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NMDA Receptor Dysfunction and Development of Translational Biomarkers for Autism and Schizophrenia

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Abstract

Autism and schizophrenia are neurodevelopmental disorders which both have highly disabling negative and cognitive symptoms with few effective treatments. A challenge to developing effective therapeutics is a dearth of pre-clinical models. Part of the difficulty in developing predictive models is that the symptoms being treated are complex, and difficult to reduce to a simple behavioral task. Therefore, the use of endophenotypes from methods such as EEG presents a new promising avenue for a model of complex human behaviors pre-clinically. New evidence suggests that autism and schizophrenia have reliable electrophysiological endophenotypes, some of which have been correlated to negative and cognitive symptoms. These endophenotypes therefore represent a possible new pathway for understanding the disrupted circuits in both diseases and developing treatments.

Evidence has been accumulating for glutamate disruption in both schizophrenia and autism; accordingly, pre-clinical models are being developed around NMDA receptor (NMDAR) disruption to examine both diseases. NMDA disruption models have been used for many behavioral tasks, but only a few possible electrophysiological endophenotypes such as ERP amplitudes have been investigated. Investigating pre-clinical models of established clinical endophenotypes could lead to better translational biomarkers of disease symptoms.

This thesis's unifying theme is the study of how glutamate disruption can recreate the electrophysiological endophenotypes present in autism and schizophrenia and develop their use as translational biomarkers in both diseases. The primary models of focus are acute NMDA antagonist administration and NMDAR knockdown of PV interneurons. I used these models to examine the relationship between dose and EEG changes, along with the perturbations present with NMDAR disruption in PV interneurons. I investigated the degree to which NMDAR antagonists recreate signal-to-noise ratio (SNR) and timing perturbations in schizophrenia, and found a dose-dependent decrease in SNR and timing consistency. I assessed the extent to which low dose NMDAR antagonism recreates latency and gamma synchrony perturbations present in autism and found latency was increased and gamma synchrony was decreased dose-dependently. I examined the extent to which Parvalbumin (PV) containing interneurons cell type selective NR1 KO mice recreate the clinical EEG profiles of autism and found selective deficits in social behavior and increases in N1 latency.

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NMDA RECEPTOR DYSFUNCTION AND DEVELOPMENT OF TRANSLATIONAL BIOMARKERS FOR AUTISM AND SCHIZOPHRENIA

To my loving family and future wife

Abstract

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John A. Saunders IV

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Autism and schizophrenia are neurodevelopmental disorders which both have highly disabling negative and cognitive symptoms with few effective treatments. A challenge to developing effective therapeutics is a dearth of pre-clinical models. Part of the difficulty in developing predictive models is that the symptoms being treated are complex, and difficult to reduce to a simple behavioral task. Therefore, the use of endophenotypes from methods such as EEG presents a new promising avenue for a model of complex human behaviors pre-clinically. New evidence suggests that autism and schizophrenia have reliable electrophysiological endophenotypes, some of which have been correlated to negative and cognitive symptoms (Light, Hsu et al. 2006; Dias, Butler et al. 2011). These endophenotypes therefore represent a possible new pathway for understanding the disrupted circuits in both diseases and developing treatments.

One set of pre-clinical models for schizophrenia that have been studied extensively revolve around glutamate disruption. This is achieved by a variety of measures, including administration of NMDA antagonists, lesion, and genetic knockout (KO) of NMDA receptors (Dias, Butler et al. 2011; Fitzgerald 2012). More recently, evidence has been discovered that glutamate disruption may underlie some of the disruptions present in autism. Glutamate disruption models have been used for many

behavioral tasks, but they are often unable to elucidate the underlying circuit disruption. Furthermore, only a few possible endophenotypes such as ERP amplitudes have been investigated. Investigation into pre-clinical models of established clinical endophenotypes could lead to better translational biomarkers of disease symptoms, and speed therapeutic development.

The unifying theme of the work that comprises this thesis is the study of how glutamate disruption can be used to recreate the electrophysiological endophenotypes present in autism and schizophrenia and develop their use as translational biomarkers in both diseases. The primary models of focus are acute NMDA antagonist administration and NMDA receptor knockdown of PV interneurons. Using these models, I examined the relationship between the dose and EEG changes, along with the perturbations present with NMDA disruption in PV interneurons. I investigated the degree to which NMDAR antagonists recreate signal-to-noise ratio (SNR) and timing perturbations in schizophrenia, and found a dose-dependent decrease in SNR and timing consistency. I assessed the extent to which low dose NMDAR antagonism recreates latency and gamma synchrony perturbations present in autism and found latency was increased and gamma synchrony was decreased in a dose-dependently. I examined the extent to which parvalbumin (PV) containing interneurons cell type selective NR1 KO mice recreate the clinical EEG profiles of autism and found selective deficits in social behavior and increases in N1 latency.

Table of Contents

Abstract……iv

Table of Contents……...……...vi

List of Tables……....ix

List of Illustrations…….....x

Chapter 1: Introduction……1

1.1 General Introduction……..1

1.2 Animals as models of Human disease……...2

1.2.1 Criteria for pre-clinical models……..……2

1.2.2 Assessing Model Specificity……...3

1.3 NMDAR disruption as a model of schizophrenia……..4

1.3.1 Clinical Evidence for Connection……...4

1.3.2 Animal models……5

1.3.2.1 NMDA Receptor Antagonist models……...5

1.3.2.2 Genetic Models……5

1.4 NMDAR disruption as a model of autism……6

1.4.1 Clinical Evidence for Connection……...6

1.4.2 Animal models……6

1.4.2.1 NMDA Receptor Antagonist models……...6

1.4.2.2 Genetic Models……6

1.5 Analysis methods……...7

1.5.1 ERP Methods……..7

1.5.2 Data Integrity Methods……...9

1.5.3 Wavelet Method advantages……11

1.5.4 Wavelet Method description……12
1.6 Current validation of EEG measures against symptoms……..19
1.6.1 ERP components……...19
1.6.2 Gamma activity……19

Chapter 2: NMDA Antagonists Recreate Signal-to-Noise Ratio and Timing Perturbations Present in Schizophrenia……22

2.1 Abstract……22
2.2 Introduction……..24
2.3 Methods……29
2.3.1 Subjects……29
2.3.2 Drugs and doses……29
2.3.3 EEG Recording……29
2.3.4 Frequencies analyzed and methods……...30
2.3.5 Factor Analysis……31
2.4 Results……..32
2.5 Discussion……35
2.5.1 Comparison of Pharmacological Models to Schizophrenia……..35
2.5.2 Potential Mechanisms for Specificity of Drug Effects……36
2.5.3 Limitations and Future Directions……37
2.6 Figure Legends……39

Chapter 3: NMDA antagonist MK-801 recreates auditory electrophysiological disruptions present in autism……..45

3.1 Abstract……45
3.2 Introduction……..46

3.3 Methods……49

3.3.1 Subjects……49

3.3.2 Drugs and doses……49

3.3.3 EEG Recording……49

3.3.4 Frequencies analyzed and methods……...50

3.4 Results……..52

3.5 Discussion……53

3.6 Figure Legends……56

Chapter 4: Knockout of NMDA Receptors in Parvalbumin Interneurons Recreates Autism like Phenotypes……...60

4.1 Abstract……60

4.2 Introduction……..61

4.3 Methods……64

4.3.1 Subjects……64

4.3.2 Behavioral Methods……..64

4.3.3 EEG Methods……65

4.3.4 USV Methods……...66

4.4 Results……..68

4.5 Discussion……69

4.6 Figure Legends……71

Chapter 5: Summary and Conclusions……..78

5.1 Summary……..78

5.2 Future Directions……81

5.3 Conclusions……..82

References……83

LIST OF TABLES

Chapter 2

Table 1: Factor Analysis of EEG measures in both MK801 and ketamine treated mice..34

LIST OF ILLUSTRATIONS

Chapter 1

Figure 1.5.1a- One epoch of a EEG stimulus response EEG trace illustration…….8

Figure 1.5.1b- Method for amplitude measurement……..8

Figure 1.5.1c- Method for latency measurement……...9

Figure 1.5.2a - Too Saturated (bad reference/electrode surgery)……10

Figure 1.5.2b- Too Regular (bad surgery)……...10

Figure 1.5.2c- Too thick (poor grounding) See line cycle noise on zoom……...10

Figure 1.5.2d- Good signals……10

Figure 1.5.4a- One trial's unit phase vector……12

Figure 1.5.4b-Example EEG epoch trace……13

Figure 1.5.4c- Example EEG slice from epoch, the width of the EEG used will vary depending on frequency……...14

Figure 1.5.4d- Wavelet transform equation applied to that slice……15

Figure 1.5.4e- Definition of wavelet transform terms……15

Figure 1.5.4f- Outcome of one calculation……..16

Figure 1.5.4g- Baseline saline plot completed from calculations……17

Figure 1.5.4h- Region averaged to obtain single gamma value……...18

Figure 1.5.4i- Summary of process to obtain results……...19

Chapter 2

Figure 2.6.1- Overview of Frequency Measures Evoked power…….39

Figure 2.6.2- High Frequency Total and Pre-stimulus Power…….40

Figure 2.6.3- High Frequency Evoked Power…….41

Figure 2.6.4- High and Low Frequency ITC……...42

Figure 2.6.5- N40 Amplitude Changes……43

Figure 2.6.6- SNR Evoked……...44

Chapter 3

Figure 3.6.1- N1 Latency……..……...56

Figure 3.6.2- P1 Latency…….57

Figure 3.6.3- Gamma ITC and Evoked Power……58

Figure 3.6.4- Overlapping Symptoms and EEG Profile……...……...59

Chapter 4

Figure 4.6.1- N1 Latency…….71

Figure 4.6.2- Sociability…….72

Figure 4.6.3- Raw Premating Vocalization Data…….73

Figure 4.6.4- USV Vocalization Power……...74

Figure 4.6.5- Mating USV Power……75

Figure 4.6.6- T-maze……76

Figure 4.6.7- N1 latency to Social Preference Correlation……..77

Chapter 1.1: General Introduction

Schizophrenia and autism are disabling illnesses which occur in 2-4% of the population. They both result in a wide variety of negative symptoms, including anhedonia, avolition, deficits in social communication, and comprehension of emotion. In addition, there are often cognitive deficits which include reduced working memory and executive control. There are few effective treatments for the negative and cognitive symptoms present in both diseases. In both diseases, there is increasing evidence that N-Methyl-D-aspartate (NMDA) receptor dysfunction is part of the driving mechanism for the untreated symptoms (Timofeeva and Levin 2011). In addition, robust electrophysiological biomarkers for both diseases have been discovered (Light, Hsu et al. 2006; Roberts, Khan et al. 2010).

In schizophrenia, there was initially interest in the dopamine system as dopamine antagonists were able to ameliorate the psychotic symptoms present in schizophrenia. Subsequent anti-psychotics were all based on a similar mechanism, though efficacy is unrelated to degree of dopamine antagonism. Also while, the drugs were effective in treating psychosis, they have little to no effect on the negative or cognitive symptoms present in schizophrenia that have the largest impact on patient quality of life. The inability to hold a job, be an active community member, and socially engaged often lead to depression, and contribute to a great extent of the disease burden on society. A new pathway for disease treatment has been explored, based on the observation that the NMDA antagonists phencyclidine and ketamine reproduced schizophrenia like psychosis, disorganization, and reduced cognitive function (Javitt 2010). Thus in an effort to attack

the as of yet untreatable symptoms, there has been extensive research into the glutamate system for ways to ameliorate the illness's effects.

Autism has recently come to the forefront with increased recognition of its existence by the general public and clinicians. While it does not possess the psychosis present in schizophrenia, it overlaps in the impaired social function with language deficits along with reduced ability to understand and interpret emotional content. Common comorbidities include cognitive deficits such as reduced working memory and executive function, along with anxiety that overlap with schizophrenia. In addition, there has been recent evidence that glutamate disruption on PV interneurons may be related to the deficits present in autism.

In both diseases treatment has been attempted using traditional anti-psychotics with little success. While anti-psychotics such as risperidone are approved for use in both diseases, it only serves to control behavior such as angry outbursts. For other comorbidities such as anxiety and ADHD (more prevalent in autism), there are some treatments. Intensive behavioral therapy appears to help with some of the functional issues, but has had limited success in treating patients and fully restoring function. Overall, there is little that can be currently done to treat the core negative and cognitive symptoms these patients face.

1.2 Animals as models of Human disease

1.2.1 Criteria for pre-clinical models

When investigating rodent models of autism and schizophrenia, it is important to establish the validity of the model before being extrapolating observed findings to the

clinical population (Crawley 2008; Chadman, Yang et al. 2009). “Construct validity” indicates that the preclinical model incorporates an analogous biological perturbation (e.g., genetic mutation, environmental insult) that is associated with the human disease. This stipulation is relatively straightforward, given the number of such insults that have been linked to autism spectrum disorders (ASDs) and schizophrenia (Betancur 2011; Greenwood, Lazzeroni et al. 2011). For genetic mutations, it is important that the orthologous genetic change is introduced into the mouse, instead of simply deleting the gene, because human disease-causing mutations can often be gain-of-function mutation (Tabuchi, Blundell et al. 2007). “Face validity” indicates that the preclinical model shows phenotypes (and endophenotypes) analogous to those seen in the human disorder. Importantly, this criterion should also incorporate specificity; that is, a valid animal model should not show neural abnormalities that are not observed in the clinical syndrome. Finally, “predictive validity” indicates that therapeutics which are effective in a preclinical setting translate successfully to the clinical population (and vice versa). Because antipsychotics like risperidone are the only FDA-approved drugs to treat some of autism’s symptoms and only psychosis in schizophrenia, but lack impact on negative symptoms in both diseases, this criterion is likely the least important. However, specificity is important here as well–a drug that has failed to improve negative symptoms in patients with autism or schizophrenia (e.g., anti-psychotics, SSRIs, or secretin) should not be effective in valid animal models (Williams, Wray et al. 2005; Williams, Wheeler et al. 2010).

1.2.2 Assessing Model Specificity

When assessing the behavioral effects of genetic or environmental perturbations relevant to autism or schizophrenia in rodents, it is important to assess the specificity of such deficits. Autism is not caused by comprehensive neurological impairment, and the negative symptoms of schizophrenia can occur independent of psychosis. Global brain dysfunction in a mouse could manifest the negative symptoms present in both diseases. Therefore, it is important to rule out nonspecific cognitive and motor deficits. Comprehensive phenotypic characterization helps rule out potential behavioral confounds that could contribute to false positive results. For example, reduced social activity could be caused by deficits in olfactory function or reduced locomotor activity. In addition, discrete T-maze tests can test whether working and spatial memory is intact. Validation to test that our induced deficits are specific to certain set of symptoms as opposed to generalized neural impairment is instrumental to creating models effective in guiding therapeutic development.

1.3 NMDAR disruption and Schizophrenia

1.3.1 Clinical Evidence for Connection

Initial evidence for glutamate disruption as the pathway for schizophrenia, was found from clinical reports that abusers of PCP or ketamine exhibited schizophrenia like symptoms. Research has also revealed that a compound's ability to recreate schizophrenia like symptoms was related to its degree of NMDA receptor binding independent of other receptor types (Javitt and Zukin 1991). In addition, conditions such as anti-NMDA receptor encephalitis induce similar symptoms. Furthermore, there is evidence that NMDA receptors are disrupted in patients with schizophrenia (Javitt 2010).

A report from the Consortium on the Genetics of Schizophrenia showed evidence that multiple proteins involved in the NMDAR pathway are highly associated with features of schizophrenia, and that the NMDAR pathway was most represented in the genetic studies (Greenwood, Lazzeroni et al. 2011).

1.3.2 Animal models

1.3.2.1 NMDA Receptor Antagonist models

Since the discovery by Javitt that abusers of NMDA antagonists ketamine and PCP exhibited schizophrenia like psychosis, and then subsequent clinical trials with the agent showed similar results NMDA antagonists have been hypothesized to be a schizophrenia model (Javitt 2010). In addition, acute administration in preclinical models recreates many schizophrenia symptoms including psychosis, working memory deficits, asociability, and reduced prepulse inhibition (PPI) of startle (van der Staay, Rutten et al. ; Javitt and Zukin 1991; Zou, Zhang et al. 2008). In addition, administration of NMDA antagonists reduces parvalbumin interneuron expression, in agreement with post-mortem findings in schizophrenia (Lewis, Curley et al. 2012). These developments have provided evidence that NMDA antagonist administration models may be useful for understanding and dissecting Schizophrenia.

1.3.2.2 Genetic Models

Genetic knockout models have been used to provide selectivity to NMDAR models, and incorporate the developmental aspects of reduced NMDAR function. Several models including neuregulin, constitutive 90% reduction of NMDA receptors, and cell type selective NMDAR knockdown of either interneurons or pyramidal cells have been

created to study the effects of NMDAR in a variety of scenarios. This allows for developmental evaluation of the effects of different receptor knockdowns.

1.4 NMDAR disruption and Autism

1.4.1 Clinical Evidence for Connection

Many genes related to autism and it cognitive disabilities are on the NMDA pathway (Marco, Hinkley et al. 2011). Genes encoding NMDA receptor subunit proteins such as *GRIN1 GRIN2A, GRIN2B, GRIN2C, and ERBB4* have been implicated autism and lie in the NMDA receptor pathway. There is also data to indicate NMDAR signaling is a key deficit in several mouse models of autism risk genes including *UBE3A, NRXN1, MeCP2, DISC1,* and *SHANK*.

1.4.2 Animal models

1.4.2.1 NMDA Receptor Antagonist models

Low level administration of NMDAR antagonists induce social and cognitive deficits similar to those found in autism. In addition, low dose administration of the NMDA antagonist MK801 recreates the robust electrophysiological endophenotype of N1 latency delay. Furthermore, administration of NMDAR antagonists can cause the reduced evoked power and inter-trial coherence present in autism.

1.4.2.2 Genetic Models

Several models of autism risk genes have been used as mentioned above. Recent evidence indicates that selective disruption of NMDARs on PV interneurons causes N1

latency delays and selective deficits in social behavior, providing evidence for NMDAR interneuron disruption as a mechanism of autism deficits.

