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Impulsive behaviour induced by both NMDA receptor antagonism and $GABA_A$ receptor activation in rat ventromedial prefrontal cortex

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Abstract
Rationale Previous work has demonstrated a profound effect of *N*-methyl-D-aspartic acid receptor (NMDAR) antagonism in the infralimbic cortex (IL) to selectively elevate impulsive responding in a rodent reaction time paradigm. However, the mechanism underlying this effect is unclear.
Objectives This series of experiments investigated the pharmacological basis of this effect in terms of excitatory and inhibitory neurotransmission. We tested several pharmacological mechanisms that might produce the effect of NMDAR antagonism via disruption or dampening of IL output.
Methods Drugs known to affect brain GABA or glutamate function were tested in rats pre-trained on a five-choice serial reaction time task (5-CSRTT) following either their systemic administration or direct administration into the IL.
Results Systemic lamotrigine administration (15 mg/kg), which attenuates excess glutamate release, did not counteract the ability of the intra-IL NMDAR antagonist 3-((*R*)-2-carboxypiperazin-4-yl)-propyl-L-phosphonic acid ((*R*)-CPP) to increase premature responding on the 5-CSRTT. Putative elevation of local extracellular glutamate via intra-IL infusions of the selective glutamate reuptake inhibitor DL-*threo*-β-benzyloxyaspartate as well as local α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor antagonism also had no effect on this task. However, intra-IL infusions of the $GABA_A$ receptor agonist muscimol produced qualitatively but not quantitatively comparable increases in impulsive responding to those elicited by (*R*)-CPP. Moreover, the $GABA_A$ receptor antagonist bicuculline blocked the increase in impulsivity produced by (*R*)-CPP when infused in the IL.
Conclusions These findings implicate glutamatergic and GABAergic mechanisms in the IL in the expression of impulsivity and suggest that excessive glutamate release may not underlie increased impulsivity induced by local NMDA receptor antagonism.

Keywords Impulsivity · Prefrontal cortex · Glutamate · AMPA · NMDA · GABA

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Introduction

Individual differences in premature responding on the five-choice serial reaction time task (5-CSRTT) predict vulnerability to the reinforcing effects of cocaine and may indicate a trait impulsivity phenotype of relevance to attention deficit hyperactivity disorder and drug addiction (Blondeau and Dellu-Hagedorn 2007; Dalley et al. 2007; Everitt et al. 2008). Understanding the contributions of excitatory and inhibitory mechanisms controlling impulsive responding in the prefrontal cortex (PFC) may thus be relevant for the

treatment of such disorders. We previously reported that localised infusions of the *N*-methyl-D-aspartic acid receptor (NMDAR) antagonist 3-((*R*)-2-carboxypiperazin-4-yl)-propyl-L-phosphonic acid; ((*R*)-CPP) produced significant and selective increases in premature responding on the 5-CSRTT when administered into the infralimbic (IL) but not the prelimbic (PL) PFC (Murphy et al. 2005). However, the precise mechanisms by which NMDAR blockade produced this effect are unclear.

NMDAR antagonism in the IL cortex may act via several mechanisms to effect behavioural changes. First, we hypothesised that premature responses provoked by intra-IL NMDAR antagonism may be a consequence of increased extracellular glutamate availability (see Ceglia et al. 2004; Moghaddam et al. 1997), which may increase and/or disrupt local glutamatergic transmission such that output signals are dysregulated and behavioural control is impaired. To test this hypothesis, we attempted to block excess glutamate release with lamotrigine (Cunningham and Jones 2000) and, in a subsequent experiment, to elevate extracellular glutamate with a selective glutamate reuptake inhibitor (DL-*threo*-β-benzyloxyaspartate). Alternatively, NMDAR antagonism may dampen local fast synaptic transmission and decrease output signals, also impairing behavioural control. To test this hypothesis, we administered an α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor antagonist to block excitatory neurotransmission and the $GABA_A$ receptor agonist muscimol to promote inhibitory neurotransmission in the IL. We report that intra-IL infusions of muscimol produce a profile of behavioural effects qualitatively similar to that of (*R*)-CPP. Thus, excessive inhibition mediated by $GABA_A$ receptors may be a candidate mechanism underlying the effects of intra-IL (*R*)-CPP on impulsivity. Therefore, in a final experiment, we tested whether blockade of $GABA_A$ transmission in the IL with bicuculline could abolish the effect of (*R*)-CPP on impulsivity.

