THE EFFECT OF NMDA RECEPTOR ANTAGONISM ON CORTICAL GAMMA POWER AND SYNCHRONISATION *IN VIVO*

by

ASHLEIGH GEORGINA WILCOX

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Neurobiology Section
School of Clinical and Experimental Medicine
College of Medical and Dental Sciences
University of Birmingham
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- ABSTRACT -

Acute administration of NMDA receptor antagonists such as PCP and ketamine are accepted models of schizophrenia in rats. Oscillatory activity in the gamma range (30-120 Hz) is known to be increased in schizophrenia and by NMDA receptor antagonism. Putative links have been made between abnormal gamma activity and cognitive deficits that are known in schizophrenia. However, the site of action of these antagonists and how they modulate gamma *in vivo* remains unclear.

In this study PCP was systemically injected into freely moving rats and ketamine systemically injected into terminally anaesthetised rats, whilst recording intracranial EEG activity in the prefrontal cortex, visual cortex and hippocampus. Ketamine was also administered locally into these same areas in anaesthetised rats and EEG activity recorded.

1 mg/kg PCP significantly decreased performance in an associative learning task, significantly increased gamma power in all recording areas and decreased inter-area gamma synchronisation. 4 mg/kg ketamine also significantly increased gamma power and decreased inter-area synchronisation. Local injection of 5 μg/μl ketamine caused no significant changes to gamma power or inter-area synchronisation.

The data presented in this study suggests that local NMDA receptor antagonism may not be the primary cause of changes to gamma activity that occurs following systemic treatments.

- ACKNOWLEDGEMENTS -

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This thesis and all work leading up to it is dedicated to Kenny, the truest friend that anyone could have ever hoped for. If "the only escape from the miseries of life are music and cats" (*Albert Schweitzer*), then all that is left is music which will never sound the same.

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- INTRODUCTION -

1i | Schizophrenia as a cognitive disorder

Schizophrenia is a serious psychiatric disorder affecting up to 1% of the population worldwide (Javitt, 2010). Symptoms associated with the condition are commonly separated into two categories: positive and negative. Positive symptoms include hallucination, hostility, delusions and disorganisation of thoughts. Whereas negative symptoms include anhedonia, withdrawal from social obligations, apathy and avolition (Jodo, 2013; Dauvermann et al, 2014). As well as these emotional and behavioural symptoms, schizophrenic individuals also have well characterised deficits in cognition. The disorder is now termed as 'widespread cortical dysfunction' which accounts for the diverse range of symptoms mentioned. Often cognitive deficits precede official diagnosis of the disease and any true first psychotic episode, and will worsen throughout progression of the condition. Also, deficits in cognitive functions such as working memory have been found to be depleted in those at high risk of developing the disorder – reinforcing the importance of cognitive ability as a core component of the illness even before any conversion to psychosis (Dauvermann et al, 2014; Javitt, 2010).

Through viewing schizophrenia as a condition of neuronal network dysfunction, two major theories of disease development were proposed - the dopaminergic and glutamatergic. The original dopamine hypothesis states that alterations in dopaminergic receptors underlie the clinical symptoms observed in schizophrenia. This theory is no longer solely believed to be true as it does not account for negative and cognitive symptoms, which are extremely important in classifying diagnoses (Howes & Kapur, 2009). The original glutamate hypothesis - first proposed by JS Kim and colleagues in 1980 (Kim et al, 1980) was based on the idea that

alterations to glutamate receptors was the key cause of schizophrenia development. This has been subsequently expanded to include various other aspects of glutamatergic transmission to account for positive, negative and cognitive symptoms occurring in schizophrenia. There are also other theories that highlight the role of the serotonin system in schizophrenia pathology, which are originally based on interactions between LSD and the serotonin receptor during drug induced psychosis (Aghajanian & Marek, 2000). It is likely that no sole theory is true or complete, and that interactions between different systems involved in neural transmission account for the overall pathology of schizophrenia. However, this study was focussed on a key element of glutamatergic transmission: the N-methyl-D-aspartate receptor (NMDAR).

1ii | The NMDAR and schizophrenia

Originally, the glutamate hypothesis of schizophrenia was based on the observation that treatment with drugs such as ketamine and phencyclidine (PCP) mimics psychosis in healthy humans and animals – which is thought to be comparable to some aspects of positive symptoms in schizophrenia. These drugs are antagonists of the NMDAR, which is a glutamate receptor subtype abundant in the central nervous system (Dauvermann et al, 2014; Javitt, 2010). Glutamate receptors mediate excitatory synaptic transmission through interactions with, amongst others, GABA (gamma aminobutyric acid)-ergic interneurons and dopaminergic neurons (Traynelis et al, 2010) and so dysfunction of this class of receptor is likely to have widespread cortical consequences to subsequent neuronal transmission systems. The NMDAR is also crucial for long-term potentiation, thus NMDAR hypofunction will lead to reduced reinforcement of synaptic connections and is implicated in schizophrenia development (Coyle

et al, 2003). NMDARs are composed of a combination of different subunits classified as NR1, NR2 and NR3; within these units there are various other subtypes known. For example, NR2A and NR2B are the dominant classes of NR2 subunit in the adult brain (Lee & Rajakumar, 2003). It is also thought that subunit composition may contribute to the pathology of schizophrenia as they convey different pharmacological properties (Cull-Candy et al, 2001).

The NMDAR is activated via binding of glutamate along with its co-agonist glycine to their respective sites. Under normal circumstances, the NMDAR is blocked by Mg^{2+} at the Mg binding site on the receptor. Depolarisation above threshold in the postsynaptic cell removes the Mg^{2+} and an influx of Ca^{2+} occurs. Competitive inhibition of NMDAR occurs via binding to the glutamate or glycine binding site, thus blocking the receptor from being activated. Non-competitive inhibition occurs via binding to a PCP binding site located in the pore of the receptor channel, thus blocking ion flow through the channel (**figure 1.1**).

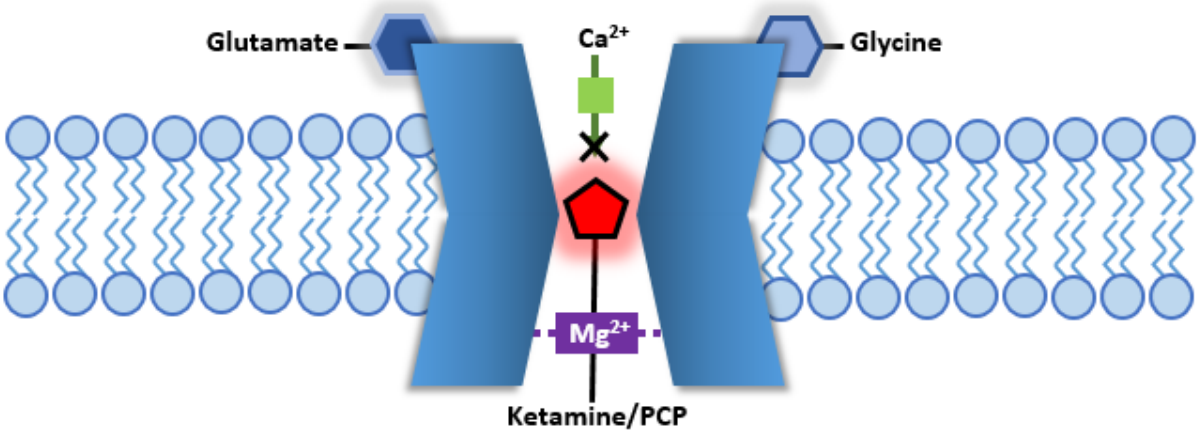

Figure 1.1| Non-competitive inhibition of NMDAR via the PCP binding site in the channel pore. Glutamate and glycine bind to activate the receptor at the sites indicated. Under normal circumstances, depolarisation of the postsynaptic cell removes Mg^{2+} block and Ca^{2+} influx occurs. This is unable to occur when a non-competitive antagonist such as PCP or ketamine is bound to the PCP binding site in the pore of the channel. Treatment with PCP or ketamine can therefore be used to mimic NMDAR hypofunction observed in schizophrenia.

Ketamine and PCP work via this non-competitive binding route to inhibit the NMDAR (Watkins, 1994; Frohlich & Van Horn, 2013). Since NMDAR hypofunction has been implicated in schizophrenia, it follows that administration of a systemic injection of PCP or ketamine can be used to create an acute model of schizophrenia. This method was used in rats to create the models for this study. It should be noted that there are also developmental, genetically manipulated and lesion animal models of schizophrenia. No animal model is considered a complete recreation, as schizophrenia is a complex heterogeneous disorder, but many are accepted replications of particularly positive symptoms and some cognitive (Jones et al, 2011).

1iii | Oscillatory activity in the brain

Neuronal networks create oscillatory activity that is abundant throughout cortical regions of the mammalian brain (Ermentrout & Chow, 2002). Changes in oscillations can be induced by sensory and/or cognitive events and are usually classified into 'natural' frequency ranges in the brain: alpha, theta, delta, beta and gamma (Basar et al, 2001). It has been long argued that these changes in rhythmic oscillatory activity have functional significance in the cortex. Oscillations within the gamma band (30-120 Hz) are almost continually present (Jasper & Andrews, 1938; Whittington et al, 2010) and occur in all cortical areas (Uhlhaas & Singer, 2010). Gamma is continually implicated in cortical processing as oscillations in this frequency band become more prevalent during cognitive task completion and are known to be induced by sensory input to different cortical areas (Wang, 2010; Bartos, Vida & Jonas, 2007).

Integration of information in the brain requires some level of synchrony; synchronisation of generated oscillations remains the most widely accepted method to

achieve this (Buzsaki & Draguhn, 2004; Uhlhaas & Singer, 2013). Importantly, the synchronisation of oscillations either between the same frequencies (e.g. gamma to gamma) or between different frequencies (e.g. theta to gamma) in different areas are thought to be responsible for communication within and between areas. This is critical for cognitive functions that require combination of sensory inputs from different cortical areas and for recall of information – i.e. in working memory. Some of the deficits stated earlier that occur in schizophrenia are due to reduced inter-area communication and therefore, it could be argued, reduced synchronisation of oscillations (Wang, 2010). One study, which analysed data from experiments conducted in awake cats and monkeys (Womelsdorf et al, 2007), concluded that gamma band synchronisation of synaptic inputs is responsible for enhancing the effectiveness of synapses by increasing strength of transmission. Many others have found general abnormal synchronisation in the gamma band (Uhlhaas & Singer, 2010) as well as changes in gamma power in both human schizophrenic patients and animal models (Hong et al, 2009). The majority of studies on gamma and schizophrenia have focussed on the lower gamma range (around 30-80 Hz), but there is also increasing evidence for impairment in higher frequency gamma oscillations (Grutzner et al, 2013). Abnormal gamma activity has been shown to occur not only in response to sensory stimuli and during cognitive task completion but also during a resting state in schizophrenic patients. Experiments conducted on twins have shown that these resting state changes are highly heritable and that changes persist despite the use of anti-schizophrenia drug treatment in patients (Uhlhaas & Singer, 2010). Thus, supporting a causal role of abnormal oscillatory activity in core schizophrenia development and pathology.

1iv | Aims and rationale

This study aimed to characterise changes across an extended gamma frequency range (30-120 Hz) in line with more recent research in the literature. The design of this study can be separated into three experimental approaches, all principally aiming to characterise changes in gamma activity in rat models of schizophrenia. In all experiments, local oscillatory activity was recorded in the visual cortex (VC), hippocampus (Hip) and prefrontal cortex (PFC). These areas were originally chosen due to their crucial involvement in completion of a visual paired associative learning task (sPAL), used for behavioural experiments.

There are a variety of *in vivo* techniques for measuring neural oscillations that have been extensively studied in both humans and model species. In rats, intracranial EEG recording is achieved via insertion of electrodes into the brain and recording directly from the area of interest. The voltage difference between this recording site and a reference electrode placed elsewhere in the brain is amplified to give a signal for neural network activity in the area of interest (del Campo et al, 2009). Intracranial measurement of EEG activity is considered to be more accurate than placement of electrodes on the skull as there is less signal loss and noise created via tissue conduction (Valiante & Carlen, 2014), making intracranial recording the method of choice for this study. The first set of experiments conducted combined intracranial electroencephalographic (EEG) recording with behavioural testing in freely moving animals following systemic PCP injection, to test the hypothesis that systemic PCP treatment increases gamma power during completion of a cognitive task but decreases gamma synchronisation.

The second set of experiments involved intracranial EEG recording after systemic treatment with an NMDAR antagonist again, but this time ketamine was used instead of PCP and experiments were conducted on terminally anaesthetised rats. Ketamine has a slightly

lower affinity for the NMDAR than PCP but is still a widely accepted antagonist when used to recreate NMDAR hypofunction (Frohlich & Van Horn, 2013); ketamine is also known to 'wash out' more quickly than PCP, making it an attractive choice for these terminal experiments. The hypothesis here was that systemic ketamine treatment causes an increase in gamma power yet also causes desynchronisation between areas in the brain – affecting inter-area communication, which could be the cause of poor cognitive task performance seen in schizophrenia. Using anaesthetised rats for this experiment eliminated electrical activity generated by muscle movement in freely moving animals from the recording situation, so only true EEG activity in recording areas should have been present after artefact removal.

The final set of experiments aimed to further characterise the specific effect of ketamine on gamma activity in local neural networks. This was achieved by local injection of ketamine to the same areas of interest in the brain as previous whilst conducting intracranial EEG recording. It is thought that systemic treatment with ketamine changes gamma activity in a neural region by causing NMDAR hypofunction in local neural networks. These experiments were designed to directly test this theory in an attempt to elucidate where ketamine is actually acting in the brain and the implications of this in schizophrenia.

Using these three experimental approaches allowed comparisons to be made between neural oscillatory activity in freely moving and anaesthetised rats and between ketamine and PCP as NMDAR antagonists, helping to further validate the use of these drugs in modelling schizophrenia. This combinational approach is necessary for the translation of basic neuroscience to cognitive and behavioural symptoms known to occur in schizophrenia.

- METHODS -

Animal husbandry and ethical statement

Adult male Lister Hooded rats (Charles-River, Margate, UK) were used for all experiments and housed in groups of 3-4 under a 12 hour light/dark cycle. All procedures carried out complied with University of Birmingham internal regulations and those set out by the Home Office and European legislation under the Animals (Scientific Procedures) Act 2013.

2i | Freely moving rat experiments

Completion of sPAL task under control and PCP conditions

Six rats were previously trained by undergraduate students (H. Fowler & W. Clarke) to complete an sPAL task in an automated Bussey-Saksida rat touch screen chamber (Campden Instruments Ltd, Loughborough, UK). This task was chosen as schizophrenia patients have been shown to have decreased ability to complete similar tasks. The task consisted of two identical pictures appearing on an infra-red touch screen in two different locations, one in the correct location learnt by the rat and the other in an incorrect location (**figure 2.1**). This test assesses the rat's ability to associate both an object and location, which requires integrative use of the PFC, Hip and VC - hence why it was chosen for this study.

