A ride in the park: Cycling in different outdoor environments modulates the auditory evoked potentials

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Abstract

Mobile EEG allows the investigation of brain activity outside of the lab and in increasingly complex environments. In this study, EEG equipment was adapted for use and transportation in a backpack while cycling. Participants performed an auditory oddball task while cycling outside either in a quiet park or near a noisy roadway. In both conditions, we were able to accurately measure reliable event related potentials (ERP). The P2 and P3 were similar in topography, and morphology, with no differences in amplitude between conditions. When biking near the roadway, an increased N1 amplitude was observed when evoked by both standards and targets compared with biking in the quiet park. This may be due to attentional processes filtering the overlapping sounds between the tones used and similar environmental frequencies. No behavioural differences were found. This study established methods for mobile recording of ERP signals. Future directions include investigating auditory ERPs in more realistic studies outside of laboratory.

1. Introduction

Cognitive neuroscience traditionally requires subjects to sit in highly controlled environments, and avoid any kind of natural light, sound, and movement during recording. This is because all uncontrolled sensations have the possibility of introducing noise into the EEG signal (Schlögl, Anderer, Roberts, Pregenzer, & Pfurtscheller, 1999; White & Van Cott, 2010), and this noise can be what determines the statistical power when analyzing EEG and ERP data (Luck, 2014). New technologies are beginning to make it easier to record laboratory-quality EEG data outside of the lab while subjects are mobile (Debener et al., 2012; Kuziek, Sheinh, & Mathewson, 2017; Scanlon et al. 2017b). While the effects of movement on EEG data are beginning to be understood, few studies have attempted to investigate the effect that different environments may have on the way the brain functions. The present study was conducted in an attempt to go beyond the limitations of measuring EEG on a mobile participant, while also investigating the ways in which different environments may affect the ERP.

In mobile EEG literature, the oddball task is commonly used due to its high signal to noise ratio in the detection of the P3, as well as for the ability to infer changes in attentional resources due to a concurrent task (Polich, 1987; Polich & Kok., 1995). Debener et al. (2012) recorded brain activity using a small consumer passive electrode wireless EEG mounted to a typical laboratory electrode cap, and had participants perform an auditory oddball task while either walking outdoors or sitting indoors. The authors demonstrated that the P3 was significantly larger when generated while sitting indoor than during outdoor walking. Using a similar wireless EEG system, de Vos, Kroesen, Emkes, & Debener (2014) found a significantly larger P3 response to a three-stimulus oddball paradigm while participants were sitting compared to walking outside. Zink, Hunyadi, Van Huffel, and de Vos (2016) used a passive wireless

mobile EEG system and asked participants to perform an auditory oddball paradigm during mobile cycling, stationary pedaling and sitting in a natural outdoor environment. The authors found no difference in RMS data noise or P3 amplitude between sitting and pedaling, but demonstrated a significant increase in noise and near-significant decrease in P3 amplitude while participants were cycling. Scanlon, Townsend, Cormier, Kuziek, & Mathewson (2017b) had participants perform an oddball task both while cycling outside and sitting inside, using a regular non-mobile EEG system with active electrodes and optimized for mobility using a tablet computer. Consistent with other mobile EEG studies mentioned, we found a significantly decreased P3 amplitude while cycling outside. Altogether these studies indicate that the P3 is reduced during purposeful movement.

Several studies have investigated how EEG can be optimized for mobile tasks. De Vos and Debener (2014) recommended that new technologies for mobile EEG be lightweight, small, and able to avoid cable motion (ideally wireless). Similarly, Makeig, Gramann, Jung, Sejnowski, Poizner (2010) recommended that ideal mobile EEG systems use small and lightweight EEG sensors to avoid hindering movement and with wireless data recording capabilities. Following these recommendations, Debener, Emkes, Gandras, de Vos and Bleichner (2015) demonstrated reliable P3 effects while using a cEEGrid electrode array, composed of a flexible sheet of electrodes, which were placed around the ear, and using a smartphone for stimulus presentation. Another study by Bleichner, Lundbeck, Selisky, Minow, et al. (2015) used a BCI speller to obtain significant P3 effects during a spelling task, with a device small enough to be concealed under a baseball cap. De Vos, Kroesen, Emkes, and Debener (2014) used a similar approach with a BCI speller task, and demonstrated equivalent results between a wireless mobile amplifier and a wired laboratory EEG system. With these new technologies, mobile EEG

can be used to ask increasingly complex questions about the way individuals process their environments.

In addition to ERPs, some studies have used oscillations measured during mobile EEG to answer novel questions about the body in motion. Storzer et al. (2016) recorded EEG in the motor cortex during walking and cycling tasks of comparable speed, to investigate cortical oscillations during these tasks. The authors showed that both walking and cycling were associated with a decrease in the power of alpha (8-12 Hz) oscillations. Similar to this, Scanlon et al. (2017b) demonstrated lower alpha power while participants cycled outside compared to sitting inside during an oddball task. Another study by Jain, Gourab, Schindler-Ivens, and Schmit (2013) used EEG to measure motor cortex oscillations during active and passive (low effort) pedaling stationary cycling. The authors demonstrated significantly more beta desynchronization during active compared to passive pedaling. These studies demonstrate the potential for measuring oscillations during mobile tasks.

