Published in final edited form as:

Eur Neuropsychopharmacol. 2014 February ; 24(2): 321–332. doi:10.1016/j.euroneuro.2013.07.005.

Characterization of electrically evoked field potentials in the medial prefrontal cortex and orbitofrontal cortex of the rat: modulation by monoamines

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Abstract

Medial prefrontal cortex (mPFC) and orbitofrontal cortex (OFC) play critical roles in cognition and behavioural control. Glutamatergic, GABAergic, and monoaminergic dysfunction in the prefrontal cortex has been hypothesised to underlie symptoms in neuropsychiatric disorders. Here we characterised electrically-evoked field potentials in the mPFC and OFC. Electrical stimulation evoked field potentials in layer V/VI of the mPFC and layer V of the OFC. The earliest component (approximately 2 ms latency) was insensitive to glutamate receptor blockade and was presumed to be presynaptic. Later components were blocked by 6,7-dinitroquinoxaline-2,3-dione (DNQX (20 μ M) and were assumed to reflect monosynaptic (latency 4–6 ms) and polysynaptic activity (latency 6–40 ms) mediated by glutamate via AMPA/kainate receptor. In the mPFC, but not the OFC, the monosynaptic component was also partly blocked by 2-amino-5-phosphonopentanoic acid (AP-5 (50–100 μ M) indicating the involvement of NMDA receptors. Bicuculline (3–10 μ M) enhanced the monosynaptic component suggesting electrically-evoked and/or glutamate induced GABA release inhibits the monosynaptic component via GABA_A receptor activation. There were complex effects of bicuculline on polysynaptic components. In the mPFC both the mono- and polysynaptic components were attenuated by 5-HT (10–100 μ M) and NA (30 and 60 μ M) and the monosynaptic component was attenuated by DA (100 μ M). In the OFC the mono- and polysynaptic components were also attenuated by 5-HT (100 μ M), NA (10–100 μ M) but DA (10–100 μ M) had no effect. We propose that these pharmacologically characterised electrically-evoked field potentials in the mPFC and OFC are useful models for the study of prefrontal cortical physiology and pathophysiology.

Keywords

glutamate; GABA; 5-HT; noradrenaline; dopamine

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1. Introduction

The prefrontal cortex (PFC) is critically involved in executive functions and behavioural control. The rat mPFC mediates attentional set shifting (Birrell and Brown, 2000) and so is regarded as homologous to the dorsolateral prefrontal cortex in primates. In both rodents and primates, the orbitofrontal cortex (OFC), appears to play a pivotal role in reversal learning (McAlonan and Brown, 2003; Rygula et al., 2010) and the motivational control of behaviour and impulsivity (Berlin et al., 2004; Rudebeck et al., 2006; Zeeb et al., 2010).

Both the mPFC and OFC are extensively innervated by 5-HT (serotonin), noradrenaline (NA) and dopamine (DA) (Agster et al., 2012; Descarries et al., 1987; Hoover and Vertes, 2007; Oades and Halliday, 1987; Slopeema et al., 1982). Electrophysiological studies have revealed that monoamines regulate firing activity in neurones of the prefrontal cortex. In the mPFC, inhibition of pyramidal cell firing activity is the predominant response to electrical stimulation of the serotonergic dorsal and median raphe nuclei (DRN and MRN) (Hajos et al., 2003; Mantz et al., 1990), the noradrenergic locus coeruleus (LC) (Mantz et al., 1988) and the dopaminergic ventral tegmental area (VTA) (Godbout et al., 1991; Mantz et al., 1988). Data from the OFC are sparser but DRN stimulation has been shown to alter the electroencephalogram (Dringenberg and Vanderwolf, 1997) and 5-HT agonists have been shown to influence OFC pyramidal cell firing (Rueter et al., 2000). The noradrenaline and DA releasing agent d-amphetamine (Homayoun and Moghaddam, 2008) and the DA reuptake inhibitor cocaine (Guillem et al., 2010), have also been shown to influence pyramidal cell firing in the OFC. Studies employing pharmacological agents that modify monoamine neurotransmission or selective monoamine neurotoxic lesions also point to important roles for the monoamines in the behavioural functions subserved by the mPFC and OFC (McGaughy et al., 2008; Robbins and Arnsten, 2009; Winstanley et al., 2006).

Many psychiatric disorders, including bipolar disorder, schizophrenia, obsessive compulsive disorder and substance abuse disorders, are characterised by deficits in prefrontally-mediated cognitive functions and/or behavioural control (Cunha et al., 2010; de Geus et al., 2007; McKirdy et al., 2009). Given the efficacy of drugs which target monoamine neurotransmission in the treatment of these disorders, it is reasonable to hypothesise that alterations in monoamine modulation of prefrontal function may underlie the functional deficits and/or the therapeutic effects of drugs. Models to assess prefrontal cortical function and the influence of monoaminergic neurotransmission in experimental animals would be of potential utility in investigating these hypotheses.