1.5 Analysis methods

1.5.1 ERP Methods

Studies from the Siegel laboratory and others have shown a high degree of similarity between human and mouse EEG measures and auditory event-related potentials (ERPs) in terms of waveform morphology, as well as physiological and pharmacological response properties. These measures offer greater translatability than behavioral phenotypes as they can be investigated using the same auditory paradigms in preclinical and clinical studies, while giving responses with analogous components such as the P20/P50 and N40/N100 between mouse/human (Connolly, Maxwell et al. 2003; Umbricht, Vyssotky et al. 2004; Metzger, Maxwell et al. 2007). We have demonstrated that schizophrenia-like auditory-ERP deficits, such as reduced P1/N1 amplitude and habituation, can be recreated using pharmacological manipulations that disrupt neurotransmitter systems involved in schizophrenia pathophysiology, including dopamine, acetylcholine, and glutamate systems (Siegel, Maxwell et al. 2005; Maxwell, Ehrlichman et al. 2006; Phillips, Ehrlichman et al. 2007). Similar abnormalities have been demonstrated in transgenic mice with mutations in schizophrenia risk genes, including *DTNBP1*, *NRG1*, and *GRIN1*, which regulate glutamatergic signaling (Carlson, Talbot et al. ; Ehrlichman, Luminais et al. 2009; Halene, Ehrlichman et al. 2009). A graphical illustration of the wave and how components are measured are as follows:

Average Many EEG Traces

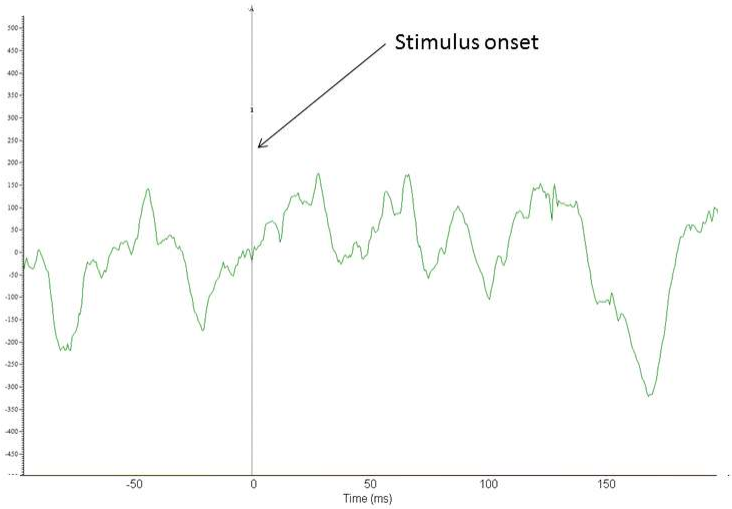


Figure 1.5.1a- One epoch of an EEG stimulus response

Amplitudes

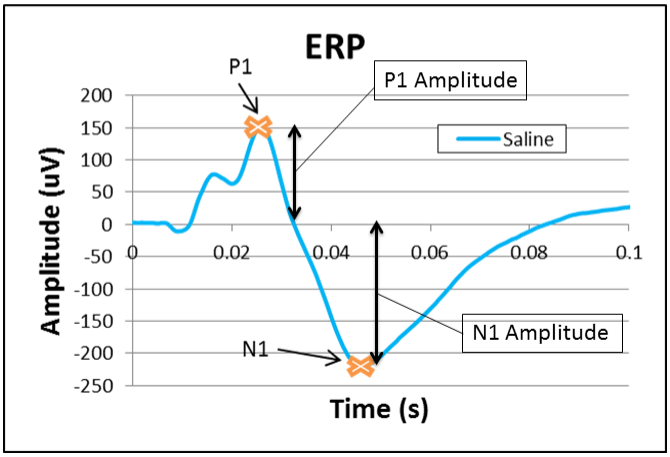


Figure 1.5.1b- Method for amplitude measurement

Latencies

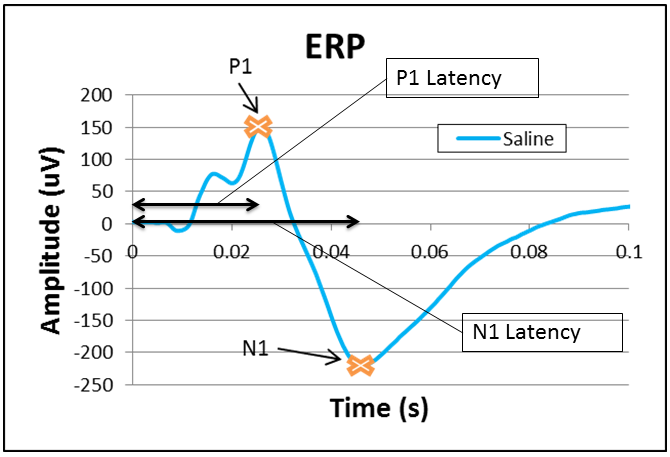


Figure 1.5.1c- Method for latency measurement

1.5.2 Data Integrity Methods

To protect data integrity, methods are implemented to reduce artifacts, and reject any that are recorded. As a first pass, the connectors to the mice are taped down to reduce motion artifacts, next the data is band pass filtered between 1 and 500 Hz to reduce DC electrical noise and high frequency aliasing. The data is then examined manually for ungrounding artifacts where the system steps between maximum voltages, see figures below.

Before a mouse is run for data collection, a test run is performed to check for the following abnormalities in the raw signal (y-axis is signal in μV and x-axis is time in seconds).

Figure 1.5.2a - Too Saturated (bad reference/electrode surgery)

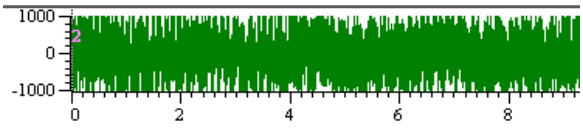


Figure 1.5.2b- Too Regular (bad surgery)

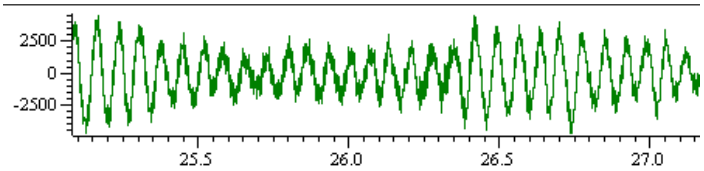
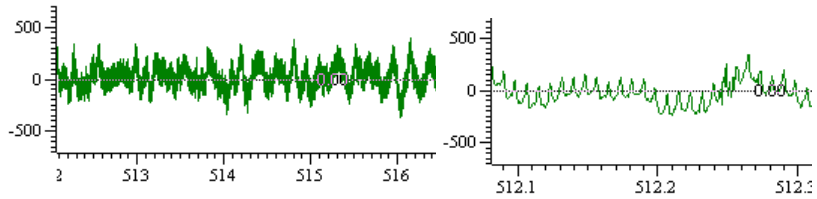
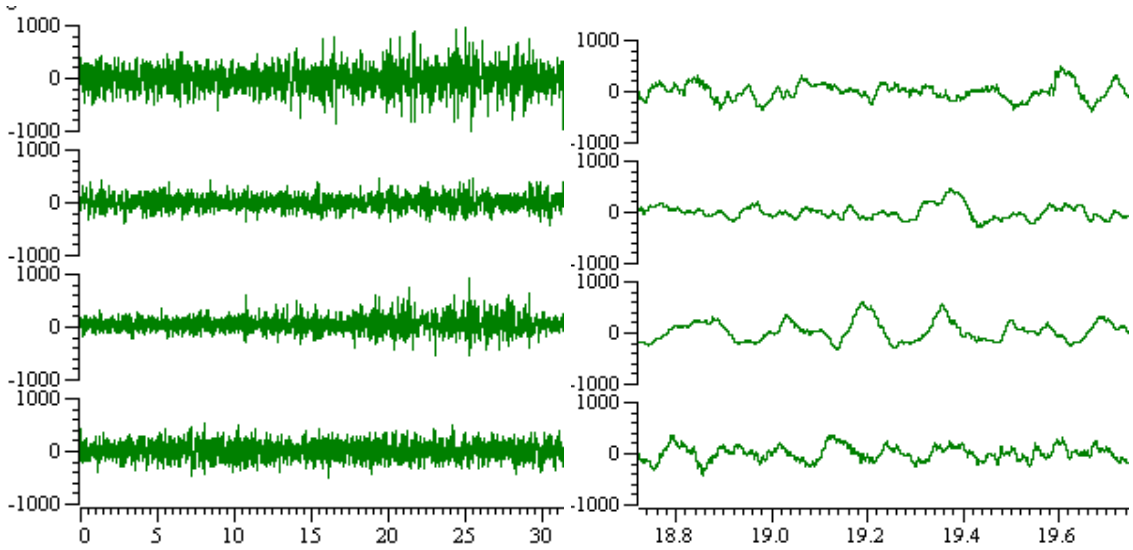


Figure 1.5.2c- Too thick (poor grounding) → See line cycle noise on zoom



If these patterns are observed in the pre-test run, the equipment is re-hooked. If this doesn't fix the problem, then it is likely the mouse has a failed surgery and is sacrificed.

Figure 1.5.2d- Good signals are as follows:



Next when the data is gated into EEGLab, a pass is made to reject trials that fall more than 2 standard deviations from the mean. After analysis of the various EEG measures, each data point is subject to outlier rejection if they fall more than two standard deviations over the mean.

1.5.3 Wavelet Method advantages

In Fourier analysis, there is always a tradeoff between time and frequency resolution. For any signal that is narrow in the time band or frequency band, it will be broadband in the other domain. In the extreme case, there is the Fourier Transform that has no time resolution but very fine frequency resolution. While this provides some useful information, many neural processes are transient, and there is interest in capturing the responses rather than all activity. One step in this direction is the use of a method called short time Fourier transform, where short windows of time are Fourier transformed, and used to track the brain's response. While this is superior to the Fourier transform in capturing transient activity, it is unable to scale based on frequency. For example, if activity from 4 Hz Theta to 100 Hz High Gamma is of interest, there is a conflict, because a window of at least 250 ms (1 second/4 Hz) is required to calculate the time-frequency information for the theta range, but when the same window is applied at 100 Hz, it unnecessarily sacrifices useful temporal information for little gain frequency information. Wavelets circumvent this problem by having higher frequency resolution and wider windows at low frequencies, and shorter time windows at higher resolutions. This allows the calculation technique to be optimized to the transient changes in each frequency domain.

1.5.4 Wavelet Method description

Formula for power calculations: $E(t, f_0) = w(t, f_o) \cdot s(t)^2$

Formula for ITC calculations: $P_i(t, f_o) = w(t, f_o) \cdot s_i(t) / |w(t, f_o) \cdot s_i(t)|$ which give the complex phases, the phases are then averaged. Phase locking factor, a measure of inter-trial coherence, is then calculated by taking the modulus of the average phase. For a complex number z=x+yi, the modulus of z is $|z| = \sqrt{x^2 + y^2}$.

The phase can be calculations can be visualized as the averaging of the phase unit vectors for each trial, then taking the modulus

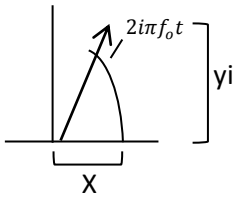


Figure 1.5.4a- One trial's unit phase vector:

To improve analysis, a range of wavelet families with cycle numbers ($\frac{f_o}{\sigma_f}$), varying linearly from 2 to 10 from the lowest frequency to the highest frequency were used. Sacrificing some frequency spread at low frequencies to improve temporal spread, while at high frequencies sacrifices temporal spread to improve frequency spread.

Term definitions:

- $s(t)$ is the signal
- $s_i(t)$ is the signal for each trial
- $w(t, f_o)$ is the wavelet equation equal to $A \cdot e^{-t^2/2\sigma_t^2} \cdot e^{2i\pi f_o t}$
- A is the normalization constant $(\sigma_t \sqrt{\pi})^{-1/2}$ so total energy is equal to 1
- σ_t is the time standard deviation, two times this is the time spread

- σ_f is the frequency standard deviation, two times this is the frequency spread
- The relationship between frequency standard deviation (σ_f) and time standard deviation (σ_t) is $\sigma_f = {}^{1}/_{2\pi\sigma_t}$

Example power calculation at 80 Hz using 10 cycles

1. The wavelet with the above parameters is: $(0.02\sqrt{\pi})^{-1/2} \cdot e^{-t^2/2(.02)^2} \cdot e^{-2i\pi(80\ Hz)t}$

2. Convolution of signal with wavelet $\int_{-\infty}^{\infty} s(t)w^*(t, f_o)$

3. Take the norm and square $\sqrt{\left(\int_{-\infty}^{\infty} s(t)w^*(t, f_o)\right)^2}^2 = w(t, f_o) \cdot s(t)^2 = E(t, f_o)$

The following is a graphical overview of how data would be processed to calculate wavelet T/F outcomes.

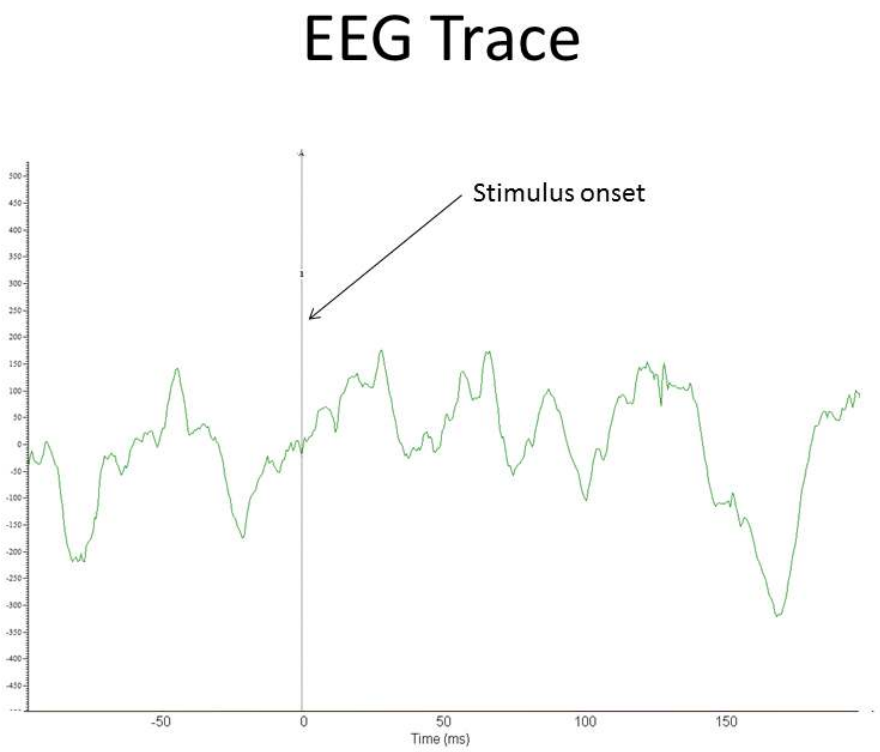


Figure 1.5.4b- Example EEG epoch trace

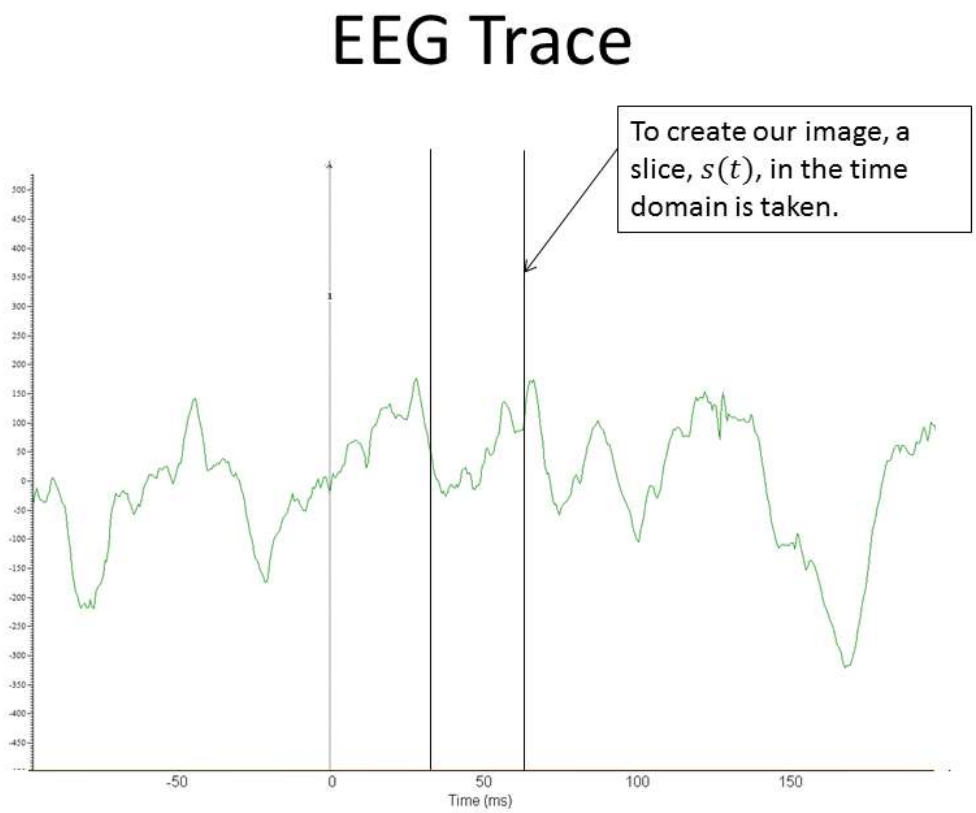
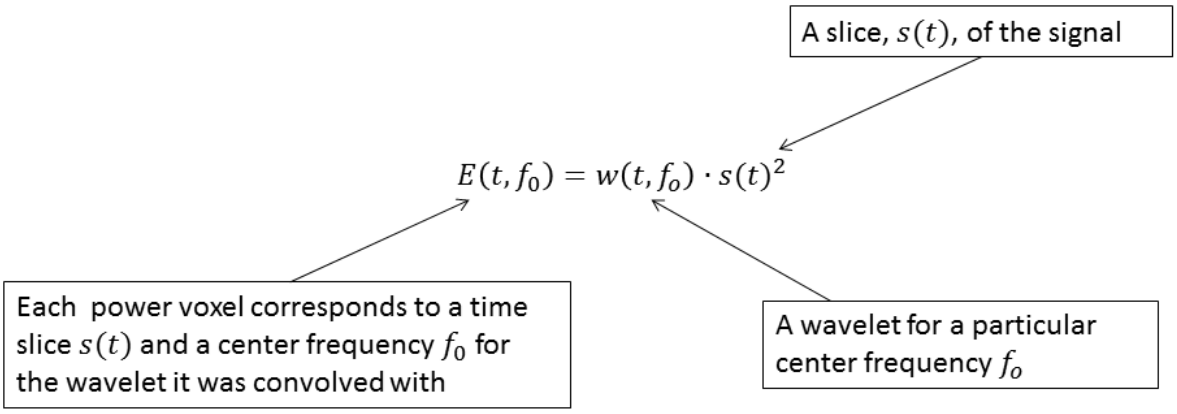