Our research group at the University of Alberta has recently found cycling to be an ideal task for mobile EEG research, due to the ability for smooth, straightforward movement while navigating the environment. Our first cycling study looked at the effects of cycling on EEG data, and had participants perform an auditory oddball task before, during and after a sub-aerobic stationary cycling task (Scanlon et al., 2017a). We found increased EEG data noise during the stationary cycling task, however despite this noise, ERP signals were reliably recorded, with no ERP component differences between the cycling conditions. In a follow-up to this study (Scanlon et al., 2017b) we used compact technologies that could be fit inside a backpack, in order to have participants perform the auditory oddball task while cycling outside. Participants were asked to listen to the oddball task through headphones both while cycling outside and while

sitting in the laboratory. Baseline data noise was increased during the cycling condition; however, we were able to collect laboratory quality ERPs in both conditions. We demonstrated significantly decreased P3 amplitude and alpha band oscillations while participants cycled outside. This study also found an unexpected increase in the N1 component and decrease in the P2 component of the ERP, during both standard and target tones while participants were cycling outside. In a follow-up study (Scanlon et al., *Under review*) we investigated this effect further by having participants perform the same headphone auditory oddball task inside the lab with different sounds playing in the background of the task. Here, we found that we were able to replicate the N1 and P2 effects while playing outdoor noises in the background of the auditory oddball task, indicating that the effect may have been due to a selective attention process of filtering background noises in order to perform the task. These studies indicate that bringing EEG into ecologically valid environments opens up the possibility of demonstrating the way mechanisms such as auditory selective attention can be observed in real world environments.

The current study builds on the findings of Scanlon et al., (2017b), and Scanlon, et al., (*Under review*), using methods and analysis established in these studies. It is of particular interest to examine variations in ERP components between path cycling in quiet and noisy outdoor areas, as well as how these conditions differ in statistical power and noise. If the N1 and P2 are in fact related to a selective attention process of filtering background noises in order to pay attention to task-relevant sounds, we would expect to see an increased N1 and decreased P2 while participants perform the same task in a noisier environment. For this experiment, each participant completed four 6-minute blocks in an auditory oddball task while both sub-aerobically cycling near a noisy roadway and in a quiet city park. Similar to Scanlon, et al. (2017b), active low-impedance electrodes were used. Levels of data noise and power spectra

were analyzed, as well as ERP magnitude, morphology and topography for the P3, P2, N1 and MMN/N2b. Our first hypothesis is that even with the noise created during movement in both conditions, we will be able to accurately record ERPs in both cycling conditions, with enough accuracy to detect possible ERP differences between the conditions. Our second hypothesis is that due to increased ambient noise in the roadway condition, there will be an increased N1 and decreased P2 while participants cycle near the roadway.

2. Materials and Methods

2.1 Participants

10 people who were part of the university community participated in the experiment (mean age = 23.4; Age range= 20-31; 4 female). Each participant received an honorarium of $20. All participants had normal or corrected to normal vision with no history of neurological problems. Experimental procedures used were approved by the Internal Research Ethics Board of the University of Alberta.

2.2 Materials

Prior to the experiment, participants selected one of two bicycles (Kona Mahuna), which differed only in frame size (17 or 19 inches) based on the participant's height. Seat height was adjusted according to the participants' comfort level. Bicycle gears were set to the 2nd gear in both the front and back derailleurs, in order to allow participants to pedal constantly and evenly throughout the trials at a sub-aerobic level. Data was collected using a Microsoft Surface Pro 3 running Brain Vision recorder (Brain Products), and powered by an Anker Astro Pro2 20000mAh Multi-Voltage External Battery or or Tzumi Extreme PocketJuice 6000mAh battery. Due to technical problems with the Anker astro battery, the Tzumi battery was used for 8

subjects. The various technologies mentioned were connected using a Vantec 4-Port USB 3.0 Hub.

To run the oddball task and mark the EEG data for ERP averaging, we used a Raspberry Pi 2 model B computer, running version 3.18 of the Raspian Wheezy operating system, using version 0.24.7 of the OpenSesame software (Mathôt, Schreij, & Theeuwes, 2012; see Kuziek, Sheinh, & Mathewson, 2017 for validation study). A 900 MHz quad-core ARM Cortex-A7 CPU was connected through a 3.5 audio connector for audio output. To mark the data for EEG averaging, 8-bit TTL pulses were sent to the amplifier through a parallel port connected to the General Purpose Input/Output (GPIO) pins. A button connected to the Raspberry Pi was affixed to the handlebar of the bike, which was used both as a start button for each block, and a response button to the target tones. Participants wore a two-pocket backpack (Lululemon; Figure 1A, C) containing all of the stimulus generating and recording materials. In the *roadway* condition, participants rode along approximately 0.65 km of a shared use path next to River Valley Road in Edmonton, Canada (Figure 1B green). During the park condition on the same day, participants rode along approximately 0.65 km of a shared use path in MacKinnon Ravine Park, also in Edmonton, Canada (Figure 1B, blue). The conditions order was randomly varied across participants.