In specific brain regions with coherent laminar structures such as the cerebral cortex and hippocampus, synaptic transmission can be measured using electrically evoked field potentials (Empson and Heinemann, 1995; Langdon and Sur, 1990; Lomo, 1971). Here we characterized intra-laminar field potentials stimulated and recorded in layer V/VI of the infralimbic region of the mPFC and in layer V of the ventral/lateral portions of the OFC. The roles of glutamate and GABA and the potential modulation of the field potentials by monoamine neurotransmitters were examined.

2. Experimental Procedures

Animals and tissue

All animal procedures were approved by the Newcastle University Ethical Review Committee and licenced by the UK Home Office. All efforts were made to minimise the numbers of animals used and the suffering of individual animals.

Adult male Lister hooded rats (250-330 g) (n=45) (Charles River, UK) were group housed in controlled conditions of light and humidity and were allowed free access to food (SDS diet) and water. Rats were killed by decapitation and the brain was rapidly removed and placed in ice-cold sucrose slush (composition: 176mM Sucrose, 9.9mM HEPES, 25mM NaHCO₃, 10mM D-Glucose, 1.2mM NaH₂PO₄, 0.5mM CaCl₂, 2.5mM KCl, 7mM MgSO₄) oxygenated with 95% O₂: 5% CO₂ (carbogen). Coronal slices (350 or 400 µm) of the PFC (containing both mPFC and OFC) were prepared using a Vibratome™. A single slice was placed on lens tissue (Whatman 105) in an interface perfusion chamber and was perfused with oxygenated artificial cerebrospinal fluid (aCSF) (0.5 ml/min). The slice and aCSF were warmed to 37°C with a water chamber and a stream of warm humidified carbogen passed continuously over the slice. Spare slices were maintained in oxygenated aCSF at room temperature for later use.

Electrophysiological recording

Stimulating electrodes, comprised two ultra-fine (0.111mm) Teflon insulated silver wires, (Advent Research Materials, England) twisted together and cut square at the end to reveal two contacts separated by approximately 100 µm. Recording electrodes were glass microelectrodes (1.5 mm OD; Clarke Electromedical, Reading, UK) pulled on a vertical pipette puller (Narashige, Japan) and filled with 2.5M NaCl coloured with 2% Pontamine Sky Blue. For the mPFC, the stimulating electrode was placed in layer V/VI of the ventral infralimbic cortex immediately medial to the white matter of the claustrum and the recording electrode was placed 500-1000 µm dorsal to the stimulating electrode in layer V/VI (Fig 1). For the OFC, the stimulating electrode was placed in layer V of ventral or lateral OFC immediately ventral to the claustrum and the recording electrode was placed 500-1000 µm lateral to the stimulating electrode also in layer V (Fig 1). It was not possible to visualise the cell layers during recordings and placement in layer V/VI (mPFC) and layer V (OFC) is presumed. Extracellular potentials were amplified (x1000) and filtered (band pass: 0.2 Hz - 15 kHz) and were fed via an interface (micro1401, CED, Cambridge, UK) to a PC and recorded at a sampling rate of 10 or 50 kHz using Spike2 software (version 4, CED, Cambridge, UK). Data sampled at 50 kHz was subsequently down sampled using a Matlab script. Potentials quoted are corrected for amplification.

Square pulses generated by a Grass S88 stimulator (Grass Instruments, USA) were delivered to the stimulating electrode via an isolated stimulator unit (Digitimer Ltd). Test stimulation pulses (3-20 V, 100 µs duration) were applied at a frequency of 0.1 Hz. Following identification of a field potential (FP), the stimulus rate was reduced to 0.03 Hz and a voltage-response relationship established. A stimulus voltage evoking an approximately half-maximal field potential was chosen, and repeated stimulations at this voltage were then

applied. Once the response was stable for at least 10 minutes, drugs were applied via the perfusion medium.

Drugs

Stock solutions of 10mM were prepared. 6,7-Dinitroquinoxaline-2,3-dione (DNQX, Tocris) was dissolved in dimethyl sulphoxide. (–) Bicuculline methiodide (Tocris), DL-2-amino-5-phosphonopentanoic acid (AP-5, Sigma), 5-hydroxytryptamine HCl (5-HT, Alfa Aesar), noradrenaline HCl (NA, Sigma) and dopamine HCl (DA, Sigma), were dissolved in aCSF. Sodium-metabisulphite ($\text{Na}_2\text{S}_2\text{O}_5$) was added to the DA and NA stock solutions to a final concentration of 0.01M and they were kept in the dark to prevent oxidation. Intermediate and final dilutions of all drugs were made in aCSF.

Drugs were applied via the perfusion for 10 min periods. In most cases, multiple drugs and/or drug concentrations were applied to each slice. Increasing concentrations of drugs were applied without any recovery period (unless otherwise indicated). Where different drugs were to be applied, the field potential was allowed to recover following washout of one drug before another drug was applied.