The rat was rewarded with a 45 mg sucrose pellet (Sandown Scientific, Hampton, UK) for each correct touch and 'punished' for wrong touches by the chamber light switching on for 5 seconds – extending the intertribal interval, and an 'incorrect tone' sounding. The task was executed and analysed using ABET II touch software (Lafayette Instrument, Lafayette, USA) and the touch screen was controlled by Whisker software (Cambridge University, UK).

Rats were kept at approximately 90% of their individual estimated ad libitum weight by rationing standard rat 'chow' in order to ensure motivation for task completion (Ward et al, 2013), water was provided ad libitum.

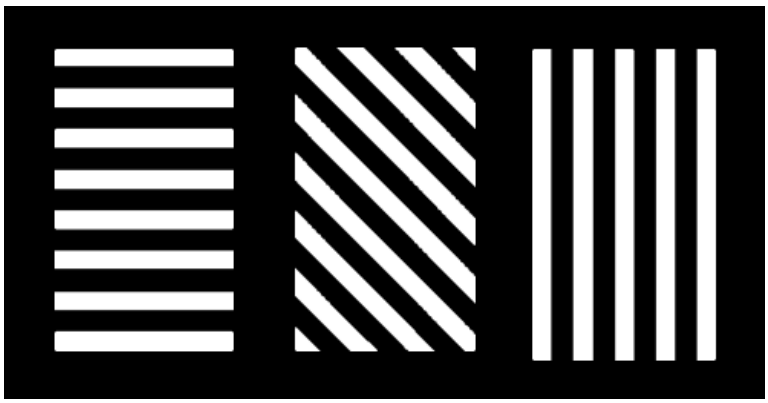

Figure 2.1| Images and layout used on the touchscreen for sPAL testing of rats in behavioural testing chamber. The correct locations shown are the ones learnt by rats; during task completion two of the same image were shown to the rat – one in the correct position, the other in an incorrect position.

Recording of neural oscillations during task completion

After rats were deemed proficient in task completion, electrodes were implanted and secured in the skull (surgery completed by M. Vreugdenhil). Implanted electrodes were made using 50 µm Formvar insulated 80% Nickel 20% Chromium wire (Advent Research Materials, Oxford, UK). Two wires were twisted and then cut to give a longer and shorter length to facilitate two separate recording channels of data in the area of implantation (**figure 2.2**), giving six overall recording channels.

Having two different recording sites in the same area of implantation can be used to calculate local activity via subtraction of the two recording channels or counteract poor recording in one channel - as there are two channels to obtain data from. For Hip and VC electrodes, the difference between the two lengths was 0.5 mm. For the PFC the electrode

length distance was 1 mm to facilitate recording from the same layer in the prelimbic cortex (PrL; longest electrode) and cingulate cortex (Cg1; shorter electrode).

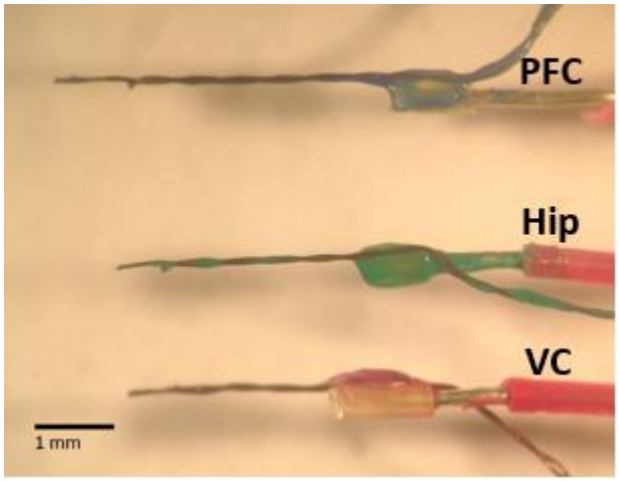

Figure 2.2| Electrodes were constructed to facilitate recording from two different areas. Image shows a typical set of twisted NiCr wire electrodes used for recording from the PFC, VC and Hip. Distance between lengths for VC and Hip – 0.5 mm, distance between lengths for PFC – 1 mm (allowing recording in PrL and Cg1 areas).

The axis of co-ordinates for surgery are typically abbreviated as listed; AP: anterior-posterior, ML: medial-lateral and DVC: dorsal-ventral. Electrode placements for connection to headstage were as follows (from Bregma); PFC: AP 2.5, ML 1.3, DVC -3.7; Hip: AP -4.0, ML 2.0, DVC -2.2; VC: AP -6.0, ML 2.5, DVC -1.2. Angles for insertion of recording electrodes were as follows: PFC 10°, VC/Hip 0°. The headset was anchored to the skull with four screws positioned at AP -1.5, ML 3; AP -1.5, ML -3; AP -10.5, ML 1.5; AP -10.5, ML -1.5. The two screws positioned at the back of the skull were used for reference and grounding (**figure 2.3**).

An 8-channel pre amplifier headstage (MP8A Multichannel systems, UK) was used to amplify voltage difference with the reference electrode. The headstage was attached to a tether mounted on a slip-ring commutator, which allowed the rat to move around the chamber unhindered. Further amplification - giving 50x total, and filtering was carried out by

AM systems 3600 16-channel amplifier and band-pass filtered at 0.5-500 Hz. The signal was digitised and sampled at 2 kHz using a CED power 1401 interface (Cambridge Electronic Design, Cambridge, UK).

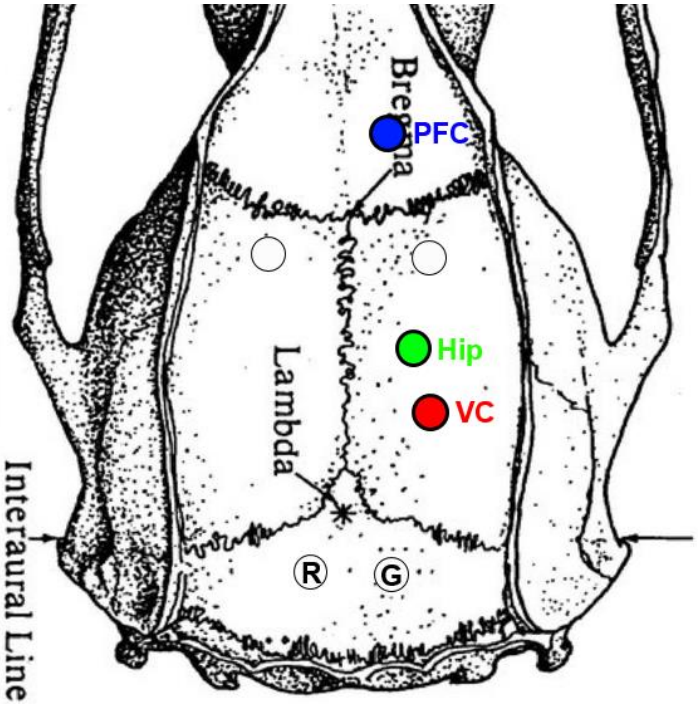

Figure 2.3| Recording electrodes were implanted in VC, Hip and PFC to record local EEG activity. Schematic diagram of rat skull showing electrode and anchor screw placements in freely moving rats. Coloured circles indicate the locations of recording electrodes: PFC (blue) – prefrontal cortex, Hip (green) – hippocampus, VC (red) – visual cortex. White circles indicate anchor screws with R being the reference screw and G being the grounding screw.

All rats completed the sPAL task under control tethered conditions and whilst under the influence of PCP. Rats were injected subcutaneously with 1 mg/ml PCP in physiological saline to give 1 mg/kg systemic dose or with 1 ml/kg saline only (vehicle) immediately prior to being connected to a tether for recording and placed in the testing chamber to start the task. For PCP trials the task ran for 180 minutes and for controls 90 minutes; recording of neural oscillations was carried out throughout the duration of all trials.

Data analysis of freely moving rat experiments

Neural oscillations were recorded from PrL, Cg1, Hip and VC using Spike software (Spike2 v7.08, Cambridge Electronic Design, UK) to give six original channels of raw data. Spike was then used for initial analysis and preparation of channel data. Traces were first 'cleaned' before data could be extracted from recordings for further analysis. In certain rats where recording of a particular channel had failed, this channel was disregarded. This was also the case for when certain channels could not be deemed consistently reliable throughout the length of recording.

EMG activity (generated by muscle movement) and any recording artefacts were removed via a software script in Spike (ArtRem) and by manual deletion. Fast Fourier transforms (FFTs) were used to produce power spectra; FFT size 2048 and Hanning window applied. Power spectra were taken from 100 s sections for remaining channels covering the entire length of recording from a control and PCP file for each rat. Spectral power was assessed in known physiological ranges for neural oscillations, namely: theta (3-8 Hz), alpha (8-13 Hz), beta (13-30 Hz), gamma (30-120 Hz) and fast (120-250 Hz). Average values for power during the 100 s sections were calculated for each data channel during control and PCP trials. From average power in the gamma range, the time of maximum PCP effect for each rat was determined and a 500 s epoch of this used for comparisons with control.

PrL, Cg1 and the most effected VC and Hip channels (where available in individual rats) were FIR (finite impulse response) band pass filtered to give low frequency gamma oscillations (LFG; 30-80 Hz) and low pass filtered to give 'slow' (<10 Hz) oscillations containing theta activity. From this, cross-correlograms were generated for the same 500 s period used for power analysis (time at which PCP had maximal effect) and the corresponding control period.

Frequencies compared were: slow, LFG and low frequency gamma power (LFGP). Gamma power was calculated by applying root mean squared amplitude process to original traces using a time constant of 0.02.

2ii | Anaesthetised rat experiments

Surgery and preparation for EEG recording under terminal anaesthesia

Twelve rats (230-355g) were used for experiments under terminal anaesthesia. Rats were initially anaesthetised in an induction chamber with 5% isoflurane in oxygen before receiving an intraperitoneal injection of 0.7 mg/kg urethane. Rats were then placed under 2% isoflurane in oxygen for 30 minutes before receiving a second intraperitoneal injection of 0.7 mg/kg urethane. A 0.01 mg/kg medetomidine bolus injection was administered 20 minutes after the second urethane injection, before attaching a subcutaneous drip filled with 0.005 mg/kg medetomidine in sterile saline. The drip was attached to a syringe driver (Graseby Medical Ltd, Watford, UK) with the speed of the driver set to deliver 0.03 mg/kg/h. The rat's head was then shaved and cleaned in preparation for surgery. Once no pedal reflex remained the rat skull was fixed into a stereotactic frame via ear bars; 5% EMLA cream (AstraZeneca, UK) was applied inside the ears before securing the bars to minimise discomfort. An incision was made along the middle of the skull and the skin held apart with wound clips for the remainder of the procedure. Membrane layers covering the skull were scraped away and any bleeding stopped before the skull was cleaned with 3% hydrogen peroxide solution and sterile saline. A thin layer of light cured resin (Ultradent Products Inc, Utah, USA) was applied to the surface of the skull in preparation for drilling of insertion holes. All insertion holes were then drilled in the skull using a 0.8 mm diameter drill bit and the dura surrounding the brain

underneath opened, this concluded surgery. After completion of surgery, the medetomidine drip speed was titrated to suit individual rats' anaesthetic depths (usually around 0 - 0.01 mg/kg/h). Depth of anaesthesia throughout surgery and experimental procedures was monitored by regular pedal withdrawal checks and taking readings for breathing rate at regular intervals. Temperature of the rat was controlled throughout surgery and experimental procedures by an automatically adjusted heating pad with core temperature being recorded via a rectal probe (ATC1000, World Precision Instruments).

Holes were drilled for insertion of recording electrodes in VC, Hip and PFC at the same co-ordinates used in freely moving rat experiments. A hole was then drilled at AP -7.8, ML 1.8 (relative to Bregma) for insertion of a stimulating electrode into the pontis oralis (PO). Holes were also drilled for insertion of guide cannulas in VC, Hip and PFC to be used for local applications of ketamine in these areas. The co-ordinates for these guide cannula holes (from Bregma) were as follows: PFC: AP 2.0, ML 0.8, DVC -1; Hip: AP -3.5, ML 1.5, DVC -1; VC: AP -6.0, ML 1.7, DVC 0. When insertion cannulas (34G silica pipette filling fibre; WPI, Sarasota, USA) were placed into guide cannulas for local injection, the actual DVC value for each area was deeper due to the cannula having an insertion stop that ensured a fixed distance of extension from the end of the guide cannula. Final DVC values were as follows: PFC: 3, Hip: 2.5, VC: 1. Angles for insertion of guide cannulas were as follows: PFC 0°, VC/Hip 10°. Guide cannulas were made from 28 gauge stainless steel. The method used for construction of electrodes was identical to as described previously. Recording electrodes were inserted to the same depths as stated previously and guide cannulas inserted alongside before both were secured with light cured adhesive resin. Recording electrodes were connected to a headset as described previously using gold connector pins and a connected grounding wire placed under

the skin of the rat. The reference screw used for terminal experiments was the same as that used in freely moving experiments described previously but was this time placed at AP -10, ML -2.5 relative to Bregma (**figure 2.4**). The headset was connected to the same preamplifier headstage as stated previously. The stimulating electrode was inserted into PO at DVC values between 5 and 6 mm at an angle of 0°.

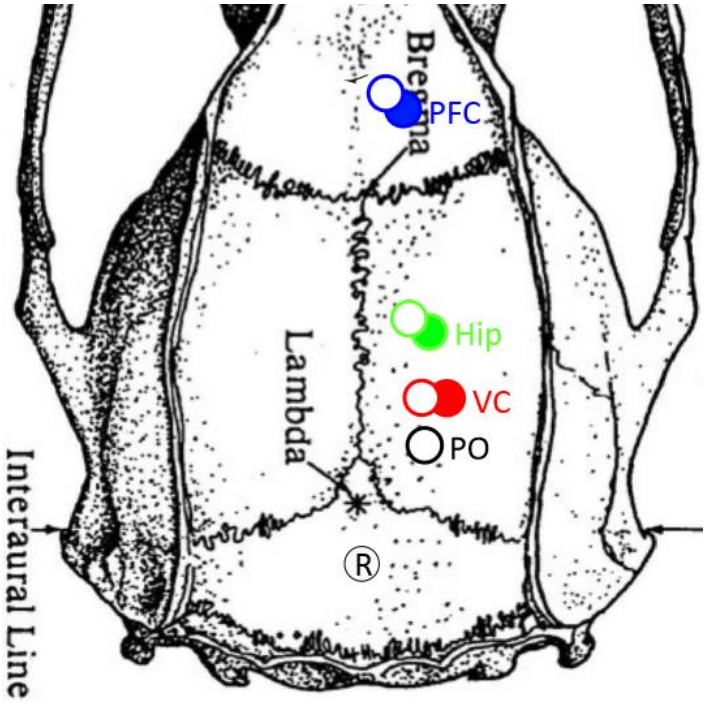

Figure 2.4| Guide cannulas were implanted proximally to recording electrodes. Schematic diagram of rat skull showing electrode and cannula placement sites for terminal experiments. Closed coloured circles indicate the locations of recording electrodes, open coloured circles indicate the locations of guide cannulas: PFC (blue), Hip (green), VC (red). Location of stimulus electrode in PO is indicated by the open black circle. White circle containing R denotes the reference screw.

