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Auditory gating in rat hippocampus and medial prefrontal cortex: Effect of the cannabinoid agonist WIN55,212-2

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ABSTRACT

Sensory gating can be assessed in rodents and humans using an auditory conditioning (C)–test (T) paradigm, with schizophrenic patients exhibiting a loss of gating. Dysregulation of the endocannabinoid system has been proposed to be involved in the pathogenesis of schizophrenia. We studied auditory gating and the effects of the cannabinoid agonist WIN55,212-22 on gating in CA3 and dentate gyrus (DG) of the hippocampus and medial prefrontal cortex (mPFC) in male Lister hooded rats using *in vivo* electrophysiology. The effects of a single dose of WIN55,212-2 on the N2 local field potential (LFP) test/conditioning amplitude ratios (T/C ratio) and response latencies were examined. In rats that demonstrated gating of N2, mPFC showed higher T/C ratios and shorter conditioning response latencies compared to DG and CA3. WIN55,212-2 disrupted auditory gating in all three areas with a significant increase in test amplitudes in the gating rats. A group of non-gating rats demonstrated higher test amplitudes and higher T/C ratios compared to gating rats. WIN55,212-2 had no effect on T/C ratios in the non-gating rats. The cannabinoid receptor (CB1) antagonist SR141716A prevented WIN55,212-2 induced disruption of gating. This study demonstrates gated auditory-evoked responses in CA3, DG and mPFC. The mPFC showed an early phase of gating which may later be modulated by CA3 and DG activity. Furthermore, cannabinoid receptor activation disrupted auditory gating in CA3, DG and mPFC, an effect which was prevented by CB1 receptor antagonism. The results further demonstrate the presence of a non-gating rat population which responded differently to cannabinoid agonists.

1. Introduction

Sensory gating has been conceptualized as a continuously active process, contributing to an individual's ability to modulate a continuous stream of sensory and cognitive information (Light and Braff, 2003). This process allows the identification of the occurrence of a stimulus, the assessment of its importance and elicitation of a response and suppression when the stimulus is redundant or repetitive (Adler et al., 1982; Boutros et al., 1999; Cromwell et al., 2005).

Sensory gating can be demonstrated in human subjects using auditory-evoked EEG responses (AERs) to a conditioning–testing paradigm, in which two identical auditory tones are presented 500 ms apart. Normal subjects have a smaller response to the second (test) tone compared to the first (conditioning) tone and the ratio of the amplitudes of the testing (T) to the conditioning (C) response (T/C ratio) is used as a quantitative measure of sensory gating. A positive wave occurring 50 ms (P50) following the auditory stimuli is the most widely used auditory-evoked response to assess gating in humans. Responses in the auditory conditioning–test paradigm have been found to be consistently abnormal in schizophrenia (Adler et al., 1982; Boutros et al., 1999; Cadenhead et al., 2000).

Studies using the auditory conditioning–test paradigm in rats have shown gating of a negative auditory-evoked potential recorded from implanted skull electrodes and from the CA3 region of the hippocampus around 40 ms after an auditory stimulus (Adler et al., 1986; Bickford-Wimer et al., 1990; Bickford et al., 1993; Miller et al., 1992; Krause et al., 2003). This wave, known as N40, is considered homologues to the P50 wave recorded from humans (Adler et al., 1986).

An early study by Bickford-Wimer et al. (1990), using intracerebral electrodes in rats under chloral hydrate anaesthesia, showed the CA3 region of hippocampus as the source of the N40 wave. Recent studies have shown involvement of other brain areas such as the medial septum (Miller and Freedman, 1993), reticular thalamus (Krause et al., 2003), amygdala (Cromwell et al., 2005), striatum (Cromwell et al., 2007), midbrain (Anstrom et al., 2007) and medial prefrontal cortex (mPFC) (Mears et al., 2006) in auditory gating.

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Studying auditory gating in areas implicated in the pathophysiology of schizophrenia is helpful to understand the neurobiology of gating. The hippocampus and prefrontal cortex (PFC) have direct reciprocal connections and depth electrode studies in pre-surgical epileptic patients have demonstrated auditory gating in the PFC and hippocampus (Grunwald et al., 2003). Sensory information from the cortical sensory areas is funnelled down to the CA3 via the dentate gyrus (DG), which receives outputs from perirhinal and entorhinal cortices. It is important to understand how auditory information is processed by these three interconnected regions (CA3, DG and mPFC) to bring about auditory gating and whether it is disturbed by pharmacological manipulations.