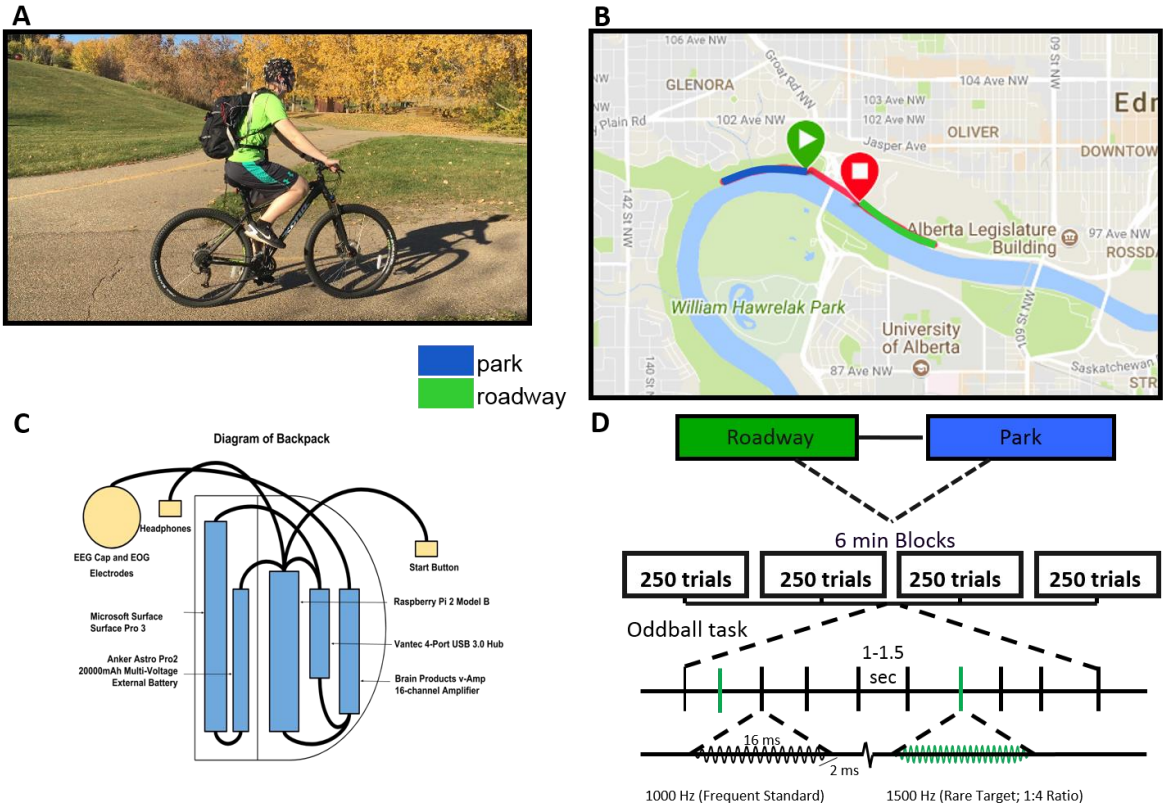

Figure 1. Biking apparatus and procedure for mobile EEG.

A: The task was performed while cycling on a bicycle, with the participant wearing an EEG cap and backpack which contained the EEG equipment. B: A map of the cycling routes. C: Schematic layout of the contents of the mobile EEG backpack. The backpack had two pockets and contained the following: Anker Astro Pro2 20,000 mAh Multi-Voltage external battery or Tzumi Extreme PocketJuice 6000mAh battery, Raspberry Pi 2 model B, Microsoft Surface Pro 3, Vantec 4-Port USB 3.0 hub, Brain Products v-Amp 16 channel amplifier. D: During both cycling by the roadway and in the park, participants performed four 6 minute blocks of an auditory oddball task, with self-paced breaks in between. Within the oddball task, 20% of the tones were rare high-pitched tones (1500 Hz) and 80% were frequent low-pitched tones (1000

Hz). Each tone played for 16 ms with a 2 ms ramp-up and down. Tones were spaced 1-1.5 seconds apart.

2.4 EEG Recording

Active wet electrodes were selected for EEG recording in this study based on previous studies by Laszlo et al. (2014) comparing active and passive amplification electrodes at differing levels of impedance, as well as by Oliviera et al. (2016) who compared active wet, passive wet and passive dry electrodes during a mobile task. Both of these studies demonstrated that active wet electrodes afford cleaner and better quality signals in non-ideal recording conditions. Fifteen Ag/AgCl pin electrodes were arranged in 10-20 positions (Fp2, F3, Fz, F4, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, and Oz). Additionally, a ground electrode was embedded in the cap at the Fpz position, along with two reference electrodes clipped to the right and left ears. To lower electrode impedance, SuperVisc electrolyte gel was used, followed by mild abrading of the skin with a blunted syringe tip. The aforementioned techniques and gel application continued until all the electrodes had impedance levels lower than 10 kΩ, as measured using an impedance measurement box (BrainProducts) and until the raw data appeared to be clean and free of excessive noise. In addition to the 15 EEG electrodes, two reference electrodes and one ground electrode, horizontal and vertical bipolar electrooculogram (EOG) was recorded through passive Ag/AgCl easycap disk electrodes placed above and below the left eye, as well as 1 cm from the outer canthus of each eye. These passive electrodes were used for the EOG because the AUX ports of our amplifier do not support active electrodes and we did not see a benefit to giving up 4 EEG channels for active-wet EOG sensors. These EOG electrodes can nonetheless record reliable signals due to their placement on the face, where there is generally less hair to impede