Amplification and recording procedures were the same as described previously for recording of neural oscillations during sPAL task completion in freely moving rats. Efforts were made to conserve implantation techniques across terminal and freely moving in vivo protocols in order to minimise recording differences and allow comparisons of data to be more valid.

Determining stimulation level to achieve cortical activation

Rats under terminal anaesthesia do not display neural activity comparable to those recorded in freely moving awake animals. The trace is typically dominated by slow 'sleep like' waveforms and can vary dramatically throughout the experimental time. To study changes in neural oscillations induced by drug treatments it is also desirable to have an 'activated' cortex, as this is the closest mimic of an awake animal. It was decided that electrical stimulation of brainstem nuclei would be used to achieve this stable activated state. A pilot experiment using one male Lister Hooded rat was undertaken to determine the most effective place for stimulation. It was found that stimulation of PO resulted in the longest level of activation (see results, figure 3.6), making it the chosen stimulation site for terminal experiments.

Once all electrodes were connected and neural activity was being recorded in every channel, electrical stimulations were started to determine the most effective stimulus electrode depth. The stimulus electrode was initially placed at DVC 5.0 and a 0.5 mA-1.0 mA stimulus applied at depths in steps of 0.25 mm down to DVC 6.0. The stimulus electrode was fixed at the depth at which the most effective level of cortical activation was achieved. Electrical stimulation was delivered via a constant current isolated stimulator (Digitimer Ltd, Welwyn Garden City, UK). A 100 Hz 50 pulse stimulus train at the lowest sufficient amperage for cortical activation was given every 60 s throughout experimentation.

Administration of ketamine under terminal anaesthesia

Ketamine, dissolved in sterile saline, was administered subcutaneously during nine of the terminal experiments; three rats received a dose of 10 mg/kg, six rats received 4 mg/kg. Sufficient washout time to re-establish a reliable baseline was allowed after systemic

ketamine treatment in rats where both systemic and local protocols were used, systemic ketamine was given either before or after local injections.

Ketamine was infused locally into VC, Hip and PFC in all rats with time being allowed for washout after this also. 5 µg/µl ketamine dissolved in artificial cerebral spinal fluid (aCSF) was used for local injection. Vehicle injections using aCSF have been performed previously in the lab and caused no change to oscillatory activity in the brain. Either an infusion pump (KDS 311CE, kd Scientific, RoYem Scientific Limited, UK) containing a Hamilton Syringe or a 'micro-pump' developed by researchers at Boise State University (S. Barker, Idaho, USA) was used to deliver local injections. Both were calibrated to deliver 1 µl of ketamine at a rate of 0.333 µl/min. Pumps were connected to an insertion cannula made of 150 micron silicon hollow fibre which fitted inside of implanted guide cannulas as described previously (**figure 2.5**).

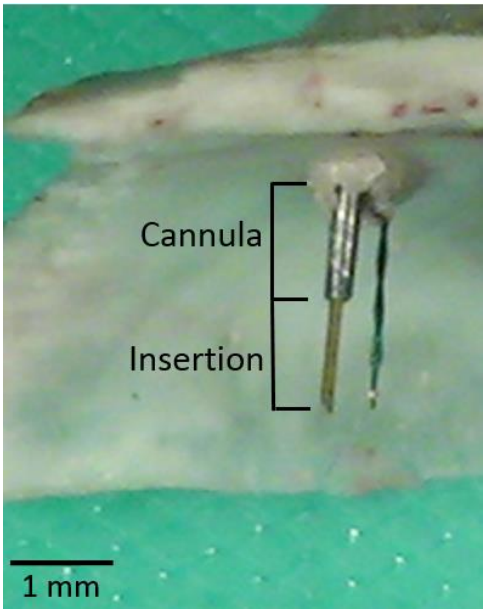

Figure 2.5| Insertion cannulas were implanted proximally to recording electrodes in the area of interest. Image shows part of rat skull containing Hip electrode and guide cannula to illustrate arrangement. 'Cannula' denotes metal guide cannula, 'Insertion' denotes silicon insertion cannula used for local injection of ketamine. The recording electrode is located to the right of the cannula in this image.

After placement of the insertion cannula into tissue, sufficient time (5-10 minutes) was allowed to re-establish a stable baseline before injections were started. The insertion cannula was left in place at least 10 minutes after the injection had finished to prevent any backflow of solution.

Data analysis of terminal experiments

Spike software was used for collection of raw data and cleaning of traces. Stimulation artefacts were removed using an artefact removal script and any obvious background artefacts removed manually. Virtual channels were created for VC and Hip by subtraction of the two recording channels in these areas. This removed shared line noise and shared non-local activity that may have been recorded through volume conduction. These virtual channels were then used in analysis of VC and Hip activity; for PrL and Cg1, original recording channels were used.

FFTs were used to produce power spectra, with FFT size 2048 and Hanning window applied. For local ketamine injections, power spectra were taken from 60 s sections before and after injection with ketamine. Average power during the 60 s sections was calculated for PrL, Cg1, VC and Hip during baseline recording and after injection. For systemic ketamine experiments, power spectra were generated from 100 s sections before and after injection – as was used for systemic PCP analysis. From the average time course for gamma power, the time of maximum ketamine effect was determined for each recording location by averaging the three data points encompassing the value furthest away from baseline readings. This corresponded to a 180 s epoch for local injections and a 300 s epoch for systemic injections, baseline values were taken for the same length of time prior to injection start.

Band pass filtering was applied to PrL, Cg1, VC and Hip channels to give LFG, and High Frequency Gamma (HFG; 80-120 Hz) oscillations; low pass filtering was applied to give 'slow'. LFGP and HFGP were generated by applying a root mean squared process to LFG and HFG traces respectively. From this, cross-correlograms were generated for the same epoch used for power analysis (time at which ketamine had maximal effect) and the equivalent baseline period before injection. Numerical values of these correlation waveforms were extracted into a spreadsheet for further analysis.

Statistics

Shapiro-Wilks test for normality was used on all data sets. Comparisons were made between control/baseline recordings and recordings conducted following drug treatments. Where Shapiro-Wilks was found to be significant, related samples Wilcoxon signed rank test was used. Related samples paired T-tests were used when data was normally distributed.

Injection of dye and TTX to validate local injection protocol

Injections were delivered as described previously for local injection of ketamine into VC, Hip and PFC. In one rat, 1 µl of blue dye was injected at a rate of 0.333 µl/min in each injection site sequentially. The brain was then dissected out, following cervical dislocation of the rat, and sliced to examine dye location. In five rats 1 µl of 10 ng/ml Tetrodotoxin (TTX) was injected at a rate of 0.333 µl/min into each injection site sequentially, whilst recording EEG activity. Both of these protocols were undertaken at the end of experiments, as electrical activity in the brain would not have recovered to a baseline situation.

Pilot experiment: local ketamine injection into Nucleus Basalis

In two rats, ketamine was injected locally into the Nucleus Basalis (NB) using the same protocol as described previously for local ketamine injection. This was an additional experiment conducted at the end of this study to form the basis of a future set of experiments. EEG was recorded and PO stimulation used as described previously also. The co-ordinates for insertion of NB cannula (from Bregma) were: AP -1.8, ML 3.0, DVC -7.8.

- RESULTS -

3i | PCP experiments in freely moving rats

PCP caused behavioural changes during sPAL task completion

The six rats used for the series of PCP experiments had been previously trained and were competent in completion of the sPAL task. Therefore, all rats were comfortable being handled and whilst in the testing chamber – importantly this meant no signs of stress were observed that may have caused changes in neural activity not related to PCP kinetics. During control trials, where the rat was injected with a vehicle immediately before being placed in the chamber, task completion was to the standard level of proficiency acquired during training and no abnormal behaviour was observed. Treatment with 1 mg/kg PCP caused profound changes in behaviour whilst rats attempted to complete the task, with the main affect being hyperactivity followed by moderate ataxia. The ability to complete the task was affected; rats regularly initiated the task, failed to select an image, yet attempted to collect a sugar pellet. Behavioural changes were observed as quickly as 10 minutes post injection and were evident in all rats until around 60 minutes, by which time movement had returned to near normal. By 90 minutes most rats had lost interest in task completion and mainly spent the remaining time resting, indicating that any major behavioural effects of PCP had worn off by this time point.

As well as induced behavioural changes by PCP treatment, changes were observed in task proficiency. The average percentage 'correct touches' value for control trials was 86.5%, as there was a 50% chance of making a correct touch (due to two images being displayed) this can be viewed as 36.5% above being correct by chance. This was significantly decreased by 23.9% during PCP trials [$n=6$, $p=0.028$], making percentage correct performance only 12.6%

greater than chance whilst under the influence of PCP. This illustrates that 1 mg/kg PCP is sufficient to decrease paired associative learning performance. Correction trials were offered to the rat after an incorrect touch was made in the task, rats usually remember their previous wrong choice and so choose the correct image in a correction trial. Correction trial number significantly increased by an average of 57.5% in PCP trials compared to controls [*n=6, p=0.027*]. This indicates that rats persistently chose the wrong image in the task when previously they were able to remember which image should be the correct one knowing they were initially incorrect. This result is consistent with impairments in working memory which, as stated earlier, is a key symptom of schizophrenia.

From raw traces to EEG data

Traces from recording channels contained many artefacts (**figure 3.1a**) and so needed to be 'cleaned' before any further power analysis could be completed (**figure 3.1b**). Clear EMG activity and recording artefacts are visible in the raw trace, which were manually removed. It was from these cleaned traces that power spectra were then generated for analysis (**figure 3.2**). Spectra were generated for PCP and control files from 'cleaned' traces for each recording area. The numerical values for these power spectra were separated into physiological ranges, e.g. 30-80 Hz for LFG, and used for statistical comparisons.

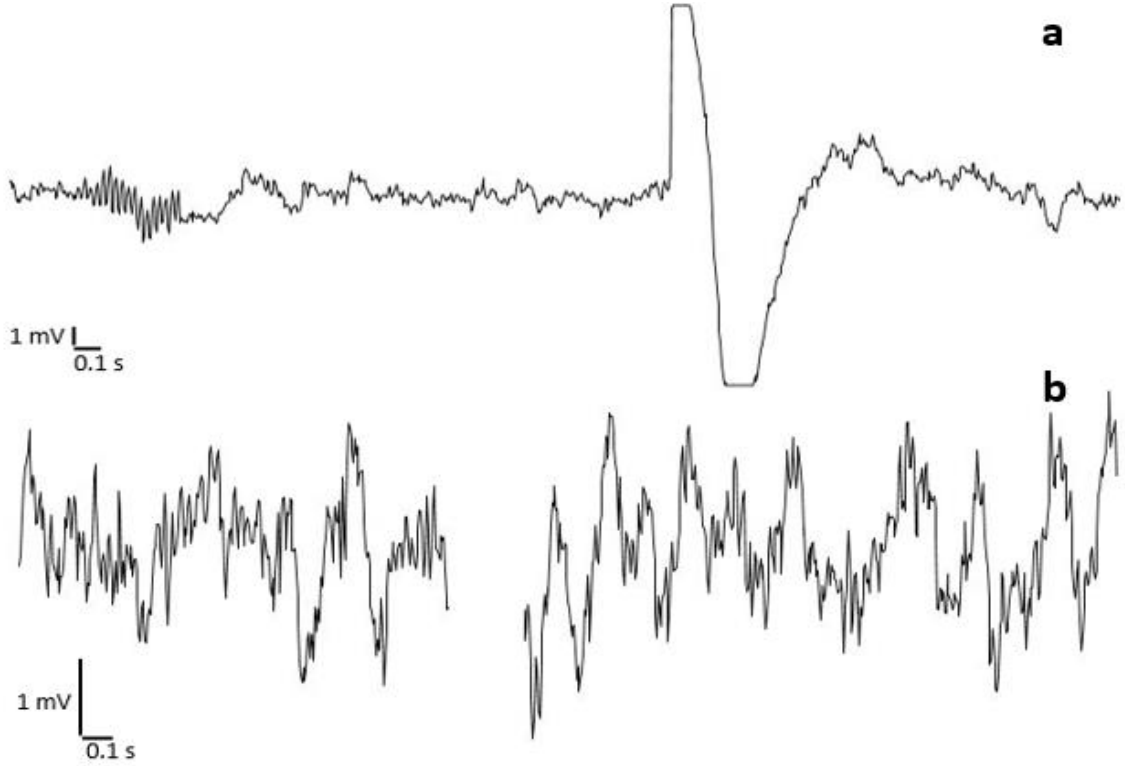

Figure 3.1| Traces had to be cleaned and movement artefacts removed to leave EEG activity. (**a**) shows 3 seconds of recording from PrL for a rat completing sPAL after injection with PCP, EMG activity from chewing is apparent at the start of the trace and a large wave recording artefact; (**b**) shows 3 seconds trace from the same channel after artefact removal, revealing gamma and theta activity. Note the scale difference between **a** and **b**.

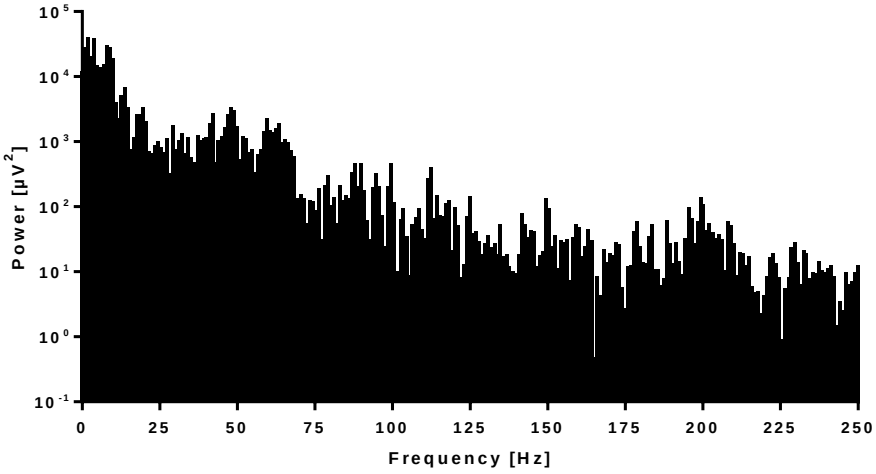

Figure 3.2| An example of the power spectra generated for analysis of changes to power following PCP treatment. Shown is a power spectrum generated from the trace in figure **1b**, which was recorded in PrL during completion of sPAL after PCP injection. Data is shown on a log power scale for recorded activity in 0-250 Hz frequency range.

PCP caused increased gamma power in all recording areas

The time of maximal PCP effect occurred firstly in Hip and VC, at around 800s post injection time. Then followed the PrL and Cg1, with their peaks occurring at around 1000s (**figure 3.3**). Gamma power remained slightly elevated above baseline levels in all areas after 120 minutes – up until the end of recording. At the 60 minute mark, the time at which behaviour appeared to have returned to normal in all rats, gamma power was still around 50% higher than control values in VC, Hip and Cg1. This indicates that changes to EEG activity caused by PCP are much longer lasting than effects on behavioural measures.