Emrich et al. (1997) proposed that the pathophysiology of schizophrenia may be related to a functional disturbance of the endocannabinoid system. The endocannabinoid system is a ubiquitous neuromodulatory system comprising cannabinoid receptors (CB1, CB2, and CB3), endocannabinoids (e.g. anandamide, 2-arachidonoylglycerol) and enzymes responsible for their transport and degradation (e.g. fatty acid amide hydrolase) (Straiker and Mackie, 2006). Most of the CNS actions are brought about by stimulation of the CB1 receptors which are found in abundance in the hippocampus and PFC. Several studies have shown that the administration of cannabinoid agonists either acutely (Emrich et al., 1997) or chronically (Patrick et al., 1999) can produce schizophrenic symptoms in healthy individuals and exacerbate symptoms in schizophrenic patients. Patrick et al. (1999) and Rentzsch et al. (2007) reported that chronic cannabis abuse, in otherwise healthy individuals, disrupts auditory gating in scalp recorded AERs.

We have shown in preliminary studies that acute administration of the cannabinoid agonist WIN55,212-2 to adult Lister hooded rats disrupted auditory gating in the hippocampal CA3 region (Dissanayake et al., 2006; Zachariou et al., 2007). In this study we conducted simultaneous recordings from the DG, CA3 and mPFC in rat and sought to determine if auditory gating in these regions could be disrupted by a single administration of the cannabinoid agonist WIN55,212-2 and whether any effects observed were reversed by the CB1 receptor antagonist SR141716A.

2. Experimental procedure

2.1. Animals

Experiments were performed on male Lister hooded rats ($n = 16$) weighing 300–400 g at the time of surgery. Animals were group housed in standard plastic cages on a 12 h:12 h light:dark cycle (lights on at 7:00 AM), light intensity 100–200 lux at cage bottom, ambient temp 22 ± 1 °C; food and water were available *ad libitum*. Experimental procedures were carried out in accordance with the UK Animals (Scientific Procedures) Act, 1986 and associated guidelines (project licence No: 40/2715).

2.2. Surgical procedure

Anaesthesia was induced with 3% isoflurane in a 50%:50% N_2O:O_2 mixture. The isoflurane level was reduced progressively and maintained at 1.5–2% throughout surgery to ensure a state of complete absence of the hind paw withdrawal reflex. Rats were mounted in a modified stereotaxic frame using hollow ear bars with connected Sony Walkman® stereo-earphones to present auditory stimuli. Core temperature was monitored and maintained at 37–38 °C using a homeothermic heating pad and controller (Harvard Instruments, UK). A scalp incision was made, and 5 mm diameter craniotomies were performed separately above the right hippocampus and right mPFC.

2.3. Recording procedure

A sixteen channel micro-wire electrode array (arranged in a 2 × 8 pattern ~0.25 × 0.7 mm, Teflon-coated stainless steel, 50 μm diameter per wire; NB Labs, Texas, USA) with a stainless steel ground electrode connected to a unity-gain headstage (Plexon Inc., Texas, USA) was used to record neuronal activity from CA3 region and dentate gyrus of the hippocampus (3.6–3.9 mm posterior, 3–3.2 mm lateral and 3.6–3.9 ventral from bregma). An eight-channel micro-wire electrode bundle (Teflon-coated stainless steel, 50 μm diameter per wire; NB Labs, Texas, USA) was used to record neuronal activity from the mPFC (3–3.2 mm anterior, 0.5–0.8 mm lateral and 2.5–2.7 ventral from bregma), according to the atlas of Paxinos and Watson (1997). Multiple single-unit activity was recorded from all the electrodes (filtered at 250 Hz–8 kHz; unpublished data), with local field potentials (LFPs) recorded from two electrodes in each region.

Auditory stimuli were presented as paired 3 kHz frequency tones, 90 dB intensity, 10 ms duration, 10 s interstimuli interval for 128 trials with auditory-evoked LFP and unit activities recorded simultaneously. Neural signals were split at the Plexon preamplifier (unit activity was processed by a Plexon MAP system) and the LFP signals amplified ×1000, filtered (0.7–170 Hz), fed via a National Instruments data acquisition NIDAQ card (PCI-6071E, 1.25 MS/s, 12-bit resolution) in the host PC and digitised at 1 kHz (Stevenson et al., 2007).