the signal. Dirt and oils at the EOG electrode sites are removed using NuPrep preparation gel, followed by cleaning the skin with an antibacterial sanitizing wipe prior to electrode placement in order to reduce impedance levels of the EOG electrodes based on visual inspection of the raw data. These bipolar channels were recorded using a pair of BIP2AUX converters plugged into the AUX ports of the V-amp amplifier, along with a separate ground electrode placed on the central forehead. EEG and EOG was recorded using a V-amp 16 channel amplifier (Brain Products) through a laptop USB port. Data were digitized at 500 Hz with a 24 bit resolution. The data were filtered using an online bandpass with 0.1 and 30 Hz cutoffs, along with a 60 Hz notch filter. These narrow filters were recommended in the actiCap Xpress manual in order to increase signal quality and reliability in mobile settings (Brain Products, 2014).

2.5 EEG Analysis

Analyses of EEG data were completed using MATLAB 2016b with EEGLAB (Delorme & Makeig, 2004), with custom scripts. Following data recording, the EEG data was re-referenced to an average of the left and right ear lobe electrodes. TTL pulses time-locked to the onset of target and standard tones were marked in the EEG data during recording, and were then used to construct epochs 1200-ms long (including a 200-ms pretrial baseline). Average voltage in the first 200-ms baseline was subtracted from the data for each trial and each electrode. To remove artifacts caused by movement, amplifier blocking, and any other non-physiological factors, any trials in either of the conditions with a voltage difference from baseline larger than ±1000 μV on any channel (including eye channels) were removed from further analysis. Following eye correction, a second threshold was applied to remove any trials with a voltage difference from baseline of ±200uV. Following this procedure, over 88% of trials were retained in each condition. Overall, artifact rejection left approximately equal trial numbers per

participant in the park (M_{targ} = 183.1; $range_{targ}$ = 127-200; M_{stand} = 722.4; $range_{stand}$ = 457-800; SD = Standard Deviation) and roadway (M_{targ} = 178.6; $range_{targ}$ = 124-200; M_{stand} = 707.9; $range_{stand}$ = 498-800) conditions, which we then used for the remaining analysis.

A regression-based procedure was used to estimate and remove variance due to EEG artifacts caused by blinks, as well as horizontal and vertical eye movements (Gratton, Coles, & Donchin, 1983). This technique uses a template-based approach to identify blinks, then computes propagation factors such as regression coefficients, and predicts the vertical and horizontal eye channel data from each electrode's signal. Then, weighted by these propagation factors, this eye channel data is then subtracted from each channel, allowing us to remove most variance in the EEG data that can be predicted by movements from the eyes. In order to keep as many trials as possible for both conditions, no further filtering or rejection was done on the data.

Data collection was completed between the months of August and November. When outside, participants sometimes wore a toque (knitted cap) over the EEG cap to prevent the electrolyte gel from drying out, goggles to prevent excessive eye blinking during the trials, and gloves, if desires due to the weather (Temperature range = -4 - 17°C; Mean temperature = 11°C). During each 6 minute block the participant would follow a research assistant on a leading bike, and travel approximately 650 meters from the starting point until the tones stopped. After which they would turn around, have the data visually inspected for noise and electrode problems by the research assistant, and complete another 6 minute block going in the opposite direction. Participants were instructed to stare straight ahead as much as possible and pedal at a slow, even pace.

3. Results

3.1 Behavioural analysis

In order to assess behavioural effects in this task, we tested for condition differences in response rate and average reaction time to the target stimuli in the task, depicted in Figure 2. A two-tailed paired samples t-test indicated a marginal difference in response rate between the two conditions, with participants responding to a higher percentage of targets in the roadway than park condition (M_{diff}=-1.6; SD_{diff}=2.4922; t(9) =-2.0302; p=0.07291). The same test was used to test for differences in average reaction time, finding no differences between conditions (M_{diff}=4.1037; SD_{diff}=26.2207; t(9) =0.4949; p=0.6325).