Methods and materials

Subjects

Subjects were male, Lister Hooded rats (Charles River, UK) weighing 300–400 g at the start of each experiment (n=31, total for whole study). During behavioural testing, animals were maintained on 18 g of rat chow per day and had ad libitum access to water. Animals were housed in pairs, and then singly following surgery under a reverse light cycle (lights on from 1900–0700 hours). Testing took place between 0900 and 1900 hours, 5 to 7 days each week. All experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 (PPL: 80/2234). Subjects included in each experiment were confirmed to have correctly located injector placements. Six subjects were used for experiment 1. Seven subjects were used for experiment 3, five of which were then used in experiment 2. For experiment 4, six subjects had injector tips located in the IL whilst seven subjects had injector tips in the PL. Five subjects were used for experiment 5.

Behavioural apparatus

A detailed description of the behavioural apparatus has been described previously (Carli et al. 1983; Murphy et al. 2005). Briefly, 20 25×25×25 cm nine-hole operant conditioning chambers (8 boxes built in the Department of Experimental Psychology, Cambridge, UK; 12 boxes from Campden Instruments, UK) were used, each contained within a ventilated and sound-attenuated chamber and illuminated by a 3-W house light. Nine evenly spaced square holes (2.5×2.5×4 cm) each containing a 3-W light were set into the curved aluminium wall at the rear of the box, 2 cm above the wire grid floor. Five of these holes were uncovered and available. An infrared beam located at the entrance to each hole enabled detection of nose-poke responses. A food magazine was located in the middle of the opposite wall into which food pellets could be dispensed (Noyes dustless pellets, 45 mg; Sandown Scientific, UK). The distance between the centre hole at the rear of the box and the magazine was 25 cm. A hinged door with a microswitch or infrared beam allowed access to the magazine and recording of entries. The boxes were controlled by Whisker software (Cardinal and Aitken 2010).

Behavioural training

Rats were trained on the 5-CSRTT as described previously (Murphy et al. 2005), and the task sequence has been described elsewhere (see Dalley et al. 2002, Fig. 2). Subjects were trained to make nose-poke responses into one of five holes in the front array upon brief stimulus illumination of the light located therein. The duration of the stimulus light was gradually reduced over 12 training stages from 30 to 0.5s. To begin a trial, the rats were required to enter the food magazine panel, and the next stimulus was illuminated after a 5-s intertrial interval. Following stimulus presentation, a correct nose-poke response was rewarded with a food pellet and illumination of the magazine light. Retrieval of the reward by entering the magazine initiated the next trial. A response prior to stimulus onset (a 'premature' response) or a failure to respond in a 5-s limited hold period after stimulus presentation (an 'omission') was punished by a 5-s timeout period in which the house light was extinguished and no reward was delivered.

Subsequent responses in any hole after the initial correct response or within the timeout period following an omitted or incorrect response were classified as perseverative responding. Rats received 5–7 sessions per week until a high level of stable performance was reached (≥80% accuracy, ≤15% omissions for the 5-CSRTT). Each session consisted of 100 completed trials and lasted a maximum of 30 min.

Surgery

Subjects were anaesthetised with either inhaled isoflurane (induced at 5%, maintained at 1–2%, flow rate 2 l/min) and secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA), fitted with atraumatic ear bars and an anaesthetic delivery nose cone fitted on the incisor bar (David Kopf Instruments) or with an intra-muscular injection of ketamine (Ketaset, 100 mg/kg; Vet Drug, Bury St Edmunds, UK) and xylazine (Rompun, 10 mg/kg, Vet Drug), and then secured in a stereotaxic frame. For all surgeries, the incisor bar was set at −3.3 mm relative to the interaural line for a flat skull position. Postoperative local anaesthetic was applied to the surgical wound.

Bilateral, 22-gauge stainless steel guide cannulae (Plastics One, UK) were implanted in the medial region of the PFC using standard stereotaxic techniques and were secured to the skull with bone screws and dental cement. The coordinates used were: AP +3.0 mm, L ±0.75 mm and DV −2.2 mm (Paxinos and Watson). After implantation, the guide cannulae were blocked with obturators and flush with the end of the guide, with the entire unit protected by a plastic dust cap. After surgery, the rats were singly housed and given ad libitum access to food and water for at least 5 days recovery before retraining on the behavioural task.

Microinfusion procedure

The guide cannulae allowed access to the PL or IL regions of the PFC. For PL infusions, 29-gauge injectors extending 1.5 mm beyond the end of the guides were used (−3.7 mm from dura). For IL infusions, 29-gauge injectors extending 3.0 mm beyond the end of the guides were used (−5.2 mm from dura).

The rats were habituated to the microinfusion procedure as previously described (Murphy et al. 2005). Intracranial infusions were always given on a 3-day cycle, with each infusion preceded by a baseline day and followed by a washout day where rats were not behaviourally tested.

