DEVELOPMENT OF A NEUROPHYSIOLOGICAL SENSORY GATING MODEL IN THE RAT TO AID IN THE PRECLINICAL IDENTIFICATION OF POSSIBLE TREATMENTS OF SENSORY FLOODING IN SCHIZOPHRENIA

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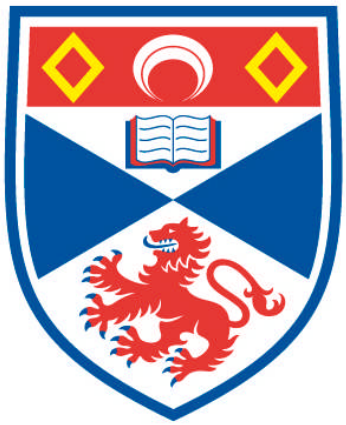

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Development of a neurophysiological sensory gating model in the rat to aid in the preclinical identification of possible treatments of sensory flooding in schizophrenia

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Acknowledgments

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I shall be telling this with a sigh
Somewhere ages and ages hence:
Two roads diverged in a wood, and I—
I took the one less traveled by,
And that has made all the difference.

-Robert Frost

Abbreviations

WHO – World Health Organization

UK, NHS – United Kingdom, National Health Services

CNS – Central nervous system

ERP – Event-related potential

EEG – Electroencephalography

P50 – Positive going electrical potential at 50msec

ISI – Inter-stimulus interval

MMN – Mismatch negativity

D-Amphetamine – Dextroamphetamine

I.P. – Inter peritoneal

Abstract

Objective

It has been widely hypothesized that a failure to properly filter, or 'gate', incoming auditory information occurs in schizophrenic patients. This can be observed in a reduced event-related potential response to the second of a pair of clicks, and this is referred to as P50 suppression after the name of the component that is attenuated to the second click. Our aim was to develop a model of gating failure in rats by measuring event-related potentials at different intervals between the clicks to validate that apparent gating in rats looks like P50 suppression in humans. We also sought to determine the relationship between two of the most commonly used assays of auditory gating: the mismatch negativity (an event-related potential evoked when a series of standard tones is followed by a *deviant* or oddball tone) and neurophysiological suppression in the double-click paradigm.

Methods

Male outbred Lister Hooded rats (N=8) were tested using electrophysiology to record P50-like event-related potentials (ERPs) to assess the overall competence of the rats' sensory gating. The rat N40 potential, thought to be equivalent to the human P50 potential, was measured after each of two 85-dBZ paired (conditioning and test) 0.1msec duration clicks separated by inter-stimulus intervals of 250msec, 500msec, 1000msec, and 2000msec presented at 10second inter-trial intervals. If the N40 potentials are similar to the human P50, then the magnitude of the potential of the second click should be attenuated (a measure of gating of the repetitive stimulus). Additionally, we were interested in whether the suppression of the rat N40 to the second click was similar to the suppression of the human P50 in being vulnerable to disruption by amphetamine. We measured N40 suppression in four conditions: pre-drug, after saline injection, after

dextroamphetamine injection, and post-drug. Finally, we correlated the N40 suppression with another neurophysiological measure of gating, the mismatch negativity.

Results

We determined that as the inter-stimulus interval increased in duration, the degree of N40 suppression decreased linearly. The administration of d-amphetamine had a non-significant effect, although our results indicate that further testing with a slightly larger sample size would be relevant. Finally, the relationship between the MMN and N40 suppression was weak, which is similar to the relationship between the human P50 suppression and mismatch negativity.

Conclusions

These data are a relevant initial step towards a neurophysiological sensory gating model to aid in preclinical identification of possible treatments of sensory flooding in schizophrenia. The characteristics of the rat N40 suppression match those of the human P50 suppression with the apparent exception of vulnerability to disruption by amphetamine.

Introduction

Background

The World Health Organization defines schizophrenia as a severe form of mental illness, affecting about 7 per thousand of the adult population, that interferes with a person's ability to recognize what is real, manage emotions, think clearly, make judgments and communicate (WHO, 2011). From a societal prospective, the annual costs associated with schizophrenia in the United States in 2002 was estimated to be $62.7 billion, with $22.7 billion excess direct health care cost and $32.4 billion excess indirect costs (Wu et al., 2005). The estimated total societal cost of schizophrenia in England was 6.7 billion pounds in 2004/05, with a direct cost for treatment of nearly 2 billion pounds on the public purse (Mangalore et al., 2007). The disorder typically afflicts patients beginning in their young adulthood and results in long-term hardship. In men, symptoms of schizophrenia usually begin between the ages of 15 and 30, while in women the onset of symptoms begins later, between the ages of 25 and 30 (UK NHS, 2011).

The clinical symptoms of schizophrenia are usually described as forming both a positive group and a negative group. The positive group represents a change in behavior or thoughts, including delusions and hallucinations; the negative group represents a withdrawal or deficiency of function observed in a healthy person, including reduced motivation, impaired emotional responses, social withdrawal and reduced speech content (UK NHS, 2011). Importantly, schizophrenic patients are also impaired in cognitive tests of memory and attention (O'Carroll, 1996). The degree of cognitive impairment in schizophrenia is associated with poor employment (McGurk & Meltzer, 2000) and low quality of life (Matsui et al., 2008). One of the major information processing impairments observed in schizophrenia is attributed to the failure to inhibit, or properly gate, the neuronal responses to incoming sensory information, notably auditory stimuli (Mayer et al., 2009). Although systematic behavioural and neurophysiological research of this symptom of schizophrenia is relatively new, Swiss psychiatrist Eugen Bleuler (famous

for coining the term “schizophrenia”) noted more than 100 years ago that in schizophrenia “the selectivity which normal attention ordinarily exercises among the sensory impressions can be reduced to zero so that almost everything is recorded that reaches the senses” (Bleuler, as quoted in Light & Braff, 2003). The inability to properly gate auditory stimuli and the resulting poor quality of life amongst schizophrenics (Huppert et al., 1999, Matsui et al., 2008) may be correlated to the greater rate of suicide and suicide attempts that occur in schizophrenic patients – it is estimated that 10% of people with schizophrenia will eventually commit suicide (Siris, 2001), a rate of suicide 1.4% higher than that recorded by individuals with severe depression (Bostwick et al., 2000).

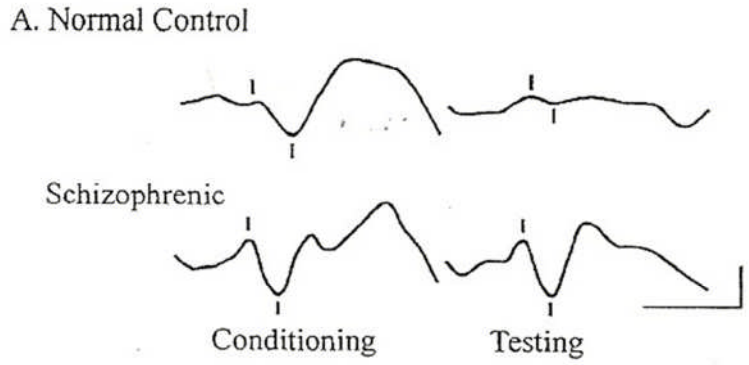
It has been suggested that a malfunctioning of central processing mechanisms of the central nervous system (the complex of nerve tissues that controls the activities of the body) underlie schizophrenics’ self-reported inability to filter or *gate* incoming sensory information (Judd et al., 1992; Waldo et al., 1994). Sensory gating refers to the central nervous system’s (CNS) ability to filter repetitive sensory inputs and can be measured in a task in which paired clicks are presented (Brenner et al., 2009). When two clicks are presented one after the other the brain will perform an action known as ‘gating’ in which the neural response to the second click is reduced compared the response to the first click. If inhibitory pathways are functioning normally, the response to the second click stimulus (test response) is diminished because of inhibitory or refractory mechanisms that are activated in response to the first stimulus (conditioning response) (Adler et al., 1986). There are two types of auditory gating performed by the brain: *gating out* refers to the neural reduction of incoming redundant input and *gating in* refers to the brain’s innate ability to respond when the stimulus changes by accentuating novel inputs (Gjini et al., 2010). The normal human brain is able to process and habituate auditory input, the failure of which is associated with the behavioural and cognitive disturbances observed in schizophrenia (Grunwald et al., 2003). In a related study, 50% of tested medicated schizophrenic patients but none of the control participants reported aversion to synthetic urban noise (Tregellas et al., 2009). Schizophrenic patients also exhibited hyperactivity of various brain areas evoked by synthetic urban noise (Tregellas et al., 2009).