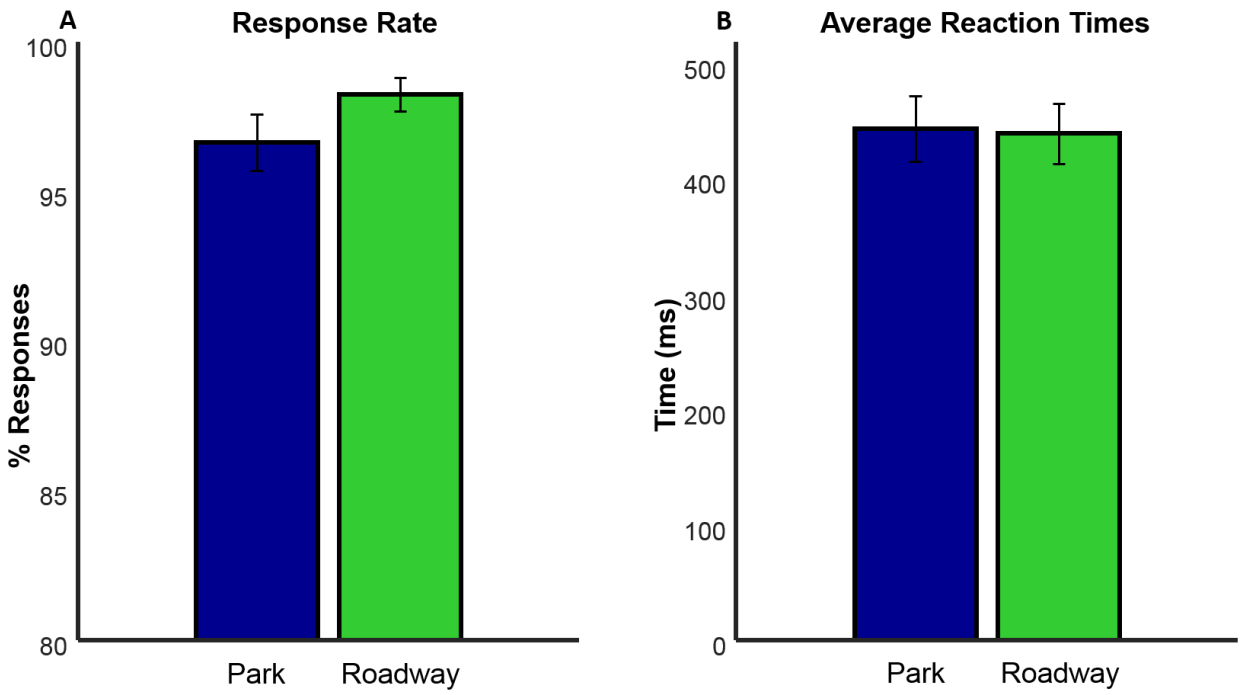

Figure 2. Behavioural analysis.

A: Bar graph depicting mean and standard error for the percentage of targets that received a response within 2 seconds after the tone onset across participants for both conditions. B: Bar graph depicting the mean and standard error for the average time taken to respond (with missed responses removed) across participants for each condition.

3.2 Data Noise

Figure 3A depicts raw data for a representative participant at the Pz electrode location. We used two separate methods to estimate the data noise on individual trials. First, we took an average of the spectra over each EEG epoch in the Fz and Pz electrode locations. Each participant's data was randomly sampled for 290 of their artefact-removed standard trials. A fast Fourier transform (FFT) was then calculated through a procedure of symmetrically padding the 600 time point epochs with zeros, making for each epoch a 1,024-point time series, which provided frequency bins of .488 Hz. Because the data were collected online with a low-pass filter of 30Hz, only frequencies measuring up to 30 Hz were plotted. We then calculated the spectra for each participant by computing the average of the 290 spectra for each participant, and then combining these into the grand average spectra, as shown in Figure 3B for the Fz and Pz channels. Shaded regions depict the standard error of the mean across participants. Evident in the plots, both conditions depict the expected 1/frequency structure in the data. Also evident is a significant increase in beta oscillations (15-30Hz) at the Fz electrode location (M_{diff}=0.06410; SD_{diff}=0.07322; t(9)= 2.7685; p=0.02181) during the park compared to the roadway condition, as tested with a two-tailed paired samples *t*-test.

3.3 Single-Trial Noise

As an additional estimate of the single-trial EEG noise, we computed the RMS value for a baseline period of each standard trial (de Vos & Debener, 2014). To avoid any interference from the evoked ERP activity while measuring RMS, the baseline period consisted of the 200 ms (100 time points) prior to each tone's onset. RMS is a good estimate of single trial noise in EEG data, as it is equivalent to the average absolute voltage difference around the baseline. To

estimate an RMS distribution for each subject in the dataset, we used a permutation test that selects a different set of 290 epochs without replacement for each participant on each of 10,000 permutations before running second order statistics (Laszlo et al., 2014; Mathewson et al., 2017). For each condition and each permutation, a grand average single-trial RMS was calculated and recorded. Figure 3C depicts a histogram of these grand averaged single-trial RMS values computed for each permutation and each condition. Below this is a bar graph of the mean and standard deviation of the grand average distributions of RMS permutations. These plots demonstrate a clear distinction in single-trial noise for the two conditions. The park condition ($M_{RMS\text{-}EEG} = 7.9101$; $SD_{RMS\text{-}EEG} = 0.028742$) clearly demonstrated larger single-trial noise levels compared to the roadway condition ($M_{RMS\text{-}EEG} = 7.4782$; $SD_{RMS\text{-}EEG} = 0.027245$; Wilcoxon signed rank test; $z = 86.6047$; $p < 0.001$).