Experiment 1: effects of NMDA receptor blockade and systemic lamotrigine

The rats were pretreated with systemic lamotrigine (LTG) prior to intra-IL administration of (*R*)-CPP. LTG was administered at 15 mg/kg (2 ml/kg) with the control group receiving the same volume of vehicle. LTG was dissolved in 50% propylene glycol in distilled water and administered by intra-peritoneal injection 2 h prior to infusion to allow for maximal drug absorption into the brain (Castel-Branco et al. 2002). The rats were tested in a counterbalanced 2×2 drug design: vehicle or LTG versus saline or intra-IL (*R*)-CPP (50 ng/side). The rats received each combination of treatments in a balanced Latin square design.

Experiment 2: effects of selective glutamate reuptake inhibition on impulsivity

In this experiment we putatively enhanced extracellular glutamate by blocking synaptic reuptake with intra-IL administered DL-*thre*o-β-benzyloxyaspartate (DL-TBOA). DL-TBOA (Tocris, UK) was dissolved in sterile phosphate-buffered saline (PBS) and administered in ascending doses on subsequent days (vehicle, 50 ng (0.388 mM), 500 ng (3.88 mM)) in a volume of 0.5 μl per side. This was done in case of excessive excitotoxicity at the highest dose, although previous findings suggest that acute increases in glutamate produced by transporter blockade are not sufficient to induce excitotoxicity (Massieu et al. 1995).

Experiment 3: effects of the AMPA receptor antagonist NBQX

In this experiment, an α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) antagonist was separately administered intra-IL prior to testing on the 5-CSRTT. NBQX di-sodium salt (2,3-dihydroxy-6-nitro-7-sulfamoylbenzo(f)quinoxaline) (Tocris, UK) was dissolved in sterile saline and administered in the IL at three doses: vehicle, 50 ng or 500 ng in 0.5 μl per side in a Latin square counterbalanced design (three levels). The doses were based on previous in vivo behavioural studies (e.g. Biondo et al. 2005; Choi et al. 2000; Ikeda et al. 2003; Nakamura et al. 2000).

Experiment 4: effects of the GABA receptor agonist muscimol on impulsivity

In experiment 4, we tested the effects of the $GABA_A$ receptor agonist muscimol in the PL or IL on the 5-CSRTT. Different subjects received either IL or PL infusions depending on cannulae location. Muscimol (Tocris, UK) was dissolved in PBS at a concentration of 1 μg/μl. Bilateral infusions aimed at either the PL or IL cortex were delivered at a dose of 0.5 μg and a volume of 0.5 μl/side over 1 min. Doses were chosen based on previous research (Coutureau and Killcross 2003).

Experiment 5: effects of bicuculline on (*R*)-CPP induced impulsivity

Bicuculline methiodide (Fluka, UK; 50 ng/side) was dissolved in PBS at a concentration of 1 μg/μl (Koya et al. 2009). Bilateral infusions aimed at the IL cortex were delivered at a concentration of 0.5 μg and a volume of 0.5 μl/side over 1 min, 5 min before the intra-IL infusion of (*R*)-CPP (50 ng/side).

Histology

After behavioural testing was completed, subjects were anaesthetised with a lethal dose of sodium pentobarbital (Euthatal, 200 mg/ml, Genus Express, UK) and perfused transcardially with 0.01 M PBS followed by 4% paraformaldehyde. The brains were removed and post-fixed in 4% paraformaldehyde overnight. Prior to being sectioned, the brains were transferred to a 20% sucrose solution in 0.01 M PBS as a cryoprotectant. Coronal sections were cut at 60 μm and every third section taken for cresyl violet staining. The locations of injector cannulae were mapped onto standardised sections of the rat brain (Paxinos and Watson 1998).

Data analysis

Data were analysed using SPSS 12.0.1 (SPSS Inc., Chicago, IL, USA). Six variables were analysed: the percentage of correct responses made (number of correct responses/total correct and incorrect responses), percentage of responses omitted (number of omissions/total number of correct, incorrect and omitted responses), percentage of premature responses (number of premature responses/total number of correct, incorrect and omitted responses), latency to make a correct response, latency to collect reward and perseverative responses. Behavioural data were subjected to analysis of variance (ANOVA) using a general linear model within-subjects design with appropriate factors and levels for the experimental design, using SPSS's type-III sum-of-squares method. Homogeneity of variance was verified using Levene's test (Levene 1960) and skewed data were subjected to appropriate transformations. All tests of significance were performed at α=0.05. Full factorial models were used unless otherwise stated. For repeated measures analysis, Mauchly's test of sphericity (Mauchly 1940) of the covariance matrix was applied and the degrees of freedom *df* were corrected to more conservative values using the Huynh–Feldt epsilon ε (Huynh and Feldt 1970) to correct any violations in the sphericity assumption (Cardinal and Aitken 2006). Significant interactions were investigated with post hoc tests using the Šidák procedure to reduce the probability of type I errors.