The processing of sensory information can be observed through event-related potentials (ERPs), which are neurophysiological responses evoked by internal or external stimuli. ERPs are measured with electroencephalography (EEG), a procedure that measures electrical activity of the brain via surface electrodes on the scalp. The positions of the electrodes are used to maximise the chance of recording the rat equivalent of P50 suppression (the N40). One pair of active electrodes was implanted bilaterally over the frontoparietal cortex (our best guess for the homotopic location of the scalp electrode that is used to record the P50 in humans) and the second pair over the primary auditory cortex (which is thought to a dipole source for the P50 in humans). Using bilateral active electrodes allowed for post-recording averaging of the signal across both sides, thereby controlling for the variable position of the rat with respect to the speaker in the testing chamber.

There are numerous components of ERP that include both positive and negative electrical potentials recorded at different times after a stimulus is presented. The human P50 is a component of the ERP and is so named because it is a positive going electrical potential that peaks at about 50 milliseconds after the onset of an external stimulus. P50 amplitude has received much attention as one of the most reliable electrophysiological markers of impaired brain function observed in schizophrenia (Bramon et al., 2004, Sidse et al., 2003). A number of studies have indicated that the P50 gating deficit in schizophrenia is caused by an inability to properly inhibit the response to the second of two identical stimuli (Siegel et al., 1984, Nagamoto et al., 1996). For instance, when two clicks are presented in rapid succession, a 61% decrease in the P50 evoked by the second click is observed in normal controls but in contrast a weak 20% P50 suppression to the second click has been observed in schizophrenics, which has been interpreted as a deficit in the sensory gating that filters out background stimuli (Patterson et al., 2008). This inability to suppress the subsequent click is considered the physiological correlate of schizophrenia patients' perception of being flooded by sensory impressions (Bachmann et al., 2010) and is correlated with the poorer community functioning observed in schizophrenia

(Dickinson, 2002). The following figure is an example of the double-click paradigm in both normal control and schizophrenic patients:

A. Normal Control and Schizophrenic P50 waveforms in humans – scale bar: vertical 5μV, horizontal 50msec



(Moxon et al., 2003)

Figure 1: Recordings of human P50 auditory-evoked potentials of a normal subject and a schizophrenic subject to click stimuli presented in the conditioning-testing paradigm (Moxon et al., 2003). The amplitude of the P50 auditory-evoked potential recorded in response to the conditioning and test stimuli was measured between the two *tick marks* above and below each waveform. The amplitude of the conditioning response (C) is reflective of the first click in the double-click paradigm while the test response (T) is reflective of the second click. In this experiment, gating is measured as the ratio of the test response to the conditioning response (T/C ratio). A small T/C ratio indicates a high degree of gating, whereas a high T/C ratio indicates improper gating. On average, normal subjects have a T/C ratio of less than 20% while schizophrenic subjects have T/C ratios greater than 85% (Adler et al., 1982) – this is an average and no exact number has yet been determined. The normal control exhibits a small T/C ratio, therefore a high degree of gating. The schizophrenic exhibits a large T/C ratio, therefore a diminished degree of gating. These data represent responses to 3 trains of 32 pairs of clicks that were averaged. *Tick marks* below each evoked potential indicate the P50 wave; marked above indicate the point from which amplitude was measured. The P50 evoked response is the positive potential (downward direction is positive) recorded 50msec after the auditory click stimulus. The auditory stimulus occurs at the beginning of each trace. Horizontal calibration is 50msec; vertical is 2.5 mV, positive polarity down (Moxon et al., 2003).

The proposed research builds on previous attempts in rodents to model the human P50 (Miyazato et al., 1999b; de Bruin et al., 2001) and the P50 suppression deficit in schizophrenia (de Bruin et al., 1999; Miyazato et al., 1999a; Swerdlow et al., 2006; Broberg et al., 2010). These efforts have been only partially successful and no rodent model of the P50 suppression deficit in schizophrenia has been fully validated. Our approach was to record event-related potentials from rats exposed to a double-click behavioural protocol as similar as possible to those used to measure P50 suppression deficits in schizophrenia. The rats were not given anaesthesia during testing and were kept lucid and awake throughout – previous studies of sensory responses under anaesthesia revealed auditory responses in *motor* cortex and secondary *visual* cortex in the cat (Thompson et al., 1960). By definition, sensory processing is fundamentally abnormal during anaesthesia. We isolated features of the ERP waveform that have

characteristics similar to those found in humans, e.g., modulation by the inter-stimulus interval (ISI) between the two clicks. We also performed tests of the mismatch negativity (MMN), which is also impaired in schizophrenia (Umbricht et al., 2005), to determine the degree to which these measures of sensory gating in the rat are related. Finally we used acute drug treatment of d-amphetamine to induce impairments in the rats' suppression of the ERP to the second click, which will model deficits observed in human preclinical models of schizophrenia (in effect reproducing normal human symptoms of schizophrenia temporarily in rats).

Effect of inter-stimulus interval (ISI)

The known data for manipulating the inter-stimulus interval (ISI) in humans differs between healthy normal controls and schizophrenics. ISI manipulation is critical when observing sensory gating, as gating abnormalities in schizophrenic patients are limited to specific inter-stimulus intervals (Nagamoto et al., 1991). Recordings made at conditioning-testing intervals of 500msec, 150msec, and 75msec show good sensory gating at all three intervals in normal subjects, whereas the schizophrenics had conditioning-testing ratios indicative of poor gaiting of the auditory P50 wave at the 500msec and 150msec intervals, but had good sensory gating at the 75msec interval (Nagamoto et al., 1989). We compared the event related potential in the rat when S1 and S2 were separated by ISI's of 250msec, 500msec, 1000msec, and 2000msec. This was done to determine first if our animal model is consistent with the current animal data, and second to observe any statistical differences in auditory gating among the differing ISI's.

P50 vs. MMN

Before administering dextroamphetamine, thereby increasing dopaminergic neurotransmission broadly similar to that observed in schizophrenics, we used the drug naïve rats to perform a comparison between two common methods of measuring auditory gating: mismatch negativity (MMN) and the double-click paradigm to measure P50-like ERP suppression. MMN is an auditory ERP component that is elicited when a sequence

of repetitive standard sounds is interrupted by deviant "oddball" stimuli (infrequent stimuli that differ in duration or pitch from the more frequently presented stimuli) (Light et al., 2005). Physiologically, MMN is the first measurable brain response component that differentiates between frequent and deviant auditory stimuli (Naatanen et al., 1989). MMN has many advantages for cognitive neuroscience, including the study of the neural substrates of schizophrenia and its treatment (Naatanen, 2003). While several studies have examined MMN deficits in schizophrenic patients, little is known about the functional correlates and consequences of this, and other, early sensory information processing deficits, like P50 suppression (Light et al., 2005). So far little evidence for either genetic or environmental association between both ERP paradigms has been found, suggesting that MMN and P50 suppression serve to evaluate different brain information processing functions that may be mediated by distinct neurobiological mechanisms (Hall et al., 2006). At a superficial level the two might be related. The standard tones in MMN are repeated and one would expect suppression just like the P50 suppression to repeated clicks at short ISI's. The ISI for the MMN deviant tone, in contrast, is long and therefore might not be subject to gating out as much as the standard tones. Although there is research suggesting the P50 difference (P50d) suppression amplitude correlates significantly with MMN amplitude in humans (Ermutlu et al., 2007), their relationship has not been examined in rats.

Effect of d-amphetamine

The effects of d-amphetamine on P50 response have been shown to be strong in people (Light et al., 1999) and in two previous studies using rats; Alder et al. (1986) and Johnson et al. (1998) both observed inhibited P50 suppression after injecting rats with inter peritoneal (i.p.) amphetamine in physiological saline. This coincides with research from Breier *et al.* (1997) where data provided direct evidence of elevated amphetamine-induced synaptic dopamine concentrations in schizophrenia patients (Breier et al., 1997). Since amphetamine enhances dopamine transmission, and since dopamine plays a role in neuroplasticity, we expected amphetamine to disrupt P50-like suppression in the rat. We

will also explore medium term effects that outlast the acute effects of amphetamine (e.g., we test during and after amphetamine exposure).

Research Questions

(1) The P50-like suppression in rats will decrement with increasing ISI, as per normal humans; (2) P50-like suppression will be weakly correlated with other measures of impaired sensory gating in schizophrenia, such as the mismatch negativity; (3) that d-amphetamine will disrupt P50-like suppression in rats as it does in humans; and that (4) the d-amphetamine might have a lasting impact given dopamine's role in plasticity.