Figure 1.5.4c- Example EEG slice from epoch, the width of the EEG used will vary depending on frequency

Wavelet Transform the EEG



- In my thesis, for each range calculated, each voxel was 1 Hz wide

Figure 1.5.4d- Wavelet transform equation applied to that slice

Wavelet Transform the EEG

Formula for power calculations: $E(t, f_0) = w(t, f_o) \cdot s(t)^2$

Term definitions:

- $s(t)$ is the signal
- $w(t, f_o)$ is the wavelet equation equal to $A \cdot e^{-t^2/2\sigma_t^2} \cdot e^{2i\pi f_o t}$
- A is the normalization constant $(\sigma_t\sqrt{\pi})^{-1/2}$ so total energy is equal to 1
- σ_t is the time standard deviation, two times this is the time spread
- σ_f is the frequency standard deviation, two times this is the frequency spread
- The relationship between frequency standard deviation (σ_f) and time standard deviation (σ_t) is $\sigma_f = {}^1/_{2\pi\sigma_t}$

Figure 1.5.4e- Definition of wavelet transform terms

One voxel of ERSP plot

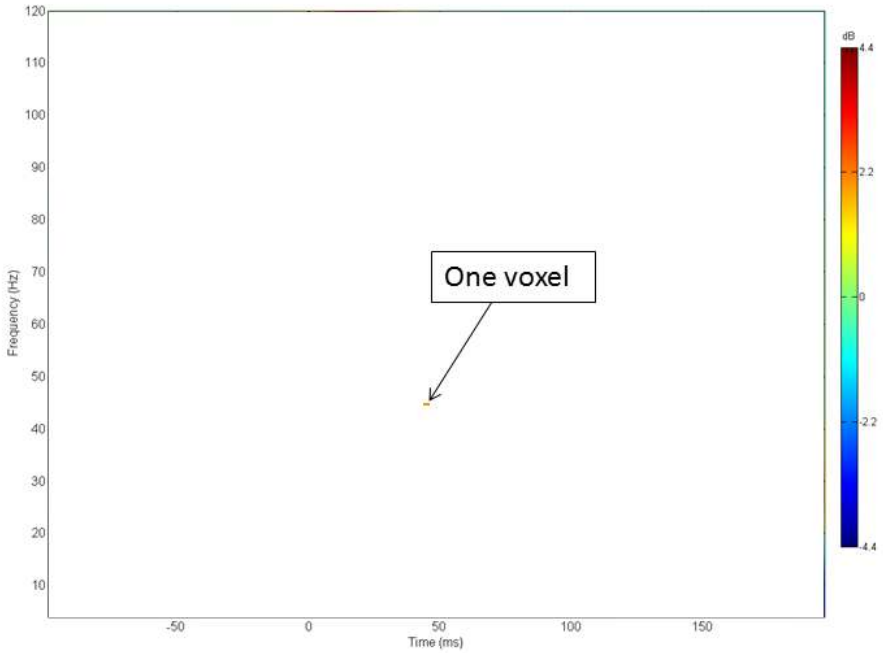


Figure 1.5.4f- Outcome of one calculation

Saline ERSP Plot

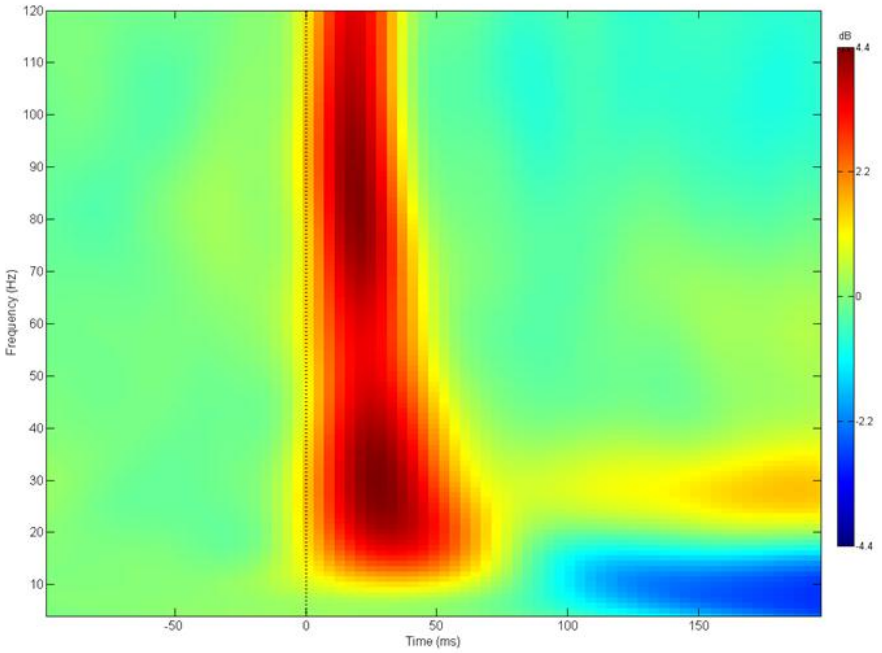


Figure 1.5.4g- Baseline saline plot completed from calculations

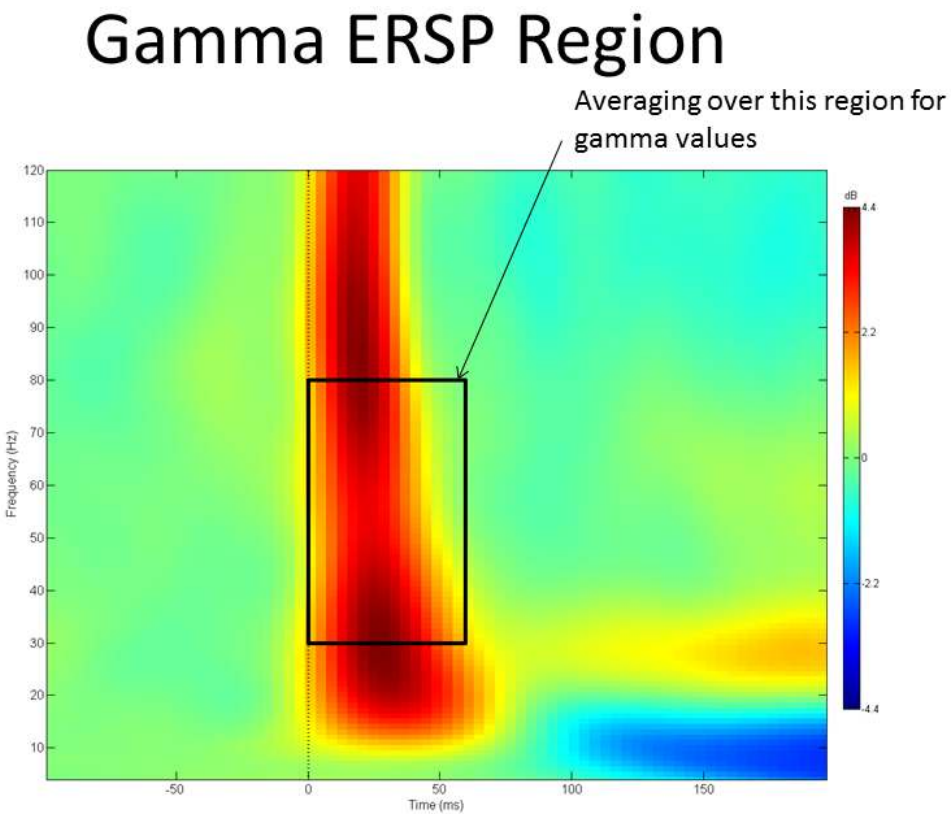


Figure 1.5.4h- Region averaged to obtain single gamma value

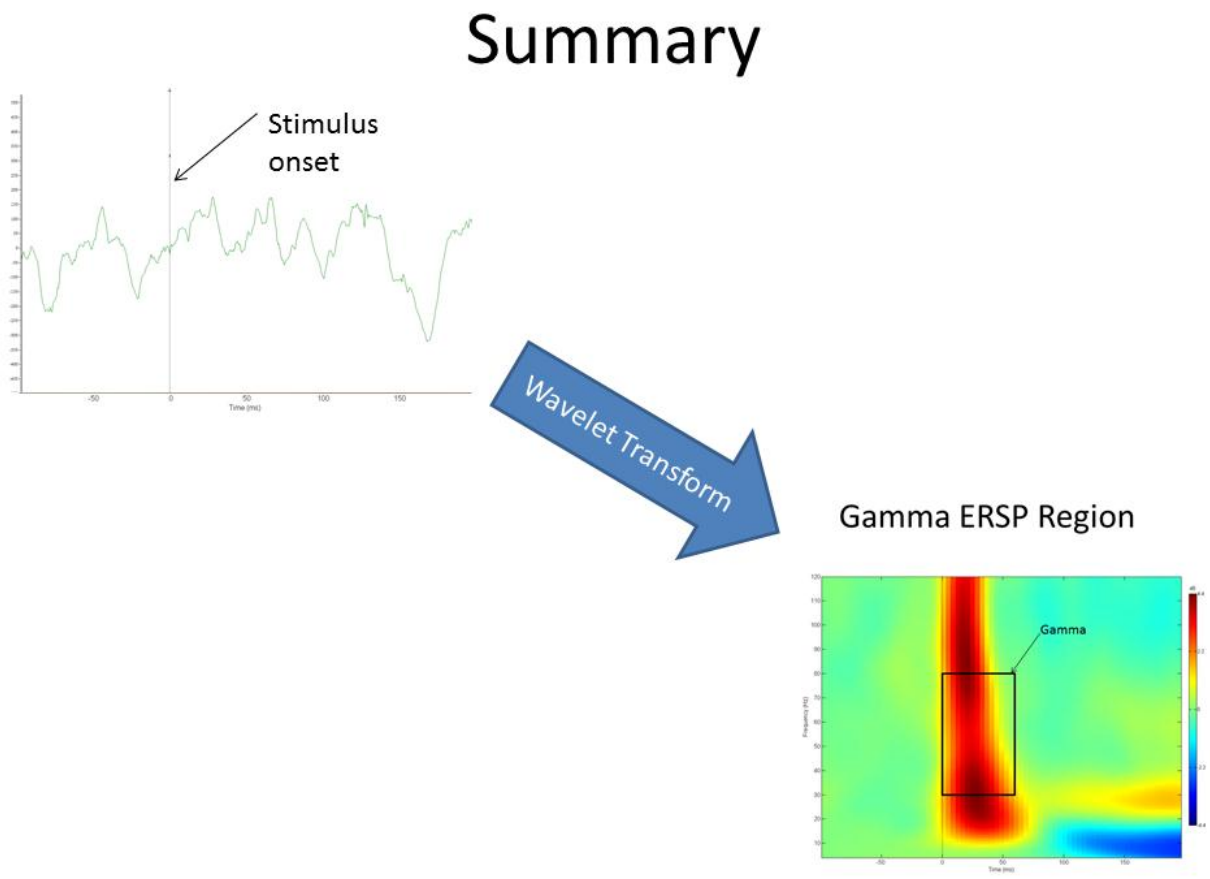


Figure 1.5.4i- Summary of process to obtain results

1.6 Current validation of EEG measures against symptoms

1.6.1 ERP components

N1 latency delay is a robust electrophysiological endophenotype for autism, able to separate patients from non-patients (Roberts, Khan et al. 2010). In addition, there is evidence latency may be a predictor of language function (Oram Cardy, Flagg et al. 2008).

1.6.2 Gamma activity

Recent work has indicated that high-frequency (e.g., gamma) oscillations are particularly important as a biomarker for the treatment-resistant symptoms of schizophrenia (Gonzalez-Burgos, Fish et al. ; Sun, Farzan et al. ; Gandal, Edgar et al. 2010; Uhlhaas and Singer 2010). Gamma oscillatory activity is known to be important for attention, working memory, sensory processing, and perceptual 'feature binding' – neurocognitive processes that are all disrupted in schizophrenia (Gandal, Edgar et al. 2010). Indeed, one study reported a disruption in the phase-locked auditory-evoked gamma band response in schizophrenia patients that was correlated with reduced working memory capacity (Light 2006). Parvalbumin-expressing, fast-spiking interneurons – a sub-population of GABAergic cells disrupted in schizophrenia – have been shown to necessary and sufficient to generate gamma rhythms *in vivo* (Hashimoto, Volk et al. 2003; Cardin, Carlen et al. 2009; Sohal, Zhang et al. 2009). There is preliminary evidence that pharmacologic reversal of gamma-band deficits in patients with schizophrenia is associated with clinical improvement in treatment-refractory domains (Lewis, Cho et al. 2008). Finally, there is a wealth of evidence that the properties of gamma rhythms -- including frequency range, cross-frequency coupling, circuit generators, cortical function, and cognitive correlates – are phylogenetically conserved across mammals (and even invertebrates), making this an attractive biomarker for translational investigation (Gray and Singer 1989; Kirschfeld 1992; Brosch, Budinger et al. 2002; Buzsaki and Draguhn 2004; Hall, Holliday et al. 2005; Colgin, Denninger et al. 2009; Sohal, Zhang et al. 2009).

In summary, recent convergence of established signal processing techniques with the wealth of electrophysiological data, and an increase in understanding about the role of glutamate in psychiatric disease present new pathways for translational biomarker

development. The rest of this thesis examines acute NMDA antagonist administration models and selective KO of NMDARs on PV interneurons, examining the EEG changes present in each. It also explores some of the interrelationships between the measures, and relates some of the measures back to behavior. This thesis hopes to incrementally improve current understanding of glutamate disruption in disease and the ramifications it poses for brain circuits.

Chapter 2: NMDA Antagonists Recreate Signal-to-Noise Ratio and Timing Perturbations Present in Schizophrenia

2.1 Abstract

Rationale: There is increasing evidence that functional deficits in schizophrenia may be driven by a reduction in the signal-to-noise ratio (SNR) and consistent timing of neural signals. This study examined the extent to which exposure to the NMDA receptor antagonists ketamine and MK801, frequently used pharmacological models of schizophrenia, recreate deficits in electrophysiological markers of disturbed brain circuits that are thought to underlie the illness. Furthermore, this study characterizes the specificity of these differences across the frequency spectrum so as to help identify the nature of selective circuit abnormalities that mediate each oscillatory response as relevant to schizophrenia.

Design: Mouse EEG was recorded during exposure to repeated auditory stimuli after injection of either vehicle or drug. The dose-response relationship for each electrophysiological measure was determined for ketamine and MK-801. Time-frequency analyses were performed to assess baseline, total, and evoked power and intertrial coherence (ITC) at low (5-10 Hz) and high (35-80 Hz)-frequencies.

Results: High frequency evoked and total power was decreased by MK-801 and ketamine in a dose-dependent fashion. High frequency baseline power was increased by MK-801 and ketamine in a dose-dependent fashion. Similar to evoked power, high frequency inter-trial coherence was dose-dependently decreased by both drugs. Low frequency ITC was only decreased by ketamine.

Conclusions: Both ketamine and MK-801 cause alterations in high-frequency baseline (noise), total (signal), and evoked (signal) power resulting in a loss of high frequency SNR that is thought to primarily reflect local circuit activity. These changes indicate an inappropriate increase in baseline activity, which can also be interpreted as non-task related activity. Ketamine induced a loss of intertrial coherence at low frequencies, indicating a loss of consistency in low-frequency circuit mechanisms. As a proportion of baseline power, both drugs had a relative shift from low to high frequencies, reflecting a change in the balance of brain activity from coordination of global regions to a pattern of discoordinated, autonomous local activity. These changes are consistent with a pattern of fragmented regional brain activity seen in schizophrenia.

2.2 Introduction

Schizophrenia is a disabling psychiatric illness that affects about 1% of the population. Currently, there are no effective treatments for the negative and cognitive symptoms associated with this disease. Developing novel therapeutics for treatment resistant symptoms requires appropriate neural biomarkers associated with these deficits and valid animal models that reflect underlying disease pathophysiology. This study examined two pharmacologically-induced models of schizophrenia based on the glutamate hypothesis of disease pathogenesis, which is based on the observation that NMDA-receptor antagonists, such as ketamine, PCP, and MK801, have been shown clinically to induce psychosis and cognitive deficits indistinguishable from that seen in schizophrenia (Javitt and Zukin 1991). Additionally, administration of NMDA-receptor antagonists to model organisms has been demonstrated to recreate many of the cognitive, sensory, motor, and electrophysiological deficits seen in schizophrenia (Shiigi and Casey 1999; Javitt, Jayachandra et al. 2000; Jackson, Homayoun et al. 2004; Swerdlow, Geyer et al. 2006). However, many of these preclinical studies have used ketamine to induce schizophrenia-like phenotypes, despite the fact that ketamine has many effects in addition to NMDA-receptor antagonism, such as activation of HCN1 channels, which make the causal interpretation of its effects more difficult (Chen, Shu et al. 2009). As such, this study investigated the effects of ketamine in addition to MK-801, a selective NMDA-receptor antagonist, to compare the effects of these two drugs. Finally, to our knowledge, no studies have investigate the effects of either drug on auditory-evoked gamma-frequency signal-to-noise, despite emerging evidence that this is an important biomarker for the treatment resistant symptoms of schizophrenia (Gandal, Edgar et al. 2010).