Results

Histology

All cannulae placements were accurately located in the appropriate brain areas. Figure 1 shows a composite schematic of PL and IL cannulae placements for all experiments.

Experiment 1: effects of NMDA receptor blockade and systemic lamotrigine

Intra-IL infusions of (*R*)-CPP significantly increased premature responding on the 5-CSRTT but this effect was not antagonised by pre-treatment with 15 mg/kg LTG. A 2×2 ANOVA revealed a main effect of (*R*)-CPP ($F_{1,5}$=19.6, p=0.007; see Fig. 2 and Table 1). LTG alone had no significant effect on any behavioural measure on the 5-CSRTT. Intra-IL (*R*)-CPP also significantly decreased accuracy (main effect; $F_{1,5}$=7.3, p=0.043) and increased

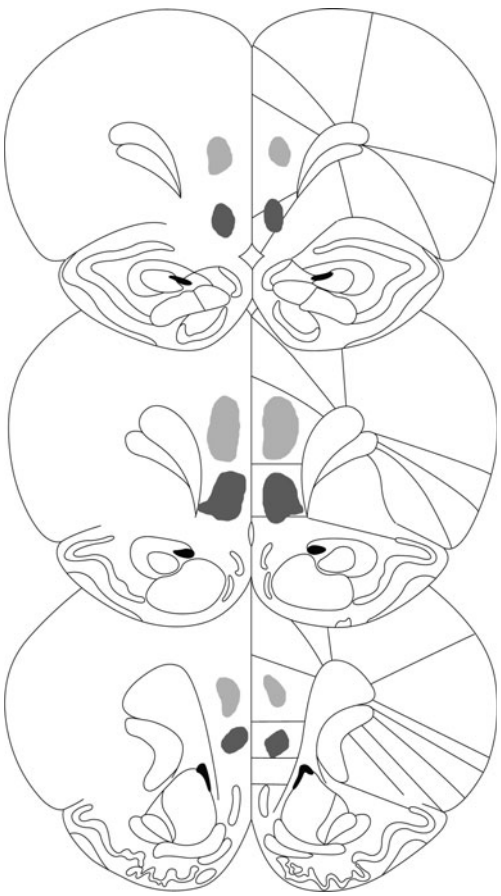

Fig. 1 Composite diagram illustrating the placement of injector tips in PL cortex (*light grey*) and IL cortex (*dark grey*) across all experiments. Adapted from Paxinos and Watson 1998 (from Bregma: *top* +3.7 mm; *middle* +3.2 mm; *bottom* +2.7 mm)

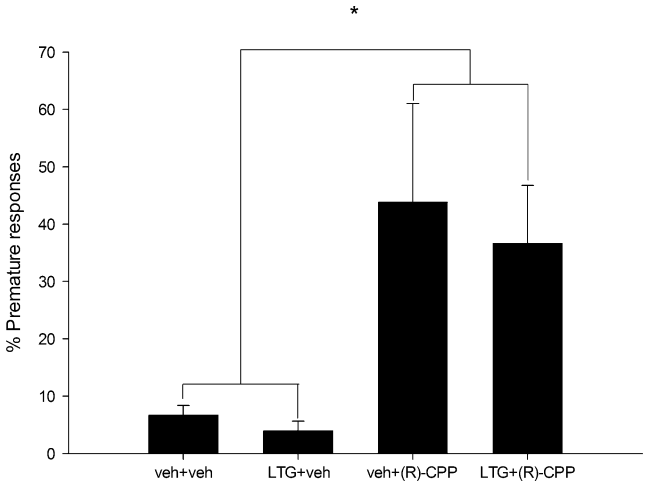

Fig. 2 Percentage of premature responding in the 5-CSRTT following the infusion of 50 ng/side (*R*)-CPP into IL cortex in rats pretreated with either 15 mg/kg LTG or vehicle (n=6). (*Asterisk*) indicates main effect of drug 2 (veh+veh, LTG+veh vs. veh+(*R*)-CPP, LTG+(*R*)-CPP)

omissions (main effect; $F_{1,5}$=87.8, p<0.001) and slowed correct response latencies ($F_{1,5}$=8.0, p=0.037).

Experiment 2: effects of selective glutamate reuptake inhibition on impulsivity

Intra-IL DL-TBOA produced no significant effects on behavioural performance (Table 2). The highest dose (500 ng) tended to increase omissions, though this effect did not reach significance ($F_{2,8}$=4.07, p=0.06). Increasing doses of DL-TBOA tended to slow correct response latency; this effect approached but did not reach significance ($F_{2,8}$=3.63, p=0.075).