Methods

Subjects

Eight healthy male outbred Lister Hooded rats were obtained from Harlan Research Laboratory for use in neurophysiological recordings. The rats were kept in a room maintained at 21°C with 60% humidity and were housed in groups of four prior to surgery in NKP RC2R cages (1575cm^2 x 22cm) filled with sawdust, a chewing block, and a cardboard house. The rats were housed singly following surgery. *Ad libitum* access to a standard pelleted (9.5mm) diet (supplied by DBM Scotland) and fresh tap water was given. Lighting (390 lux) was cycled at 12-hr intervals (lights on at 7:00 a.m.) All rats were given ten days after arrival to the colony from the supplier to gain weight and adjust to the new surroundings before the experiment. The rats weighed 315-350 grams at the beginning of the experiment. Each rat was habituated to handling and weighing prior to surgery. In order to reduce neophobia to the food treats used in the behavioural testing procedure (see below), Honey Nut Cheerios were placed in the rats' home cages for several days. Throughout testing the rats' weight was monitored to confirm that postoperative weight loss never exceeded 15% of the free-feeding maximum body weight. The research reported here was conducted under Home Office Project Licence # 60/4040 and complied with the U.K. Scientific Procedures Act of 1986.

Procedure

Surgery

Each rat was weighed and placed into a sealed anaesthetic box. Isoflurane and oxygen were delivered via connected tubing into the box. The isoflurane concentration levels were increased from 1% to 5% over a seven-minute period and the oxygen (O_2) flow rate was 4L/min. The eyeblink and foot-withdrawal reflexes were checked as a way of

confirming the depth of anaesthesia. Once anaesthesia was confirmed the rat was secured in the stereotaxic frame. The rat's nose was placed into an anaesthetic delivering system and covered in Parafilm to form a seal with the anaesthetic mask. A 0.05mL injection of the nonsteroidal anti-inflammatory Rimadyl was injected subcutaneously into the rat's hindquarter to aid post-operative recovery. The isoflurane level was reduced from 5% to 3% and the oxygen level was reduced from 4L/min to 1.6L/min. Once the rat was secured in the ear bars, the skull was leveled so that bregma and lambda were at equal dorsoventral positions with respect to the stereotaxic frame. The dorsal surface of the rat's head was shaved; the skin around the intended incision was wiped down with 70% ethanol; and a 1-inch midline incision was made anterior to posterior across the skull. Muscle tissue overlying the dorsal temporal bone over primary auditory cortex was retracted gently by atraumatic blunt dissection using cotton-tipped swabs. Once this procedure was completed the Isoflurane level was reduced to 2% and the oxygen level was reduced to 1.4L/min.

Six stainless steel wires, diameter 0.200mm and length 25mm (PlasticsOne E363/1), were used as epidural electrodes, with four active electrodes (negative), a reference electrode (positive), and a ground electrode. The electrodes were connected to a plastic 7.7mm diameter, 6.9mm height multi-channel electrode pedestal (PlasticsOne MS363). Two active electrodes were placed on the frontoparietal junction; 1.6mm posterior and 1.2mm lateral from bregma. Two more active electrodes were placed bilaterally over the auditory cortices; 5mm posterior and 6mm ventral from bregma (no lateral component required because these electrodes were placed bilaterally on both sides of the skull). The reference electrode was placed over the posterior cerebellum on the midline 4.5mm posterior to lambda, and the ground electrode was placed between the reference electrode and the four active electrodes; 2mm posterior to lambda on the midline. Two structural screws were placed in front of the frontoparietal junction.

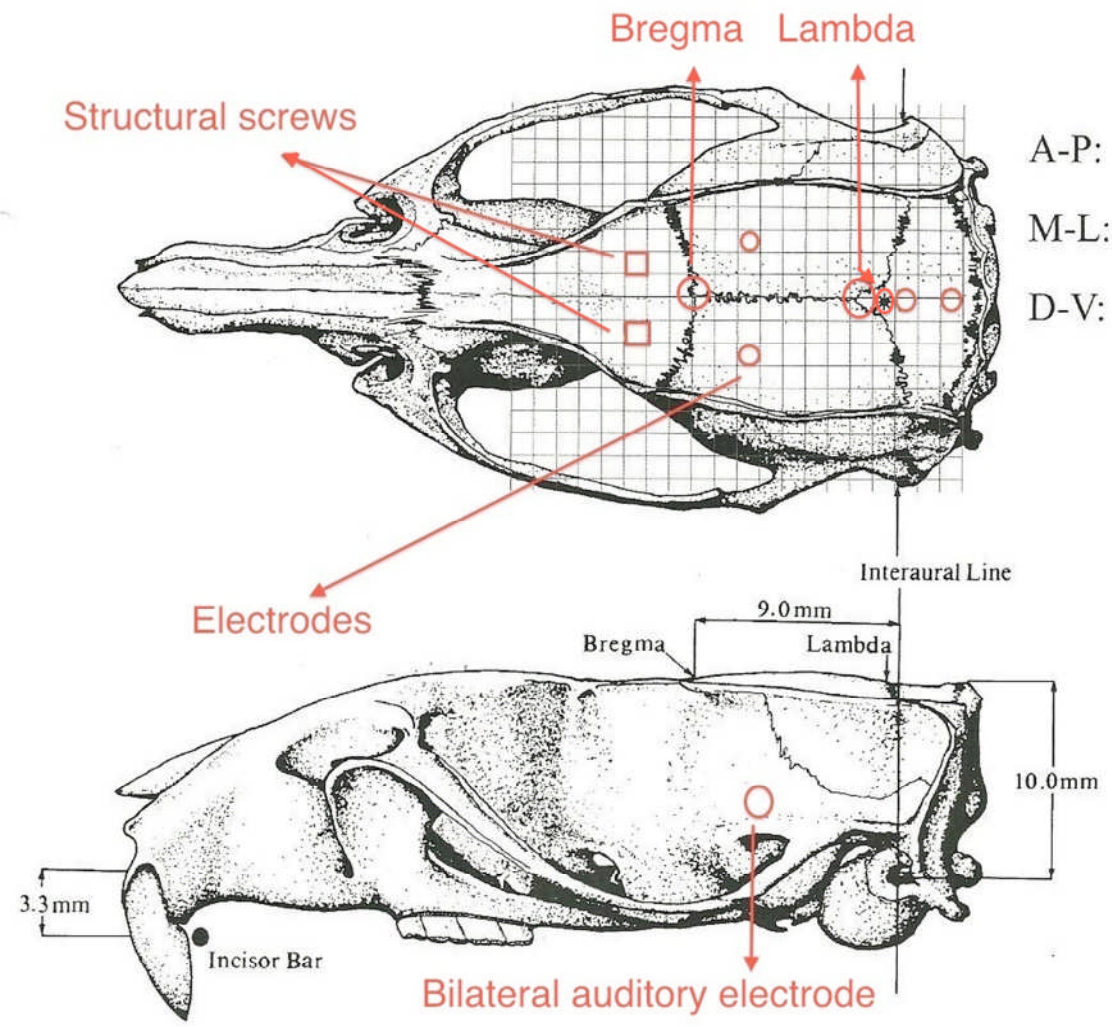


Figure 2: Placement of electrodes

The reference, ground, and two frontoparietal electrodes were fastened via 0-80 threaded hex stainless steel head screws, 6.35mm in length (Travers Tool Co., Flushing, NY, USA). The two auditory electrodes were fastened with 0-80 threaded nylon pan head machine screws, approximately 1mm in length (Micro Fasteners, Lebanon, NJ, USA). Simplex Rapid Powder (shade S28/1 Pink) was mixed with Simplex Rapid Liquid to form a self-cure (autopolymerising) acrylic resin, which was used to cement the headcap onto the rat's skull. Two Ethicon-coated Vicryl sutures were placed behind the headcap; one was placed in front of the headcap. The rat was then placed in a postoperative box set on top of a heating pad, and was monitored until conscious. The rats were given seven days of recovery, during which they were gently handled daily, before

neurophysiological recording began. Metacam (Meloxicam;1 drop per 50 grams of the rat's body weight) was administered for two days post-operatively in order to reduce inflammation. Drawing adapted from the Paxinos stereotaxic atlas.

Neurophysiological procedures

The rats were tested in a large melamine sound-attenuating cabinet (S.A.C, Med Associates ENV-016M-025) in which there was an inner modular Perspex test chamber (Med Associates ENV-007-CT). Dimensions of the sound-attenuating cabinet are:
The inner chamber is the Med Associates ENV-007.

Dimensions:

- Base: 21.0" L x 13.75" W x 0.50" H (53.3 cm x 34.9 cm x 1.3 cm)
- Interior: 12.0" L x 9.5" W x 11.5" H (30.5 cm x 24.1 cm x 29.2 cm)
- Exterior: 12.5" L x 10.0" W x 13.5" H (31.8 cm x 25.4 cm x 34.3 cm

The outer cubicle is the Med Associates ENV-018MD-W.