Data analysis

The slice was defined as the experimental unit. The baseline was defined as the average of the ten field potentials in the 300s prior to drug application and the drug response as the average of the ten field potentials in the last 300 s of the drug application. Field potentials were averaged, and points of inflection were identified. These points of inflection were used to calculate the magnitude of components PC and C1-C4 (see results below). Data were subject to ANOVA (using a within subjects design) and where appropriate post hoc paired t tests were performed. Because PC was not present in all recordings, PC data were analysed separately. PC values were subject to one way ANOVA or paired t test.

3. Results

Field potentials in the mPFC contain multiple components

Electrically-evoked field potentials in layer V-V1 of the mPFC showed a consistent shape (Fig 2a). Following the stimulus artefact, five distinct points of inflection were observed. The first point was a small negative deflection with a very short latency around 2 ms. This putative presynaptic component (PC) was not always present or distinguishable from the stimulus artefact. Next there was a large negative peak (A) with latency around 4 ms suggesting a monosynaptic response, followed by a positive peak (B) around 8 ms, a negative trough (C) at 14 ms and a final positive peak (D) at around 22 ms.

In layer V of the OFC the electrically-evoked field potential was also highly consistent (Fig 2b). The field potential comprised four distinct points of inflection: the putative presynaptic component had a very short latency around 2 ms and was not always present. The second negative deflection (A) had a latency of around 4 ms, suggesting a monosynaptic response. This was followed by a positive deflection (B) with a latency of close to 8 ms, a much later

negative deflection (C) at around 16 ms. Often later positive and negative deflections were present but these were not consistent.

For analysis of the effects of drugs, the points of inflection were resolved into absolute voltages by subtraction (Figs 2a and 2b). Thus, where present, the presynaptic component (PC) was defined as the absolute value of this point. Component (C1) was defined as the absolute value of point A. C2 was defined as the difference between points A and B. C3 was defined as the difference between points B and C. C4 (mPFC only) was defined as the absolute difference between points C and D.

Role of glutamate in field potentials in the mPFC and OFC

To investigate the contribution of glutamate to the field potential, the AMPA/kainate receptor antagonist DNQX and the NMDA receptor antagonist AP5 were applied.

mPFC—In the mPFC, DNQX (20 μ M) markedly reduced the later components of the field potential ($n=7$) (Fig 3a). The PC component was distinguishable in 6/7 recordings. Paired t test showed that DNQX failed to alter PC (0.4079 ± 0.0912 mV vs 0.4064 ± 0.1160 mV (control vs DNQX); paired t test). Two way ANOVA showed a significant main effect of DNQX ($F_{1,6}=36.0$; $P<0.001$) and a significant main effect of component ($F_{3,18}=8.2$; $P<0.001$) as well as a significant DNQX $\times$ component interaction ($F_{3,18}=5.1$; $P<0.01$). Post hoc paired t tests showed that DNQX significantly reduced C1, C2, C3, and C4 ($P<0.01$).

AP-5 (50 and 100 μ M), was applied with a control period between the two concentrations ($n=6$). The PC component was apparent in 5/6 recordings. There was no significant effect of either concentration on the PC (0.2402 ± 0.0560 mV vs 0.2600 ± 0.0782 mV (control vs 50 μ M AP-5) and (0.2273 ± 0.0553 mV vs 0.2386 ± 0.0732 mV (control vs 100 μ M AP-5); paired t tests). At the lower concentration of AP-5 there were significant main effects of AP-5 ($F_{1,5}=57.0$; $P<0.001$) and component ($F_{3,15}=6.9$; $P<0.01$) and a significant interaction ($F_{3,15}=7.1$; $P<0.05$). Only C1 ($P<0.05$) was significantly reduced (post hoc paired t test) (data not shown). For the higher concentration, again there were significant main effects of AP-5 ($F_{1,5}=95.3$; $P<0.001$) and component ($F_{3,15}=8.9$; $P<0.001$) and a significant interaction ($F_{3,15}=6.8$; $P<0.01$). Both C1 and C2 ($P<0.05$) were significantly attenuated by AP-5 (100 μ M) (Fig 3b).

OFC—DNQX (20 μ M) markedly reduced the amplitude of the later parts of the field potential in the OFC (Fig 4a). The earliest component (PC) was distinguishable in 8 of the 12 recordings. As in the mPFC, the PC was not reduced by DNQX (0.3285 ± 0.1035 mV vs 0.2890 ± 0.0654 mV (control vs DNQX); paired t test) however later components were markedly reduced. Thus there was a significant main effect of DNQX ($F_{1,11}=24.9$; $P<0.001$) and a significant main effect of component ($F_{2,22}=26.8$; $P<0.001$) as well as a significant DNQX $\times$ component interaction ($F_{2,22}=18.2$; $P<0.000$). DNQX significantly reduced C1, C2 and C3 ($P<0.01$, post hoc paired t tests).