Experiment 3: effects of the AMPA receptor antagonists NBQX

There were no significant effects of NBQX on behavioural performance on the 5-CSRTT when administered directly in the IL (Table 3).

Experiment 4: effects of the GABA receptor agonist muscimol on impulsivity

Infusions of muscimol into the IL, but not PL, significantly increased premature responding on the 5-CSRTT (Fig. 3 and Table 4). Percentages of premature responses were subjected to an arcsine transformation to satisfy homogeneity of variance. The ANOVA revealed a significant main effect of the drug ($F_{1,11}$=11.2, p=0.007), a between subject effect of brain region ($F_{1,11}$=10.8, p=0.007) and a significant drug × brain region interaction ($F_{1,11}$=5.14, p=0.045). Pair-wise comparisons revealed that muscimol significantly increased premature responding when administered in the IL (p=0.002) but had no effect on premature responding when administered in the PL (p=0.477).

Muscimol also produced statistically significant but qualitatively moderate effects on accuracy, omissions, perseverative responding and reward collection latency, but none of these effects were modulated by the site of drug infusion in the mPFC (see Table 4). The decline in accuracy after muscimol infusion was qualitatively similar in both brain areas (main effect of drug, $F_{1,11}$=13.64, p=0.004) but there was no main effect of brain region nor interaction, (F ratios <1).

Muscimol also increased the percentage of omitted responses when administered to either brain area (main effect of drug ($F_{1,11}$=24.28, p<0.001), but there was no significant main effect of brain region and no interaction, F ratios<1). Muscimol also increased perseverative responding when infused into the PL and IL ($F_{1,11}$=8.7, p=0.013) whilst slowing reward collection latencies ($F_{1,11}$=9.882, p=0.009). However, correct response latency was not significantly affected by muscimol, indicating that animals were not slowed in choosing the correct stimulus and that the slowing of magazine latencies was unlikely to be a sedative or a motor effect.

Experiment 5: effects of bicuculline on (*R*)-CPP induced impulsivity

As observed in experiment 1, premature responses increased following intra-IL infusions of (*R*)-CPP. This effect

Table 1 Effects of 50 ng/side (*R*)-CPP in IL cortex and 15 mg/kg LTG (i.p.) on the 5-CSRTT

Drug	Correct (%)	Omissions (%)	Correct latency (s)	Collection latency (s)	Persev nose pokes
Veh+veh	81.9±3.6	15.8±6.7	0.56±0.03	4.06±2.4	61.5±12.6
Veh+LTG	80.2±3.5	16.1±5.5	0.58±0.04	3.08±1.27	55±11.4
CPP+veh	63.8±6.1[a]	31.1±6.4[a]	0.68±0.06[a]	2.14±0.84	64.2±9.6
CPP+LTG	71.1±5.8	41.3±5.4	0.81±0.08	4.24±1.92	78.2±16.2

Values are means±SEM

[a] Significant with respect to veh+veh

Table 2 Effects of intra-IL DL-TBOA on 5-CSRTT performance

Drug	Correct (%)	Omissions (%)	Premature (%)	Correct latency (s)	Collection latency (s)	Persev nose pokes
PBS	92.6±1.7	4.4±0.9	4.0±1.4	0.47±0.04	1.40±0.08	50.2±8.0
50 ng	91.1±1.3	3.4±0.8	5.6±3.4	0.50±0.03	1.49±0.09	56.2±8.1
500 ng	90.0±0.6	9.2±2.5	4.0±1.3	0.56±0.03	1.46±0.10	46.2±13.6

Values are means±SEM

PBS phosphate-buffered saline

was antagonised by pre-treatment with intra-IL infusions of bicuculline (Fig. 4 and Table 5). The ANOVA revealed main effects of bicuculline ($F_{1,5}$=14.56, p=0.012), (*R*)-CPP ($F_{1,5}$=14.68, p=0.01) and a non-significant interaction, p=0.069. As a significant omnibus interaction is not required if family-wise error is controlled (Cardinal and Aitken 2006), we conducted pair-wise comparisons using Šidák's correction, which revealed an increase in premature responding following IL infusions of (*R*)-CPP (p=0.024). This effect was prevented by prior bicuculline infusion (p=0.015) at a dose which itself also reduced premature responding (p=0.019).

Consistent with the results of experiment 1, intra-IL (*R*)-CPP increased omitted responses (main effect of (*R*)-CPP; $F_{1,6}$=13.06, p=0.015). This effect was neither antagonised nor potentiated by intra-IL bicuculline. Measures of accuracy, perseverative responding and response latencies (correct and collection latencies) were not significantly affected (see Table 5) by these manipulations. Although marginal main effects of (*R*)-CPP were observed for correct (p=0.065) and collection (p=0.074) latencies, these did not interact with the prior infusion of bicuculline (both ps>0.15).