Studies from our group and others have shown a high degree of similarity between human and mouse EEG measures and auditory event-related potentials (ERPs) in terms of waveform morphology, as well as physiological and pharmacological response properties. These measures offer greater translatability than behavioral phenotypes as they can be investigated using the same auditory paradigms in preclinical and clinical studies, while giving responses with analogous components such as the P20/P50 and N40/N100 between mouse/human (Connolly, Maxwell et al. 2003; Umbricht, Vyssotky et al. 2004; Metzger, Maxwell et al. 2007). We have demonstrated that schizophrenia-like auditory-ERP deficits, such as reduced P1/N1 amplitude and habituation, can be recreated using pharmacological manipulations that disrupt neurotransmitter systems involved in schizophrenia pathophysiology, including dopamine, acetylcholine, and glutamate systems (Siegel, Maxwell et al. 2005; Maxwell, Ehrlichman et al. 2006; Phillips, Ehrlichman et al. 2007). Similar abnormalities have been demonstrated in transgenic mice with mutations in schizophrenia risk genes, including *DTNBP1*, *NRG1*, and *GRIN1*, which regulate glutamatergic signaling (Carlson, Talbot et al. ; Ehrlichman, Luminais et al. 2009; Halene, Ehrlichman et al. 2009).

Recent work has indicated that high-frequency (e.g., gamma) oscillations are particularly important as a biomarker for the treatment-resistant symptoms of schizophrenia (Gonzalez-Burgos, Fish et al. ; Sun, Farzan et al. ; Gandal, Edgar et al. 2010; Uhlhaas and Singer 2010). Gamma oscillatory activity is known to be important for attention, working memory, sensory processing, and perceptual 'feature binding' – neurocognitive processes that are all disrupted in schizophrenia (Gandal, Edgar et al. 2010). Indeed, one study reported a disruption in the phase-locked auditory-evoked

gamma band response in schizophrenia patients, which was correlated with reduced working memory capacity (Light 2006). Parvalbumin-expressing, fast-spiking interneurons – a sub-population of GABAergic cells disrupted in schizophrenia – have been shown to necessary and sufficient to generate gamma rhythms *in vivo* (Hashimoto, Volk et al. 2003; Cardin, Carlen et al. 2009; Sohal, Zhang et al. 2009). There is preliminary evidence that pharmacologic reversal of gamma-band deficits in patients with schizophrenia is associated with clinical improvement in treatment-refractory domains (Lewis, Cho et al. 2008). Finally, there is a wealth of evidence that the properties of gamma rhythms -- including frequency range, cross-frequency coupling, circuit generators, cortical function, and cognitive correlates – are phylogenetically conserved across mammals (and even invertebrates), making gamma rhythms an attractive biomarker for translational investigation (Gray and Singer 1989; Kirschfeld 1992; Brosch, Budinger et al. 2002; Buzsaki and Draguhn 2004; Hall, Holliday et al. 2005; Colgin, Denninger et al. 2009; Sohal, Zhang et al. 2009).

Since NMDA receptor antagonists have been able to recreate many of the cognitive, sensory, motor, and electrophysiological deficits of schizophrenia, these pharmacologic agents are among the leading methods for recreating schizophrenia-like deficits in animals (Shiigi and Casey 1999; Javitt, Jayachandra et al. 2000; Jackson, Homayoun et al. 2004; Swerdlow, Geyer et al. 2006). However, the electrophysiological consequences of disrupted glutamate signaling have only been studied for limited number of outcomes, P1, N1, P2, amplitude and latency as well as mismatch-negativity deficits following ketamine (Maxwell, Ehrlichman et al. 2006; Turetsky, Calkins et al. 2007; Amann, Halene et al. 2009). Furthermore, the mechanism by which high-frequency

oscillations are perturbed by NMDA disruption is less studied, even though evidence suggests these oscillations reflect deficits in higher order cognitive functioning in schizophrenia (Light, Hsu et al. 2006). Therefore, it is important to understand how well these pharmacological models reflect the true endophenotypes of the disease in order to assess their face and predictive validity. This study examines how different NMDA antagonist agents influence low- and high-frequency oscillations to determine the extent to which they recreate the perturbations in SNR present in schizophrenia.

For calculating SNR, morlet wavelets were used to create a time and frequency resolved map of event related spectral perturbations (ERSP), as shown in Figure 2.6.1A. This method allows evoked, baseline, and total power changes to be observed as they change in both the time and frequency domains in contrast to the traditional ERP and FFT methods that only have resolution in one domain. This enables more comprehensive analysis of transient stimulus related responses and by extension, understanding of neural circuit response. This method has been previously established in clinical studies of schizophrenia and autism and to examine power changes in those disorders. To evaluate the state of SNR in this model, baseline, evoked and total power were examined in high and low frequency ranges to determine if these two well established pharmacological models cause perturbations in the noise or stimulus-related signal similar to those found in schizophrenia (Hall, Taylor et al. ; Leicht, Kirsch et al. ; Reinhart, Mathalon et al. ; Lifshitz, Lee et al. 1987; Kessler and Kling 1991; Schellenberg and Schwarz 1993; Dierks, Strik et al. 1995; Jensen and Lisman 1996; Yamamoto 1997; Canive, Lewine et al. 1998; Kirino and Inoue 1999; Kwon, O'Donnell et al. 1999; Doheny, Faulkner et al. 2000; Kissler, Muller et al. 2000; Koukkou, Federspiel et al. 2000; Behrendt 2003;

Krause, Hoffmann et al. 2003; Behrendt and Young 2004; Fujisawa, Matsuki et al. 2004; Hong, Summerfelt et al. 2004; Spencer, Nestor et al. 2004; Light, Hsu et al. 2006; Ford, Roach et al. 2008; Teale, Collins et al. 2008; Brenner, Kieffaber et al. 2009; Gaspar, Bustamante et al. 2009; Hall, Taylor et al. 2009; Krishnan, Hetrick et al. 2009). Additionally, inter-trial coherence, a measure for determining the trial to trial response consistency, was examined in low and high frequency regions to determine whether or not a loss of response consistency was associated with changes in power. This measure has also been used in clinical studies to examine deficits in circuit response timing in schizophrenia clinical trials. These measures will give a better understanding of the circuit disruptions driven by the NMDA system and how they match those found in schizophrenia.

2.3 Methods

2.3.1 Subjects

Forty-five male C57BL/6Hsd (B6) mice were obtained at 7–8 weeks of age from Jackson Labs (Bar Harbor, ME, USA). All testing was conducted between 10 and 18 weeks of age. Mice were acclimated to the animal facility for 7 days before experimentation began and were housed four to five per cage until surgical implantation of the recording electrode, after which they were single-housed for the remainder of the study. All subjects were maintained in a standard 12-h light/dark cycle with free access to food and water. Experiments were performed during the light phase between 9:00 AM and 4:00 PM. All protocols were conducted in accordance with University Laboratory Animal Resources (ULAR) guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania. All efforts were undertaken to minimize the number of animals used in the experiment and their suffering.

2.3.2 Drugs and doses

MK801 was obtained from Sigma-Aldrich (St. Louis, MO) and ketamine was obtained from Hospira (Lake Forest, IL). MK801 was dissolved in saline at doses of 0.1, 0.4, 0.7, and 1 mg/kg prior to recording. Ketamine was dissolved in saline at doses of 5, 10, 20, 40 mg/kg. All drugs and saline controls were administered via i.p. injection 5-10 minutes prior to initiation of recording.

2.3.3 EEG Recording

Electrode Implantation

At 12 weeks of age, animals underwent stereotaxic implantation of electrode assemblies (PlasticsOne Inc., Roanoke, VA, USA) for non-anesthetized recording of auditory ERPs as previously reported (Connolly, Maxwell et al. 2003; Siegel, Connolly et al. 2003; Connolly, Maxwell et al. 2004; Maxwell, Liang et al. 2004). Animals were anesthetized with isoflurane and unipolar recording electrodes were placed in the CA3 hippocampal region (1.8 mm posterior, 2.65 mm lateral and 2.75 mm deep relative to bregma) and referenced to the ipsilateral frontal sinus to reflect whole brain electrical activity. ERPs recorded from this electrode configuration are characteristically similar to human recordings from the Cz scalp location as illustrated in a prior publication by our group (Siegel, Connolly et al. 2003). The electrode pedestal was secured to the skull using ethyl cyanoacrylate (Loctite, Henkel, Germany) and dental cement (Ortho Jet, Lang Dental, Wheeling, Illinois). EEG was recorded 1 week later, as described below.

Electrophysiological Recordings

EEG was recorded with Power1401 hardware and Spike6 software (CED, Cambridge, UK) as published previously (Ehrlichman, Gandal et al. 2009). A total of 1000 white noise clicks were presented with a 1000 ms interstimulus interval at 85 dB.

2.3.4 Frequencies analyzed and methods

Intertrial coherence (ITC), evoked (total stimulus-related activity minus induced and baseline), baseline (pre-stimulus power), and event-related spectral perturbation (ERSP also known as baseline corrected total power) values were calculated with Morlet wavelet decomposition using EEGLab (Delorme and Makeig 2004). Single-trial epochs -250 and 750 ms relative to the auditory stimulus were extracted from the continuous data.

For each epoch (trial), the ITC, baseline power, evoked power, and baseline corrected total power were calculated at 75 linearly spaced frequencies from 5 to 80 Hz, with wavelet cycles increasing from 2 (at low frequencies) to 10 (at high frequencies). ITC is expressed as a unitless ratio between 0 and 1, where 1 represents complete phase synchrony at a given frequency and time across trials. Baseline, evoked, and total power were expressed in dB (10 * loguV^2). For each subject, high-frequency ITC and the power measures were calculated in a window around the peak evoked response, defined as the region from 0-60 ms poststimulus and from 35-80 Hz. The 0-60 ms range was also examined for perturbations in low frequency (5-10 Hz) activity. N40 amplitude was calculated from the ERPs as the difference between the lowest point between 30 and 80 ms and the average of the baseline amplitude 50 ms pre-stimulus.

2.3.5 Factor Analysis

One way ANOVAs were performed to investigate the effect of each pharmacologic agent on EEG measures. Significant effects were followed by Tukey's post-tests where appropriate. Factor analysis was performed using principle component analysis (PCA) on the individual subject data, examining the relationship between different measures across all drug and saline conditions.

2.4 Results

Baseline EEG

Baseline, pre-stimulus auditory-evoked EEG power was calculated at low and high frequencies using EEGLab. Both MK801 and ketamine dose-dependently increased baseline high-frequency power (MK801: $F(1,4)=6.523$, $P=0.0001$, ketamine: $F(1,4)=11.92$, $P<0.0001$) (Fig 2.6.2). MK801 and ketamine had no significant effects on baseline low frequency power.

Post-stimulus Total Power

Total power (e.g., event-related spectral perturbation, ERSP) was measured from 0-60 ms following auditory stimuli using Morlet wavelet decomposition in EEGLab. MK-801 dose-dependently decreased total power at high frequencies (35-80 Hz) (Fig 2.6.2 $F(1,4)=15.15$, $P<0.0001$). Ketamine had a similar effect, reducing high frequency total power (Fig 2.6.2; $F(1,4)=18.48$, $p<0.0001$) . MK801 and ketamine had no effect on low frequency total power.

Post-Stimulus Evoked Power

Evoked (e.g., phase-locked) power was calculated within the same time and frequency ranges. MK801 and ketamine reduced high-frequency evoked power (Fig 2.6.3; MK801: $F(1,4)=7.533$, $P<0.0001$, ketamine: $F(1,4)=6.727$, $P<0.0001$).

Intertrial coherence (ITC) and evoked power are related measures that reflect the degree of phase-locking of an oscillation with a repeated external stimulus. ITC is thought to be a more pure measure of neural synchrony, however, as it is not biased by

the amplitude of an oscillation (CITE). Ketamine reduced low and high frequency ITC (low frequency ITC: $F(1,4)=4.417$, $P=0.002$, high frequency ITC: $F(1,4)=7.573$, $P<0.0001$). MK-801 did not change low frequency ITC but did reduce high-frequency ITC (Fig 2.6.4; $F(1,4)=6.99$, $p<0.0001$).

N1 Amplitude

Patients with schizophrenia have reduced auditory-evoked response amplitudes, especially for the N1 component, which is thought to reflect glutamatergic signaling (Dias, Butler et al. 2011). Validating that this effect was also present in our mouse models, MK801 and ketamine dose-dependently reduced N1/N40 amplitude. An ANOVA for the effects of each drug on N40 amplitude revealed that MK801 and ketamine significantly lowered N40 amplitude (Fig 2.6.5; MK801: $F(1,4)=10.36$, $p<0.0001$, ketamine: $F(1.4)=15.01$, $p<0.0001$).

Signal-to-Noise Ratio (SNR)

A change in SNR can be driven (1) a decrease in signal, (2) an increase in noise, or (3) both. In this study, decreased evoked and total power can be thought of as a reduction in signal, whereas increased baseline power likely reflects an increase in noise. To more explicitly calculate SNR, we divided post-stimulus evoked power by the preceding baseline levels in the same frequency range. As expected, both pharmacologic agents significantly reduced high frequency SNR, consistent with deficient sensory encoding (Fig 2.6.6; MK-801: $F(1,4)=7.564$, $P<0.0001$; ketamine: $F(1,4)=7.57$, $P<0.0001$).

Factor Analysis

Factor	1	2
High-frequency evoked power:	0.95	0.12
High-frequency ITC:	0.35	0.60
High-frequency baseline:	-0.60	-0.11
High-frequency baseline-corrected total power:	0.24	0.77
Low-frequency ITC	0.13	0.77
High-frequency SNR evoked:	0.96	0.12
N40 amplitude from baseline:	-0.01	-0.79

Table 1: Factor analysis is used to understand the relationships between different variables within a dataset. Variables that concurrently have high loadings on the same factor indicate that they are related or depend on common causes. We separated the measures for all saline and drug conditions on to different factors using a threshold of 0.5 for each factor loading, meaning one quarter the variance of each EEG measure can be accounted for by the factor. Using this process high-frequency evoked power, baseline power, and SNR grouped on factor 1. High-frequency and low-frequency coherence, high-frequency baseline-corrected total power, and N40 amplitude grouped together, on factor 2.

2.5 Discussion

2.5.1 Comparison of Pharmacological Models to Schizophrenia

Mice treated with MK-801 showed reductions in high-frequency evoked power, total power and intertrial coherence, similar to the deficits observed in schizophrenia (Koukkou, Federspiel et al. 2000; Roach and Mathalon 2008; Hall, Taylor et al. 2009; Krishnan, Hetrick et al. 2009). There was also an increase in high frequency baseline power similar to schizophrenia (Hong, Summerfelt et al. 2008). Unlike schizophrenia, there were no decreases in low-frequency evoked power, intertrial coherence, or increases in low-frequency baseline power (Kessler and Kling 1991; Schellenberg and Schwarz 1993; Canive, Lewine et al. 1998; Ford, Roach et al. 2008). Similarly, ketamine administration caused reductions in high-frequency intertrial coherence, evoked, and total power, along with decreased low frequency intertrial coherence, similar to findings reported in schizophrenia (Koukkou, Federspiel et al. 2000; Roach and Mathalon 2008; Hall, Taylor et al. 2009; Krishnan, Hetrick et al. 2009). Across drugs, we observed an increase in high frequency baseline power similar to schizophrenia (Hong, Summerfelt et al. 2008). In contrast, ketamine did not mimic the schizophrenia-like EEG signatures of reduced total low-frequency power, decreased low-frequency evoked power, and increased low-frequency baseline power (Kessler and Kling 1991; Nakagawa, Takeda et al. 1991; Schellenberg and Schwarz 1993; Canive, Lewine et al. 1998; Ford, Roach et al. 2008).

Ketamine decreased low frequency intertrial coherence across trials, indicating reduced global consistency of response. Neither agent significantly changed baseline low frequency activity. With both agents, there was a reduction in high-frequency SNR caused by both a reduction of stimulus response signal and an increase in baseline noise. The relative increase in high compared to low frequency baseline activity reflects the shift from low to high frequencies reported in patients with schizophrenia. Taken together, these data suggest that disruption of NMDA-receptor mediated neurotransmission caused schizophrenia-like deficits in global coordination and consistency of responses.

Factor analysis demonstrated the expected relationship between SNR and its constituent measures (factor 1). Factor analysis also indicated a pattern of disruptions in intertrial coherence, N40 amplitude reductions, and baseline-corrected total power (factor 2) that could be hypothesized as a deficit in ability to mount a timely response to a salient stimulus. Furthermore, this analysis indicates that the SNR component parts vary independently of the timing measures. This could be thought of as two separate patterns of system disruption, one in the strength of the signal over noise, factor 1, and one in the timing of the response, factor 2.

2.5.2 Potential Mechanisms for Specificity of Drug Effects

The NMDA-receptor is a cation channel that produces excitatory postsynaptic potentials when activated and is important for learning and synaptic plasticity. Although both NMDA- receptor antagonists are non-competitive inhibitors, MK801 has a much lower reversibility than ketamine. Additionally, MK801 has 161 times the affinity of

ketamine, necessitating higher ketamine doses for similar drug effect (Wong, Kemp et al. 1986). Unlike MK801, ketamine also has activity at mu opioid receptors, the targets of morphine and codeine among other opiates, as well as at HCN channels that are thought to mediate its anesthetic effect. Furthermore, administration of mu-opioid receptor targeting drugs alters low frequency EEG (Greenwald and Roehrs 2005). Therefore, high doses of ketamine may be altering low frequency oscillations via the mu-opioid receptor, while MK-801 may provide more specific activity at NMDA-receptors.

2.5.3 Limitations and Future Directions

The current study employed acute MK-801 and ketamine administration. Alternatively, chronic administration may have resulted in a different pattern of deficits. This is relevant as schizophrenia is thought to be a chronic developmental disease, in which both reduced NMDA- receptor function and compensatory changes likely contribute to observed phenotypes. This limitation is more applicable to ketamine where repeated administration is required for PV interneuron disruption as opposed to MK801 where only a single dose is required (Romon, Mengod et al. ; Behrens and Sejnowski 2009).