Only the higher concentration of AP-5 was tested in the OFC ($n=9$) (Fig 4b). The PC component was present in only 4 of the 9 recordings. Paired t test showed no effect of AP-5 on the PC (0.4605 ± 0.1843 mV vs 0.4559 ± 0.1632 mV (control vs AP5)). AP-5 (100 μ M)

reduced the later parts of the field potential; however the magnitude of this effect was very small. ANOVA showed a significant main effect of AP5 ($F_{1,8}=5.4$; $P<0.05$) significant effect of component ($F_{2,16}=21.1$; $P<0.001$) but no significant interaction.

Role of GABA in field potentials in the mPFC and OFC

mPFC—To investigate the role of GABA in the mPFC field potential, the GABA_A receptor antagonist bicuculline was applied at two concentrations (3 μ M and 10 μ M) with a washout between ($n=7$). Bicuculline had dramatic effects on the field potential in the mPFC. The lower concentration (3 μ M) significantly increased C1 and caused a shift to positive potentials in the later parts of the field potential (Fig 5a). The higher concentration (10 μ M) also significantly increased C1 and caused a gradual drift to negative potentials in the later part of the field potential (Fig 5b). Because the appearance of the field potential changed so much in response to bicuculline, C2-C4 could not be confidently identified and so were not analysed statistically. The effect of bicuculline on the pre-synaptic component (PC) was not analysed as this component was only identifiable in 3 experiments.

OFC—Bicuculline 3 μ M also had dramatic effects on the field potential in the OFC. Although C1 was unaffected (paired t test), later components were massively altered. In 5/8 cases there was a switch to much more positive potentials after C1 (Fig 6a): a very similar pattern to that observed with 3 μ M bicuculline in the mPFC. In the 3 remaining cases, the overall effect of bicuculline was less marked and was a gradual change to more negative potentials in the later part of the field potential (Fig 6b). This was similar to the response to 10 μ M bicuculline observed in the mPFC. It was also noted that in many cases, in the presence of bicuculline a second sharp negative deflection like a recurrence of C1 appeared in the field potential apparent in Fig 6b. Because the pattern of the field potential changed so much it was not possible to confidently identify C2 and C3, hence the later components were not analysed statistically.

Field potentials in the mPFC and OFC are attenuated by 5 HT

mPFC—5-HT (10-100 μ M) ($n=6$) attenuated the amplitude of the field potential in the mPFC in a concentration-dependent manner (Fig 7). ANOVA revealed a significant effect of 5-HT ($F_{3,15}=10.8$; $P<0.001$), a significant effect of component ($F_{3,15}=25.2$; $P<0.001$), and a significant interaction ($F_{9,45}=8.6$; $P<0.001$). The concentration dependence of the effect of 5-HT was then assessed by individual one way repeated measures ANOVA for each component and post hoc paired t tests between concentrations where indicated. For C1 there was a significant effect of 5-HT ($F_{3,15}=9.6$; $P<0.001$); C1 in the presence of 100 μ M 5-HT was significantly decreased compared to control, 10 μ M, and 30 μ M 5-HT. For C2 there was a significant effect of 5-HT ($F_{3,15}=11.5$; $P<0.001$); C2 in the presence of both 30 and 100 μ M 5-HT was significantly reduced compared to both control and 10 μ M 5-HT. For C3 there was a significant effect of 5-HT ($F_{3,15}=8.8$; $P<0.001$; all concentrations (10, 30 and 100 μ M) had a significant effect compared to control. In addition, responses in the presence of 30 and 100 μ M 5-HT were both significantly different to 10 μ M 5-HT and to each other. There was no significant effect of 5-HT on C4.

OFC—5-HT (10-100 μ M) had a small but significant inhibitory effect on the field potential in the OFC (n=8) (Fig 8). PC was present in 5 of the 8 recordings. One way repeated measures ANOVA on the PC showed no effect of 5-HT. ANOVA showed a significant main effect of 5-HT ($F_{3,21}=3.3$, $P<0.05$), a significant effect of component ($F_{2,14}=25.7$, $P<0.001$) and a significant 5-HT $\times$ component interaction ($F_{6,42}=3.3$, $P=0.01$). One way ANOVA for each component showed a significant effect of 5-HT on C1 ($F_{3,21}=3.8$, $P=0.05$), C2 ($F_{3,21}=3.1$, $P<0.05$) but not C3. Post hoc t tests showed that for C1, 100 μ M was significantly different from 10 and 30 μ M; but for C2 no individual concentration of 5-HT was different from another or from baseline.

Field potentials in the mPFC and OFC are attenuated by noradrenaline

mPFC—In the mPFC, two concentrations of NA were tested independently. NA (30 μ M; n=8) inhibited the postsynaptic components of the field potential (Fig 9a). Two way ANOVA showed a significant main effect of NA ($F_{1,7}=6.2$; $P<0.05$) a significant main effect of component ($F_{3,21}=6.4$; $P<0.01$) and a significant NA $\times$ component interaction ($F_{3,21}=8.2$; $P<0.001$). NA (30 μ M) significantly reduced C2 ($P<0.01$) and C4 ($P<0.05$). The effect on C1 was marginal ($P=0.056$).