Baseline analysis

Baseline accuracy and omissions performance levels for all rats included in the final analysis was stable, as assessed over 12 different sessions: 3 preoperative training sessions immediately prior to surgery, 4 postoperative restabilization sessions (including 2 mock infusions) and the baseline session immediately prior to each infusion day (no significant effects of day on attentional accuracy ($F_{11,132}$=1.290, NS) or omissions ($F_{3.257,39.089}$=0.805, ε=0.296, NS)). Baseline accuracy and omissions were also stable as assessed over 11 sessions (3 preoperative, 4 postoperative (including a mock infusion) and the baseline day prior to each infusion) in the two combined cohorts used for the bicuculline study (n=6) $F_{10,60}$=1.319, p=.241 and $F_{10,60}$=1.604, p=.127, respectively.

Discussion

The main findings may be summarized as follows: enhancing GABAergic transmission via infusions of the $GABA_A$ receptor agonist muscimol into the IL but not the PL dramatically increased premature responding, with a relative lack of effect on other performance variables; a profile that was qualitatively similar to that previously shown following the NMDAR antagonist (*R*)-CPP and replicated here. In addition, we show that blockade of $GABA_A$ receptor transmission in the IL is sufficient to prevent the increase in impulsivity induced by (*R*)-CPP.

Previous findings suggest that the effects of the NMDAR antagonist may be a direct result of excess extracellular glutamate (see Ceglia et al. 2004; Moghaddam et al. 1997). However, we could not find any evidence to substantiate this conclusion using direct pharmacological manipulation of glutamate levels; (1) the systemically administered glutamate release blocker LTG failed to block premature responding despite presumably decreasing glutamate release in the PFC and (2) intra-IL DL-TBOA failed to increase premature responding despite its putative action to elevate extracellular glutamate. Furthermore, the effect of NMDAR antagonism in the IL was not mimicked by intra-IL AMPA receptor antagonism. However, some degree of caution is necessary in the interpretation of these results as

Table 3 Effects of intra-IL NBQX on 5-CSRTT performance

Drug	Dose	Correct (%)	Omissions (%)	Premature (%)	Correct latency (s)	Collection latency (s)	Persev nose pokes
NBQX (n=7)	Saline	91.5±1.0	4.7±1.8	6.7±2.4	0.53±0.03	1.6±0.2	50.7±8.8
	50 ng	92.9±1.4	6.6±2.5	5.7±1.7	0.49±0.02	2.4±1.0	53.7±9.7
	500 ng	90.7±1.8	7.1±2.4	6.4±1.4	0.53±0.04	1.4±0.1	49.6±6.4

Values are means±SEM

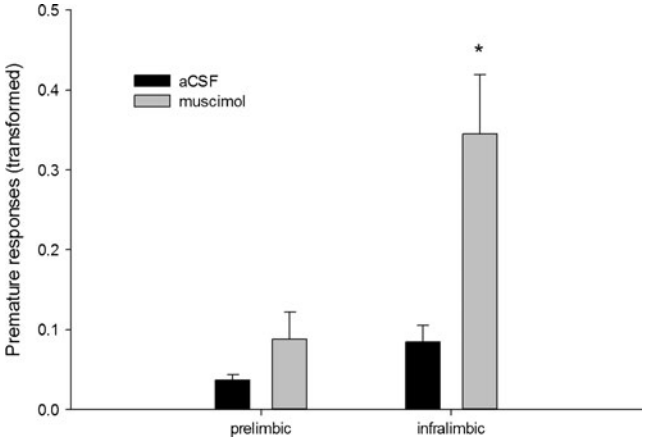

Fig. 3 Proportion of premature responses (arcsine transformation) following either the administration of PBS or muscimol into the PL (n=6) or IL (n=7). *Asterisk* indicates $p<0.05$ compared to the PBS control condition. Mean raw scores (SEM) of percentage premature responding. PL, percentage premature responding: mean=3.7% (0.07); muscimol, percentage premature responding: mean=8.8% (3.4). IL, percentage premature responding: mean=8.4% (2.1); muscimol, percentage premature responding: mean=33.9% (7.3)

the assumption that changes in extracellular levels of glutamate necessarily reflect changes in neuronal firing may not be valid in all cases (Obrenovitch et al. 2000). Thus, although extracellular glutamate levels increase after NMDA blockade, the resultant behavioural effects may be independent of changes in extracellular glutamate.