3.4 ERP Baseline Analysis

We ran a similar permutation analysis to test for noise that was not effectively averaged out across trials on noise levels within trial averaged ERPs. We again used a permutation test of the baseline RMS values to quantify the amount of noise in the participant average ERPs. This analysis is complementary to the above single-trial analysis, as the computation estimates the amount of phase-locked EEG data noise which is not averaged out over the trial with respect to tone onset. The computation randomly selected and averaged 290 of each participant's standard artefact-removed trials without replacement. The obtained RMS values were then averaged over EEG electrode channels to create a grand average of all participants. This made 10,000 permutations after averaging each participant's data together to compute second-order statistics. Figure 3F shows a bar graph of the means of these distributions, with error bars to indicate the standard deviation of the permutation mean distributions. The histogram in Figure 3E depicts the

grand average RMS values calculated from these 10,000 permutations in each condition. The park condition ($M_{RMS\text{-}ERP}$ = 0.52253; $SD_{RMS\text{-}ERP}$ = 0.020995) had a higher RMS value compared to the roadway conditions ($M_{RMS\text{-}ERP}$ = 0.48798; $SD_{RMS\text{-}ERP}$ = 0.019317; Wilcoxon signed rank test: z = 79.3401; p <0.001).

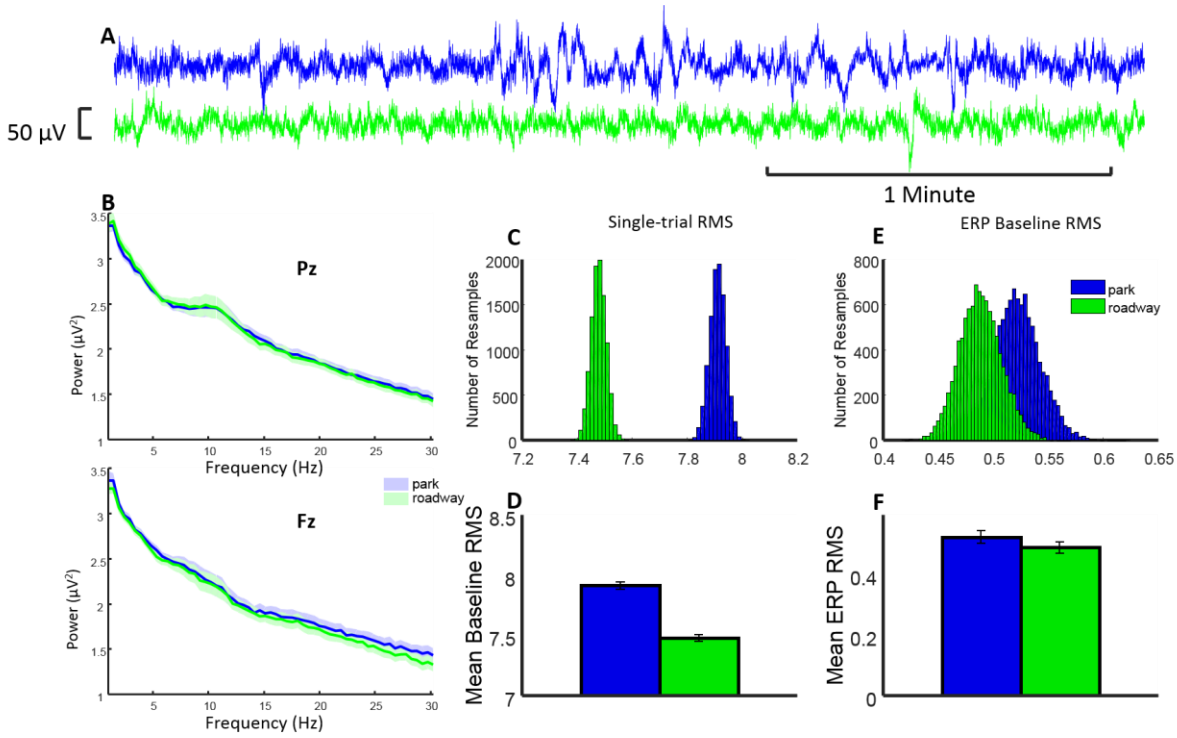

Figure 3. Data noise levels.

A: Raw EEG data (with online band-pass and notch filters) for several minutes for a representative subject and for each condition, shown at the Pz electrode location. B: Single-trial EEG spectra from electrodes Pz and Fz, calculated with zero-padded FFTs on 290 auditory standard trial epochs for each subject, averaged over trials, then subjects. Shaded regions depict the standard error of the mean. C: Histogram of average single-trial root mean square (RMS) grand average values collected during a 200 ms baseline period before tone onset, for 10,000 permutations of 290 randomly chosen standard trials for each subject. RMS values are averaged

first over all electrodes within each trial, then over trials, then over subjects. D: Bar graph of these 10,000 permuted single-trial RMS values. Error bars depict standard deviation of the permuted distributions. E: Histogram of average ERP baseline RMS values, computed using 10,000 randomly selected permutations of 290 standard trials for each subject. For each permutation RMS of the baseline period is computed and the data is averaged over trials. F: Bar graph of these 10,000 permuted ERP baseline RMS values. Error bars depict standard deviation of the permuted distributions.