A higher concentration of NA (60 μ M, n=18) also decreased the amplitude of the field potential in the mPFC (Fig 9b). PC was observed in 9/18 recordings. Paired t test showed no significant effect of NA on the PC (0.5345 ± 0.0928 mV vs 0.05308 ± 0.0947 mV (control vs NA), n.s.). However, a two way ANOVA showed a significant main effect of NA ($F_{1,17}=21.8$; $P<0.001$), a significant effect of component ($F_{3,51}=25.9$; $P<0.001$), and a significant component $\times$ NA interaction ($F_{3,51}=15.3$; $P<0.001$). NA (100 μ M) significantly reduced C1 and C2 ($P<0.001$).

OFC—In the OFC, increasing concentrations of NA were applied (n=14). NA (10-100 μ M) attenuated the amplitude of the field potential (Fig 10). PC was distinguishable in only 5/14 recordings. One way ANOVA showed a main effect of NA ($F_{4,16}=3.5$, $p<0.05$). NA 60 μ M caused a small but significant reduction in PC (0.2474 ± 0.0867 mV vs 0.2022 ± 0.0848 mV (control vs NA), $P<0.05$). A two way ANOVA showed a significant main effect of NA ($F_{4,52}=19.4$; $P<0.001$), a significant effect of component ($F_{2,26}=9.9$; $P<0.001$), and a significant component $\times$ NA interaction ($F_{8,104}=4.5$; $P<0.001$). Further one-way ANOVAs for each component, with post-hoc t tests where indicated, showed that all components were significantly inhibited by NA (significant main effect of NA) in a concentration-dependent manner (C1:- 30, 60, 100 μ M different to control and 10 μ M, 30 and 60 μ M significantly different to each other; C2:- 10, 30, 60, 100 μ M different to control; 30, 60, and 100 μ M different to 10 μ M; C3, 30 and 100 μ M different to control).

Field potentials in the mPFC are inhibited by DA whilst those in the OFC are not

mPFC—Two concentrations of DA (30 and 100 μ M) were tested independently in the mPFC. Figure 11 shows the average field potential in the absence and presence of DA (100 μ M). Only the higher concentration of DA affected the amplitude of the field potential. Thus, for 30 μ M DA there was a significant effect of component ($F_{3,9}=25.0$; $P<0.001$), but there was no significant effect of DA, and no component $\times$ DA interaction. However, for

100 μ M DA there was a significant effect of DA ($F_{1,7}=5.6$, $P<0.05$), and component ($F_{3,21}=32.3$; $P<0.001$), and a significant interaction ($F_{3,21}=5.3$; $P<0.01$). C1 was significantly reduced. PC was not distinguishable in enough recordings to be analysed statistically.

OFC—In the OFC increasing concentrations of DA were applied consecutively ($n=7$). DA (10-100 μ M) failed to significantly alter the field potential in the OFC. Figure 12 shows the average OFC in the absence and presence of 100 μ M DA. The PC was distinguishable in 5/7 recordings. One way ANOVA showed no significant effect of DA on the PC (0.3011 ± 0.0484 mV (control) 0.2814 ± 0.0435 mV (10 μ M DA) 0.2301 ± 0.0273 mV (30 μ M DA) and 0.1513 ± 0.0136 mV (100 μ M DA), n.s.). DA also failed to alter postsynaptic components of the OFC field potential. Thus, two-way ANOVA revealed a significant main effect of component ($F_{2,12}=4.3$ $P=0.039$) but no significant main effect of DA, and no DA $\times$ component interaction.

4. Discussion

Here we have characterized electrically-evoked field potentials in two distinct regions of the prefrontal cortex; the mPFC and the OFC. In both regions the field potential was dependent on glutamate acting on AMPA/kainate receptors and in the mPFC there was some involvement of NMDA receptors. Field potentials were attenuated by 5-HT, NA and DA (mPFC only) consistent with a modulatory role for these monoamine neurotransmitters on excitability in the mPFC and OFC.

Basic characteristics of the mPFC and OFC field potentials

Field potentials evoked and recorded in layer V/VI of the infralimbic region of the mPFC were highly consistent. When stimulating and/or recording electrodes were incorrectly placed, there was no measurable field potential. In all cases, the amplitude of the field potential was dependent on the applied voltage with a clear threshold voltage. The first negative deflection of the field potential was unaffected by the AMPA/kainate antagonist DNQX, indicating that it results from a presynaptic rather than postsynaptic event (i.e. it does not rely on glutamate release). This presynaptic component (PC) results from the synchronous firing of a large group of axons in direct response to the electrical stimulus (Varela et al., 1997). In the mPFC, all later components of the field potential were strongly inhibited by DNQX, indicating that they are postsynaptic in origin and result from glutamatergic excitation. The first of these later deflections, denoted C1, was quite a sharp spike with a short latency (4-5ms) indicative of a monosynaptically induced potential. It seems likely that this reduction in the field potential results from excitatory activity - a combination of population spike i.e. the simultaneous firing of action potentials by many (presumed pyramidal cells) in layer V/VI and excitatory postsynaptic potentials (EPSPs). Thus previous studies have shown that the early negative deflection is co-incident with the occurrence of action potentials (Chapman et al., 1998; Gemperle et al., 2003). The action potentials that comprise the population spike are generated in the cell body of pyramidal neurones, and surrounding regions source current to this site, creating a prominent current sink reflected as a reduction in field potential. The next deflection C2 was positive and

