the mPFC can produce complex cognitive impairments (e.g., Savelli et al. 1995, Stefani et al. 2003), our findings suggest that some of the behavioral abnormalities are associated with modified downstream neuronal activity in multiple subcortical structures.

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