2.4. Drug administration

(*R*)-(+)-(2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo(1,2,3-de)-1,-4-benzoxazin-6-yl)-1-naphthalenylmethanone mesylate (WIN55,212-2) was purchased from Tocris Cookson Ltd (Avonmouth, UK) and stored in a tightly sealed container in the refrigerator at 4 °C.

WIN55,212-2 was dissolved in a vehicle containing propylene glycol (0.5%), Tween 80 (0.2%) and 0.9% (w/v) saline. The drug solution was prepared on the day of the experiment and was administered as a single dose of 1.2 mg/kg intraperitoneally (Schneider and Koch, 2002, 2003; Drews et al., 2005; Schneider et al., 2005) in an injection volume was 1 ml/kg.

Following basal recording of the auditory responses to 128 trials of auditory stimuli, the vehicle was administered (1 ml/kg, i.p.; $n = 10$) and the effect on auditory gating assessed by recording the responses to 128 auditory conditioning–test trials 15 min after administration. WIN55,212-2 was administered to the same animals 40 min after the vehicle and the effect on auditory gating was again assessed by recording auditory responses to 128 stimuli trials, begining15 and 45 min after drug administration.

N-piperidono-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (SR141716A) was supplied by the National Institute of Mental Health Chemical Synthesis and Drug Supply Program (contract N01-MH-32005). SR141716A was dissolved in 100% ethanol (4.64 mg/1 ml) to make a 10 μM stock solution and stored at −20 °C. At the time of the experiment 100 μl of stock was dissolved in 364 μl of 0.9% saline (w/v) to make up a working concentration of 1 mg/ml.

Following basal recording of the auditory responses to 128 trials of auditory stimuli, the vehicle for SR141716A was administered (1 ml/kg, i.p.; $n = 6$ rats) and the effect on auditory gating assessed by recording the responses to 128 auditory conditioning–test trials 15 min after administration. SR141716A was then administered as a single dose (1 mg/kg, i.p.; Brodkin and Moerschbaecher, 1997) 40 min after the vehicle. The effect of SR141716A on auditory gating was assessed by recording the responses to 128 auditory conditioning–test trials 15 min after administration. Then WIN55,212-2 (1.2 mg/kg, i.p.) was administered to the same animals 40 min after SR141716A and the effect on auditory gating assessed over 128 stimuli trials, 15 and 45 min following WIN55,212-2 administration.

2.5. Histological verification of the recording sites

At the end of each experiment a 10 μA current was passed for 10 s (D.C. constant current lesion marker; Grass instruments, Quincy, MA) through the recording electrodes to deposit ferric ions. Rats were perfused transcardially with 0.9% (w/v) saline followed by 4% potassium ferrocyanide. Brains were removed and stored overnight in a 4% paraformaldehyde. Tissue blocks were sectioned transversely at 200 μm on a vibratome (Campden Instruments, UK). Recording sites were identified as blue deposits with reference to the rat brain atlas of Paxinos and Watson (1997). Only the animals with electrodes identified as placed in the CA3, DG and mPFC were used for analysis.

2.6. Data analysis and statistics

Data were analysed off-line using NeuroExplorer (v3; NEX technologies Inc., USA) with evoked response amplitudes, latencies and T/C ratios computed using a custom-designed Matlab script (v7.0 with associated Matlab Signal Processing, Neural Network, Statistics and Image Processing Toolboxes; The Mathworks). Statistical analysis used Prism (v4.03; GraphPad, USA) and within region changes of all the parameters at different time points were analysed using one-way analysis of variance (ANOVA) for repeated measures with post hoc Tukey *t*-test. Data are expressed as mean ± SEM (standard error of mean), statistical significance was taken when $P < 0.05$.

2.6.1. LFP analysis

Auditory-evoked potentials were initially visualized as peri-event averaged LFPs (Fig. 1). The AERs were identified according to the polarity and the order of occurrence, i.e. N1, P1, N2, P2, N3 peaks (Van Luijtelaar et al., 2001; Boutros et al., 2004). Identification of AERs based on their order of occurrence in a specified latency range excluded the error of identifying N1 as N2. We used the N2 wave, identified as the second negative peak during 100 ms period following the stimulus onset, for further analysis. Amplitudes were measured from the preceding positive peak (P1) to the negative peak of the N2 response and the latencies were measured from the time of