occurred around 8 ms and was broader than C1. This was also abolished by DNQX as were the subsequent wide negative (C3) and final even wider positive (C4) deflections at 14 and 22 ms respectively. Given the relatively long latency of these later components and the fact that they were broader in shape than C1, it seems likely that they are polysynaptic in nature. Positive deflections can reflect depolarization in distant neuronal populations or repolarization or hyperpolarization in neurones closer to the recording site. Later negative events are likely to be the result of further excitatory events in neurones close to the recording site. Although we quantified only four postsynaptic components, in reality the evoked field potential displayed a recurrent negative-positive pattern of over several cycles with decreasing amplitude suggestive of a recurrent excitatory loop.

In the OFC a field potential of consistent shape could be induced in layer V by electrical stimulation in the same layer. As in the PFC, the response was voltage dependent with a clear threshold. When either stimulating or recording electrode was misplaced no field potential could be evoked. The first negative deflection had a very short latency (2-3 ms) and was unaffected by DNQX suggesting that it resulted from a presynaptic fibre volley as a direct result of the electrical stimulation. As in the mPFC, this component, denoted PC, was not always distinguishable from a negative deflection at around 4-5 ms from the stimulus. This negative deflection (denoted C1), a positive deflection at around 8 ms (C2) and the final negative deflection (C3) which had rather variable latency, were all completely abolished by DNQX. Thus C1, 2 and 3 appeared to be postsynaptically mediated and reliant on glutamate neurotransmission. Because of its short latency and sharp shape, C1 was assumed to be monosynaptic and to represent a combination of a population spike and subthreshold EPSPs. C2 and C3 which had much longer and more variable latencies and broader shapes were assumed to be polysynaptic. This is the first report of an evoked field potential in layer V of the OFC but it is consistent with the known anatomy of the region and in particular the arrangement of pyramidal cells within layer V (Van De Werd and Uylings, 2008).

Role of NMDA receptors

In the mPFC, the first monosynaptic component C1 was partially inhibited by the NMDA antagonist AP-5 although the effect was much less pronounced than the effect of DNQX suggesting a relatively minor role for NMDA receptors compared to AMPA receptors. The finding that early (putative monosynaptic) components of the field potential are partially NMDA mediated is consistent with previous studies in other regions of the mPFC (Gemperle and Olpe, 2004; Gemperle et al., 2003; Orozco-Cabal et al., 2006). Interestingly, in the present study the polysynaptic components were unaffected by NMDA receptor antagonism. This is in contrast to the observations of Orozco-Cabal et al. (Orozco-Cabal et al., 2006) and Gemperle et al. (Gemperle et al., 2003) who found NMDA antagonism also inhibited the long latency polysynaptic components of inter and intra-laminar field potentials in the neighbouring prelimbic region of mPFC. In the OFC, although there was a statistically significant effect of AP-5, the magnitude of the effect was very small suggesting a minimal role for NMDA receptors. Thus it appears that the field potential in layer V of OFC is almost entirely mediated via AMPA/kainate receptors. The difference in NMDA responses in the field potential in mPFC and OFC is not easily explained. *In vivo* NMDA receptor

blockade causes similar excitatory responses in putative pyramidal cells in medial PFC and OFC (Homayoun and Moghaddam, 2007, 2008) suggesting tonic activation of NMDA receptors restrains pyramidal cell firing in both regions through GABAergic interneurons. If such a mechanism were operative in the slice, it would be expected that NMDA receptor antagonism would increase the magnitude of C1 (and later components) and would do so similarly in the mPFC and OFC: this was not the case. It is possible that those glutamatergic terminals synapsing with GABAergic interneurons emanate from outside the immediate region and are lost in the slice preparation. The fact that in the mPFC C1 was attenuated by AP-5 would suggest NMDA receptors on pyramidal cells (rather than on GABAergic interneurons) are activated by the glutamate released by the stimulus.

The Role of GABA in the field potential

Having established that the field potentials in both mPFC and OFC are mediated by glutamate, we examined the potential role of GABA. In the mPFC blockade of GABA_A receptors by bicuculline resulted in an increase in C1 suggesting that the monosynaptic component is under some tonic inhibitory regulation by GABA. This is likely to be mediated by GABA_A receptors on the postsynaptic pyramidal cells. The effect of bicuculline to increase C1 is also consistent with our conclusion that C1 results from excitatory events. Later parts of the field potential were also affected by blockade of GABA_A receptors but in a more complex manner. However, in all cases the points of inflection became broader likely indicating a loss of synchrony in recurrent activity.