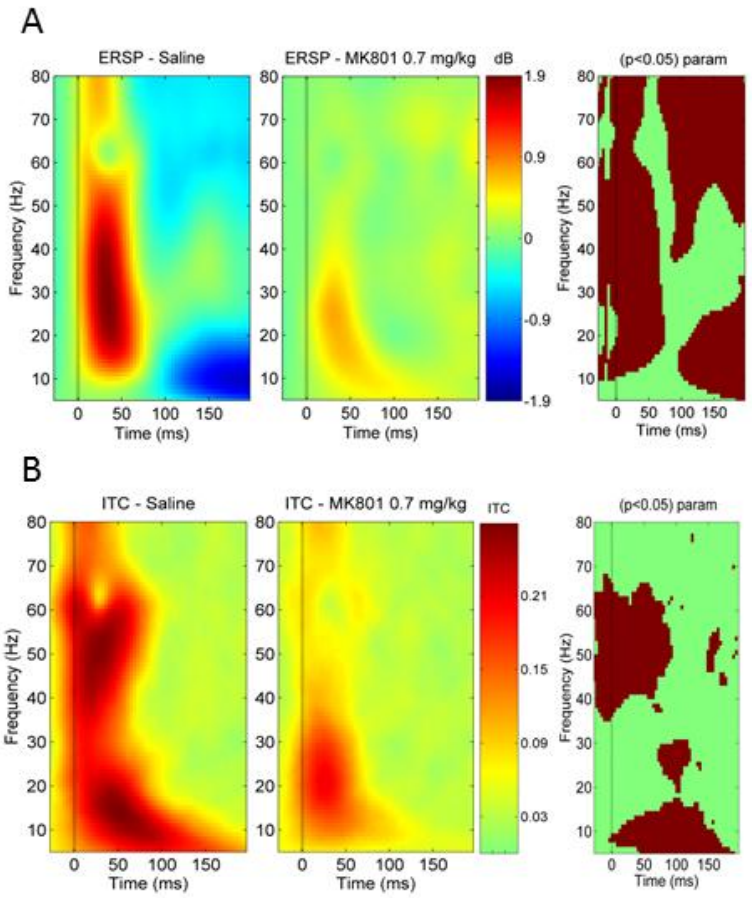
This study limited its EEG analysis to the region 60 ms post-stimulus. While there are relevant changes outside this range reported, this study focused its analysis to the obligatory ERP region and EEG changes related to that range (Gallinat, Winterer et al. 2004). This allowed for more cohesive analysis of a constellation of related features, and an examination of the relationship between these early related features.

In addition, it would be preferable to know the relative disruption of interneurons and pyramidal cells following NMDA-receptor blockade, however it is not possible to quantify that distribution using the systemic administration drug model. Some have suggested that there are brain region specific differences in high-frequency activity when treated with NMDA antagonists (Roopun, Cunningham et al. 2008). Although this has been noted in slices, *in-vivo* data suggests that the disruption is generalized (Hakami, Jones et al. 2009). There are many possible reasons for these apparently disparate results, many likely arising from the confounds inherent in slice work where physiologic conditions and connections are difficult to replicate. In addition, while similar results were recently published in human subjects following ketamine exposure, we believe this strengthens the current work by showing consistency across species after administration of similar drugs (Hong, Summerfelt et al. 2010). We have also extended previous work by performing multi-factor analysis between the different disruptions of neural circuit operations and additionally used a more specific NMDA antagonist, MK-801, to confirm that the cause of the disruptions is primarily glutamate related.

Despite these potential limitations, the current study suggests that a reduction of NMDA-receptor signaling contributes to the pattern of reduced high-frequency SNR observed in schizophrenia, as well as the shift from low frequency to high frequency activity in schizophrenia. These changes are consistent with the hypothesis that schizophrenia is characterized by a functional disconnection of distant brain regions, with a coincident increase in autonomous, local activity (Schmitt, Hasan et al.).

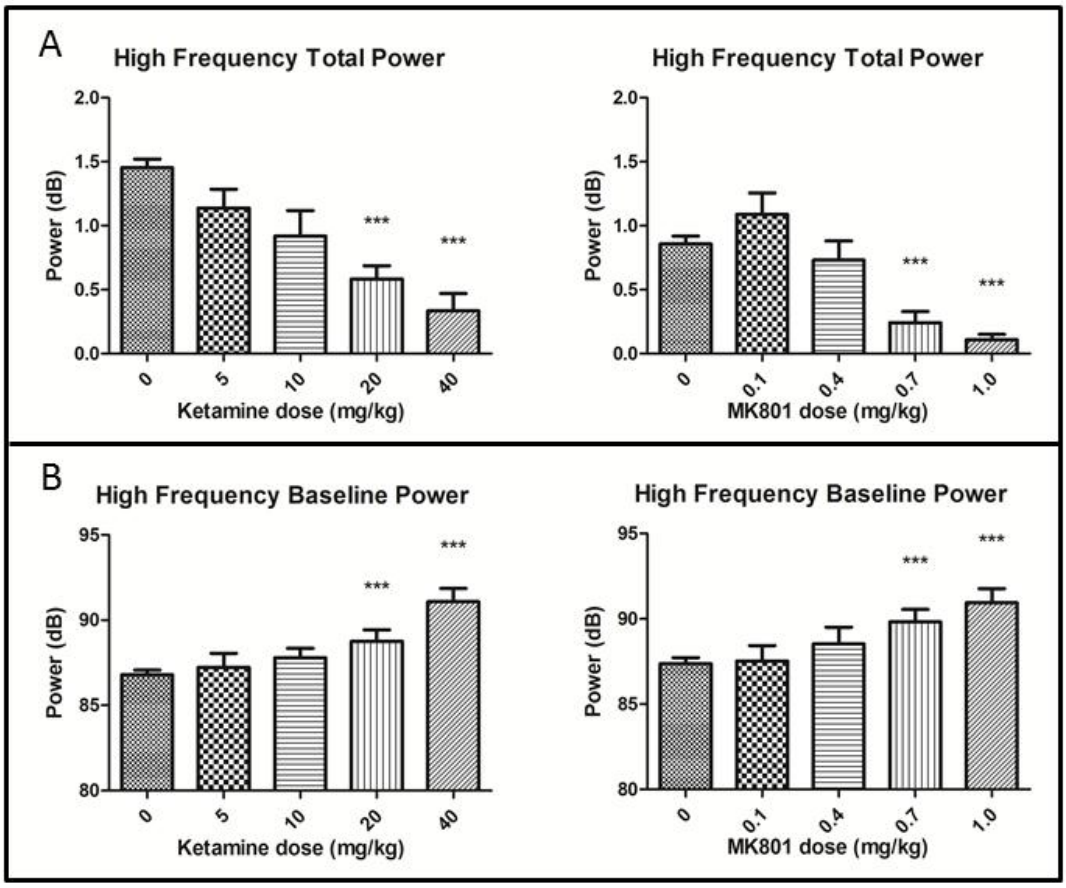
2.6 FIGURE LEGENDS

Figure 2.6.1- Overview of Frequency Measures



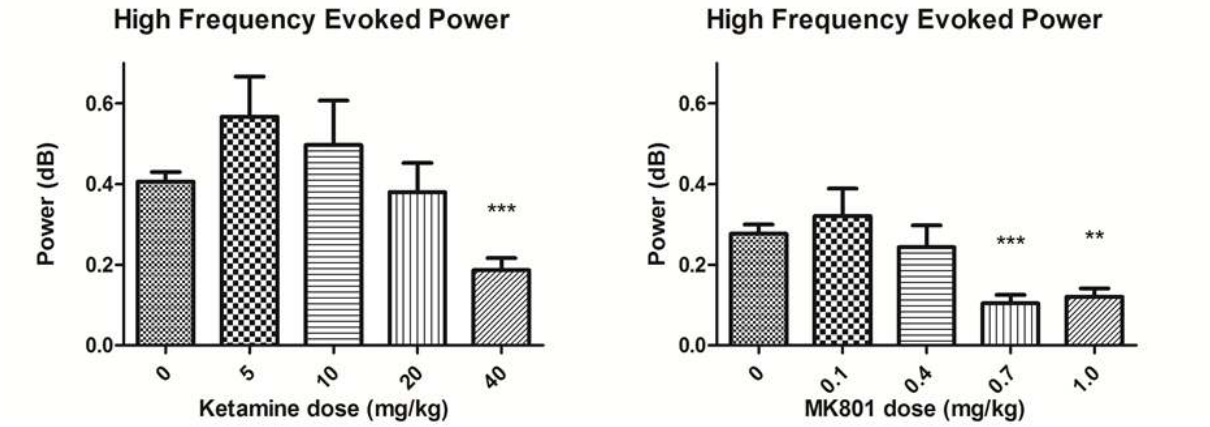
Grand-Average Time-Frequency Plots of 0.7 mg/kg MK801. The effect of MK801 on auditory-evoked power and intertrial coherence (ITC) is shown. A) Event-related spectral perturbation (e.g., ERSP, in dB relative to pre-stimulus-baseline) is demonstrated for saline and MK-801 (0.7 mg/kg) conditions. The right panel shows statistically different time-frequency regions (P<0.05). B) Intertrial coherence (e.g., ITC) is plotted across time and frequency for saline and MK-801 conditions. The right plot highlights statistically different regions (P<0.05).

Figure 2.6.2- High Frequency Total and Pre-stimulus Power



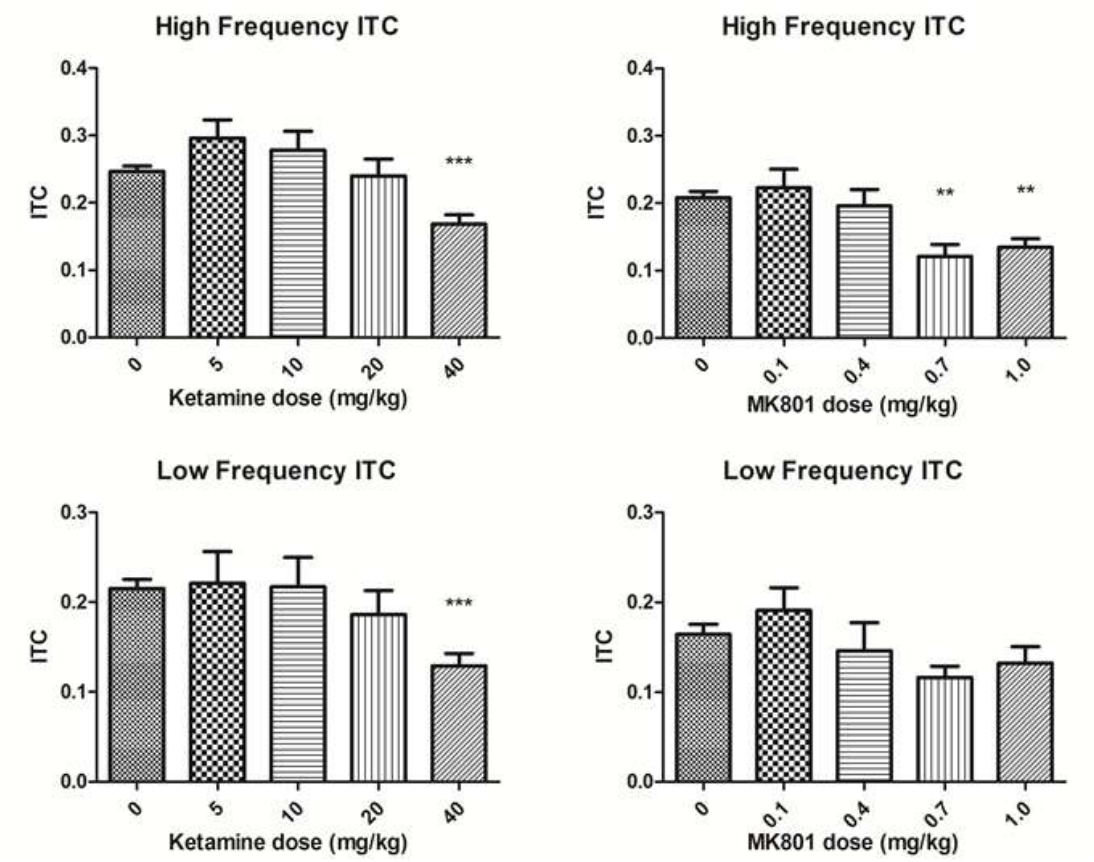
Effects on total and baseline power in the high frequency range following ketamine and MK-801. A) Total high-frequency power was dose-dependently reduced following both ketamine and MK-801. B) Both agents dose-dependently increased high-frequency baseline power, which can be thought of as non-task related activity or noise if it was increased over the reference state. Figures show mean + S.E.M. (*** $P<0.001$)

Figure 2.6.3- High Frequency Evoked Power



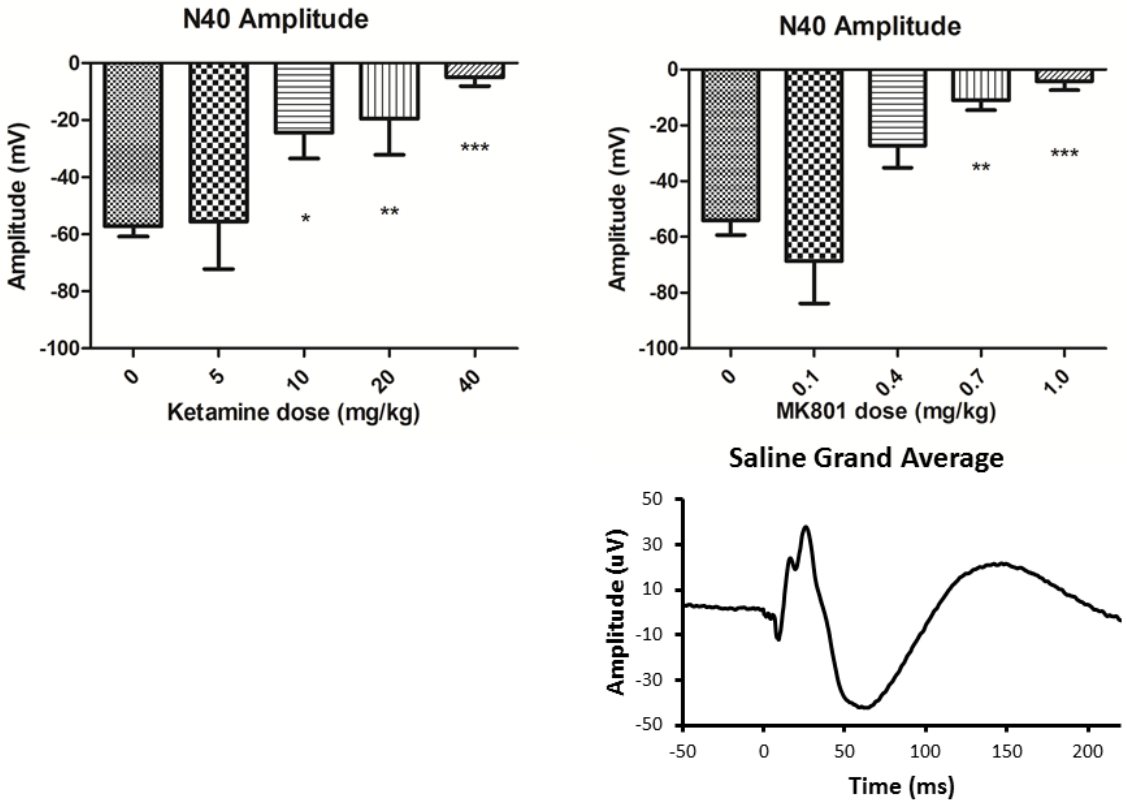
Effects on high-frequency evoked power following ketamine and MK-801 administration. Both agents dose-dependently reduced high-frequency evoked power, which can be thought of as the specific signal response to an external stimulus. Figures show mean + S.E.M. (**P<0.01, *** P<0.001)

Figure 2.6.4- High and Low Frequency ITC



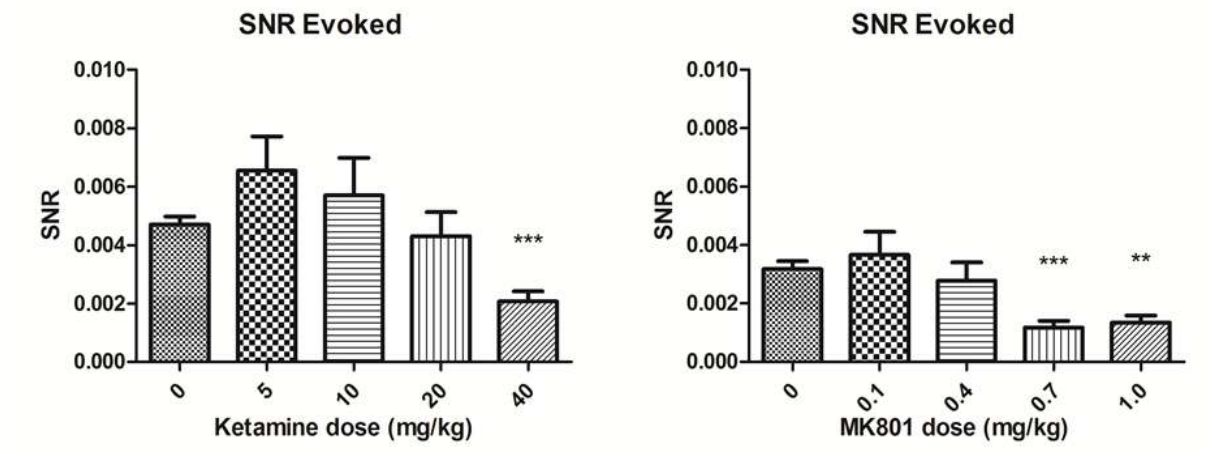
Effects of ketamine and MK-801 on low and high-frequency intertrial coherence. Both ketamine and MK-801 dose-dependently reduced high and low-frequency ITC which measures the precision of response timing in the brain. Figures show mean +/- S.E.M. (**P<0.01, *** P<0.001)

Figure 2.6.5- N40 Amplitude Changes



Effects on N40 amplitude following ketamine and MK-801 administration. Both agents dose-dependently reduced N40 amplitude, replicating previous studies of NMDA-receptor antagonists and consistent with EEG results observed in schizophrenia patients. Grand average saline ERP shown on left. Figures show mean + S.E.M. (**P<0.01, *** P<0.001)

Figure 2.6.6- SNR Evoked



Signal-to-noise ratio (SNR) as calculated by evoked power divided by baseline activity. Both ketamine and MK-801 dose-dependently decreased high-frequency SNR as defined by the ratio of evoked power divided by prestimulus baseline power. Figures show mean +/- S.E.M. (**P<0.01, *** P<0.001)

Chapter 3: NMDA antagonist MK-801 recreates auditory electrophysiological disruptions present in autism

3.1 Abstract

Autism is a highly disabling neurodevelopmental disorder characterized by social deficits, language impairment, and repetitive behaviors. There are few effective biological treatments for this disorder, partly due to the lack of translational biomarkers. However, recent data suggest that autism has reliable electrophysiological endophenotypes, along with evidence that some deficits may be caused by NMDA receptor (NMDAR) dysfunction. Similarly, the NMDAR antagonist MK-801 has been used in behavioral animal models of autism. Since MK801 has also been used as a model of schizophrenia, this paper examines the independent and overlapping ways in which MK-801 recreates the electrophysiogical changes present in both diseases. Mouse EEG was recorded in response to auditory stimuli after either vehicle or MK-801 and the dose-response relationship for each measure was determined. ERP component amplitude and latency analysis was performed along with time-frequency analysis of gamma frequency inter-trial coherence and evoked power. Evoked gamma power and ITC were decreased by MK-801 at the highest dose. P1 and N1 latency were increased in dose dependent fashion following MK-801. There were no amplitude changes in P1 or N1. MK801 caused alterations in evoked gamma activity, gamma ITC, P1 and N1 latency similar to findings in autism. These data provide evidence indicating that NMDAR dysfunction may contribute to deficits specific to autism and some that overlap with other disorders such as schizophrenia. Such observations could be important for developing novel

therapeutics, as electrophysiological endophenotypes are associated with functional measures and therefore may provide early biomarkers for efficacy in clinical trials.