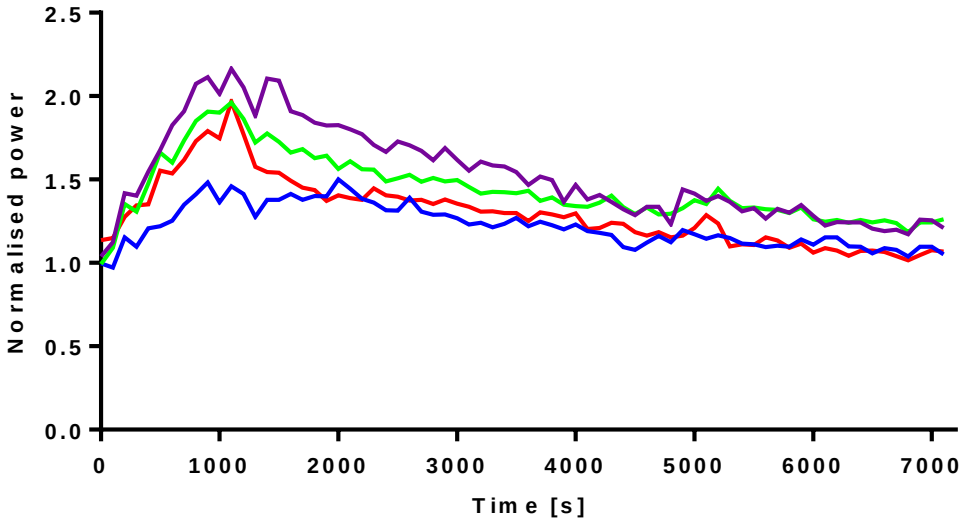

Figure 3.3| Systemic PCP injection caused an increase in gamma power in all recording areas throughout sPAL task completion. Graph shows mean changes in gamma band power in all recording areas throughout EEG recording during PCP trials in all rats [n=6]. Line colours: purple – Cg1, green – Hip, red – VC, blue – PrL. Gamma power was normalised to control recordings for each rat.

Total gamma power (sum of power in 30-120 Hz range) at the peak of PCP effect was significantly higher than during control trials for all rats. PCP power values were normalised to

a baseline by dividing values by their control counterpart at the same time point. Normalising allowed data from each recording area to be more easily comparable and accounted for natural changes occurring in EEG over the length of task completion. Following PCP injection: gamma power in PrL was increased by 49% [$t(4)=3.930,\ p=0.017$], VC increased by 86% [$t(4)=5.938,\ p=0.004$] and Hip increased by 98% [$t(4)=5.028,\ p=0.007$]. The largest change caused by PCP in all areas of interest was recorded in Cg1, which increased by 115% [$t(4)=10.877,\ p<0.001$].

Recorded traces were filtered before completion of cross correlation analysis

Simultaneous recording of different neural regions allows changes in synchronicity between areas to be studied. In order to complete cross-correlation analysis, traces were initially filtered to leave oscillations in relevant ranges. These were LFG, fluctuations in LFGP and slow (<10 Hz). FIR digital filtering was used to achieve this (**figure 3.4**). Cross-correlograms were then generated from these filtered traces of different frequencies, comparing the phase relationships between activity in recording areas. An example is shown comparing the relationship between LFG in Hip (used as reference) and LFG in PrL (**figure 3.5**). For the example shown, the correlation peak is not exactly 0, this indicates that any measured correlation is a true relationship and not shared line noise. The peak of correlation is slightly negative – indicating that that peak of oscillation in PrL occurs slightly before that in the Hip. The max correlation value of a cross-correlogram is a measure of the strength of correlation between the two areas compared; a higher value indicates greater phase synchrony. For The example correlograms shown, the max correlation of the peak was higher during the control

recording [0.17] than during the PCP recording [0.09]. This change was observed frequently after PCP treatment and will be discussed in more depth with other changes in the next section.

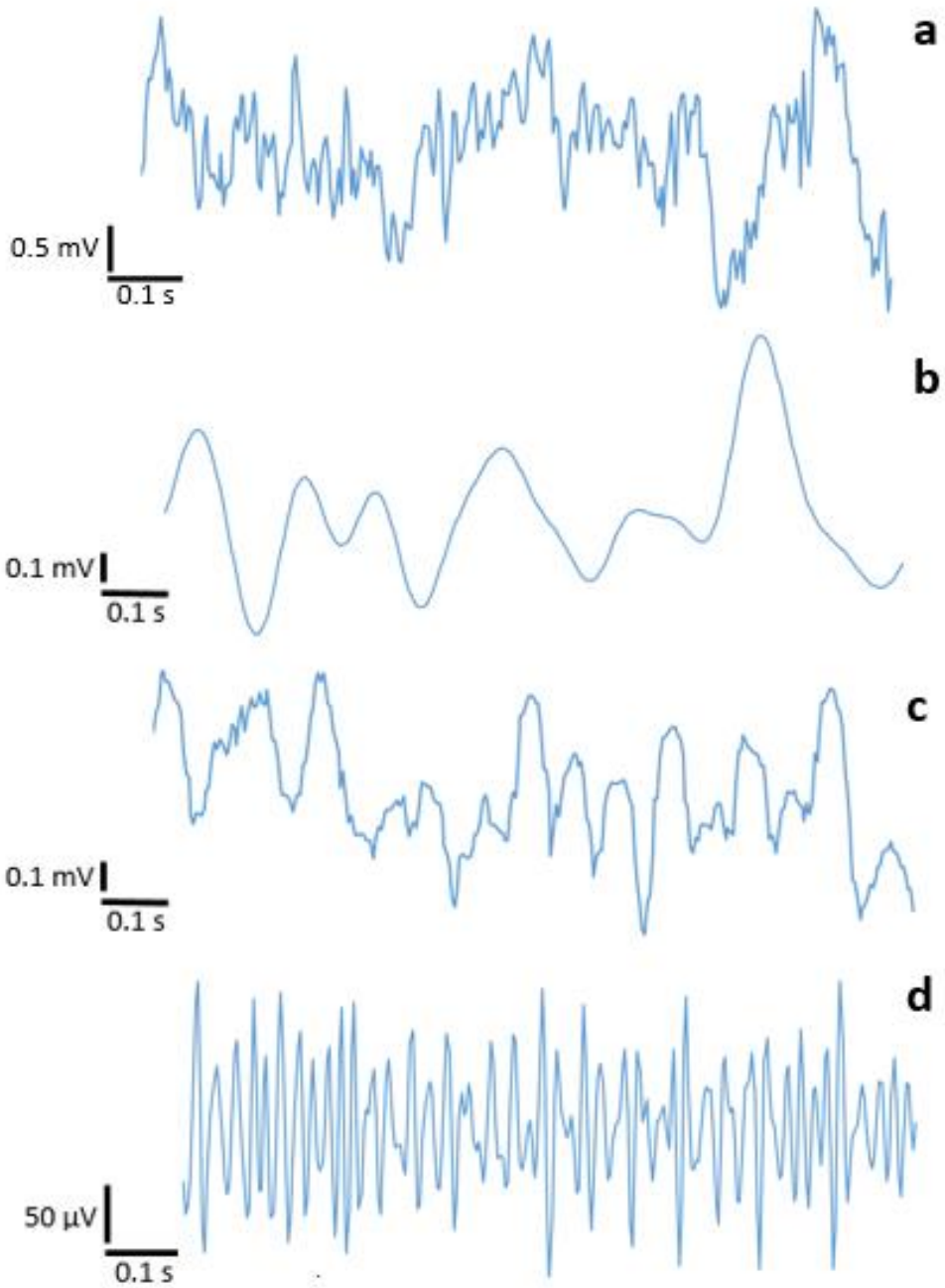

Figure 3.4| Raw traces were filtered to leave activity in the relevant frequency bands needed for cross correlation analysis. Example filtered traces taken from 1 s of PrL recording; **a** – raw clean trace containing all recorded frequencies; **b** – slow oscillations, low pass filtered <10 Hz; **c** – LFG oscillations, band pass filtered 30-80 Hz; **d** – LFGP, root mean square amplitude process applied to trace in **c**.

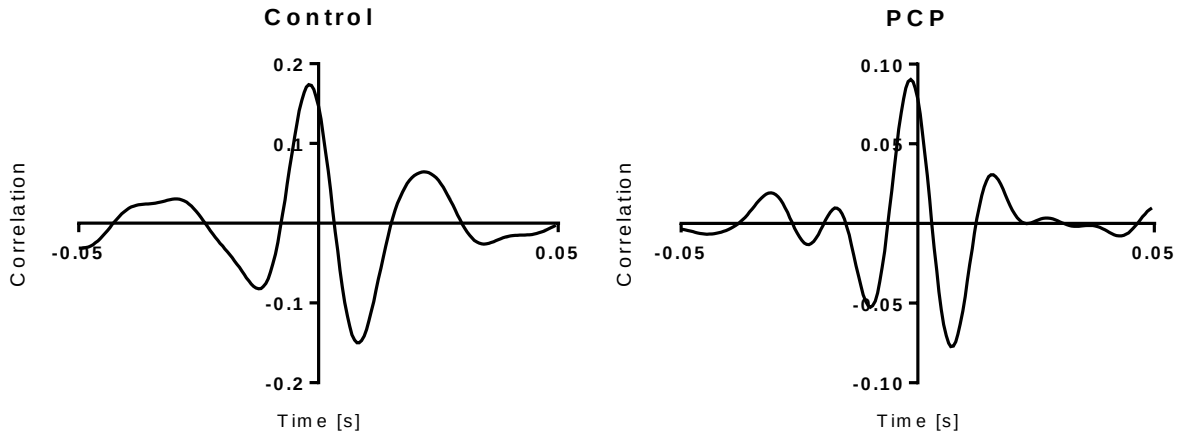

Figure 3.5| From filtered traces in relevant frequency bands, cross-correlograms were generated showing the phase relationship between recording areas. Shown are typical cross-correlograms taken from LFG recorded simultaneously in Hip and PrL, here Hip was taken as the reference and the phase of PrL compared with it. Control refers to a control trial recording where the rat completed the sPAL task without intervention, PCP refers to a recording in which the rat received an injection of 1 mg/kg PCP prior to starting the sPAL task. In this example, correlation between the two areas decreased following PCP treatment – as indicated by the decreased amplitude of the correlogram for PCP.

PCP treatment caused changes to correlations between recorded areas

Cross correlation analysis can be used to determine how synchronised activities in areas of the brain are with each other, this reflects the phase relationship between oscillations generated in each area. Phase synchronicity can be interpreted to reveal changes in the ability of the areas analysed to communicate effectively, as synchronisation is required for the transmission of information in the cortex (Vinck et al, 2013). Lower frequency oscillations such as theta are also thought to modulate gamma power, thus facilitating synchronisation and communication (Doesburg et al, 2012). Therefore, disruption to these relationships induced by drug treatment can be viewed as detrimental to communication in the cortex.

When cross-correlations of LFG between all recorded areas were analysed, all areas showed a PCP induced decrease in the max correlation value (**table 3.1**). Generally, the greatest decreases occurred between the areas located furthest apart (e.g. Hip and PrL/Cg1) and the smallest decreases between areas located close together in the brain (e.g. PrL and Cg1). As six comparisons were made of LFG correlation between recorded areas, it was decided that a Bonferroni correction would be appropriate – giving a new alpha value of 0.008. The decrease in max correlation between Hip and Cg1 was the only change still deemed statistically significant under the new corrected significance level (see **table 3.1**).

Correlations	**Max correlation**		**Significance**		
Areas compared	Control (sem)	PCP (sem)	*t* value	*p* value	df
Hip-PrL	0.157 (0.018)	0.107 (0.015)	-3.349	0.029*	4
Hip-Cg1	0.164 (0.009)	0.091 (0.005)	-5.036	0.007*	4
Hip-VC	0.473 (0.132)	0.451 (0.134)	1.235	0.305	3
PrL-Cg1	0.593 (0.075)	0.590 (0.076)	0.158	0.882	4
VC-Cg1	0.213 (0.058)	0.160 (0.060)	-2.466	0.069	4
VC-PrL	0.176 (0.065)	0.136 (0.032)	-0.852	0.457	3

Table 3.1| Systemic PCP caused a decrease in inter-area LFG correlation during sPAL task completion. Changes in max correlation value between LFG in recorded areas during control and PCP treated trials for all available rats. PCP refers to 500 s of data encompassing highest peak in gamma power effect determined previously, control refers to the same 500 s time point in a control recording. The first channel listed in 'areas compared' was used as the reference that the second channel listed was compared to this. * denotes significant decrease in max correlation value when $\alpha = 0.05$; * denotes significant when $\alpha = 0.008$. Data is shown as the mean value with the sem (standard error mean) for these values in brackets.

The same trend of decrease for LFGP inter-area correlation occurred as for LFG; max correlation decreased and the largest decreases were observed between those areas furthest apart spatially. Two significant changes were revealed during analysis of LFGP-LFGP relationships (**table 3.2**), these were between Hip-PrL and Hip-Cg1 – the same two correlations

that were also initially significant when comparing LFG. As the same number of statistical comparisons were made as previous, the adjusted alpha value after correction was 0.008. Again, the decrease in max correlation between Hip-Cg1 was the relationship deemed still significant after correction.

Correlations	**Max correlation**		**Significance**		
Areas compared	Control (sem)	PCP (sem)	*t* value	*p* value	df
Hip-PrL	0.073 (0.009)	0.039 (0.006)	-3.165	0.034 *	4
Hip-Cg1	0.058 (0.009)	0.047 (0.006)	-6.365	0.003 *	4
Hip-VC	0.324 (0.106)	0.273 (0.103)	2.497	0.088	3
PrL-Cg1	0.400 (0.061)	0.394 (0.078)	0.198	0.852	4
VC-Cg1	0.070 (0.017)	0.038 (0.009)	2.492	0.067	4
VC-PrL	0.111 (0.020)	0.071 (0.020)	1.834	0.164	3

Table 3.2| Systemic PCP caused a decrease in inter-area LFGP correlation during sPAL task completion. Changes in max correlation value between LFGP in recorded areas during control and PCP treated trials for all available rats. PCP refers to 500 s of data encompassing highest peak in gamma power effect determined previously, control refers to the same 500 s time point in a control recording. The first channel listed in 'areas compared' was used as the reference that the second channel listed was compared to this. * denotes significant decrease in max correlation value when $\alpha = 0.05$; * denotes significant when $\alpha = 0.008$. Data is shown as the mean value with the sem (standard error mean) for these values in brackets.

As stated earlier, Cg1 showed the greatest increase in gamma power after injection with PCP. The fact that the most significant decrease in correlation value occurred in correlations containing Cg1 indicates that although gamma power is increased by PCP, the synchronisation of Cg1's gamma power with other areas – such as Hip, is decreased. As explained earlier, this may indicate disruption in communication between these areas. Also, when looking at LFG and LFGP the two significant changes found in both occurred when comparing prefrontal areas to Hip. This may have contributed to the decreased performance in sPAL as both of these areas are key for task completion. No clear change in phase

relationship was observed for slow wave to LFGP oscillations (**table 3.3**), indicating that the modulation of gamma power by slower frequencies was unaffected by PCP treatment.