Dimensions**:**

- Interior: 22.0" W x 22.0" H x 16.0" D (55.9 cm x 55.9 cm x 40.6 cm)
- Exterior: 25.0" W x 23.5" H x 17.5" D (63.5 cm x 59.7 cm x 44.4 cm)
- Walls: 0.75" (1.9 cm) thick
- Window: 7.5" x 8.0" (19.0 cm x 20.3 cm)

At the beginning of each session the rat was placed in the inner testing chamber and offered a Honey Nut Cheerio. When the rat ate the food treat we assumed that its stress levels were sufficiently low to allow the connection of the EEG apparatus via a shielded cable (PlasticsOne 363-363 W/ MESH) and electrical six-channel commutator with double brushings **(**PlasticsOne SL6C). Additional food treats were given throughout each session to maintain the alertness of the rats. The EEG signal was recorded through a cable (PlasticsOne 363-441/6) between the commutator and pre-amplifier. The amplifier cable was attached via a swivel assembly to the top of the recording chamber to allow for full freedom of movement. The voltage from each electrode was amplified (gain 20,000x, inverted) using a NL844 pre-amplifier (Digitimer Neurolog system) and processed

through high-and low-pass Neurolog filters (model NL900D) to attenuate frequencies <1Hz and >200Hz, and digitally filtered to remove 50Hz AC electrical noise using four Quest Scientific 'Humbugs'. A Tektronix (TDS 224) four-channel digital real-time oscilloscope was used to monitor EEG signals online. A Cambridge Electronic Design (CED) data acquisition system was used to digitize the filtered EEG signals on the four channels at 500Hz per channel, and CED Spike2 version 7.0x software was used to collect and analyze the event related potentials (ERP). Signals were averaged across hemispheres to reduce error.

Auditory stimulation procedures

To acquire the P50 response, EEG was recorded in trials in which pairs of clicks were presented. The CED system was used to generate the waveform of each click via its digital to analogue converter (digital to analogue output frequency of 50kHz), and the resulting 0-5V output was fed into an interface box (School of Psychology Workshop, University of St. Andrews) and then an audio amplifier (Kramer Electronics NL844). The tweeter used to present clicks (Med Associates ENV-224BM) was positioned in the upper far right-hand corner of the inner testing chamber. The loudness of the clicks, the temporal gap between them (inter-stimulus intervals), the inter-trial interval, and the duration of the clicks were controlled via Spike2 software controlling the CED system. This allowed for performing parametric manipulations to establish the sensitivity of the putative rat P50 suppression to variables that influence human P50 suppression. The loudness of the clicks was measured with a calibrated microphone (iAudioInterface, StudioSix Digital) and the Energy Time Curve (ETC) routine of AudioTools (StudioSix Digital) on an Apple iPod Touch.

In the initial experiment in which the rats were drug-naïve, a series of 85-dBZ paired 0.1msec duration clicks separated by inter-stimulus intervals (ISI) of 250msec, 500msec, 1000msec, and 2000msec were presented at 10second inter-trial intervals (ITI). Each recording session was 30 minutes; with a range of 112-218 click pairs per session. Three sessions using an ISI of 500msec were conducted to determine the reliability of the

measurements in the configuration used typically in human P50 research. We also conducted one session for each of the remaining ISI's to determine if the rat P50 suppression is reduced at longer ISI's as it is in humans.

Drug administration

Seven of our drug-naïve rats (one was euthanized due to electrode malfunction) were used to validate the P50 model. All rats were tested for normal P50 without injections using our auditory stimulation procedures with a 500 msec ISI as the baseline. After observing a normal P50, half of the animals (3/7) were given saline solution and half (4/7) were given dextroamphetamine sulphate (the animals injected with saline were later given d-amphetamine, and the animals that received the d-amphetamine were later given saline once behavioural effects had dissipated over a 48-hour period). The AMPH dates from 2004. However, it was stored in a cool, dark, dry drugs cabinet and there is no expiry date listed on the bottle. The shelf life of amphetamine (90% potency) is about 18 years @ 5 °C (which admittedly is a lower temperature than room temperature ~19.5 °C in the histology lab where the drugs are stored). The pH of the saline and d-amphetamine solutions was adjusted to 7.4 as per Johnson *et al.* (1998). In order to prevent severe stereotypy, a low dose 0.5mg/kg of d-amphetamine was administered and testing began 30-60 minutes post-injection (during peak amphetamine effects as observed by Johnson et al. (1998)). Neurophysiological and auditory stimulation procedures were performed as described above. P50 testing (ISI 500msec) without injection was later performed (48-hours post injection) to determine lasting effects, if any, from the administration of d-amphetamine.

Data analysis

The data analysis routines determined the size of the putative rat P50 (the rat N40) for the first and second click. We've used difference waveforms to reveal the suppression (1^{st} click – 2^{nd} click) as commonly reported in human behavioural procedures. Microsoft

Excel 2011 pivot tables were used to determine local minima and maxima within points defined by peaks of the waveforms comparing the responses of S2 to S1. Excel was also used to visualize event-related potentials evoked by the clicks, as well as calculate the difference waveforms by subtracting on a point-by-point basis the mean voltage of the second click from the mean voltage of the first click. PASWStatistics18.0 was used to analyze (t-tests, repeated measures ANOVA) the characteristics of the local maxima and minima revealed by analysis in Excel. Effects of different ISI's for the P50 were measured both for auditory and frontal electrode sites. The same analyses was used for testing the variant ISI's, the MMN vs paired clicks, as well as the drug trials. We used G*Power 3.13 (Faul et al., 2007,2009; http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/download-and-register, accessed 22/07/11) to calculate the sample size required to detect the nonsignificant results in the d-amphetamine experiment, using a specified statistical power of 0.80 and a specified critical p-value of 0.05.

Results

Raw auditory / frontal event-related potentials (ERP's) for S_1 and S_2 at 500msec ISI

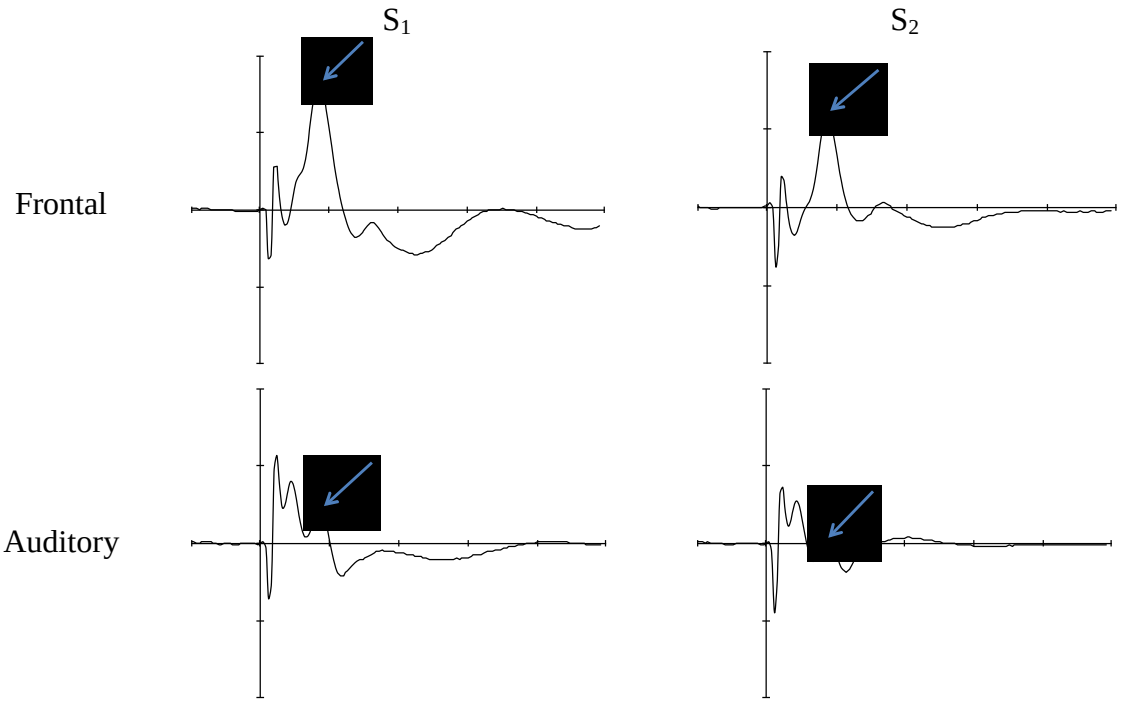


Figure 3: Effects of the first click (S_1) and second click (S_2) in the double-click paradigm from signals at both frontal-parietal and auditory recording sites (with a 0.5-s interval). The amplitude (tick marks of 75μV) of the evoked potential is represented on the *ordinate* (note that in all figures the voltage is inverted). The time (from 50msec prior to click onset to 250msec afterward; 50msec per tick) after the click is represented on the *abscissa*. N=8.

The figure above illustrates the significant decrease of auditory gating for both frontal and auditory ERP's. S_1 signifies the onset of the first click in the double-click paradigm (ISI of 500msec), and S_2 represents the second click in the series. The N40 (the putative functional equivalent of the human P50) amplitude for both the frontal and auditory ERP's are depicted with arrows. The apparent decrease in amplitude (μV) for both frontal and auditory ERP's indicates a normal level of gating by the rats.