The effect of GABA_A receptor blockade was similar in the OFC, in that bicuculline increased the C1 revealing a tonic inhibition on the monosynaptic response. However, this effect of bicuculline was of greater magnitude than that seen in the mPFC. Interestingly in several of the recordings it was noted that addition of bicuculline to the perfusion resulted in a second sharp negative deflection immediately following C1. Thus it appeared that in the absence of inhibitory GABAergic tone, the electrical stimulation and subsequent release of glutamate was able to evoke a pair of spikes rather than a single spike and suggests that C1 was largely accounted for by a population spike rather than subthreshold EPSPs. Later components of the OFC evoked field potential were also affected by bicuculline but the response was dichotomous. In some cases the field potential showed a massive late rise, in other cases there was a trend to more negative potentials following bicuculline.

Modulation of the mPFC and OFC field potentials by monoamines

We examined potential modulatory effects of the major monoamine neurotransmitters 5-HT, NA, and DA on the evoked field potentials in the mPFC and OFC. In the mPFC 5-HT had a striking concentration-dependent inhibitory effect on the field potential. Whilst PC was unaffected, C1 was reduced as were C2 and C3: overall the evoked field potential showed a flattened profile in the presence of 5-HT. 5-HT innervation in this region is particularly dense and a number of different 5-HT receptor subtypes are densely expressed. The inhibitory effects of 5-HT are potentially mediated through multiple receptor subtypes, located on both glutamatergic and GABAergic neurones. We (Gartside et al., 2000; Hajos et al., 2003) and others (Amargos-Bosch et al., 2004) have previously observed facilitatory and inhibitory effects of 5-HT in this region mediated by different receptor subtypes, and

anatomical data indicates that multiple receptor subtypes are expressed here and in some cases are found on the same neurone (Amargos-Bosch et al., 2004; Varga et al., 2001).

5-HT also inhibited the OFC field potential however the effect was much smaller than in the mPFC. Only 100 μ M 5-HT evoked a consistent response and this was largely limited to C1. The lower magnitude of effect of 5-HT in layer V of the OFC may reflect the fact that at least some 5-HT receptors are expressed in lower density in layer V of OFC than layer V/VI of mPFC (see (Adlersberg et al., 2000; Johnson et al., 2009; Meller et al., 2000)).

The evoked field potential in both the mPFC and OFC was inhibited by NA. Comparison of concentration-related effects suggested that the OFC is slightly less sensitive to NAergic modulation than the mPFC. The inhibition of C1 is consistent with the reported inhibitory effect of endogenous NA on mPFC pyramidal cells (Mantz et al., 1988). There do not appear to be any equivalent data on the effects of NA on OFC neurones but our finding that NA has similar effects on the field potential in mPFC and OFC would suggest that the effects on pyramidal cell firing would also be similar.

Finally, we found that DA also attenuated the field potential in the mPFC in a concentration dependent manner. *In vivo* mPFC pyramidal cells are reportedly inhibited by VTA stimulation (Godbout et al., 1991; Mantz et al., 1992) or iontophoretic DA (Pirot et al., 1992). Thus it seems likely that exogenous DA is acting via the same mechanism to decrease excitability in pyramidal cells of mPFC a response which was reflected in a reduction in C1.

Interestingly DA had no significant effect in the OFC. Although there is DA innervation of OFC, a detailed examination of the distribution shows DA and tyrosine hydroxylase staining is concentrated laterally to our recording site (agranular insular cortex dorsal) (Paxinos, 1999; Van De Werd and Uylings, 2008). DA levels measured by microdialysis in the mPFC are reportedly more than twice those in the OFC again suggesting that DA may have a lesser role to play in the OFC than the mPFC. Unfortunately to our knowledge there are no data published on the effects of DA or DA ligands on the activity of OFC neurones.

Here we found that field potentials in the mPFC and/or OFC were attenuated by 5-HT, NA and DA. Although we used relatively high concentrations of the transmitters, the effective concentrations of these endogenous compounds are reduced by high affinity uptake and metabolism within the slice. We did not attempt to block the effects of the monoamines, however the effects of similar concentrations of 5-HT, NA and DA in brain slices have previously been shown to be blocked by selective receptor antagonists (Arvanov et al., 1999; Judge and Gartside, 2006; Liu and Herbison, 2013; Yang et al., 2008). Thus it is likely that the effects observed here are mediated by cognate monoamine receptors rather than through non-specific actions. Further studies with selective antagonists and agonists will clarify this.