Correlations	**Max correlation**		**Significance**
Areas compared	Control (sem)	PCP (sem)	*p* value
Hip-PrL	0.038 (0.016)	0.040 (0.016)	0.949
Hip-Cg1	0.023 (0.002)	0.029 (0.005)	0.135
Hip-VC	0.079 (0.015)	0.058 (0.016)	0.156
PrL-Cg1	0.018 (0.004)	0.020 (0.005)	0.661
VC-Cg1	0.028 (0.003)	0.037 (0.009)	0.340
VC-PrL	0.037 (0.016)	0.038 (0.019)	0.905

Table 3.3| Systemic PCP caused no significant changes to slow-LFGP inter-area correlation during sPAL task completion. Changes in max correlation value for slow-LFGP in recorded areas during control and PCP treated trials for all available rats. PCP refers to 500 s of data encompassing highest peak in gamma power effect determined previously, control refers to the same 500 s time point in a control recording. The first channel listed in 'areas compared' was used as the reference that the second channel listed was compared to this. Data is shown as the mean value with the sem (standard error mean) for these values in brackets.

3ii | Systemic ketamine experiments in anaesthetised rats

Electrical stimulation was used to create an active cortex recording situation

Neural activity recorded from rats under terminal anaesthesia were dominated by slow waves and showed a typical fluctuating 'deep sleep like' pattern of activity (**figure 3.6a**). For the purpose of this study, an activated cortex was required that was more comparable to that of awake rats used for PCP experiments. To achieve this, 0.5 - 1 mA electrical stimulation (detailed in methods) via the PO was used. The PO is known to have a role in control of REM sleep and electrical PO stimulation has been previously found to generate theta (Brown et al, 2012). As well as activating the cortex to a state where changes in gamma activity could be induced and recorded, stimulation created a reproducible baseline situation comparable

among all rats used (**figure 3.6b**). It was from this initial 'activated baseline' state that all following terminal ketamine experiments were conducted.

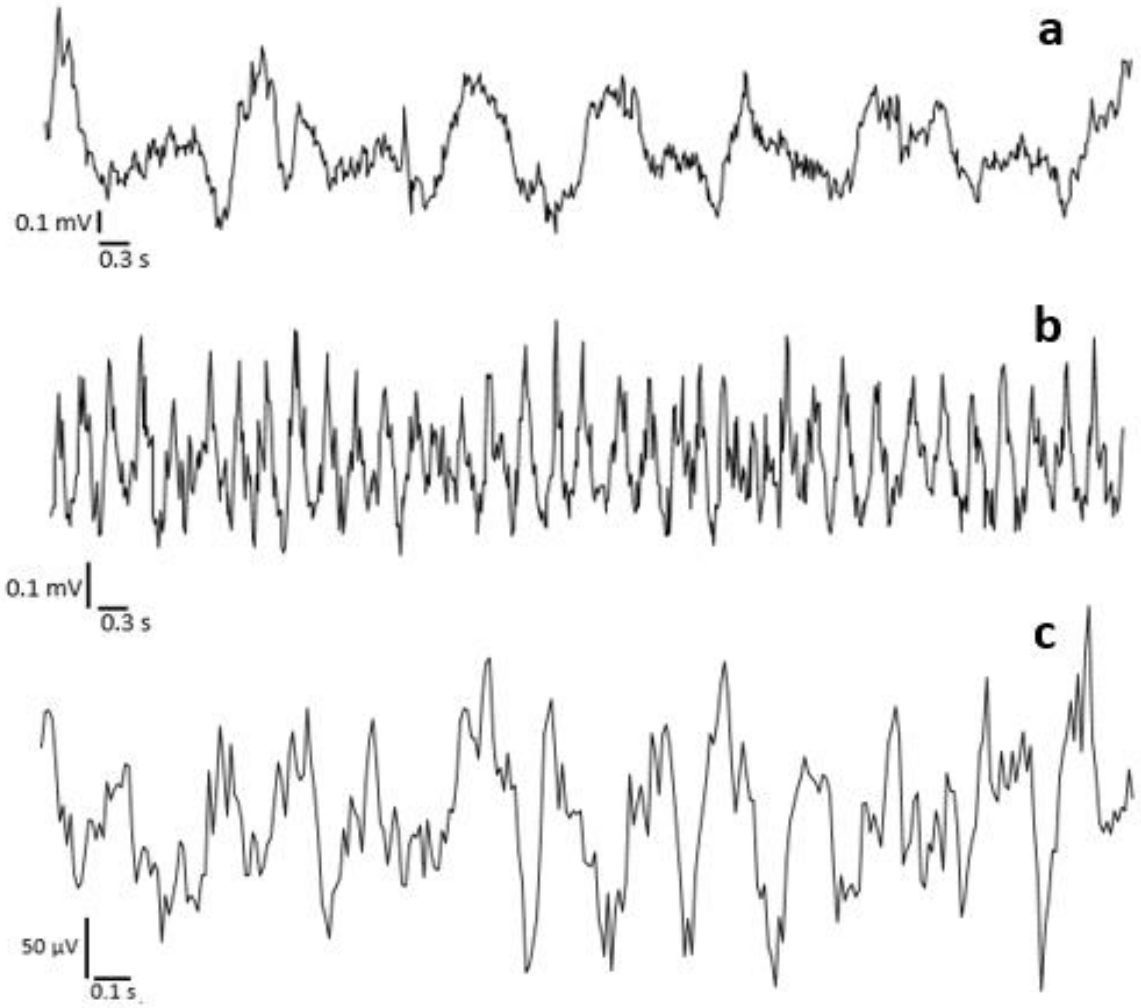

Figure 3.6| Electrical stimulation via the pontis oralis created an active cortex which could be deemed a stable baseline for terminal experiments. Traces show typical effect of electrical stimulation via PO on recording areas. **a** - 7 seconds of trace from Hip before stimulation: slow waves dominated recording, **b** - 7 seconds of trace from same Hip channel following stimulation: slow waves were supressed and a stable baseline created, **c** - 3 seconds trace from PrL following stimulation (same time scale used as **figure 3.1** in awake rats, for comparison of recording): gamma and theta activity created by stimulation mimics an awake rat.

Changes to power were different for recording areas

The frequency distribution of power spectra generated for each recording area differed during stimulated baseline recordings (**figure 3.7**). These differing baseline power levels account for differences in absolute power values following ketamine treatment for each recording area and highlight the need for normalisation of data. As well as increasing power across the entire gamma range (30-120 Hz), systemic ketamine treatment increased power in lower frequency bands such as theta (3-8 Hz). For Hip and VC, a subtraction of the two recording channels in these areas was used to create a local area activity channel (detailed in methods).

Systemic ketamine treatment significantly increased gamma power

As mentioned previously, for terminal recordings a subtraction channel for VC and Hip was created – data from these subtraction channels were used for analysis of these areas for the following experiments. Gamma power for terminal experiments denotes the power of oscillations across 30-120 Hz frequency range, LFGP and HFGP refer to previously explained lower and higher power bands within this range. For systemic ketamine experiments, the value for peak ketamine effect on gamma power was determined by averaging 300 s of data encompassing the highest power value recorded after injection in each rat and then averaging these values for all rats.

In three rats, 10 mg/kg ketamine was given systemically (subcutaneously) under terminal anaesthesia whilst recording EEG. Following systemic injection with ketamine: gamma power peaked first in Hip and VC, and was then followed by Cg1 and PrL. Peak effect

occurred earlier under 10 mg/kg ketamine than 4 mg/kg and 10 mg/kg also caused a greater overall increase in gamma power in all channels. Gamma power remained elevated above baseline levels under both doses at the end of recording (**figure 3.8**).

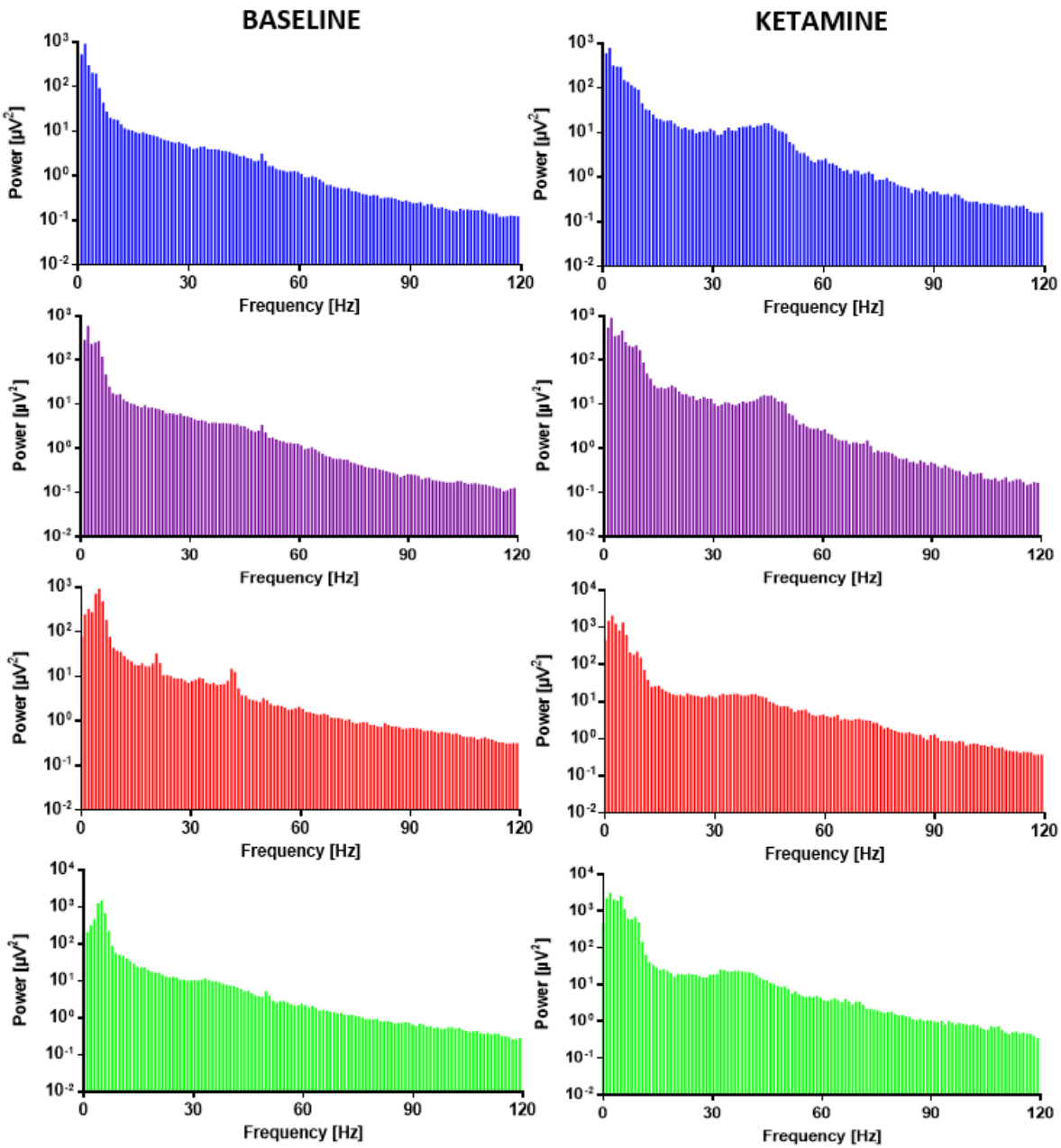

Figure 3.7| Power spectra from different areas showed different distributions of power frequencies before and after systemic injection with ketamine. Example power spectra for baseline and systemic ketamine recordings for all areas of interest, showing power of oscillations recorded between 0-120 Hz. Blue - PrL, purple – Cg1, red – VC, green – Hip. Systemic ketamine increased power in gamma and lower frequencies in all areas.

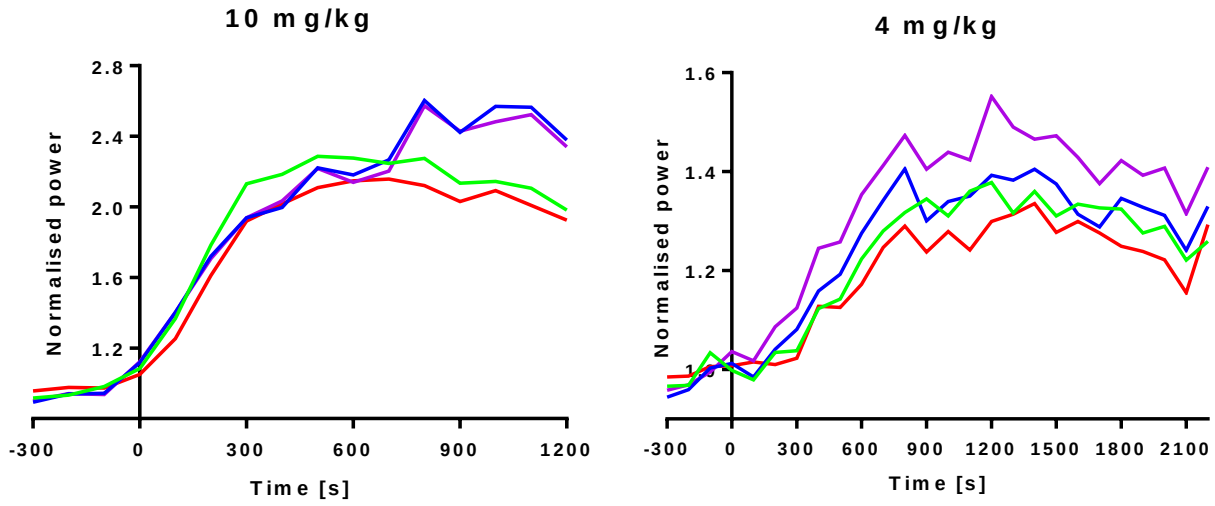

Figure 3.8| Gamma power was increased by systemic injection with 4 mg/kg and 10 mg/kg ketamine. Graphs show mean changes to gamma power in all recording areas after systemic ketamine treatment [10 mg/kg: n=3, 4 mg/kg: n=6]. Line colours: purple – Cg1, green – Hip, red – VC, blue – PrL. Data is shown for 10 mg/kg encompassing the peak of effect, data is shown for the length of recording for 4 mg/kg - both were normalised to baseline recordings for each rat before averaging.

Data was normalised to the arithmetic mean of values for 300 s - deemed a stable baseline, before injection start point. The peak of ketamine effect was taken as an average of 300 s of data encompassing the highest power value recorded after injection. Following systemic treatment with 4 mg/kg ketamine: peak gamma power in Cg1 increased by 50% [*t(5)=4.568, p=0.006*], PrL was increased by 41% [*t(5)=3.367, p=0.020*] and VC increased by 31% [*t(5)=3.349, p=0.020*]. The only increase deemed not significant following 4 mg/kg ketamine injection was in Hip, which increased by 35% [*t(5)=2.407, p=0.061*].

Following systemic treatment with 10 mg/kg ketamine: peak gamma power in Cg1 increased by 150% [*t(2)=4.610, p=0.044*], PrL was increased by 153% [*t(2)=4.760, p=0.041*], Hip increased by 127% [*t(2)=6.096, p=0.026*] and VC increased by 114% [*t(2)=12.704,*

p=0.006]. These increases in gamma power were all deemed significant when α=0.05, but as the n number for these data was just 3 (compared to n=6 for 4 mg/kg), significant values should not be deemed as robust as those for 4 mg/kg.