We cannot be certain what the traces before and after the N40 are, however, it is likely either that these are brainstem auditory responses picked up by the reference electrode (along the pathway leading from the cochlea to the cortex) or they might be due to EMG from an orienting reflex of the pinnae (the cervicoauricular reflex). Such orienting of the ears at a very early time after sounds has been noted before (Li et al., 1996).

In general we used 1-tailed analysis because we had an a priori reason to believe that the differences we would observe would be in a particular direction. The reason was based on previous research. A 2-tailed test checks for *any* difference (e.g., mean 1 < mean 2 *or* mean 1 > mean 2). The 1-tailed test looks at only one specified direction (e.g. mean 1 > mean 2 *only*).

S_1-S_2 difference waveforms at the standard 500msec ISI

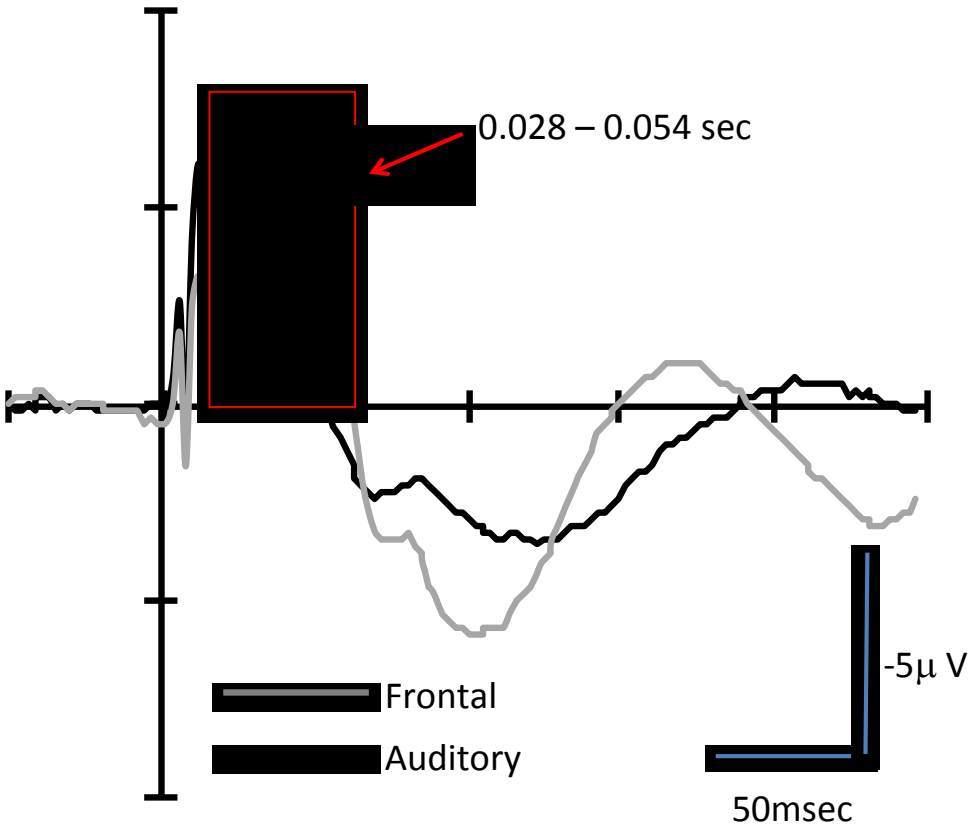


Figure 4: Difference waveforms ($S_1 – S_2$) at frontal-parietal (grey) and auditory (black) recording sites (with a 0.5-s interval). The amplitude (tick marks for the difference waveform are 5μV) of the evoked potential is represented on the *ordinate* (note in these figures the voltage is inverted). The time (50 μsec/tick) after the stimulus onset is represented on the *abscissa*. The $S_1 –S_2$ difference at the latency of the N40 response in our data was measured as the local minimum between 0.028 – 0.054 seconds post-stimulus (see Figure 1 above for ERP waveforms that indicate position of N40). Previous authors have claimed an equivalence of the human P50 and the rat N40 (Alder et al., 1986). N=8

To calculate the degree of auditory gating performed at both frontal and auditory recording locations (ISI of 500msec), it was necessary to calculate the difference waveforms ($S_1 – S_2$). While some laboratory groups calculate S_2/S_1 ratios, we decided to calculate the difference by measuring $S_1 – S_2$ difference waveforms. We used the epoch from 0.028 – 0.054msec because that is where we measured the lowest voltage during the epoch and we believed that was where the rat N40 is in our data. The amplitudes of each ERP signify the degree of gating performed by the rats, thus we see a greater degree of gating in the frontal recording sites than the auditory. However, at this particular ISI of 500msec, a strong degree of gating is seen.

Difference waveforms ($S_1 – S_2$) at varying ISIs

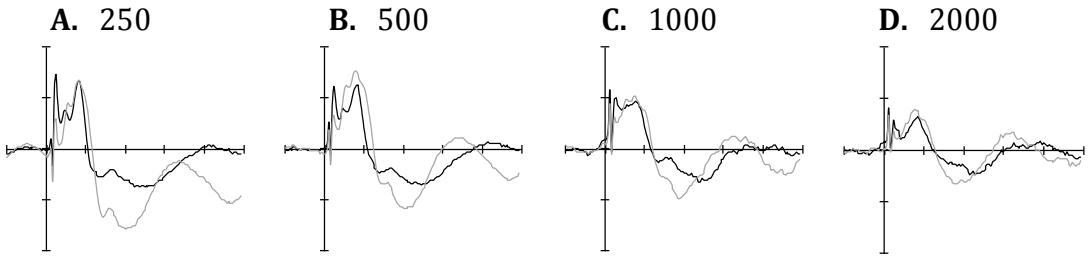


Figure 5: The difference (ΔV) between S_1 and S_2 at the latency of the N40 decreases as the ISI increases. The amplitude (Δ 5μV ticks) of the evoked potential is represented on the *ordinate* (note in these figures the voltage is inverted). Peaks above the abscissa indicate the mean voltage evoked by S_1 was more negative than S_2. The time (units per click) after the click is represented on the *abscissa.* As the ISI increases in duration a marked decrease (inverse response) in the difference amplitude is observed (Frontal, $F_{(3,21)}=6.89$, $p<0.01$; Auditory, $F_{(3,21)}=14.98$, $p<0.001$). These results indicate that as the inter-stimulus interval decrease in increases, the auditory gating decreases. This agrees with previous studies in humans (Nagamoto et al., 1989) that indicate a similar decrease in auditory gating as the ISI becomes larger. N=8

The figure above depicts the difference waveforms (ΔμV) for all four ISI's tested. There is an observed trend as the inter-stimulus interval (ISI) increases in duration, the amount

of auditory gating (amplitude of the difference waveform) decreases. The decrease in auditory gating is small between 250msec and 500msec, but becomes larger at 1000msec. At 2000msec only a small degree of auditory gating is observed at both frontal and auditory ERP's.

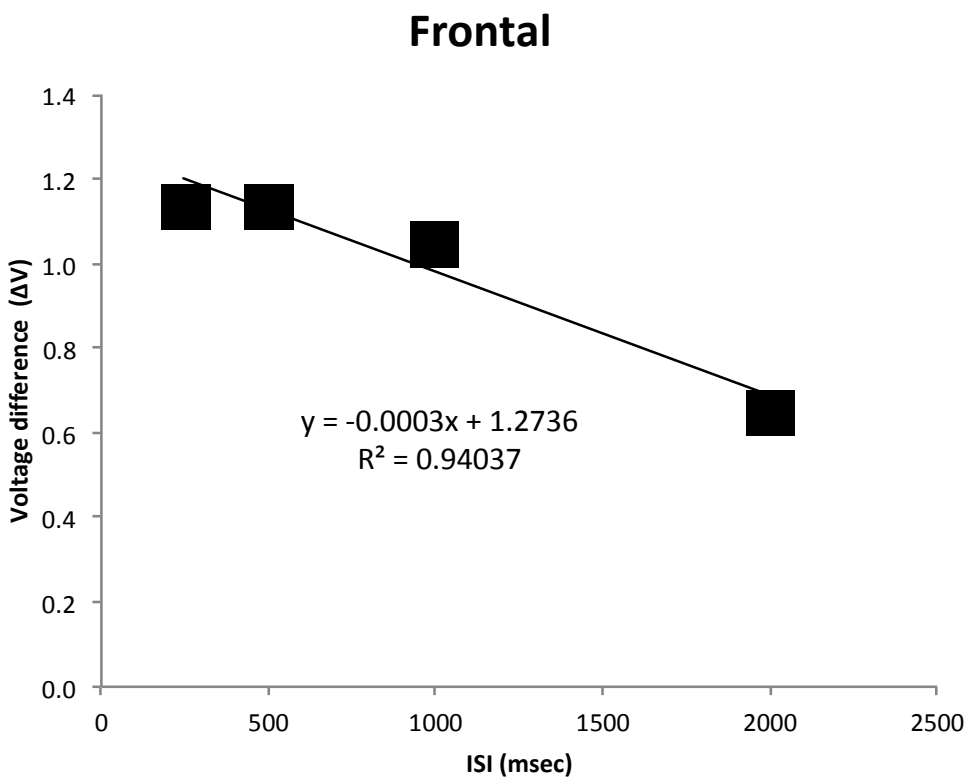


Figure 6: Linear model of ISI (msec) vs. mean voltage difference (ΔV) for frontal electrodes. As the ISI increases, the voltage difference decreases. The R^2 value is very high (close to 1), indicating a very systematic trend in this graph of frontal analysis ($p<0.05$, one-tailed). For each point the data were averaged from N=8 rats

The figure above depicts the difference waveform of the frontal recording electrodes as the ISI increases. The linear trend is strong, since the R^2 value is very close to 1. As the ISI (msec) increases in duration, the voltage difference (ΔV) for the frontal ERP decreases, indicating an indirect relationship between ISI duration and auditory gating at the frontal recording sites.