3.5 ERP Morphology and Topography

Grand average ERPs calculated from each participant's corrected and artefact-removed standard and target tones at electrode Pz are depicted in Figure 4A. Similar error levels can be observed within the two conditions. Evident from the plots is the expected P3 amplitude increase during target trials in the posterior topographical locations (Pz; Figure 4B). The expected oddball difference in the P3 is shown with an increased positive voltage between 300 and 430 ms following the infrequent target tones, compared with the common standard tones. We used this time window for all further analysis of the P3. Additionally evident in the Figure 4A plots is a target-standard difference in the MMN/N2b time window, with increased negative voltage from 175-275 ms following infrequent targets tones compared to common standard tones. This time window was used for any further MMN/N2b analysis, using the Fz electrode, as the effect is visibly maximal in frontal regions (Figure 4B).

Figure 4B shows topographies of these target-standard differences within the MMN/N2b and P3 time windows. The MMN/N2b time window topography demonstrates the expected frontal scalp activation distribution for both conditions, while the P3 topographies reveal the

expected activation distribution toward the back of the head. Figure 4C plots the ERP difference waves at electrode Pz, which are created by subtracting the standard tone ERPs from target tone ERPs for each subject. The shaded bars represent the standard-error of the difference, and estimation of error is therefore an equivalent to that used in the t-test of the target-standard difference from zero (Loftus & Masson, 1994).

Both conditions demonstrated a clear negative peak at approximately 230 ms at the Fz electrode. To test for the MMN/N2b effect, we ran a one-tailed, paired samples *t*-test comparing the MMN/N2b averaged over the 175-275 ms time window centered around this peak at electrode Fz to zero. This test revealed a significant MMN/N2b effect for both the park condition (M_{diff}=-2.4188; SD_{park}=2.3427; $t(9)$ =-3.2649; p=0.0048811) and roadway condition (M_{diff}=-2.2506; $SD_{traffic}$=1.9142; $t(9)$ =-3.718; p=0.0023928). Additionally, a clear positive peak was observed as expected at approximately 380 ms at electrode Pz. A one-tailed paired samples t-test was used to compare this P3 difference averaged over the 300-430ms time window at electrode Pz to zero revealed a significant P3 effect in both the park (M_{diff}=2.8799; SD_{park}=3.37; $t(9)$ =2.7024; p=0.012148) and roadway (M_{diff}=2.8932; $SD_{roadway}$=3.3644; $t(9)$ =2.7194; p=0.011815) conditions.