Additional filtering was needed to obtain high frequency gamma oscillations

High frequency gamma oscillations (HFG, 80-120 Hz) and HFGP (root mean squared applied to HFG) were used in cross correlation analysis of terminal ketamine data – in addition to LFG, LFGP and slow activity described previously in PCP experiments (**figure 3.9**).

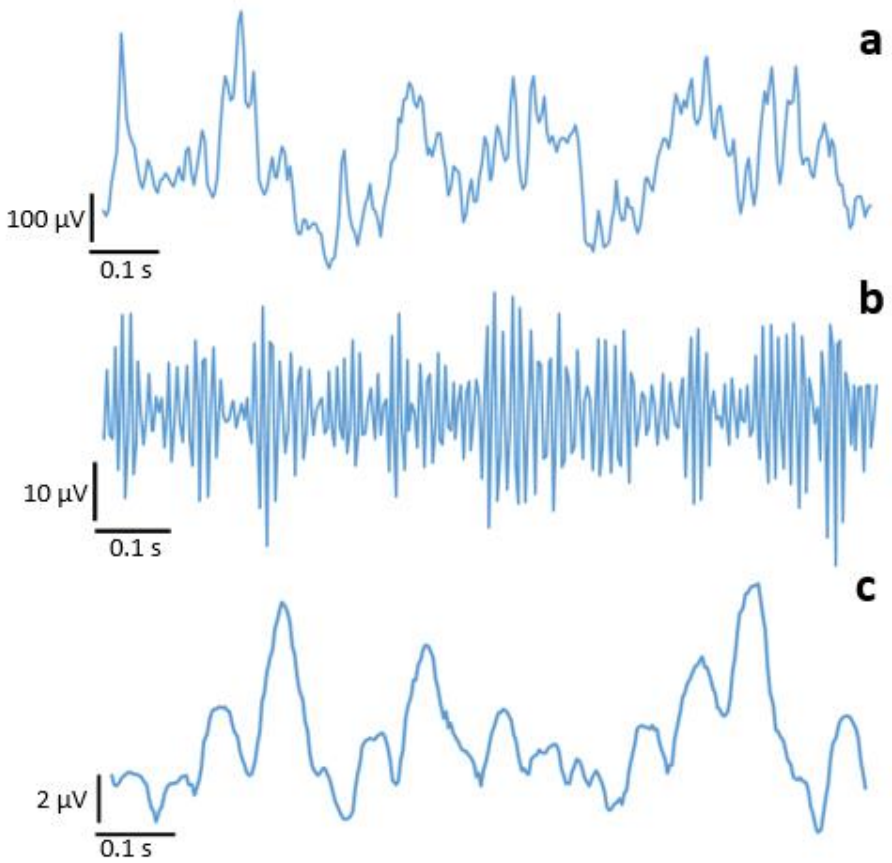

Figure 3.9| Band pass filtering was used to give HFG and HFGP for cross-correlation analysis of terminal experiments. Example filtered traces for cross-correlation analysis: **a** - 1 second of raw Hip recording; **b -** HFG taken from same trace as in **a**, band pass filtered 80-120 Hz; **c -** HFGP, amplitude root mean squared applied to HFG trace.

Systemic ketamine treatment caused changes to inter-area correlations

For cross correlation analysis, data from 4 mg/kg dose experiments were used due to higher n number [n=6] and thus greater reliability. An average value was taken for maximum correlation during baseline and peak ketamine effect for all rats. The max correlation value for HFG between areas was decreased for all correlations after systemic ketamine treatment except for between VC-Hip, where correlation slightly increased. This was also the case for LFG inter-area correlations, although the decreases were more modest (**table 3.4**). In both cases, the greatest effect on inter-area synchronisation occurred between areas located furthest apart. As described previously, a Bonferroni adjusted α value was applied to any changes found to be significant when α=0.05. There were no significant changes remaining when the adjusted α was applied.

Correlations		**Max correlation**		**Significance**
Areas compared	Frequencies	Baseline (sem)	Ketamine (sem)	*p* value
Cg1-Hip	LFG-LFG	0.301 (0.068)	0.260 (0.051)	0.209
Cg1-VC	LFG-LFG	0.370 (0.125)	0.328 (0.126)	0.120
PrL-Cg1	LFG-LFG	0.867 (0.035)	0.857 (0.045)	0.430
PrL-Hip	LFG-LFG	0.285 (0.065)	0.255 (0.043)	0.917
PrL-VC	LFG-LFG	0.370 (0.110)	0.332 (0.109)	0.181
VC-Hip	LFG-LFG	0.436 (0.126)	0.462 (0.103)	0.305
Cg1-Hip	HFG-HFG	0.212 (0.052)	0.143 (0.048)	0.050
Cg1-VC	HFG-HFG	0.309 (0.077)	0.204 (0.084)	0.028 ∗
PrL-Cg1	HFG-HFG	0.701 (0.043)	0.659 (0.063)	0.173
PrL-Hip	HFG-HFG	0.212 (0.046)	0.135 (0.043)	0.051
PrL-VC	HFG-HFG	0.308 (0.072)	0.201 (0.067)	0.046 ∗
VC-Hip	HFG-HFG	0.465 (0.068)	0.473 (0.074)	0.520

Table 3.4| Systemic ketamine induced changes to inter-area LFG and HFG correlations. Mean changes in max correlation value for LFG and HFG between areas during baseline and systemic ketamine recordings [n=6]. Ketamine refers to 300 s of data encompassing highest peak in gamma power effect determined previously, baseline refers to 300 s baseline recording before injection. The mean value for all rats is shown with the sem for these data in brackets. * denotes significant decrease in max correlation value when α=0.05, no changes were significant when α=0.008.

Changes to HFGP correlations between areas followed the opposite trend to those for HFG: max correlation value increased between every area analysed. Max correlation value for LFGP between all areas of interest also increased under systemic ketamine (**table 3.5**). This is in contrast to systemic PCP injection in freely moving rats, where a decrease in inter-area LFGP was observed. However, none of the changes to LFGP or HFGP in these terminal systemic ketamine experiments were deemed significant.

Correlations		**Max correlation**		**Significance**
Areas compared	Frequencies	Baseline (sem)	Ketamine (sem)	*p* value
Cg1-Hip	LFGP-LFGP	0.153 (0.056)	0.179 (0.080)	0.463
Cg1-VC	LFGP-LFGP	0.218 (0.147)	0.287 (0.143)	0.345
PrL-Cg1	LFGP-LFGP	0.787 (0.580)	0.805 (0.068)	0.311
PrL-Hip	LFGP-LFGP	0.138 (0.044)	0.183 (0.069)	0.173
PrL-VC	LFGP-LFGP	0.210 (0.138)	0.284 (0.133)	0.345
VC-Hip	LFGP-LFGP	0.342 (0.105)	0.377 (0.105)	0.174
Cg1-Hip	HFGP-HFGP	0.114 (0.041)	0.154 (0.051)	0.235
Cg1-VC	HFGP-HFGP	0.183 (0.088)	0.211 (0.096)	0.753
PrL-Cg1	HFGP-HFGP	0.592 (0.060)	0.601 (0.076)	0.722
PrL-Hip	HFGP-HFGP	0.119 (0.033)	0.160 (0.045)	0.304
PrL-VC	HFGP-HFGP	0.184 (0.077)	0.209 (0.085)	0.685
VC-Hip	HFGP-HFGP	0.290 (0.065)	0.380 (0.085)	0.058

Table 3.5| Systemic ketamine treatment increased inter-area LFGP and HFGP correlation for all areas. Mean changes in max correlation value for LFGP and HFGP in areas during baseline and systemic ketamine recordings [n=6]. Ketamine refers to 300 s of data encompassing highest peak in gamma power effect determined previously, baseline refers to 300 s baseline recording before injection. No significant changes were found at α=0.05, sem refers to the standard error mean for these data.

Finally, any changes to the modulation of gamma by slow wave oscillations was studied using cross correlation analysis. Systemic ketamine caused significant decreases to the max correlation value between slow wave activity in both prefrontal areas (PrL and Cg1) and LFGP in Hip. Similar to the effect on slow-LFGP following PCP injection in freely moving rats, no real

changes to correlation value for slow-HFGP occurred (**table 3.6**). Correlations between slow activity in all areas and LFGP in Hip showed a clear decrease, this indicates that modulation of LFGP in Hip by lower frequency oscillations is the most affected relationship following systemic ketamine treatment. The modulation of Hip HFGP by slow oscillations was less affected by ketamine, and the modulation of HFGP in other areas was even less affected.

Correlations		**Max correlation**		**Significance**
Areas compared	Frequencies	Baseline (sem)	Ketamine (sem)	*p* value
Cg1-Hip	Slow-LFGP	0.124 (0.021)	0.057 (0.011)	0.049 *
Cg1-VC	Slow-LFGP	0.087 (0.032)	0.090 (0.032)	0.753
PrL-Cg1	Slow-LFGP	0.087 (0.029)	0.105 (0.029)	0.463
PrL-Hip	Slow-LFGP	0.119 (0.013)	0.050 (0.009)	0.018 *
PrL-VC	Slow-LFGP	0.088 (0.028)	0.080 (0.028)	0.345
VC-Hip	Slow-LFGP	0.146 (0.026)	0.110 (0.037)	0.276
Cg1-Hip	Slow-HFGP	0.051 (0.011)	0.055 (0.026)	0.917
Cg1-VC	Slow-HFGP	0.064 (0.179)	0.078 (0.021)	0.464
PrL-Cg1	Slow-HFGP	0.087 (0.206)	0.099 (0.021)	0.187
PrL-Hip	Slow-HFGP	0.059 (0.013)	0.087 (0.305)	0.323
PrL-VC	Slow-HFGP	0.072 (0.016)	0.064 (0.016)	0.684
VC-Hip	Slow-HFGP	0.051 (0.013)	0.048 (0.016)	0.799

Table 3.6| Systemic ketamine treatment caused significant decreases to slow-LFGP correlations between PrL/Cg1 and Hip but caused none for slow-HFGP correlations. Mean changes in max correlation value for LFGP and HFGP in areas during baseline and systemic ketamine recordings [n=6]. Ketamine refers to 300 s of data encompassing highest peak in gamma power effect determined previously, baseline refers to 300 s baseline recording before injection. ***** denotes significant changes at α=0.05, sem refers to the standard error mean for these data, no significant results remained so at corrected α=0.008.

3iii | Local ketamine experiments in anaesthetised rats

Local injection of ketamine caused no significant changes to gamma power

1 µl of 5 µg/µl ketamine was injected over 3 minutes into VC, Hip and PFC and changes in oscillatory activity measured in VC, Hip, PrL and Cg1 - as previous. Data for all local injections

was normalised to an average value for 180 s before injection start point - deemed a stable baseline. Peak change in gamma power after injection was taken as 180 s of data encompassing the value furthest away from 1 on this normalised baseline. An average effect was taken for all individual rat data by calculating the arithmetic mean for all individual rat values (**table 3.7**).

Injected	Recorded	% change	*p* value
Hip	Cg1	+23	0.478
	PrL	+16	0.500
	VC	+8.1	0.103
	Hip	+60	0.225
PFC	Cg1	+1.3	0.741
	PrL	+4.5	0.418
	VC	+5.7	0.917
	Hip	-7.9	0.069
VC	Cg1	+1.1	0.800
	PrL	-4.8	0.418
	VC	+27	0.136
	Hip	+6.3	0.571

Table 3.7| Local injection of ketamine caused no significant changes to gamma power. Table shows percentage change in gamma power (30-120 Hz) following local ketamine injection in terminal experiments [PFC injection: n=6; VC/Hip injection: n=5]. Injected refers to the site of ketamine injection, recorded refers to the area in which gamma power was taken from following this injection. No significant changes were found.

As is shown in **table 3.7**, no significant changes to gamma power occurred following local injection into Hip, PFC and VC. The majority of changes calculated showed no real change in power, however some values appear to show a reasonably large effect on gamma power despite not being deemed significant. In all of these cases, it is the contribution of mainly one data set on the mean change that results in a greater value for percentage change. Although

these data were not deemed outliers, they have altered the peak gamma deviation value that is used to calculate percentage change. For example, the greatest effect induced by local ketamine treatment occurred in Hip following Hip injection; average gamma power increased to a clear peak before returning to baseline. This pattern of change and the level of increase was not dissimilar to that observed after 4 mg/kg systemic ketamine injection. However, the difference between peak effect and baseline was not deemed statistically significant as in the majority of rats a clear peak of change did not occur. Typically following local ketamine injection, power fluctuated around baseline values and no clear change was observed (**figure 3.10**). Therefore, although percentage change calculated using peak gamma values may appear to show substantial increases post injection, the average effect shows no stable overall increase in power. These results indicate that local changes in gamma power elicited by systemic ketamine treatment are not due to ketamine acting on local networks directly.

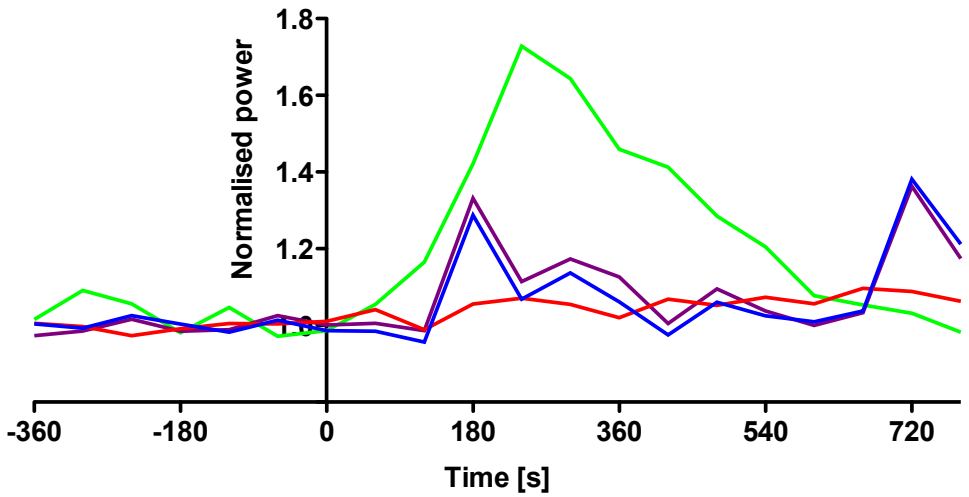

Figure 3.10| Local injection of ketamine into Hip caused percentage increases in recording areas when using peak gamma values but none were deemed significant. Average effect on gamma power in all recording areas following injection of 1 µl of 5 µg/µl locally into Hip [n=5]. Injection start point = 0 s on x-axis, data is normalised to average value for 180 s of baseline before injection start. Line colours: Hip – green, VC – red, PrL – blue, Cg1 – purple.

Local injection of ketamine did not alter inter-area correlation

Cross-correlation data presented for local ketamine injection focusses on the frequencies that were found to be significantly altered by systemic ketamine treatment, namely HFG-HFG (**table 3.8**) and slow-LFGP (**table 3.9**). Peak effect was taken as the max correlation value for 180 s that encompassed the greatest deviation in gamma power from baseline values; 180 s of stable data before injection start was taken for baseline.