Refer to figure 4 – for each animal the voltage difference between S1 and S2 was determined for each time point from -50 to +250 msec relative to the stimulus. Then the most negative point in this difference waveform from +28-54 msec was found. This local

minimum was averaged across rats to provide the y-axis value for each point in Fig. 6 and Fig. 7.

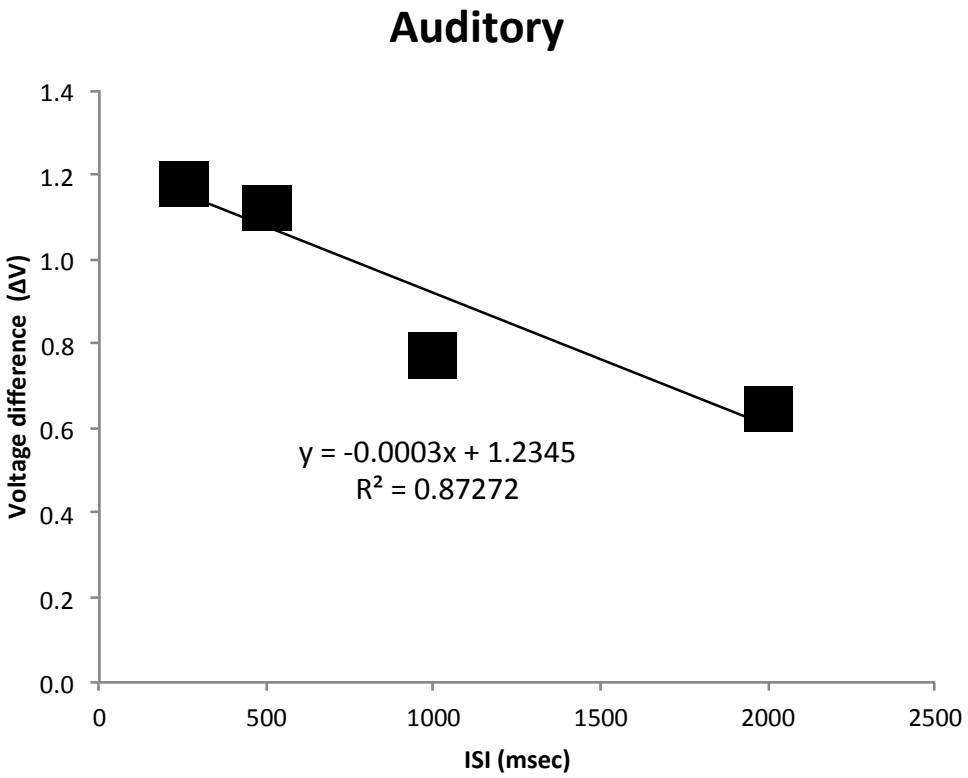


Figure 7: Linear model of ISI (msec) vs. mean voltage difference (ΔV) for auditory electrodes. As the ISI increases, the voltage difference decreases (concurrent with the data from frontal electrodes). The R^2 value is high (close to 1), indicating a strong trend ($p<0.05$, one-tailed). For each point the data were averaged from N=8 rats.

The figure above depicts N40 suppression dissipating as the ISI increases. The linear trend mirrors the strength observed in the data from the frontal electrodes. As the ISI (msec) increases in duration, the voltage difference (ΔV) for the auditory ERP decreases, indicating an indirect relationship between ISI duration and auditory gating at the auditory recording sites.

MMN difference waveforms

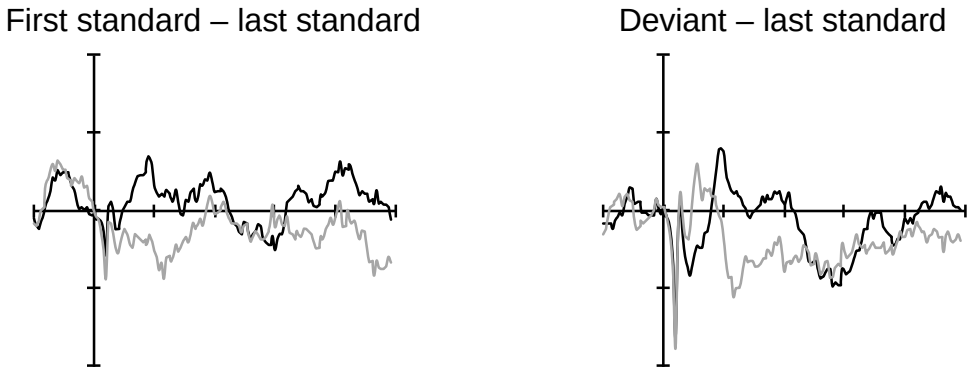


Figure 8: Mismatch-negativity difference waveforms for the frontal-parietal (grey) and auditory (black) recording sites. The amplitude (Δ 5V ticks) of the difference waveform is represented on the *ordinate* (note in these figures the voltage is inverted so that positive values indicate the ERP of interest was more negative than the last standard tone). The time (50 msec ticks) relative to the stimulus onset is represented on the *abscissa.* The left hand figure is a measure of first standard tone subtracted by the last standard tone – this is a control comparison to account for simple habituation. The right hand figure depicts the deviant tone subtracted by the last standard tone, which is our operational definition of the MMN. The early downward spike, which represents a positive ΔV, probably represents the cervicoauricular reflex. N=7

The figure above depicts two different MMN difference waveforms. The figure on the left is the first standard tone – the last standard tone. When performing the MMN, ten tones are presented in quick succession before the eleventh, *deviant* tone. By subtracting the last standard tone from the first standard tone allows us to account for simple habituation by the rats before the deviant tone. What we are most interested in is the deviant, or oddball, tone minus the last standard tone. It is at this point where the rat's ability to gate new incoming auditory stimuli is observed.

Correlations

Pearson's *r* *p*-value (*n*=7)	P50 Auditory A1 28-54 msec	P50 Frontal F1 28-54 msec	MMN Frontal F1 4-14 msec	MMN Frontal F2 42-92 msec	MMN Frontal F3 92-190 msec	MMN Auditory A1 4-14 msec	MMN Auditory A2 48-140 msec
P50 Auditory A1 28-54 msec		**0.410** 0.361	**0.450** 0.311	**-0.42** 0.056	**0.503** 0.250	**0.146** 0.540	**-0.651** 0.113
P50 Frontal F1 28-54 msec			**0.306** 0.504	**-0.51** 0.181	**-0.029** 0.951	**0.269** 0.560	**-0.329** 0.410
MMN Frontal F1 4-14 msec				**-0.290** 0.528	**0.698** 0.081	**0.699** 0.081	**-0.366** 0.420
MMN Frontal F2 42-92 msec					**-0.244** 0.598	**0.092** 0.844	**0.650** 0.096
MMN Frontal F3 92-190 msec						**0.206** 0.658	**-0.260** 0.562
MMN Auditory A1 4-14 msec							**0.220** 0.636
MMN Auditory A2 48-140 msec							

Table 1: Effects of correlation between the mismatch negativity (MMN) and double-click auditory recordings. None of the correlations are significant. The diagonal entries in the table are omitted because they all must have correlations = 1; and the cells below the diagonal have been left blank because the information is redundant with the other half of the table. The ΔV's from the epochs recorded in the MMN and P50 paradigms did not correlate, indicating the neural signals evoked in MMN paradigm are not related to the signals evoked in the double-click paradigm. We do not have sufficient numbers of rats to perform linear mixed models, which would accomplish including all of the data points. However, if we consider the unit of analysis the average of the rats rather than the values from individual rats, the analysis is fine; Fig. 5 also supports it. N=7

The table above summarizes the correlations between the mismatch negativity and the double-click paradigm used to test for the rat's P50 ERP suppression. The times of the epochs for the MMN are given just as the times for the putative P50. The epochs for measuring the MMN were determined from a much larger data set (Wilson, Marsden,

Brown & Bowman, unpublished observations). It is evident from the statistical analysis that the difference waveforms observed from the double-click paradigm and those from the MMN paradigm suppression do not strongly covary across rats.