3.2 Introduction

Autism spectrum disorders (ASD) are characterized by deficits in social, cognitive, and language function. Despite emerging evidence for genetic and molecular contributions to the disorder, behavioral measures remain the primary method of diagnosis. However, electrophysiological and neural biomarkers could potentially provide robust measures to quantify the degree of impairment and provide more focused guidance for treatment development (Edgar JC 2007). These measures have advantages in their translatability between mice and humans. For instance, human auditory evoked responses have a P50 (P1) and N100 (N1) which display similar morphology and drug responses as the mouse P20 (P1) and N40 (N1) (Amann, Gandal et al. 2010). These measures can also be examined using magnetoencephalography (MEG) which has magnetic analogues including the M50 and M100. In addition, there is evidence that gamma inter-trial coherence and evoked power are related to similar cognitive and sensory processes across species (Gandal, Edgar et al. 2011).

Several recent findings provide guidance for pre-clinical model development for ASD. First, genetic and postmortem studies indicate that the pathophysiology of ASD may include a component of NMDA receptor (NMDAR) disruption (Bangash, Park et al. 2011). Additional support for this idea has been provided by studies using MK-801 to recreate autism-like behavioral deficits in mice (Wu, Zou et al. 2005; Zou, Zhang et al. 2008). Studies in children with ASD using MEG have elucidated novel neural

biomarkers for the disorder which could be translated into animal EEG models (Roberts, Khan et al. 2010). Specifically, children with ASD show a delay in the M100 in superior temporal gyrus (STG), with this latency prolongation providing a high degree of accuracy for ASD classification. There is also evidence that neural synchrony is disturbed in ASD, particularly in the gamma-band (Gandal, Edgar et al. 2010). Such oscillations are important for sensory integration and functional connectivity, which are disrupted in ASD (Sohal, Zhang et al. 2009). There is also evidence that P1 and N1 latency are predictive of language impairment (Oram Cardy, Flagg et al. 2008; Roberts, Cannon et al. 2011).

The current study examines a potential pre-clinical model for several types of electrophysiological biomarkers, P1 and N1 latency prolongation, reduced gamma inter-trial coherence and gamma evoked power, following exposure to a NMDAR antagonist. We propose that quantifying the extent to which clinical ASD markers are recapitulated in this model will be a critical step towards evaluating its predictive validity for therapeutic development. This is important because NMDAR disruption models have also been closely linked to schizophrenia, another disabling but distinct disease. For example, gamma inter-trial coherence and evoked power disruptions have been shown in both diseases. In contrast, there is evidence that P1 and N1 latency is increased in ASD, while most studies in schizophrenia have shown no change in either latency (Hanlon, Miller et al. 2005). Alternatively, N1 amplitude is reduced in schizophrenia, but remains unaffected in ASD (Roberts, Khan et al. 2010; Mazhari, Price et al. 2011). Therefore, parsing which aspects of the model map to ASD versus schizophrenia are important for

determining the precise mechanisms of deficit, and corresponding therapeutic approaches in the different disorders.

The MK801 dose-response relationship to schizophrenia- and autism-like symptoms may be related to the degree of glutamate signaling disruption in each disorder. We observed that low levels of MK801 caused EEG changes that are more autism-like with delays in P1 and N1 latency accompanied by reduced gamma coherence and evoked power. Alternatively, high dose MK801 caused reductions in N1 amplitude, gamma inter-trial coherence, and evoked power, similar to schizophrenia (Saunders, Gandal et al. 2012). Such findings suggest a common glutamatergic circuit disruption in ASD and schizophrenia that varies in degree.

3.3 Methods

3.3.1 Subjects

Fifteen male C57BL/6Hsd (B6) mice were obtained from Jackson Laboratories at 7–8 weeks of age. All testing was conducted between 10 and 18 weeks of age. Mice were acclimated to the animal facility for at least 7 days before experimentation began and were housed four to five per cage until implantation of the recording electrode, after which they were single-housed for the remainder of the study. All subjects were maintained in a standard 12-h light/dark cycle with free access to food and water. Experiments were performed during the light phase between 9:00 AM and 4:00 PM. All protocols were conducted in accordance with University Laboratory Animal Resources (ULAR) guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania. All efforts were undertaken to minimize the number of animals used in the experiment and their suffering.

3.3.2 Drugs and doses

MK801 was obtained from Sigma-Aldrich (St. Louis, MO). MK801 was used at doses of 0.1, 0.2, 0.3, and 0.4 mg/kg i.p. All drugs and saline controls were administered 5-10 minutes prior to initiation of recording. MK801 has a half-life of two hours in rodents, ensuring consistent drug effect over a recording session.

3.3.3 EEG Recording

Electrode Implantation: At 12 weeks, animals underwent stereotaxic implantation of a stainless steel tripolar electrode assembly (Plastics1, Roanoke, Virginia)

as published (Gandal, Ehrlichman et al. 2008). Animals were anesthetized with isoflurane, and a low-impedance (5 k, 1000 Hz) macro-electrode was stereotaxically positioned between auditory cortex and auditory thalamus (1.8 mm posterior, 2.65 mm lateral, 2.75 mm deep relative to bregma) and referenced to frontal sinus. This configuration captures both early and late components of the auditory evoked potential (AEP), including the midlatency P20 (e.g., human P50/M50) and N40 (e.g., human N100/ M100) (Siegel, Connolly et al. 2003). The electrode pedestal was secured to the skull using ethyl cyanoacrylate (Loctite, Henkel, Germany) and dental cement (Ortho Jet, Lang Dental, Wheeling, Illinois). EEG was recorded 1 week later, as described below.

Electrophysiological Recordings: EEG was recorded with Micro1401 hardware and Spike6 software (CED, Cambridge, UK) as published previously (Ehrlichman, Gandal et al. 2009). A total of 250 white noise clicks were presented with a 4 s interstimulus interval at 85 dB.

3.3.4 Frequencies analyzed and methods

Electrophysiology Analysis: The amplitude and latency were calculated for P1 (defined as the most positive deflection between 10 and 40 ms) and N1 (defined as the most negative deflection between 30 and 200 ms). These regions are important because the characteristic positive and negative deflections of the EEG recording occur at approximately 40% the latency but similar morphology to the equivalent human components (Siegel, Connolly et al. 2003; Umbricht, Vyssotky et al. 2004). Therefore, the P20 and N40represent ERP deflections in mice analogous to the P50 and N100, respectively, in humans (Amann, Gandal et al. 2010).

Inter-trial coherence (ITC), evoked power (total power minus induced and baseline power), baseline (total power occurring -100 to -400 ms prestimulus), and baseline corrected total power (total power poststimulus minus baseline) values were calculated with Morlet wavelet decomposition using EEGLab (Delorme and Makeig 2004). In particular, single-trial epochs -250 and 750 ms relative to the auditory stimulus were extracted from the continuous data. For each epoch (trial), the ITC, baseline power, evoked power, and baseline corrected total power were calculated using 69 linearly-spaced frequencies from 12 to 80 Hz, with wavelet cycles increasing from 2 (at low frequencies) to 10 (at high frequencies). ITC is expressed as a unitless ratio between 0 and 1, where 1 represents complete phase synchrony at a given frequency and time across trials. Evoked, baseline, and baseline corrected total power was expressed in dB (10 $\log_{10}$). For each subject, the ITC and power measures were calculated in a window around the peak gamma response, defined as the region from 0-60 ms poststimulus and from 30-80 Hz. In addition, P1 and N1 latency was calculated in milliseconds after stimulus.

Statistical Analysis: Repeated measures ANOVAs were performed to investigate the effect of each pharmacologic agent on EEG measures. Significant effects were followed by Tukey's post-tests where appropriate.

3.4 Results

N1 latency changes had a significant positive association with dose of MK-801 (Fig 1 $P<0.0001$, $F(4,56)=8.657$). P1 latency changes had a significant positive association with dose of MK-801 (Fig 2 $P<0.0001$, $F(4,56)=9.855$). Gamma inter-trial coherence (Fig 3 $P<0.001$, $F(4,56)=5.738$) and evoked gamma power (Fig 3 $P<0.001$, $F(4,56)=5.372$) had a significant negative relationship with MK-801 dose.

After Tukey's post-test for multiple comparisons, there were significant differences in N1 latency between saline and 0.2, 0.3, or 0.4 mg/kg MK-801 conditions ($P<0.05$). There were significant differences in P1 latency between saline and 0.2, 0.3. 0.4 mg/kg MK-801 conditions. There were significant differences in gamma inter-trial coherence and evoked power between saline and 0.4 mg/kg MK-801 along with 0.1 mg/kg and 0.4 mg/kg MK-801 ($P<0.01$) conditions.

There were no significant associations between dose and P1 or N1 amplitude ($P=0.8553$, $P=0.1703$).

3.5 Discussion

The current study demonstrates that P1 latency, N1 latency, evoked gamma power and gamma inter-trial coherence changes following MK-801 mimic electrophysiological endophenotypes of ASD. N1 latency delays of 10% matched those found in ASD MEG studies which in turn were able to discriminate between patients and controls (Gandal, Edgar et al. 2010; Roberts, Khan et al. 2010). The ERP alterations following MK-801 were specific to P1 and N1 latency, with no amplitude changes observed for P1 or N1 components, also similar to findings in ASD. In addition, while latency changes are small, significant differences existed because measurements had very low variability, indicating tight physiological control of response latency. These electrophysiological data are consistent with previous studies in which MK-801 has been used to model the core deficits of ASD in rodents including stereotypies, social deficits and withdrawal (Wu, Zou et al. 2005; Zou, Zhang et al. 2008).

One limitation of the current study is that the disruptions in gamma inter-trial coherence and evoked power disruptions match recordings done in ASD, but are also present in schizophrenia. Similarly, stereotypies, social deficits and withdrawal created by MK-801 are also found in schizophrenia (Burket, Cannon et al.). Qualitative, but nonsignificant reduction of N1 amplitude was found in the current data set, consistent with the direction but not extent of changes in schizophrenia. Additionally, drugs like MK-801 and phencyclidine (PCP) can induce pre-pulse inhibition (PPI) deficits and hyperlocomotion at the doses tested, indicating that deficits are not selectively tied to social function. Thus, MK-801 is not specific as a model of ASD and reduced NMDAR-mediated glutamate transmission appears to model the overlap between ASD

and schizophrenia. It therefore may be helpful in determining the common, rather than distinguishing aspects of their pathophysiology.

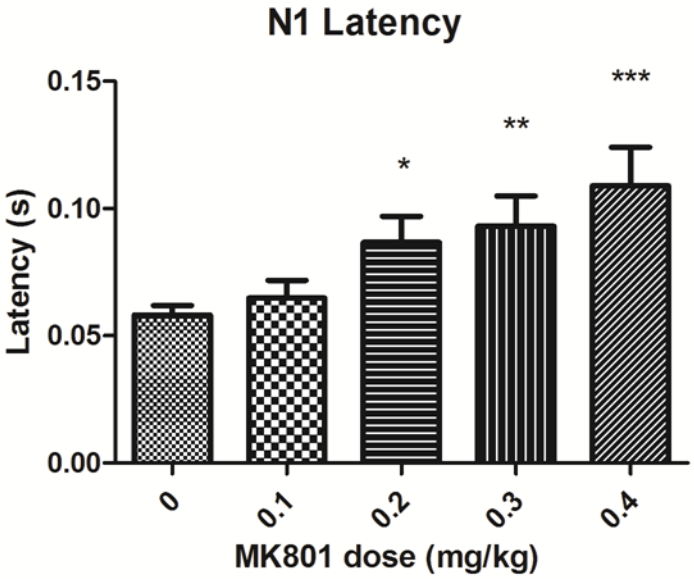
The pattern of endophenotypes that are shared by schizophrenia and ASD illustrate the relative extent of glutamatergic contributions towards the two disorders (Fig 4). There is evidence to suggest that both disorders have a glutamate component and several studies have shown a connection between autism-like symptoms and glutamate disruption. For example, Anti-NMDA receptor encephalitis has been reported to mimic late onset autism (Creten, van der Zwaan et al. 2011). Additionally, glutamate has been shown to be related to interpersonal skills (Montag, Schubert et al. 2008). This may indicate that there are localized areas of glutamatergic dysfunction in ASD that give rise to the change in electrophysiological response latencies, gamma coherence, and gamma evoked power without psychosis. In contrast, the glutamatergic dysfunction of schizophrenia may be the driver of psychosis, negative symptoms, and cognitive deficits. Another possible hypothesis is that low levels of glutamatergic disruption may be more ASD like, while greater dysfunction is more schizophrenia like. The qualitative but non-significant reduction in N1 at the MK-801 dosages used supports this view because higher doses have previously been reported to reduce N1 amplitude similar to those found in schizophrenia (Saunders, Gandal et al. 2012). However, P1 and N1 latency changes are typically not reported in schizophrenia, suggesting the electrophysiological disruptions may be from different domains. Given that patients with schizophrenia have EEG timing deficits, as measured by ITC, and that latency shifts in the current study and ASD are significant because their variability is extremely low, the reduction in

consistency of EEG response timing in schizophrenia may mask any changes in latency from being measured.

In summary, the current study indicates that acute pharmacological disruption of NMDAR signaling recapitulates several aspects of ASD, as well as those of schizophrenia. Furthermore, previous studies suggest that the electrophysiological endophenotypes induced by MK-801 are linked to functional measures in both ASD and schizophrenia, this model may be useful for testing novel therapeutics for language impairment among other targets (Oram Cardy, Flagg et al. 2008). For example, behavioral effects of NMDA disruption have been reversed with mGluR5 agonist MPEP. Similar findings in future electrophysiological and behavioral studies could suggest that reversal EEG deficits would provide an early indication that novel compounds could be effective in autism and/or schizophrenia.

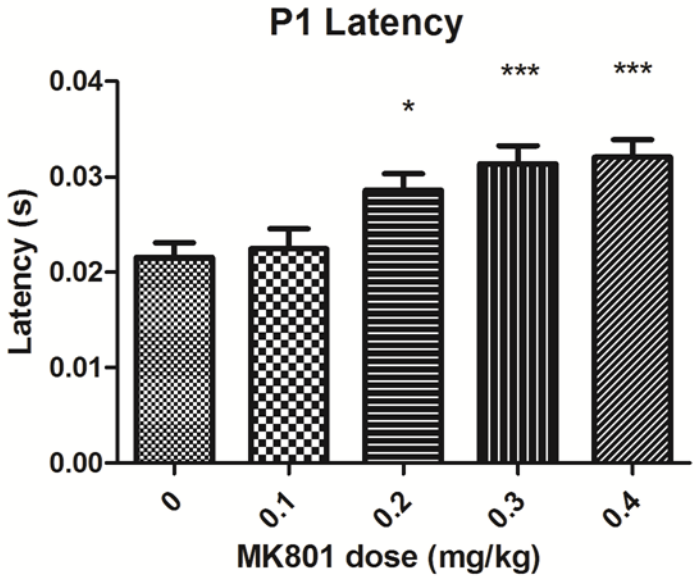
3.6 FIGURE LEGENDS

Figure 3.6.1- N1 Latency



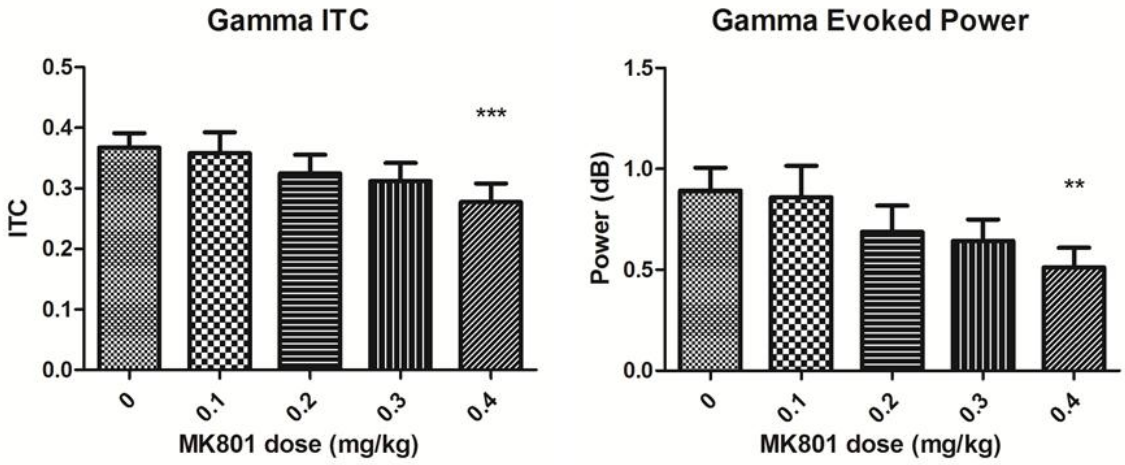
N1 latency was examined following MK-801 at 0.1, 0.2, 0.3, 0.4 mg/kg. There was a significant relationship between increasing MK-801dose and longer N1 latency. This increase in N1 latency is similar to the pattern found for the corresponding M100 in children with ASD using MEG. Figure shows mean + SEM (* $P<0.05$, ** $P<0.01$, *** $P<0.001$ after Tukey's test for multiple comparisons between saline and conditions).