Correlations		**Max correlation**		**Significance**
Local site	Areas compared	Baseline (sem)	Ketamine (sem)	*p* value
PFC	Cg1-Hip	0.059 (0.027)	0.063 (0.024)	0.643
	Cg1-VC	0.017 (0.005)	0.017 (0.003)	0.945
	PrL-Cg1	0.698 (0.080)	0.713 (0.074)	0.267
	PrL-Hip	0.057 (0.023)	0.056 (0.022)	0.891
	PrL-VC	0.014 (0.004)	0.017 (0.002)	0.418
	VC-Hip	0.111 (0.058)	0.100 (0.040)	0.917
Hip	Cg1-Hip	0.090 (0.042)	0.078 (0.027)	0.483
	Cg1-VC	0.024 (0.006)	0.025 (0.006)	0.837
	PrL-Cg1	0.688 (0.056)	0.729 (0.071)	0.438
	PrL-Hip	0.079 (0.036)	0.079 (0.020)	0.998
	PrL-VC	0.023 (0.006)	0.025 (0.001)	0.679
	VC-Hip	0.048 (0.010)	0.048 (0.007)	0.893
VC	Cg1-Hip	0.067 (0.022)	0.059 (0.020)	0.406
	Cg1-VC	0.015 (0.003)	0.030 (0.013)	0.365
	PrL-Cg1	0.759 (0.047)	0.745 (0.054)	0.489
	PrL-Hip	0.062 (0.020)	0.060 (0.021)	0.689
	PrL-VC	0.013 (0.003)	0.020 (0.005)	0.269
	VC-Hip	0.030 (0.001)	0.032 (0.006)	0.760

Table 3.8| Local injection of ketamine into PFC, Hip and VC did not significantly alter inter-area correlation. Table contains mean changes to max correlation value for inter-area HFG during baseline and following local injection of ketamine into PFC [n=6], VC and Hip [n=5]. Ketamine refers to max correlation value encompassing the greatest deviation in gamma power from baseline, baseline refers to max correlation value during baseline recording before injection, sem refers to the standard error mean.

Correlations		Max correlation		Significance
Local site	Areas compared	Baseline (sem)	Ketamine (sem)	*p* value
PFC	Cg1-Hip	0.165 (0.038)	0.150 (0.032)	0.459
	Cg1-VC	0.062 (0.020)	0.058 (0.013)	0.787
	PrL-Cg1	0.075 (0.013)	0.071 (0.009)	0.750
	PrL-Hip	0.152 (0.040)	0.014 (0.033)	0.548
	PrL-VC	0.069 (0.021)	0.069 (0.015)	0.753
	VC-Hip	0.113 (0.044)	0.096 (0.039)	0.116
Hip	Cg1-Hip	0.157 (0.029)	0.100 (0.027)	0.259
	Cg1-VC	0.029 (0.009)	0.036 (0.005)	0.539
	PrL-Cg1	0.087 (0.024)	0.070 (0.027)	0.263
	PrL-Hip	0.153 (0.027)	0.116 (0.024)	0.162
	PrL-VC	0.028 (0.010)	0.030 (0.004)	0.873
	VC-Hip	0.070 (0.011)	0.065 (0.012)	0.551
VC	Cg1-Hip	0.108 (0.021)	0.096 (0.021)	0.568
	Cg1-VC	0.035 (0.001)	0.045 (0.014)	0.606
	PrL-Cg1	0.068 (0.012)	0.073 (0.009)	0.608
	PrL-Hip	0.088 (0.014)	0.098 (0.020)	0.405
	PrL-VC	0.030 (0.013)	0.081 (0.037)	0.164
	VC-Hip	0.074 (0.015)	0.071 (0.021)	0.701

Table 3.9| Local injection of ketamine into PFC, Hip and VC caused no clear pattern of change to slow-LFGP inter-area correlations. Table contains mean changes to max correlation value for inter-area slow-LFGP during baseline recording and following local injection of ketamine into PFC [n=6], VC and Hip [n=5]. Ketamine refers to max correlation value encompassing the greatest deviation in gamma power from baseline, baseline refers to max correlation value during baseline recording before injection, sem refers to the standard error mean.

Similarly to changes in gamma power following injection of 5 µg/µl ketamine into the PFC, Hip and VC; no significant changes to inter-area correlation occurred for HFG-HFG or slow-LFGP. Not only were no changes found significant, but there was no clear pattern of change to correlations elicited by local ketamine injection.

Results for local ketamine injection are clearly in contrast to both systemic ketamine treatment under terminal anaesthesia and systemic PCP treatment in freely moving awake rats – both of which showed changes in gamma power and correlations. Taken collectively, it

can be concluded that local ketamine treatment was unable to recreate the effects on inter-area synchronisation recorded following systemic treatment.

TTX and dye injection were used to validate local cannula placement

At the end of a terminal experimental protocol, blue dye was injected into each cannula position to visualise where ketamine would have been injected locally. Injected dye remained local to the area of interest, which confirms that there is minimal (if any) leakage of locally injected substances (**figure 3.11**). This confirms that any changes to gamma power or correlation would affect only the area of injection.

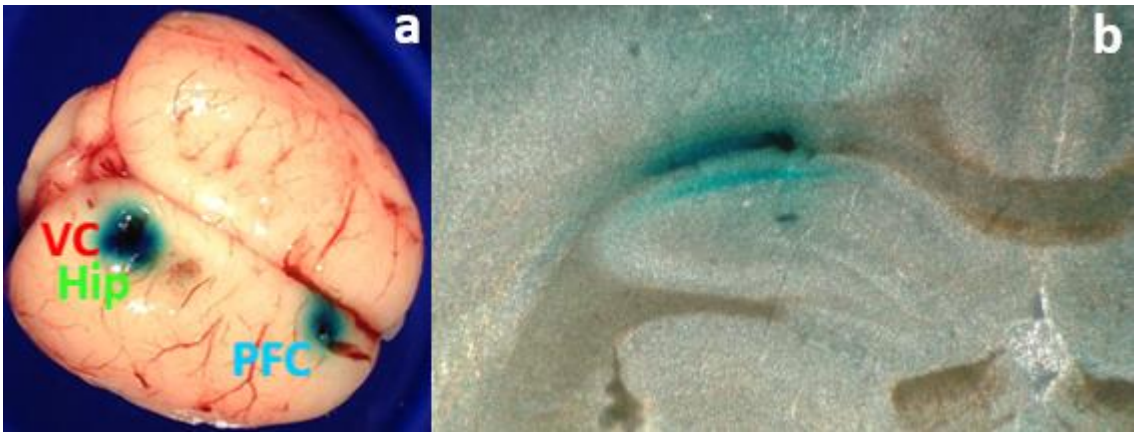

Figure 3.11| Local injection of dye remained in the desired injection area. a – Image of rat brain injected with dye in positions where cannula were placed for local ketamine injection: PFC – prefrontal cortex, VC – visual cortex, Hip – hippocampus indicate injection sites. **b –** Image of hippocampal slice taken from brain in **a** showing injection of dye remained within the CA1 layer of the hippocampus following injection. Both images indicate that ketamine when injected locally remained within and only affected the area of interest

Injection of TTX was also used to validate local injection sites and the local injection protocol. TTX is a potent neurotoxin which acts by blocking sodium channels on neurons, thus

blocking action potential firing. Therefore, sufficient delivery to a neural area of interest should decrease oscillatory activity to a level not much above 0 µV. In five rats, 1 µl of 10 ng/ml TTX was injected into each local site in sequence, using the same protocol as was used for local ketamine injections, whilst recording neural oscillations. In all cases, TTX caused gamma to decrease to a level well below baseline recordings (**figure 3.12**). Furthermore, oscillatory activity in other frequency bands never recovered to baseline values in the injected area but remained unaffected in other recording areas. This demonstrated that the method of delivery used for local ketamine injection was successful, that the drug was delivered only locally and that results for local ketamine injection could be treated as reliable.

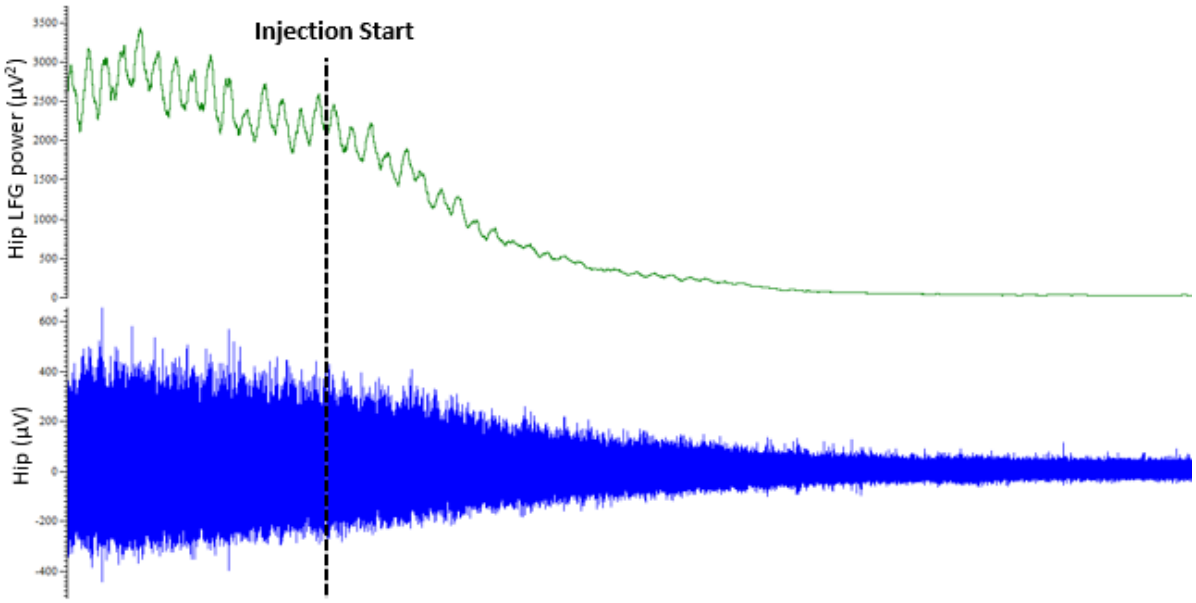

Figure 3.12| Local injection of TTX abolished local oscillatory activity in Hip following Hip injection. 1 µl of 10 ng/ml TTX was locally injected into Hip at a rate of 0.333 µl/min. Figure shows example effects**:** Top trace shows LFGP in Hip, bottom trace shows all local oscillatory activity in Hip. Entire length of trace is 5200 s, injection start point is indicated; before this start point can be considered as baseline. After around 2000 s LFGP is reduced to approximately 26 μV^2.

3iv | Comparison of systemic and local injection of ketamine

Local injection of ketamine had a different signature of effect than systemic ketamine

Average power spectra following ketamine treatment were generated and then normalised to baseline power to show changes in the distribution of all oscillation frequencies. These spectra revealed a different signature of change in local application vs systemic (**figure 3.13**). After local injection of ketamine, there was a broad peak increase covering the start of low frequency gamma that is not apparent following systemic ketamine treatment. The spectrum for systemic ketamine shows an increase in power across a wider range of frequencies than for local ketamine, encompassing the entire gamma range. However, higher frequency power (>120 Hz) is reduced after systemic ketamine injection whereas it is unchanged following local injection. A large peak in low frequency power is also apparent in the spectrum for systemic ketamine yet not in the spectra for local application, indicating that lower frequency oscillations such as theta may be altered by systemic ketamine treatment but not by local ketamine injection.

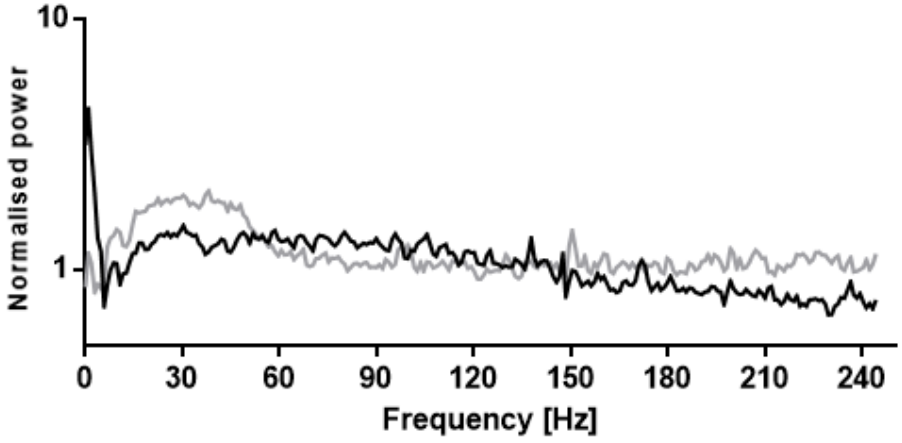

Figure 3.13| Systemic injection of ketamine produced a different effect signature to local injection. Average normalised power spectra [n=5] generated from Hip recordings after local (grey line) and systemic (black line) ketamine injection. The area of injection for the local spectrum shown was also Hip.

The fact that the two different methods of ketamine application showed two different spectral effect signatures indicates that the way in which gamma power is changed is also different. Systemic ketamine caused a shift from higher frequency oscillations to increase power across the entire gamma range, whereas local ketamine caused a shift from lower frequency oscillations to increase power overlapping the lower end of LFG. After local ketamine injection, the gamma range was not much affected above 50 Hz and higher frequency oscillations were unaffected.

Local application of ketamine to deep brain structures increased gamma power

Due to local application of ketamine in VC, Hip and PFC being unable to elicit an increase in gamma power comparable to that obtained with systemic ketamine treatment, deeper brain structures were proposed as an alternative site of effect. The protocol for delivery of ketamine and analysis of data was the same as that for local applications described previously. Results presented here are for two rats only and act as a preliminary grounding for the theory that it is the action of ketamine on deeper brain structures that elicits the majority of changes in gamma power and not action on local circuits in the area of elicited change.

1 µl of 5 µg/µl ketamine was injected over 3 minutes into deep brain structures known to have neuromodulatory effect in the brain. The most effective site found for increase in gamma power after local ketamine injection was the nucleus basalis (NB). As this data is from an experiment using only two rats, no statistical analysis is available to determine significance and full data analysis has not yet been completed. However, the signature effect following ketamine NB injection (**figure 3.14**) resembles more that seen for systemic treatment as

opposed to local area injection (see **figure 3.13**), with an increase in power occurring across the whole of the gamma range. These initial results for NB injection are promising and increasing n number for these deeper brain structure injections may elude to the local site of action of ketamine in the brain.