Difference waveforms at standard 500msec ISI with d-amphetamine administration

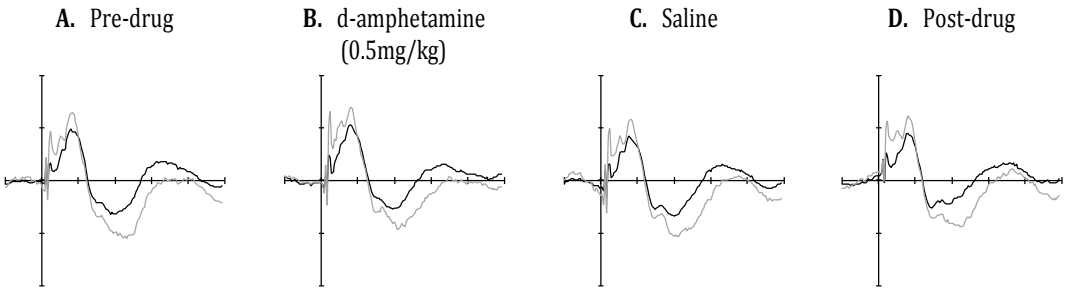


Figure 9: Difference waveforms ($S_1 - S_2$) at frontal-parietal (grey) and auditory (black) recording sites (with a 0.5-s interval) before and after intraperitoneal (i.p.) injections of saline and dextroamphetamine. The amplitude (∆ 5µV ticks) of the evoked potential is represented on the *ordinate* (note in these figures the voltage is inverted). The time (units per click) after the click is represented on the *abscissa.* A. Pre-drug recordings were done as a control. B. d-amphetamine (0.5mg/kg i.p.) was injected into the rats and neurophysiological recording was performed during 30-60 minutes post injection for optimal results. No change in gating was observed. C. A saline injection was also used to control for potential injection stress in the amphetamine condition (the order of injections was counter-balanced). A slight, nonsignificant decrease in gating deficiency was observed (Frontal, $F_{(3,18)}$=0.90, p>0.05; Auditory, $F_{(3,18)}$=0.76, p>0.05), contrasting with the results of exposure to amphetamine. D. Post-drug recordings were performed 48 hours after final injections. There was no detectable difference in the difference waveforms of pre-drug versus post-drug. N=7

The figure above illustrates the difference waveforms at the standard inter-stimulus interval of 500msec. The difference waveforms from pre-drug, exposure to d-amphetamine, saline, and post-drug recordings were all graphed and analyzed. The greatest degree of auditory gating occurred in the pre-drug scenario. Exposure to d-amphetamine had a minimal effect on the degree of auditory gating. Exposure to saline narrowly decreased the amount of auditory gating recorded. The post-drug recordings were taken 48 hours after the last i.p. injections were administered, and the amount of auditory gating recorded is less than the pre-drug recordings, and most resembles the difference waveforms from i.p. injection of saline. The degree of change in auditory gating is so minimal that is it statistically not significant.

Discussion

Hypotheses

To reiterate the research questions we intended to test: (1) The P50-like suppression in the rat N40 will decrement with increasing ISI, as per normal humans; (2) like the P50 suppression in humans, the rat N40 will be weakly correlated with other measures of impaired sensory gating in schizophrenia, such as the mismatch negativity; (3) that d-amphetamine will disrupt N40 suppression in rats as it does in the P50 of humans; and that (4) the d-amphetamine might have a lasting impact on rat N40 suppression given dopamine's role in plasticity.

Effect of inter-stimulus interval (ISI)

The results of this study indicate that the P50-like suppression in the rat N40 does decrement with increasing ISI, as per normal humans. While the P50 was the target of our model, the corresponding feature in the auditory ERP has been hypothesized to be the N40 in spite of the fact the rat N40 is of opposite sign to the human P50 (Alder et al., 1986). Suppression of the N40 auditory ERP in rats is viewed (by some) to be homologous to the P50 suppression observed in humans (Alder et al., 1986; Adler et al., 1998; Stevens et al., 1991, 1995). Like Alder *et al.,* we observed a negative potential during the double-click paradigm approximately 40msec after the stimulus onset. When the ISI was reduced to 250msec, a rather large difference was observed in the response to the second click, as seen in the $S_1 - S_2$ difference waveform. We believe that that this is a manifestation of sensory gating. As the ISI increased in duration from 500msec to 1000msec to 2000msec, the difference waveform substantially decreased. This reveals that as the ISI increases, the apparent auditory gating performed by the rat's nervous system decreases, and is consistent with human data.

N40 suppression vs. putative MMN in the rat

The results of this study indicate that there is little correlation in the rat between the amplitudes of the peaks in difference waveforms for the putative mismatch negativity (MMN) and N40 suppression in the double-click paradigm (with the signals averaged across both hemispheres). These results are similar to previous research performed in monozygotic and dizygotic twin pairs by Hall et al., (2006) who noted that MMN and P50 suppression serve to evaluate different brain information processing functions that may be mediated by distinct neurobiological mechanisms (Hall et al., 2006). The apparent gating effect we observed appears to be a simple form of habituation. The lack of correlation of the N40 suppression with the putative mismatch negativity implies that the apparent mismatch negativity observed in rats does not rely on simple sensory habituation or adaptation, but probably includes an element of stimulus specificity.

Effect of d-amphetamine

The results of this study indicate that d-amphetamine does not significantly disrupt N40 suppression in rats as it does in P50 suppression in humans. The difference waveforms indicate that a statistically non-significant change occurred from the drug naïve rats and the rats that were given the i.p. amphetamine injection. The results also indicate that there is no detectable lasting impact of the amphetamine's increase in dopamine neurotransmission and therefore neuroplasticity, as no significant change in the difference waveforms was demonstrated in pre- *versus* post-injection data. We can therefore conclude that either d-amphetamine does not affect the neural system underlying N40 suppression in the rat or that we simply had too few subjects to detect a seemingly subtle effect.

In the latter regard, using G*Power (Faul et al., 2007,2009; http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/download-and-register, accessed 22/07/11) we determined that more animals would be necessary to detect statistically the small effect of

amphetamine observed in our data. A total sample size of 13 rats would be required to detect an effect at the auditory electrode recordings and a total sample size of 11 rats were required for the frontal electrode recordings (using a specified statistical power of 0.80 and a specified critical *p*-value of 0.05; see Appendix 5). Due to attrition and limited time we were able to test only 7 rats and thus this issue might be worth revisiting with larger sample sizes.

What were our limitations?

Because of our time constraints, we were limited to using a fairly small sample size (n=8; a single rat was euthanized before MMN and drug testing). If more time had been permitted, an additional six rats would have made our results more statistically powerful (*p*-value < 0.05). As well, we were constrained to solely examining our main area of interest, being the corresponding area of the human P50 in rats, the N40. It would have been interesting to analyze and correlate our N40 data with other neurophysiology impairments observed in schizophrenia, such as the P300; reduction of the amplitude of the P300 component of the ERP is a reliable biological marker of schizophrenia (Ford, 1999). Finally, we were unable to create a preclinical model of schizophrenia because we did not identify a method of impairing the normal functioning auditory gating in rats that could mimic the auditory gating deficits observed in schizophrenic humans.

Next logical step and possible refinements

If we were to continue our research, we would have to perform surgery on at least six more rats to implant electrode caps. Verifying for normal auditory gating would then be required at differing ISI's (250msec, 500msec, 1000msec, and 2000msec). Because our results when administering a low dose 0.5mg/kg of d-amphetamine were negligible, we could elevate the concentration of d-amphetamine to see if it would induce the symptom of poor auditory gating observed in schizophrenics. We could increase the dose to 0.75mg/kg of d-amphetamine and observe the corresponding results. If the results

indicate that the d-amphetamine does disrupt auditory gating, we could then administer other medications in an attempt to reverse the gating deficit caused by d-amphetamine. We could also attempt testing other drugs to induce the auditory gating deficiency in rats that can parallel this symptom of schizophrenia in humans. Ketamine has also been shown to have a similar impairing effect on the gating of auditory evoked potentials in rats (de Bruin et al, 1999). We could further test the hypothesis that a deficiency in nicotinic cholinergic neurotransmitter systems might underlie the auditory gating deficits in schizophrenia patients, a hypothesis that is now being intensively investigated (Hughes et al., 1986; Adler et al., 1992; Stevens et al., 1995; Leonard et al., 1996; Chen et al., 2011).