Figure 3.6.2- P1 Latency



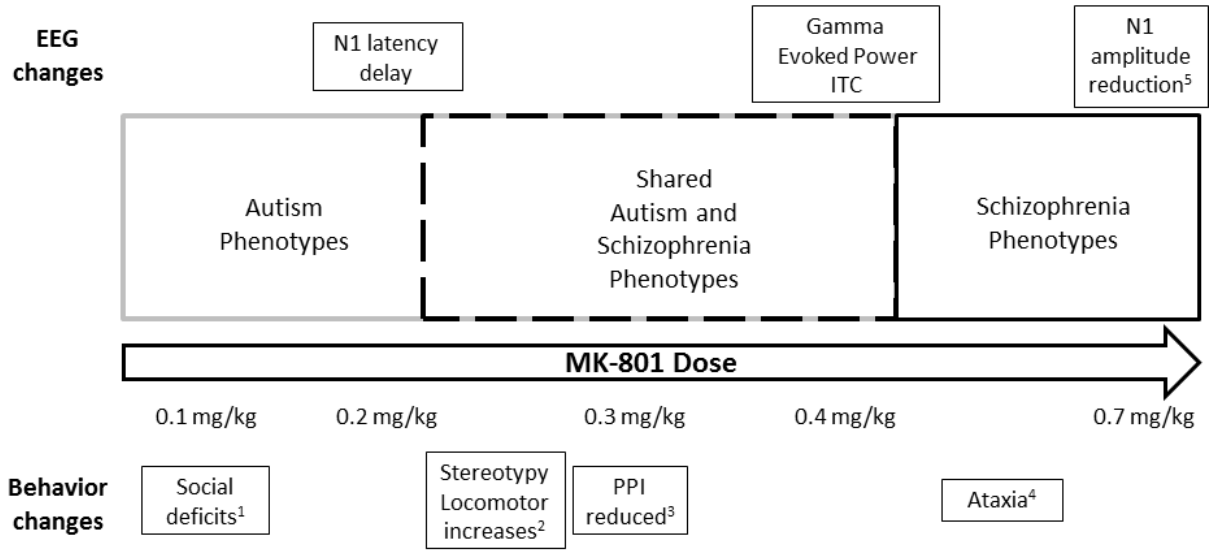
P1 latency was examined following MK-801 at 0.1, 0.2, 0.3, 0.4 mg/kg. There was a significant relationship between increasing MK-801dose and longer P1 latency. This increase in P1 latency is similar to the pattern found for the corresponding M50 in children with language impairment using MEG. Figure shows mean + SEM (* $P<0.05$, *** $P<0.001$ after Tukey's test for multiple comparisons between saline and conditions).

Figure 3.6.3- Gamma ITC and Evoked Power



Gamma ITC and evoked power was examined following exposure to MK-801 at 0.1, 0.2, 0.3, and 0.4 mg/kg. There was a significant relationship between increasing dose of MK-801and reduction in gamma ITC and evoked power. Thus, MK-801 recapitulates the overlapping pattern of reduced gamma ITC and evoked power found among patients with either ASD or schizophrenia. Figure shows mean + SEM (** P<0.01 *** P<0.01 after Tukey's test for multiple comparisons between saline and conditions).

Figure 3.6.4- Overlapping Symptoms and EEG Profile



Graphical illustration of the continuum of EEG and behavioral deficits along different doses of MK-801. The autism specific and overlapping with schizophrenia phenotypes are elucidated at the doses used in this study. (1) (Zou, Zhang et al. 2008) (2) (Ninan and Kulkarni 1998) (3) (Ralph-Williams, Lehmann-Masten et al. 2002) (4) (de Oliveira, Dall'Igna et al. 2005) (5) (Saunders, Gandal et al. 2012)

Chapter 4: Knockout of NMDA Receptors in Parvalbumin Interneurons Recreates Autism like Phenotypes

4.1 Abstract

Autism is a disabling neurodevelopmental disorder characterized by social deficits, language impairment, and repetitive behaviors with few effective treatments. New evidence suggests that autism has reliable electrophysiological endophenotypes, and that these measures may be caused by NMDA receptor (NMDAR) disruption on Parvalbumin (PV) containing interneurons. These findings could be used to create new translational biomarkers. Recent developments have allowed for cell-type selective knockout of NMDA receptors in order to examine the perturbations caused by disrupting specific circuits. This study examines several electrophysiological and behavioral measures disrupted in autism using a PV-selective reduction in NMDA R1 subunit. Mouse EEG was recorded in response to auditory stimuli. Event related potential (ERP) component amplitude and latency analysis, social testing and pre-mating ultrasonic vocalizations (USV) recordings were performed. Correlations were examined between the ERP latency and behavioral measures. The N1 ERP latency was delayed, sociability was reduced and mating USVs were impaired in PV-selective NR1 KO as compared to Wild type (WT) mice. There was a significant correlation between N1 latency and sociability, but not between N1 latency and premating USV power or T-maze performance. The increases in N1 latency, impaired sociability and reduced vocalizations in PV-selective NR1 KO mice mimic similar changes found in autism. Electrophysiological changes correlate to reduced sociability indicating the local circuit mechanisms controlling N1 latency may be utilized in social function. Therefore, we

propose that behavioral and electrophysiological alterations in PV-selective NR1 KO mice may serve as a useful model for therapeutic development in autism.

4.2 Introduction

Autism spectrum disorders (ASD) are characterized by deficits in social, cognitive, and language function. Despite sustained efforts, many of the deficits remain recalcitrant to treatment. One reason for the lack of effective treatments is the lack of predictive translational disease models. Therefore, having more predictive models that recreate the behavioral and electrophysiological changes present in autism would help aid development of new therapeutics.

Several recent findings provide guidance for pre-clinical model development for ASD. First, genetic and postmortem studies indicate that the pathophysiology of ASD may include a component of NMDA receptor (NMDAR) disruption (Bangash, Park et al. 2011). Studies in children with ASD using magnetoencephalography (MEG) have elucidated novel neural biomarkers for the disorder, which could be translated into animal EEG models (Roberts, Khan et al. 2010). Specifically, children with ASD show a delay in the M100 in superior temporal gyrus (STG), with this latency prolongation providing a high degree of accuracy for ASD classification. There is also evidence that these electrophysiological markers are predictive of language impairment (Oram Cardy, Flagg et al. 2008; Roberts, Cannon et al. 2011).

EEG measures could potentially provide behavior-independent biomarkers that quantify the degree of impairment along with providing more focused guidance for

treatment development (Edgar JC 2007). These measures have advantages in their translatability between mice and humans. For instance, human auditory evoked responses have a P50 (P1) and N100 (N1) which display similar morphology and drug responses as the mouse P20 (P1) and N40 (N1) (Amann, Gandal et al. 2010). These measures can also be examined using MEG, which has magnetic analogues including the M50 and M100.

In addition, animal models of social behaviors have been developed as translational platforms for examining human sociability. The social choice task, examines the preference of a test mouse for same sex mouse, as compared to an inanimate object. Given the reduced preference for social interactions in autism, this task attempts to model such behaviors. Additionally there are typically language impairments in patients with autism. In mice, one model of this language impairment is examining the characteristic patterns of 70 kHz ultrasonic pre-mating vocalizations. In this task, a male mouse that is exposed to a target female will vocalize at 70 kHz prior to mating.

The current study examines a potential pre-clinical model for characteristic autism-like alterations in N1 latency, sociability, and situation-appropriate (pre-mating) USVs. We propose that quantifying the extent to which clinical ASD markers are recapitulated in this model will be a critical step towards evaluating its predictive validity for therapeutic development. This is important because NMDAR disruption models have also been closely linked to schizophrenia, another disabling but distinct disease (Javitt, Jayachandra et al. 2000). Although NMDA receptor signaling is also implicated in autism, there are several distinctions in the patterns of EEG abnormalities in each

disorder. For example, there is consistent evidence that N1 latency is increased in ASD, while most studies in schizophrenia have shown no change in N1 latency (Hanlon, Miller et al. 2005). Alternatively, N1 amplitude is reduced in schizophrenia, but remains unaffected in ASD (Roberts, Khan et al. 2010; Mazhari, Price et al. 2011).

Previous studies suggest that systemic administration of NMDA receptor antagonists and non-specific genetic disruption of NMDA receptor signaling leads to an overall increase in EEG activity (Fitzgerald 2012). It has been widely proposed that this increase in activity represents a selective reduction of excitatory drive on interneurons (Gonzalez-Burgos and Lewis 2012). However, the cellular specificity of this response has not been directly tested. The current study tests the hypothesis that reduction of NMDA receptor activity only in PV containing interneurons will disrupt circuits involved in mid-latency evoked response regulation. Furthermore, the observed alterations will be related to behavioral phenotypes that are relevant to ASD.

4.3 Methods

4.3.1 Subjects

Homozygous Parvalbumin-cre/cre mice (B6;129P2-*Pvalb*$^{tm1(cre)Arbr}$/J) as well as a homozygous NMDA-Receptor1-flox/flox mice (B6.129S4-*Grin1*^{tm2Stl}/J) were purchased from The Jackson Laboratory (Bar Harbor, Maine). Homozygous parvalbumin-cre/cre (PV-cre/cre) mice were bred with homozygous NMDA-receptor1-flox/flox (NR1-fl/fl) mice. The offsprings, heterozygous PVcre/+;NR1-fl/+, were backcrossed with homozygous PV-cre/cre mice in order to obtain mice homozygous PV-cre/cre and heterozygous NR1-fl/+. The PV-cre/cre;NR1-fl/+ mice were then crossed with one another in order to obtain the following genotypes: PV-cre/cre;NR1fl/fl (referred to as PV-selective NR1 KO), PV-cre/cre;NR1-+/+ (referred to as wild type or WT). These mice have been characterized in a previous publication by Carlen *et al* (Carlen, Meletis et al. 2011). All testing was conducted between 10 and 18 weeks of age. Mice were acclimated to the animal facility for at least 7 days before experimentation began and were housed four to five per cage until implantation of the recording electrode, after which they were single-housed for the remainder of the study. All subjects were maintained in a standard 12-h light/dark cycle with free access to food and water. Experiments were performed during the light phase between 9:00 AM and 4:00 PM. All protocols were conducted in accordance with University Laboratory Animal Resources (ULAR) guidelines and were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Pennsylvania. All efforts were undertaken to minimize the number of animals used in the experiment and their suffering.

4.3.2 Behavioral Methods

Sociability Testing: We previously demonstrated that constitutive reduction of NMDAR1 and the NMDA receptor related protein Neuregulin 1 (NRG1) result in reduced social interactions (Ehrlichman, Luminais et al. 2009; Halene, Ehrlichman et al. 2009). As such, we and others have suggested that impaired NMDAR signaling may contribute to social deficits in autism. Social interactions were assessed as previously published (Ehrlichman, Luminais et al. 2009).

T-maze: PV-selective NR1 KO and WT mice were assessed for working memory function according to published methods for discrete T-maze (Deacon and Rawlins 2006). Data were collected using a 1 second delay, which has been shown to require hippocampal, as well as frontal contributions. Unpaired t-tests were used to compare effects between groups.

4.3.3 EEG Methods

Electrode Implantation: At 12 weeks of age, animals underwent stereotaxic implantation of a stainless steel tripolar electrode assembly (Plastics1, Roanoke, Virginia) as published (Gandal, Ehrlichman et al. 2008). Animals were anesthetized with isoflurane, and a low-impedance (5 k, 1000 Hz) macro-electrode was stereotaxically positioned between auditory cortex and auditory thalamus (1.8 mm posterior, 2.65 mm lateral, 2.75 mm deep relative to bregma) and referenced to frontal sinus. This configuration captures both early and late components of the auditory evoked potential (AEP), including the midlatency P1, (human P50/M50) and N1 (human N100/ M100) as published (Siegel, Connolly et al. 2003). The electrode pedestal was secured to the skull

using ethyl cyanoacrylate (Loctite, Henkel, Germany) and dental cement (Ortho Jet, Lang Dental, Wheeling, Illinois). EEG was recorded 1 week later, as described below.

Electrophysiological Recordings: EEG was recorded with Micro1401 hardware and Spike6 software (CED, Cambridge, UK) as published previously (Ehrlichman, Gandal et al. 2009). A total of 200 white noise clicks were presented with an 8 second interstimulus interval at 85 dB.

Electrophysiology Analysis: The amplitude and latency were calculated for P1 (defined as the most positive deflection between 10 and 30 ms) and the N1 (defined as the most negative deflection between 30 and 80 ms). These components of the mouse ERP recording occur at approximately 40% the latency but occur with similar morphology, pharmacology and psychophysiology to the equivalent human components (Siegel, Connolly et al. 2003; Umbricht, Vyssotky et al. 2004). Therefore, the P1, and N1 represent ERP deflections in mice analogous to the P50, and N100 in humans (Amann, Gandal et al. 2010). Amplitude was calculated relative to zero, and latency was calculated in milliseconds after stimulus onset.

4.3.4 USV Methods

USV Testing: Adult mice do not emit ultrasonic vocalizations spontaneously, but do so when placed in certain contexts involving social interaction (Scattoni, Crawley et al. 2009). When paired with a female mouse, male mice will emit “pre-mating” and “mating” vocalizations (Ricceri, Moles et al. 2007). During the pre-mating phase prior to mount, the male emits predominantly 70 kHz vocalizations.

For the adult mating paradigm, the test mouse was paired with an unfamiliar, sexual-receptive female wildtype mouse in a clean cage within a sound attenuated chamber for 5 minutes, as has been investigated in other mouse models of ASDs (Jamain, Radyushkin et al. 2008). For the mating task, USVs were recorded with an ultrasonic-range microphone suspended 10 cm above the cage.

USV Analysis: Data from ultrasonic vocalization paradigms were analyzed according to spectral power of the calls, as previously done (Maggio and Whitney 1985; White, Prasad et al. 1998; Gourbal, Barthelemy et al. 2004; Ricceri, Moles et al. 2007). Spectral analysis was done as follows. Vocalization data were analyzed in the frequency domain by computing the FFT of the entire epoch. Power was computed in dB and the sum averaged from 40-80 kHz. Significance was assessed with an unpaired t-test. To address the association between receptive auditory processing and expressive communicative functioning, dependent measures from ultrasonic vocalization paradigms were correlated with AEP indices within each mouse. Pearson R^2 correlations were calculated for AEP and USV measures. Correlations were assessed within electrophysiological and behavioral measures, assessing the relationship, for example, between adult mating vocalizations and N1 latency.

4.4 Results

The PV-selective NR1 KO mice showed a significant delay in N1 latency compare to WT mice (Figure 4.6.1, $P<0.001$). There was no reduction in N1 amplitude in the PV-selective NR1 KO compared to WT mice (Data not shown). The PV-selective NR1 KO mice showed significantly reduced sociability compared to WT mice (Figure 4.6.2, $P<0.01$). The PV-selective NR1 KO mice showed reduced pre-mating USV power compared to WT mice (Figure 4.6.3, 4.6.4. and 4.6.5, $P<0.05$). There was no difference between T-maze results for the two groups of subjects (Figure 4.6.6). There was a significant correlation between N1 latency and sociability across all mice (Figure 4.6.7 $R^2=0.38$ and $P<0.05$) but not between N1 latency and USV or T-maze measures (data not shown). There were no significant alterations for amplitude or latency of the P20 ERP component in PV-selective NR1 KO mice ($P>0.05$ for both measures, Data not shown).

4.5 Discussion

The current study demonstrates that the N1 latency, sociability, and pre-mating USV changes present in the PV-selective NR1 KO model mimic the phenotypes present in ASD. N1 latency delays matched those found in ASD MEG studies that in turn were able to discriminate between patients and controls (Gandal, Edgar et al. 2010; Roberts, Khan et al. 2010). Latency alterations in the PV-selective NR1 KO mice were specific to the N1, with no latency changes observed for the P1 component, also similar to findings in ASD. Additionally PV-selective NR1 KO mice showed selective behavioral deficits in non-mating social interactions and mating vocalizations without working memory deficits. Furthermore, there was a correlation between N1 latency and sociability, providing possible evidence of N1 latency as a biomarker for sociability.