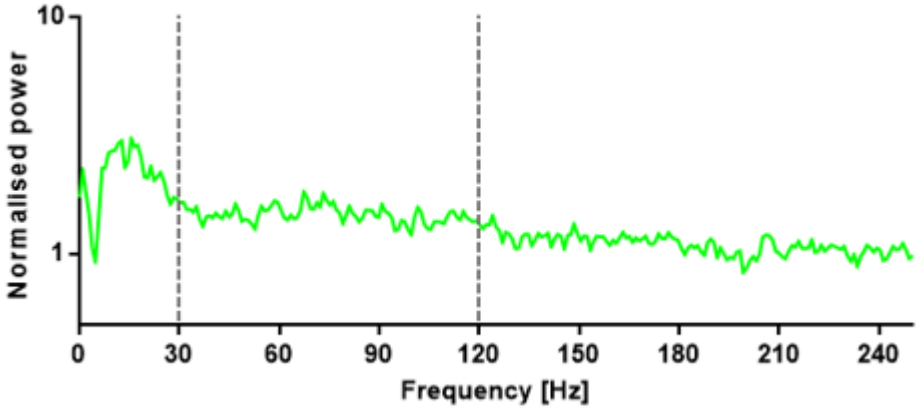

Figure 3.14| Local injection of ketamine into nucleus basalis caused an increase in power across the entire gamma range. Signature average power spectrum generated from Hip recordings following injection of ketamine into NB [n=2]. Dashed lines depict the gamma range (30-120 Hz) in which an increase in average power occurred. Data for power was normalised to baseline values before averaging.

- DISCUSSION -

4i | Acute PCP model of schizophrenia in freely moving rats

PCP caused a decrease in percentage correct performance in the sPAL task used, indicating that the acute PCP model reproduces deficits in associative learning known to effect schizophrenia sufferers (Serra et al, 2001). It could be argued that PCP, as a dissociative agent, was impairing the rats' ability to actually complete the task, rather than actually effecting associative learning – which would result in decreased performance. Indeed, behavioural changes such as ataxia did occur in the first 60 minutes following PCP injection. However, after this time point behavioural effects of PCP had alleviated and even during the time when ataxia was present, rats still carried out the task – which was shown by there being no significant change in trial number when compared to controls. In a recent study which profiled current NMDAR antagonist models of schizophrenia (Talpos et al, 2014), it was also found that systemic PCP significantly altered percentage correct in a PAL task without altering trial number. Both of these findings show that rats are not physically impaired in task completion but that it is an induced cognitive impairment in associative learning that causes decreased task performance. As well as decreased percentage correct, rats also showed a decreased ability to respond appropriately to correction trials, often continuing to choose the incorrect image that had been chosen previously. This deficit in feedback learning has been shown to consistently occur in schizophrenia patients when compared to matched controls (Yilmaz et al, 2012). Being unable to correct a wrong decision during task completion is indicative of a deficit in working memory, as stated earlier this has been found many times in humans at all stages of schizophrenia development. Taken collectively, these results suggest that the PCP

rat model of schizophrenia used in this study is a suitable experimental recreation of some of the cognitive deficits observed in schizophrenics.

Systemic injection of 1 mg/kg PCP in freely moving awake rats caused a significant increase in gamma power. As mentioned in the results section, changes recorded in gamma power outlasted any behavioural changes induced by PCP - further validating that changes occurring at a neuronal level were the key reason for decreased task performance. Also in support of this, it has been shown that treatment with ketamine and MK-801 – which block NMDARs via non-competitive binding at the same site as PCP, cause an increase in gamma activity that is unrelated to behavioural changes such as ataxia (Hakami et al, 2009). Gamma power is consistently implicated in cognition and is particularly prevalent during completion of mental tasks (Fitzgibbon et al, 2004). As gamma is associated with shifting the cortex to an attentive awake state, it would be logical to think that increased gamma power should increase attention and induce a higher cognitive state. However, in this study and in many previous studies, it has been found that increased gamma activity correlates with poor performance in cognitive tasks (Basar-Eroglu et al, 2007). This makes it more likely that it is the inter-area synchronisation of gamma that is relevant in cognition. Indeed, inter-area synchronisation within the gamma band is known to be abnormal in schizophrenia patients (Uhlhaas & Singer, 2010). In this study, cross-correlation analysis was used to determine changes in synchronisation between recording areas. Following systemic PCP treatment, the most significant decrease in maximum correlation occurred between the hippocampus and the prefrontal cortex. The hippocampus is known to be responsible for associating an object and its location (Rolls, 1996) whereas the prefrontal cortex is known to be involved in working memory and coordination of goal orientated behaviours (Basar-Eroglu et al, 2007; Miller &

Cohen, 2001). Therefore, it is feasible that reduced synchronisation between these two areas will result in disrupted communication between two vital areas for sPAL completion. It is likely that in schizophrenia patients, inefficient communication between neural areas is a significant contributor to poor performance in working memory tasks compared to healthy control individuals.

4ii | Systemic ketamine model of schizophrenia in terminally anaesthetised rats

Terminally anaesthetised rats were used in these experiments to study the effects of local ketamine application *in vivo* and also allowed systemic NMDAR antagonism to be conducted without movement artefacts. The stimulation used during terminal experiments was successful in shifting the cortex from an unstable 'sleep-like' state containing mainly slow wave activity to a state that was more relatable to that recorded in awake animals, containing regular gamma activity and reduced slow wave. It is extremely important to have a stable baseline for experimentation and also to make experimental procedures as relevant as possible; by creating this recording situation, initial measurements for gamma activity following ketamine injection could be deemed reliable. However, following systemic ketamine injection in some rats, small amounts of slow wave activity appeared to return in all recording channels. This could be indicative of the effect of the stimulus wearing off somewhat – i.e. the stimulus no longer being sufficient in activating the cortex. However, this occurred at least 30 minutes after injection and following ketamine washout the stimulus worked as well as previously. Therefore, this could be a secondary effect of ketamine itself. More work may need to be done on analysis of changes in the lower frequency oscillatory bands following

systemic ketamine injection and on further validation of the stimulus strength/position used for activating the cortex. Despite this, electrical stimulation of the cortex via deep brain structures appears to be a promising method of creating a stable baseline for terminal *in vivo* experiments in rats following further development.

As expected, 10 mg/kg ketamine caused a larger increase in gamma power in all recording areas than 4 mg/kg when given systemically. However, PrL and Cg1 were the greatest affected channels under both doses. This implies that ketamine, when given systemically, has the greatest effect in the prefrontal cortex irrespective of dose. As there are questions over whether the ketamine model of schizophrenia is recreating cognitive deficits or whether it is simply causing hallucinogenic effects (i.e. recreating positive symptoms), it is interesting to note that the visual cortex was the least affected area in terms of gamma power even under a high dose of ketamine. Comparing the normalised power increase between systemic PCP and ketamine injections, it appears that 1 mg/kg PCP in awake rats causes a change comparable to somewhere between the two doses of ketamine used in anaesthetised rats. Also, the order of channel increase under PCP influence is slightly different to that of ketamine. Cg1 showed the largest increase in gamma power following PCP injection, whereas PrL showed the smallest. The increase in gamma power in the visual cortex following 1 mg/kg PCP injection was of a similar magnitude to that following 10 mg/kg ketamine injection, this implies that systemic PCP has a greater effect on the local circuitry of the visual cortex than ketamine does. This may translate to a better manifestation of positive symptoms when using the PCP model of schizophrenia compared to the ketamine model. However, it should be noted that the rats used for ketamine experiments would have had very little sensory input to the visual system compared to the rats completing sPAL under PCP.

Unfortunately data for HFG range recorded in the freely moving PCP experiments could not be obtained as there were many artefacts contained in the range that, when removed, left very little reliable trace for analysis. This is most likely due to noise created by the recording environment of the testing chamber. As the chamber itself is undergoing development by the manufacturer, it would be desirable for this to be eliminated for future experiments and reliable HFG data obtained to complement the HFG data from terminal experiments in this study. Following systemic ketamine treatment, inter-area HFG correlation was decreased except for between the visual cortex and hippocampus. This indicates that communication between areas furthest apart in the brain is most disrupted by systemic ketamine treatment, and that communication between areas that overlap spatially may actually be enhanced. An increase in synchronisation between the visual cortex and hippocampus may also be the reason for dissociative effects induced by ketamine in humans, as spatial awareness of a perceived visual perception (hallucinations) could be being facilitated by this enhanced inter-area communication. The effects of ketamine on HFG (80-120 Hz) are relatively understudied in the literature. Systemic ketamine treatment induced significant decreases in inter-area HFG correlation whereas no significant changes were found for inter-area correlations in LFG. Also, significant changes to slow-LFGP inter-area correlations were induced by systemic ketamine but inter-area slow-HFGP synchrony was relatively unchanged. These findings highlight the need for studies to incorporate an extended gamma range, encompassing 30-120 Hz. Support for the inclusion of HFG in experimental analysis has also been concluded previously elsewhere in the literature (Grutzner et al, 2013).

It may be desirable to expand on the systemic ketamine data obtained in this study by increasing the n number of the 10 mg/kg cohort to the same as that used for 4 mg/kg. This

would allow reliable cross-correlation analysis for 10 mg/kg ketamine and comparison between the two doses. As 10 mg/kg caused greater increases in gamma power, it would be expected that this dose would have also caused more significant changes to cross-correlations. Work from previous studies suggests that the minimum systemic dose of ketamine required to produce cognitive deficits comparable to schizophrenia is 5 mg/kg (Kocsis et al, 2013), reinforcing the idea that greater changes in inter-area cross-correlation would occur if a higher dose were used.

4iii| Local application of ketamine in terminally anaesthetised rats

As far as the author is aware, this is the first time local application of ketamine *in vivo* has been conducted whilst recording local EEG activity in the same area. The protocol used for local injection into the visual cortex, prefrontal cortex and hippocampus was deemed successful due to vehicle injections causing no change in local oscillations but injection of TTX abolishing almost all recorded activity in the injected area without affecting other recording areas.

No significant changes to gamma power were found following local injection of 5 µg/µl ketamine. As mentioned in results, data from one rat for local hippocampal injection altered the average peak gamma value which resulted in apparent substantial percentage increases in gamma power. As stated these data were not deemed statistical outliers however removing them from the data set dramatically alters the average percentage increase. When the data for this rat is removed from the calculation for average percentage change in gamma power following local hip injection the new values are as follows; VC: +5%, Cg1: -5%, PrL: -6%, Hip:

+8%. These new values reinforce the conclusion that no substantial change to gamma power was elicited following local ketamine treatment. Increasing the n number for local ketamine injection would confirm whether the response elicited in this one rat is indeed not typical and would limit the effect of this data on the final average values.

Taking these results collectively, it can be concluded that increased local gamma power in the recorded areas induced by systemic ketamine treatment was not generated by local ketamine binding in those areas. This is contrary to much of the literature, where many groups have assumed that ketamine has a direct effect on local neural circuitry. However, it should be noted that the majority of support for this concept comes from *in vitro* studies in which application of ketamine has been used on isolated local circuits. But there is still some dispute even amongst *in vitro* studies as to whether ketamine acts directly on local circuitry to modulate change. For example, ketamine has been found to have direct local effects on hippocampal preparations (Greene, 2001), but it has also been found not to have any affect at all in this area in other experiments (Roopun et al, 2008). Roopun et al also found that ketamine altered oscillatory activity in *in vitro* preparations from the auditory cortex despite being ineffective in hippocampal slices. This may mean that ketamine has varying local effects dependent on the area of application. Although it is currently difficult to identify the direct cause of increased gamma activity using *in vivo* experiments, this study highlights it is not conclusive that ketamine primarily acts locally in the area of interest to increase gamma oscillations.

It has been previously commented that there is much inconsistency between *in vivo* and *in vitro* experiments with regards to evoked gamma activity following ketamine treatment

and that conclusions from *in vitro* studies should not be made in isolation (Kocsis et al, 2013). Whilst useful experiments, *in vitro* electrophysiology studies may be over-simplifying what actually occurs in the brain following ketamine treatment. This could be due to the loss of afferent connections that occurs during slice preparation for *in vitro* studies. In line with this, *in vivo* studies should be deemed more valid when considering the implications of results with respect to schizophrenia. One *in vivo* study did find that local application of an NMDAR antagonist to the prefrontal cortex in freely moving rats was sufficient to induce stereotypic behavioural changes, local changes in oscillatory activity and increase c-fos expression in cortical areas – which is a biomarker for neuronal activity (Nowak et al, 2012). However, the authors used MK-801 - a more potent NMDAR antagonist than ketamine. Also, the dose of MK-801 that was deemed sufficient to achieve this was 4 μg/μl; given the higher binding affinity of MK-801 to the NMDAR, perhaps this indicates that a local dose of 5 μg/μl ketamine is not sufficient to induce oscillatory changes. Further experiments using local application of a higher ketamine dose could be undertaken to validate this. Alternatively, injection of MK-801 into the visual cortex and hippocampus, as well as the prefrontal cortex, could be undertaken in future experiments to observe the local effects on gamma activity of a more selective NMDAR antagonist. This would further elucidate whether selective local NMDAR antagonism affects local generation of gamma oscillations.

As well as changes to recorded power within the gamma band, it was expected that - should ketamine be acting primarily at the local circuit level, the effect signature would be similar to that of systemic ketamine. This was not the case following local injection of ketamine in the hippocampus – the area where the clearest change in gamma power was induced (albeit not significant). Systemic ketamine caused a broad increase across the entire gamma range

accompanied by a decrease in higher frequency oscillations, whereas local ketamine caused a decrease in lower frequency oscillations and a peak increase encompassing the lower end of LFG without altering higher frequency oscillations. This again indicates that ketamine does not act locally in the same manner in which it does systemically and it is therefore unlikely that ketamine is acting locally in the recorded areas to produce significant changes observed following systemic ketamine injection.

The nucleus basalis consists of cholinergic neurons that project to the cortex and ablation studies have previously implicated this are in the control of cognition (Wenk, 1997). Therefore it is feasible that projections from the nucleus may be able to alter oscillatory activity in local neural networks. Preliminary results from injection into the nucleus basalis showed a modest broad increase in power across the entire gamma range – not dissimilar to that induced by systemic ketamine. In light of this, it is much more likely that ketamine is acting locally on neuromodulatory systems (such as the nucleus basalis) to elicit the increases in gamma power observed following systemic injection. It could be that simultaneous binding of ketamine to several deep brain structures has a synergistic effect on increasing gamma power. Of course, at this point in time these comments are rather speculative and future studies will need to be conducted on local applications to deep brain structures to firstly, increase the n number for nucleus basalis data and secondly, to examine other injection areas. It is clear from the results of this study that much more work is required to characterise local activity of ketamine in the brain using *in vivo* animal models.

4iv| Concluding remarks

It has been shown that systemic treatment with both PCP and ketamine is sufficient in eliciting a significant increase in gamma power in the visual cortex, prefrontal cortex and hippocampus whilst decreasing synchronisation between these areas – further validating the use of NMDAR antagonists in modelling schizophrenia in rats. However, this study provides evidence to suggest that these drugs are not acting on the local neural networks in these recorded areas in order to effect gamma. Local application to neuromodulatory nuclei appears to be a promising target for elucidating the effects of local NMDAR antagonism on gamma activity and this is the recommended focus for future experiments. If key local sites were identified that significantly impacted on gamma activity, it would be desirable to utilise these for ketamine/PCP injection during behavioural experiments such as those used in systemic PCP application in this study. Also, it would be useful for more work to be done on validating the local dose of ketamine needed to induce a significant oscillatory change, or the relative dose of ketamine that occurs in neural regions following systemic injection - as knowledge of this is not abundant in the literature.

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