The present data shed light on two parallel questions: (1) the role of IL in inhibitory control as measured in the 5-CSRTT, and (2) glutamatergic-GABAergic interactions in the IL cortex that modulate its inhibitory control function. The most striking result was that $GABA_A$ receptor activation in the IL (but not PL) produced similar elevations of premature responding as did infusions of the NMDAR antagonist (*R*)-CPP. The restriction of this effect to the IL supports the anatomical dissociation reported earlier by Murphy et al. (2005), and recent reports of limited diffusion of muscimol support the anatomical selectivity of this effect (Allen et al. 2008). The effect of muscimol infusions was most pronounced with respect to premature responding. Accuracy and omissions were also impaired by muscimol in the mPFC; however, the relatively subtle reduction in performance suggests there was no gross behavioural impairment.

The similarity of $GABA_A$ agonist and NMDAR antagonist behavioural effects is intriguing given that these manipulations superficially have opposing neurochemical effects; for example, whereas muscimol acts to augment inhibitory GABAergic neurotransmission in the PFC (Matsumoto et al. 2003), NMDAR antagonists increase the tonic firing activity in mPFC neurons (Suzuki et al. 2002). However, NMDA and GABA receptor functions are closely linked, especially in the PFC. GABAergic neurons in the PFC are exclusively interneurons shown to be dominant in regulating the activity of projecting pyramidal cells (Benes and Berretta 2001). Therefore, the most straightforward explanation for the present data in terms of cortical microcircuitry is that GABAergic interneurons mediate the increase in premature responses observed after NMDA blockade. Excess glutamate after NMDA blockade may stimulate GABAergic interneurons, resulting in inhibition of output pyramidal neurons. $GABA_A$ receptor activation thus appears to act as a 'reversible lesion' (e.g. Amat et al. 2005; Coutureau and Killcross 2003; de Wit et al. 2006; Fuchs et al. 2004; McFarland et al. 2004; Yin et al. 2006) inhibiting transmission in the IL such that output is disrupted or attenuated. Indeed, fibre-sparing, cell body lesions of the IL cause persistent deficits in inhibitory control on the 5-CSRTT (Chudasama et al. 2003) consistent with that hypothesis. In addition, $GABA_A$ receptor blockade by bicuculline reversed the effects of NMDA blockade. It should be noted that bicuculline reduced premature responding when administered alone in the IL. However, since this effect was behaviourally selective with no significant effects on other behavioural measures, we conclude that blocking endogenous GABAergic tone at $GABA_A$ receptors in the IL is sufficient to reduce impulsivity.

It is possible that a 'shutdown' of IL output similarly accounts for the NMDAR antagonism findings of enhanced impulsivity. However, a problem with this interpretation is that AMPAR antagonism with NBQX in the IL cortex had no effect on task performance. This may have been due to an inadequate dose; however, the doses used are reportedly

Table 4 Effects of intra-PL or intra-IL muscimol on 5-CSRTT performance

PFCArea	Drug	Correct (%)	Omissions (%)	Correct latency (s)	Collection latency (s)	Persev nose pokes
PL	PBS	88.2±2.1	5.8±2.6	0.50±0.02	1.07±0.03	30.3±5.7
	Muscimol	69.1±8.8[a]	19.2±3.4[a]	0.68±0.15	1.80±0.34[a]	84.3±27.2[a]
IL	PBS	84.6±3.6	4.9±1.3	0.59±0.04	1.50±0.06	62.9±20.2
	Muscimol	71.7±4.1[a]	29.4±5.6[a]	0.67±0.05	1.94±0.19[a]	94.7±33.5[a]

Values are means±SEM

[a] Significant with respect to PBS

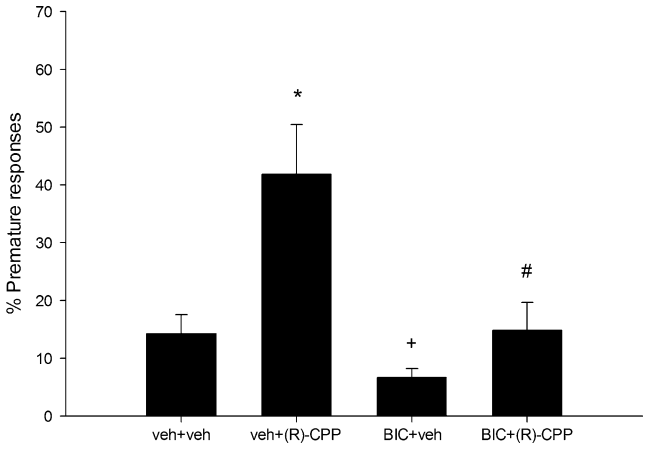

Fig. 4 Percentage of premature responding in the 5-CSRTT following the infusion of 50 ng/side (*R*)-CPP into IL cortex in rats pretreated with either 50 ng/side bicuculline or vehicle (n=6). *Asterisk* indicates main effect of drug 1 (veh+veh, veh+(*R*)-CPP vs. BIC+veh, BIC+(*R*)-CPP) and *number sign* indicates main effect of drug 2 (veh+veh, BIC +veh vs. veh+(*R*)-CPP, BIC+(*R*)-CPP). *Plus sign* indicates significance with respect to veh+veh