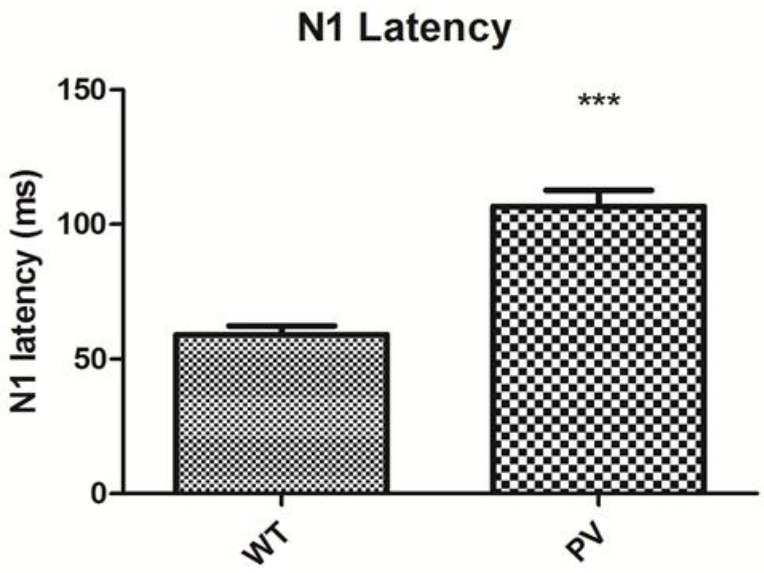
Integration of Sensory and Social function: The N1 latency delays in the current model of ASD could become an important translational biomarker, with evidence that circuits that control N1 latency are also utilized in control or initiation of social interactions. The relationship between N1 latency and social function present in mice with selective deficits in NMDAR-mediated signaling on PV interneurons could also provide insight into possible neural circuits involved with social function. Subsequent studies will determine whether normalization of N1 latency relates to increased social function. If there is evidence for N1 normalization coinciding with normalization of social function, N1 could provide a continuous biomarker for examining the severity of social deficits. However, if evidence indicate that it is only associated with the degree of initial impairment, then it may be still useful as an early diagnostic guide for disease

classification and initial treatment course in pre-language toddlers. This is useful because early consistent intervention may result in better outcomes and reduced disability (Dawson 2008).

Implications for Cognitive function: The current study does not provide direct evidence that NMDAR signaling on PV interneurons impact cognitive function. While there is a qualitative reduction in T-maze performance for the PV-selective KO mice, the variance in these subjects results in statistically similar performance. This may suggest that the current study lacked sufficient power to detect mild cognitive deficits in PV-selective NR1 KO mice. However, the sample size was sufficient to detect social deficits, suggesting a relatively selective deficit in this domain. Therefore, this work advances our understanding of autisms, and suggests that it may be a set of diseases with disrupted glutamate innervation of interneurons. Further, we propose that N1 latency has the potential to be an index of social function that may be used in development of novel therapeutics.

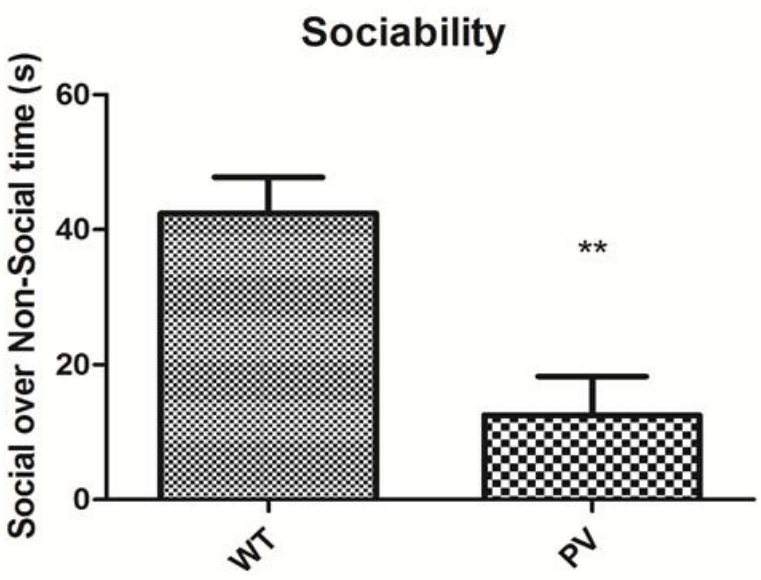
4.6 FIGURE LEGENDS

Figure 4.6.1- N1 Latency



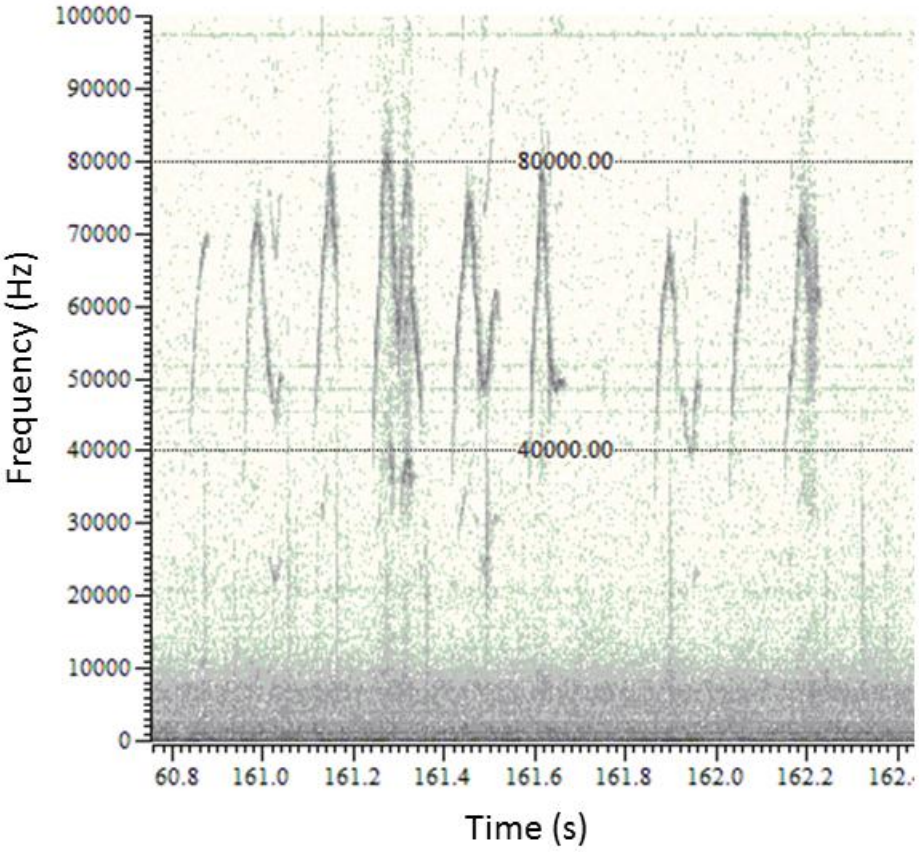
Effect of PV-selective NR1 knockout on N1 latency. PV-selective NR1 KO mice exhibit significantly delayed N1 latencies compared to WT mice. N1 latency delay is a robust marker of autism and combined with sociability specific deficits, indicates these mice have a constellation of autism like phenotypes. Figures show the mean + SEM. (*** $P<0.001$).

Figure 4.6.2- Sociability



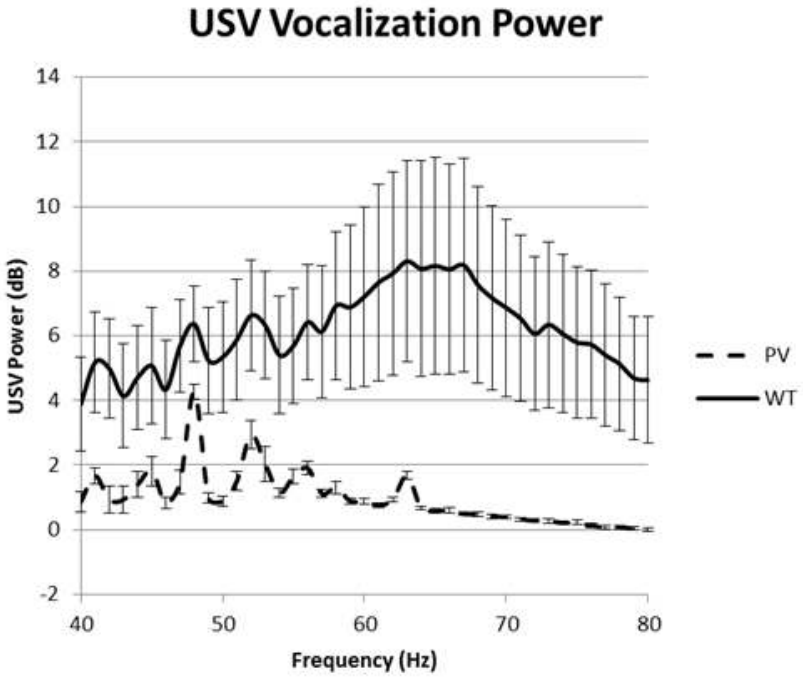
Effect of PV-selective NR1 knockout on Sociability. In a two cylinder social task, PV-selective NR1 KO mice showed significantly reduced preference for the social cylinder compared to WT mice. This indicates a reduction in sociability for the PV-selective NR1 KO mice compared to WT. Figures show the mean + SEM. (** $P<0.01$).

Figure 4.6.3- Raw Premating Vocalization Data



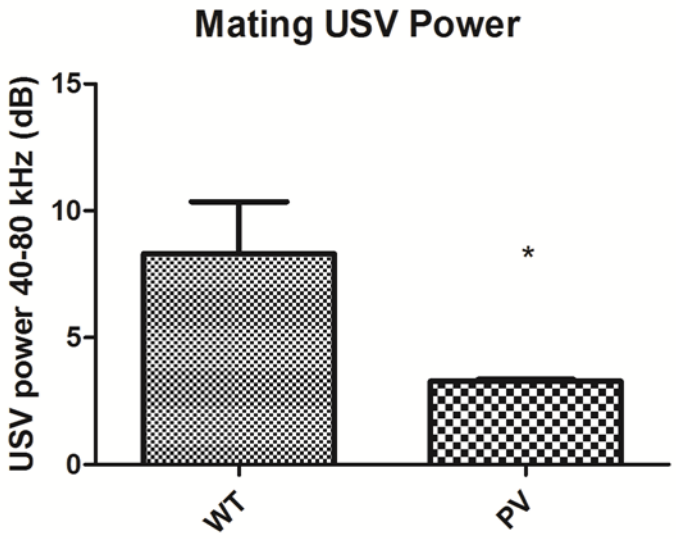
Example raw data for premating vocalizations. Example demonstrates spectrograms of calls for analysis of call frequency range.

Figure 4.6.4- USV Vocalization Power



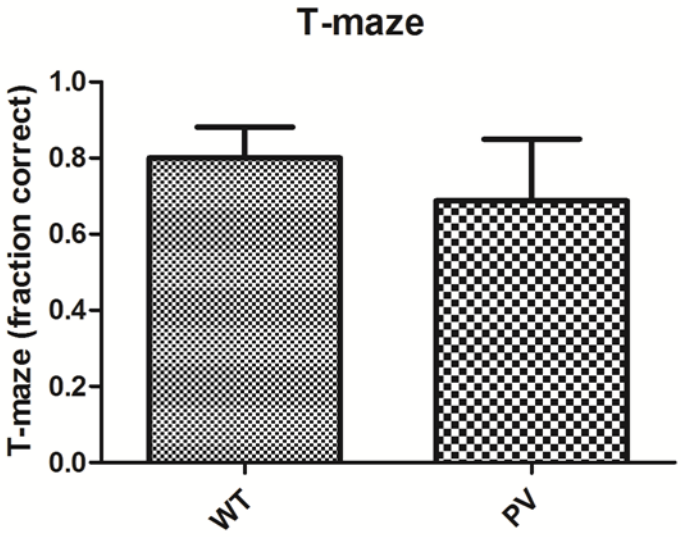
USV vocalization spectrum. PV-selective NR1 KO mice showed significantly reduced mating USVs in the presence of female mice as compared to WT mice.

Figure 4.6.5- Mating USV Power



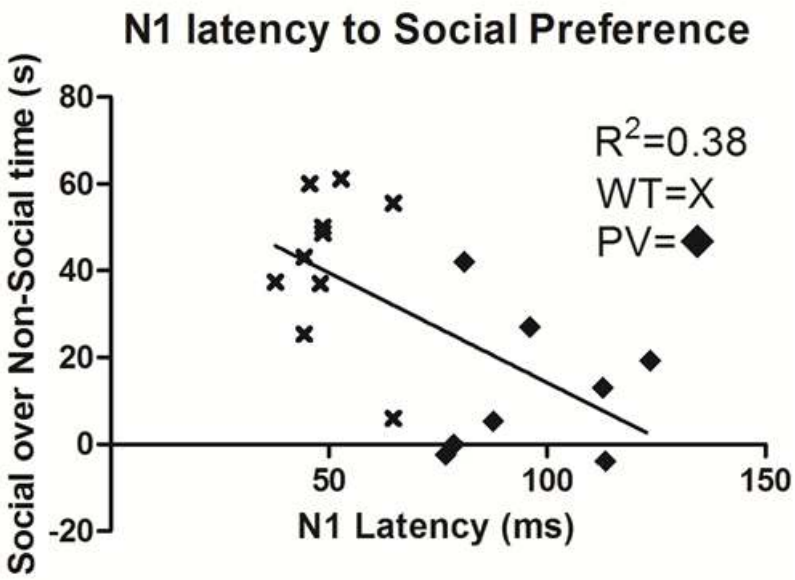
Effect of PV-selective NR1 knockout on USV pre-mating call power. PV-selective NR1 KO mice showed significantly reduced pre-mating USVs in the presence of female mice as compared to WT mice. This indicates that in both non-mating (Figure 4.6.2), and mating scenarios, PV-selective NR1 KO mice show reduced social interaction phenotypes compared to WT mice. Figures show the mean + SEM. (* $P<0.05$).

Figure 4.6.6- T-maze



Effect of PV-selective NR1 knockout on T-maze task performance. WT and PV-selective NR1 KO mice showed similar T-maze performance, indicating a lack of cognitive deficits. This indicates that alterations in NMDA receptor expression on PV interneurons do not lead to reductions in working memory. Figures show the mean + SEM.

Figure 4.6.7- N1 latency to Social Preference Correlation



Correlation for between N1 latency and Sociability among all mice. The combined PV-selective NR1 KO and WT groups show a correlation between N1 latency and social time (R^2=0.38, P<0.05). This supports the hypothesis that N1 latency is a possible marker for sociability, and the severity of autism symptoms. Furthermore, combined with selective socially deficits, and autism specific electrophysiological changes, this supports the PV interneuron NMDAR knockout mouse as a model for autism.

Chapter 5: Summary and Conclusions

5.1 Summary

This thesis explores several pre-clinical glutamate disruption mouse models that mimic the electrophysiological changes, negative and cognitive deficits present in autism and schizophrenia. Using glutamate disruption as a model we demonstrate how EEG can provide an independent measure to examine different aspects of circuit function. Furthermore, a main advantage of EEG techniques is that various signal processing methods allowing for much richer examination of new and existing EEG data frequency content such as the theta and gamma frequencies, which are thought to reflect separate brain processes. There are also works showing that different EEG measures have associations to disease symptoms(Olichney, Iragui et al. 1998; Gruzelier, Richardson et al. 1999; Light, Hsu et al. 2006; Evans, Gray et al. 2007).

The benefit of investigating glutamate disruption models with improved analyses is a more thorough understanding of the circuits disrupted in both diseases. For example, analyzing the data's inter-trial coherence gives a measure of circuit firing consistency, and gives a measure of the firing precision in the circuit. Inter-trial coherence has been related to working memory in subjects, providing evidence that loss of circuit timing consistency has functional outcomes (Light, Hsu et al. 2006). Additionally, examining evoked power provides data on the ability of the circuit to mount a response in different frequency bands, and in tandem with analyzing the baseline data can be combined to understand the signal to noise ratio of the circuit (Gandal, Edgar et al. 2011).

In this thesis, high levels of glutamate disruption via high dose NMDA antagonist administration shows evidence of mimicking schizophrenia like electrophysiological symptoms in gamma activity, and ERP components. Additionally, factor analysis was performed to understand the relationship between different circuit measures. The outcome was that the circuits that drive gamma band signal to noise were found to be disrupted independently of coherence, total power, and ERP amplitude circuits. Low level glutamate disruption using MK801 was used as a model for autism. This study revealed N1 latency prolongation, reduced gamma ITC, and evoked power in line with autism electrophysiological endophenotypes. Previous studies using low dose NMDA antagonist administration that have shown a plethora of negative and cognitive symptoms, including social impairments and working memory disruption (van der Staay, Rutten et al. ; Zou, Zhang et al. 2008). PV NMDA receptor knockdown mice were also explored, with observations showing lengthening of N1 latency in line with autism. Behavioral tests were performed in tandem, revealing the mice had selective sociability and pre-mating vocalization deficits, in the absence of statistically significant cognitive deficits. This suggests that the NMDA receptors on PV interneurons are selectively involved in EEG circuits involved in latency control and social function. As a whole, NMDAR disruption of PV interneurons appears to recreate the electrophysiological and behavioral phenotypes of negative symptoms. In addition, the electrophysiological and social interaction deficits appear to be caused by similar PV interneuron-based circuit disruptions, and this work provides evidence that in at least some cases the EEG is a direct index of function. This work provides evidence that EEG measures could be used

as specific measures of functional outcomes, with future work including clinical comparisons.

5.2 Future Directions

Patch studies examining parvalbumin interneuron excitability would be productive in showing what aspects of cellular function are changing when NMDA receptors are knocked out, to understand what the change is in the basic unit of circuit that causes system wide changes. Correlating the markers with further behavioral data, possibly comparing the dose response curves of EEG changes post NMDA antagonist disruption with similar curves in behavioral studies could help validate the markers as indices of function. Testing existing ineffective treatments that do not ameliorate symptoms as negative controls will be necessary to establish the markers validity as predictive markers. Further clinical study of the markers to understand their relationship to functional outcomes and limits as indices of symptoms, is required before they can be used as translational biomarkers for therapeutic development.

5.3 Conclusions

NMDAR disruption is a promising model of the negative and cognitive symptoms present in autism and schizophrenia based on accumulating evidence that NMDAR disruption underlies the negative and cognitive deficits present in autism and schizophrenia. Using these models and the techniques being established clinically and pre-clinically to examine the EEG endophenotypes, non-invasive, circuit-based, translational biomarkers are being developed to combat the diseases. While further development is required to fully utilize these measures in advancing clinical development, they are possible tools for understanding brain circuit dysfunction and aid in therapeutic development.

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