Appendices

Appendix 1: Data files used for ISI testing

Rat #	250 msec	500 msec	1000 msec	2000 msec
10-327	P50_121317_010611_000	P50_163104_170511_000 P50_140639_200511_000 P50_160942_230511_000	P50_154306_070611_000	P50_114815_080611_000
11-001	P50_124224_020611_000	P50_114413_180511_000 P50_145049_200511_000 P50_131046_240511_000	P50_125613_060611_000	P50_132606_010611_000
11-002	P50_125124_030611_000	P50_123639_180511_000 P50_154204_200511_000 P50_152130_240511_000	P50_141950_010611_000	P50_132245_020611_000
11-003	P50_133702_060611_000	P50_153738_190511_000 P50_134647_230511_000 P50_140331_250511_000	P50_115806_020611_000	P50_133337_030611_000
11-004	P50_150952_010611_000	P50_162921_190511_000 P50_144159_230511_000 P50_144633_250511_000	P50_141630_030611_000	P50_131854_070611_000
11-005	P50_140254_020611_000	P50_131820_200511_000 P50_152742_230511_000 P50_152804_250511_000	P50_135856_070611_000	P50_155118_010611_000
11-006	P50_154442_030611_000	P50_144041_070611_000 P50_113227_160611_000 P50_142917_160611_000	P50_133232_080611_000	P50_150652_090611_000
11-007	P50_142753_090611_000	P50_125209_080611_000 P50_165806_150611_000 P50_121239_160611_000	P50_131642_100611_000	P50_160736_090611_000

Appendix 2: Files containing the raw data for multiple ISI testing. A total of eight rats were tested at the various ISI's. More testing was done at the targeted ISI of 500msec.

Appendix 2: Data files for MMN testing

Rat #	MMN
11-001	MMN_123704_010711_000
11-002	MMN_132503_010711_000
11-003	MMN_140154_010711_000
11-004	MMN_143948_010711_000
11-005	MMN_151655_010711_000
11-006	MMN_155514_010711_000
11-007	MMN_163534_010711_000

Appendix 3: Names of the data files containing the raw data for the mismatch negativity testing. One rat (10-327) was euthanized before testing, so total sample size was seven.

Appendix 3: Data files and schedule for d-amphetamine testing

Rat #	ISI 500 msec (drug naïve)
10-327	Euthanized
11-001	P50_124439_280611_000
11-002	P50_135608_280611_000
11-003	P50_143343_280611_000
11-004	P50_151321_280611_000
11-005	P50_123148_290611_000
11-006	P50_131212_290611_000
11-007	P50_135227_290611_000

Rat #	Weight (grams)	Saline (1ml/kg)	Amph (0.5ml/kg)	Time injected	Start time	End time	Exp #
1100 1	452.22	0.46	0	10:43 a.m.	11:16 a.m.	11:46 a.m.	P50_111605_040711_000
1100 2	430.59	0	0.43	11:20 a.m.	11:53 a.m.	12:23 p.m.	P50_115317_040711_000
1100 3	440.59	0.44	0	11:56 a.m.	12:30 p.m.	1:00 p.m.	P50_123044_040711_000
1100 4	435.2	0	0.44	12:31 p.m.	1:09 p.m.	1:40 p.m.	P50_130946_040711_000
1100 5	401.69	0.4	0	1:12 p.m.	1:46 p.m.	2:18 p.m.	P50_134619_040711_000
1100 6	473.65	0	0.47	1:51 p.m.	2:28 p.m.	2:58 p.m.	P50_142827_040711_000
1100 7	440.53	0.44	0	2:25 p.m.	3:05 p.m.	3:35 p.m.	P50_150506_040711_000

Rat #	Weight (grams)	Saline (1ml/kg)	Amph (0.5ml/kg)	Time injected	Start time	End time	Exp #
1100 1	452.22	0	0.46	10:25 a.m.	10:55 a.m.	11:25 a.m.	P50_105509_060711_000
1100 2	430.59	0.43	0	11:15 a.m.	11:45 a.m.	12:15 p.m.	P50_114501_060711_000
1100 3	440.59	0	0.44	11:50 a.m.	12:20 p.m.	12:50 p.m.	P50_122000_060711_000
1100 4	435.2	0.44	0	12:25 p.m.	12:55 p.m.	1:26 p.m.	P50_125556_060711_000
1100 5	401.69	0	0.4	1:01 p.m.	1:31 p.m.	2:01 p.m.	P50_133143_060711_000

11006	473.65	0.47	0	1:41 p.m.	2:12 p.m.	2:42 p.m.	P50_141205_060711_000
11007	440.53	0	0.44	2:16 p.m.	2:29 p.m.	3:19 p.m.	P50_144929_060711_000

Rat #	ISI 500 msec (post-drug)
11001	P50_112923_080711_000
11002	P50_120523_080711_000
11003	P50_124202_080711_000
11004	P50_132118_080711_000
11005	P50_141433_080711_000
11006	P50_145104_080711_000
11007	P50_152821_080711_000

Appendix 4: Testing regime for the drug-naïve, d-amphetamine, saline, and post-drug testing conditions. Doses of saline and amphetamine were dependent on the rat's weight, which was measured pre-injection. The rats were tested 30-60mins post injection for optimal results, as shown in the previous literature (Johnson et al., 1998). 48 hours post injection each rat was tested in the double-click P50 paradigm with an ISI of 500msec, to test for lasting effects.

Appendix 4: Descriptive Statistics for MMN and N40 (P50-like) suppression

Epoch	Mean	Std. Deviation	N
P50 Auditory A1 28-54msec	0.733	0.238	7
P50 Frontal F1 28-54msec	0.981	0.167	7
MMN Frontal F1 4-14 msec	-0.165	0.110	7
MMN Frontal F2 42-92 msec	0.125	0.082	7
MMN Frontal F3 92-190 msec	-0.166	0.127	7
MMN Auditory A1 4-14 msec	-0.179	0.108	7
MMN Auditory A2 48-140 msec	-0.211	0.112	7

Appendix 5: Summary statistics of the mismatch-negativity (MMN) and the P50 recordings from frontal-parietal and auditory recording sites. The name provided in the left-hand column indicates the given epoch (e.g., 'MMN Frontal F2 42-92 msec' denotes the average from epoch F2 lasting from 42-92 msec after the stimulus on frontoparietal electrodes in the mismatch negativity experiment). N=7

Appendix 5: Sample size required to detect changes in N40 suppression due to d-AMPH

For auditory electrodes

F tests - ANOVA: Repeated measures, within factors

Analysis: A priori: Compute required sample size
Input: Effect size f = 0.3569254
α err prob = 0.05

	Power (1-β err prob)	=	0.80
	Number of groups	=	1
	Number of measurements	=	4
	Corr among rep measures	=	0.5
	Nonsphericity correction ε	=	1
Output:	Noncentrality parameter λ	=	13.2491571
	Critical F	=	2.8662656
	Numerator df	=	3.0000000
	Denominator df	=	36.0000000
	Total sample size	=	13
	Actual power	=	0.8361294

For frontoparietal electrodes

F tests - ANOVA: Repeated measures, within factors

Analysis:	A priori: Compute required sample size		
Input:	Effect size f	=	0.3882628
	α err prob	=	0.05
	Power (1-β err prob)	=	0.8
	Number of groups	=	1
	Number of measurements	=	4
	Corr among rep measures	=	0.5
	Nonsphericity correction ε	=	1
Output:	Noncentrality parameter λ	=	13.2658242
	Critical F	=	2.9222772
	Numerator df	=	3.0000000
	Denominator df	=	30.0000000
	Total sample size	=	11
	Actual power	=	0.8276766

Appendix 6: Analysis of the sample size required to detect statistically the magnitude of changes in N40 suppression that were evoked by amphetamine in our data. The G*Power analysis was set with parameters using a specified statistical power of 0.80 and a specified critical *p*-value of 0.05. In order for our drug research to be statistically relevant, a larger total sample size should be used; auditory electrodes total sample size = 13; frontoparietal electrodes total sample = 11.The effect sizes, ('Effect size f'; quantified as Cohen's *f*'s), for auditory and frontoparietal electrodes sites were taken from our sample. Sample size in our data: N=7.

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I, Andrew Bloomfield, hereby certify that this thesis, which is approximately 10,643 words in length, has been written by me, that it is the record of work carried out by me and that it has not been submitted in any previous application for a higher degree.

I was admitted as a research student in September, 2010 and as a candidate for the degree of M. Phil in September, 2010; the higher study for which this is a record was carried out in the University of St Andrews between 2010 and 2011.

Date: August 31, 2012

Signature of candidate: _____

Supervisor's declarations

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate for the degree of M. Phil in the University of St Andrews and that the candidate is qualified to submit this thesis in application for that degree.

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