active at other brain sites in different behavioural paradigms (Biondo et al. 2005; Burns et al. 1994; Choi et al. 2000; Ikeda et al. 2003; Nakamura et al. 2000; Winters and Bussey 2005). NBQX is a selective, competitive AMPA/kainate receptor antagonist (Sheardown et al. 1990). In principle, AMPAR blockade should attenuate fast excitatory synaptic transmission and NMDAR-mediated transmission concurrently (see Robbins and Murphy 2006). However, recent work with genetic knock-out animals indicates possible AMPA-independent activation of NMDA receptors (Bannerman et al. 2003; reviewed by Bannerman et al. 2006; Schmitt et al. 2003), challenging the prevailing view of the functional coupling of NMDA and AMPA receptors in controlling pyramidal cell output. The present results indicate that non-NMDA receptor blockade in the mPFC is insufficient to induce failed response inhibitory control.

The lack of behavioural effect of LTG in this study contrasts with evidence that LTG is beneficial in other cognitive tasks disrupted by NMDAR antagonism, including reversal learning (Idris et al. 2005), despite the use of similar doses. However, the present results are supported by recent work demonstrating that a broader range of doses of LTG have no effects on any measure of the 5-CSRTT (Shannon and Love 2005). It is thus possible that different mechanisms mediate NMDAR antagonist-impaired performance in reversal learning and 5-CSRTT task paradigms, though both clearly involve a component of inhibitory control. This difference may lie in the nature of the failure of a specific type of inhibitory control unique to each task. Taken together, the lack of behavioural effects of both DL-TBOA and LTG lead us to reject the hypothesis that increased impulsive responding was simply due to excess glutamate release in the IL cortex and resulting hyperexcitation.

Conclusions

The equivalent effects of NMDAR antagonism and $GABA_A$ receptor activation in the IL cortex on the control of impulsive responding do not fit with the notion that NMDAR antagonism 'disinhibits' GABAergic interneurons, leading to an excess of glutamate release (Konradi and Heckers 2003), and that it is the excess glutamate that is the primary cause of neuronal dysfunction. Indeed, $GABA_A$ receptor activation by muscimol *increases* GABAergic tone, and $GABA_A$ receptor antagonism prevented the effect of NMDAR antagonism on impulsivity. These results suggest that NMDA and $GABA_A$ receptors exert opposing effects on pyramidal cell output, with respective antagonism and agonism producing similarly dysregulated inhibitory control. The lack of effects of AMPAR antagonism argues against a hypothesis of 'fast excitatory shutdown' of the cortical microcircuitry—but does not preclude the possibility of diminished and dysregulated efferent transmission from pyramidal neurons as the cause of failure of inhibitory control.

These results highlight the complexity of the functional microcircuitry of the corticocortical glutamate and GABA systems in the PFC (Benes and Berretta 2001) as illustrated by the fact that different subpopulations of interneurons receive different strengths of glutamatergic

Table 5 Effects of 50 ng/side (*R*)-CPP in IL cortex, after 50 ng/side of bicuculline (BIC) or veh on the 5-CSRTT

Drug	Correct (%)	Omissions (%)	Correct latency (s)	Collection latency (s)	Persev nose pokes
Veh+veh	65.3±9.9	22.3±12.0	0.69±0.09	1.36±0.06	112.0±37.2
Veh+(*R*)-CPP	67.9±3.5	43.2±4.8*	0.79±0.12	2.70±0.96	100.0±15.2
BIC+veh	76.9±6.0	24.2±5.4	0.75±0.09	2.17±0.72	146.7±62.8
BIC+(*R*)-CPP	62.6±9.3	57.3±9.7	0.95±0.11	13.4±5.6	96.5±18.9

Values are means±SEM

[a] Significant with respect to veh + veh

drive and express unique complements of glutamate receptor subunits (Lewis and Moghaddam 2006). For example, overactivation of GABAergic chandelier cells may disrupt the timing of inhibition necessary to synchronize pyramidal neuron firing, thus disrupting cortical output transmission (Benes and Berretta 2001; Lewis and Moghaddam 2006). Future work should explore the apparently critical role of cortical pyramidal output in the IL with respect to inhibitory response control in the 5-CSRTT and related tasks.

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