Conclusions

Here we have pharmacologically characterised electrically-evoked field potentials in the mPFC and OFC and examined their modulation by monoamine neurotransmitters. As noted in the Introduction, the mPFC and OFC appear to play important roles in learning and goal

directed behaviours. Several recent behavioural studies have reported modulation of these functions by administration of drugs which target monoamine neurotransmission (McGaughy et al., 2008; Nikiforuk et al., 2013; Winstanley et al., 2006). The present data suggest a mechanism by these behaviours might be modulated by monoamines. The mPFC and OFC are involved in cognition and behavioural control. Given that it has been hypothesised that and that glutamate, GABA, and/or monoaminergic dysfunction in these regions may underlie some of the symptoms of psychiatric disorders, we propose that these field potentials are useful models with which to study prefrontal cortical physiology and pathophysiology.

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Figure 1.

Plate from The Rat Brain Atlas (Paxinos and Watson, 1986) showing the approximate placement of stimulating electrodes (triangles) and recording electrodes (circles) in the mPFC and OFC.

a

Figure 2.

Drug naïve field potentials in (a) layer V-VI of the IL of the mPFC and (b) layer V of OFC. Data are mean $\pm$ SEM (grey shading) of field potentials from 17 and 25 slices, respectively. Letters mark the distinct points of inflection and arrows show how these were converted into components PC and C1-C4.

Figure 3.

Field potentials in the mPFC in the absence and presence of (a) DNQX (20 μ M) and (b) AP-5 (100 μ M). Histograms show mean + s.e.m. (n=7 and 6). Insets shows the mean waveform of the field potential in the absence (control; black line) and presence (grey line) of the drug. Note that DNQX markedly reduces the later components of the field potential but has no effect on PC. AP-5 also slightly reduces C1 without affecting PC. * $p < 0.05$ (paired t test following significant ANOVA; see text for full details).

Figure 4.

Field potentials in the OFC in the absence and presence of (a) DNQX (20 μ M) and (b) AP-5 (100 μ M). Histograms show mean + s.e.m. (n=12 and 9 respectively). Inset shows the mean waveform of the field potential in the absence (control; black line) and presence (grey line) of the drug. Note that DNQX markedly reduces the later components of the field potential but has no effect on PC. AP-5 has no effect on any component of the field potential. * $p < 0.05$ (paired t test following significant ANOVA; see text for full details).

Figure 5.

Field potentials in the mPFC in the absence and presence of (a) bicuculline (3 μ M) and (b) bicuculline (10 μ M). Histograms show mean + s.e.m. (n=7). Insets shows the mean waveform of the field potential in the absence (control; black line) and presence (grey line) of the drug. *p<0.05 (paired t test).

Figure 6.

Field potentials in the OFC in the absence and presence of bicuculline (3 μ M). Part (a) histogram shows the mean data from 5 slices in which bicuculline increased the potential from around 10 ms after the stimulus (n=5). Part (b) histogram shows the mean data from 3 slices in which bicuculline caused a downward drift in the potential from 10 ms onwards. Inset shows the mean waveform of the field potential in the absence (control; black line) and presence (grey line) of the drug. *p<0.05 (paired t test).

Figure 7.

Field potentials in the mPFC in the absence and presence of 5-HT (10, 30 and 100 μ M).

Histograms show mean + s.e.m. (n=6). Inset shows the mean waveform of the field potential in the absence (control; black line) and presence (grey line) of 5-HT (100 μ M). * $p < 0.05$

(paired t test vs control following significant ANOVA); see text for full statistical analysis.

Figure 8.

Field potentials in the OFC in the absence and presence of 5-HT (10, 30 and 100 μ M).

Histograms show mean $\pm$ s.e.m. (n=8). Inset shows the mean waveform of the field potential in the absence (control; black line) and presence (grey line) of 5-HT (100 μ M). * p <0.05 (paired t test vs control following significant ANOVA); # p <0.05 one-way ANOVA. See text for full statistical analysis.

Figure 9.

Field potentials in the mPFC in the absence and presence of (a) NA (30 μ M) and (b) NA (100 μ M). Histograms show mean $\pm$ s.e.m. ($n=8$). Inset shows the mean waveform of the field potential in the absence (control; black line) and presence (grey line) of NA (30 and 100 μ M). * $p<0.05$ (paired t test vs control following significant ANOVA).

Figure 10.

Field potentials in the OFC in the absence and presence of NA (10-100 μM). Histograms show mean + s.e.m. (n=14). Inset shows the mean waveform of the field potential in the absence (control; black line) and presence (grey line) of NA (100 μM). *p<0.05 (paired t test vs control following significant ANOVA). See text for full statistical analysis.

Figure 11.

Field potentials in the mPFC in the absence and presence of DA (100 μM). Histograms show mean + s.e.m. (n=8). Inset shows the mean waveform of the field potential in the absence (control; black line) and presence (grey line) of DA (100 μM). *p<0.05 (paired t test vs control following significant ANOVA).

Figure 12.

Field potentials in the OFC in the absence and presence of DA (10-100 μ M). Histogram shows mean + s.e.m. (n=7). Inset shows the mean waveform of the field potential in the absence (control; black line) and presence (grey line) of DA (100 μ M).