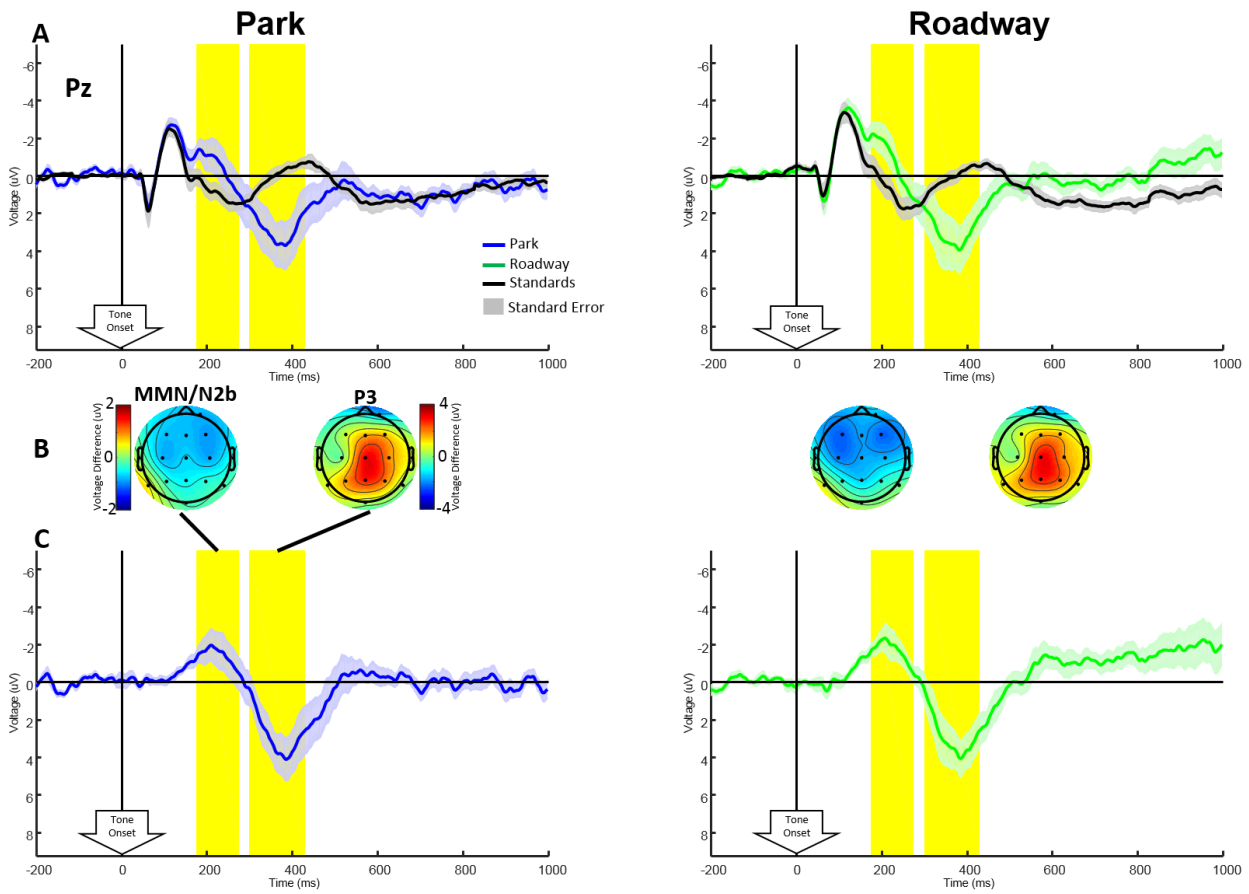

Figure 4. ERP grand averages.

A: Grand-average ERPs computed at electrode Pz for all artefact-removed and eye movement corrected trials, for both standard (black) and target (colour) tones. B: Scalp topographies for grand-average ERP difference between standard and target tones in the MMN/N2b and P3 time windows (indicated in yellow), 175-275 ms and 300-430 ms after the tone, respectively. C: ERP difference wave from electrode Pz for both conditions, with shaded regions depicting within-subject standard error of the mean for this difference, with between-subjects differences removed (Loftus & Masson, 1994). Yellow highlighted regions represent the time window for the MMN/N2b and P3 analysis as well as topographic plots.

3.6 ERP Differences and Statistical Power

Figure 5A shows differences waves plotted for both conditions at electrodes Pz and Fz. Evident from the graphs is no significant MMN/N2b or P3 differences between the two conditions at Fz and Pz, respectively. A two-tailed t-test between the park and roadway conditions was performed to test for differences in the targets-standards P3 difference between conditions at electrode Pz, finding no significant difference (M_{diff}=-0.013282; SD_{diff}=1.5997; $t(9)$ =-0.026255; p=0.97963). The same test was performed to test for any differences between conditions in targets-standards difference waves within the averaged 175-275 ms time-window at electrode Fz, and found no significant differences (M_{diff}=-0.16821; SD_{diff}=1.3555; $t(9)$ =-0.39241; p=0.70389).

As there is a possibility that the MMN and N2b contribute differentially to the MMN/N2b effect (Scanlon et al., 2017), we separated this window in order to analyze averages of the early MMN time period (100-200 ms) and the later N2b time period (200-300 ms). We found no significant differences between conditions in the targets-standards difference when the t-test was applied to either the MMN (at Fz: M_{diff}=0.021454; SD_{diff}=0.80884; $t(9)$ =0.083877; p=0.93499; at Pz: M_{diff}=0.21574; SD_{diff}=0.75063; $t(9)$ =0.90885; p=0.38712) or N2b time window (at Pz: M_{diff}=0.19951; SD_{diff}=1.487; $t(9)$ =0.42427; p=0.68133; at Fz: M_{diff}=-0.2088; SD_{diff}=1.4541; $t(9)$ =-0.45407; p=0.66053). It is likely that both the MMN and N2b were elicited within this time window, however with the current design we were unable to disentangle these components from each other. Therefore from here on, we will focus on the combined MMN/N2b time window between 175 and 275 ms.

Following the evidence of increased trial-averaged and single-trial noise during the park conditions, one may expect this to result in lower statistical power in the park condition. In order

to test this prediction, we used an analysis procedure similar to those used by Kappenman & Luck (2010), Laszlo et al. (2014) and Mathewson et al. (2017). a permutation procedure was used in which the 4:1 ratio of standard to target trials was kept constant while increasing the number of these trials that are able to contribute to the ERP average. Number of trials increased from 4 standard and 1 target trial, by 20 standard trials, up to 280 standard and 70 target trials. We then chose a random selection of this number of trials from each participant's data with replacement, and averaged over subjects to obtain grand averages. This random replacement procedure was used for both the MMN/N2b analysis at Fz and P3 analysis at Pz, and separately for each condition. For each number of trials, 10,000 permutations of this procedure was done. Note that this procedure cannot be used to examine changes in ERP morphology over time on the task due to habituation or attention, and assumes that these influences would be observed equally across conditions and stimuli (Scanlon et al., 2017).

The selected single trials for each permutation were averaged together to create separate participant ERPs for target and standard tones. Target-standard differences were computed between 175 and 275 ms at electrode Fz and between 300 and 430 ms at electrode Pz in order to measure average MMN/N2b and P3 values, respectively. We then used a one tailed paired samples t-test to test for differences between targets and standards (df = 9, α=0.05). Figure 5B demonstrates plots of the proportion of the 10,000 permutations in which the obtained p-value was passed the significance threshold as a function of the number of standards and targets selected in each permutation. Evident from this plot, the MMN/N2b for roadway condition required fewer trials (120 standard/30 target trials) to reach significance in 80% of permutations (gray line depicts 80% power) than the park condition (140 standard/35 target trials). The opposite effect is shown for the P3, in which the park condition appears to require fewer trials

(200 standard/50 target trials) than the roadway condition (240 standard/60 target trials) to reach 80% permutation significance.

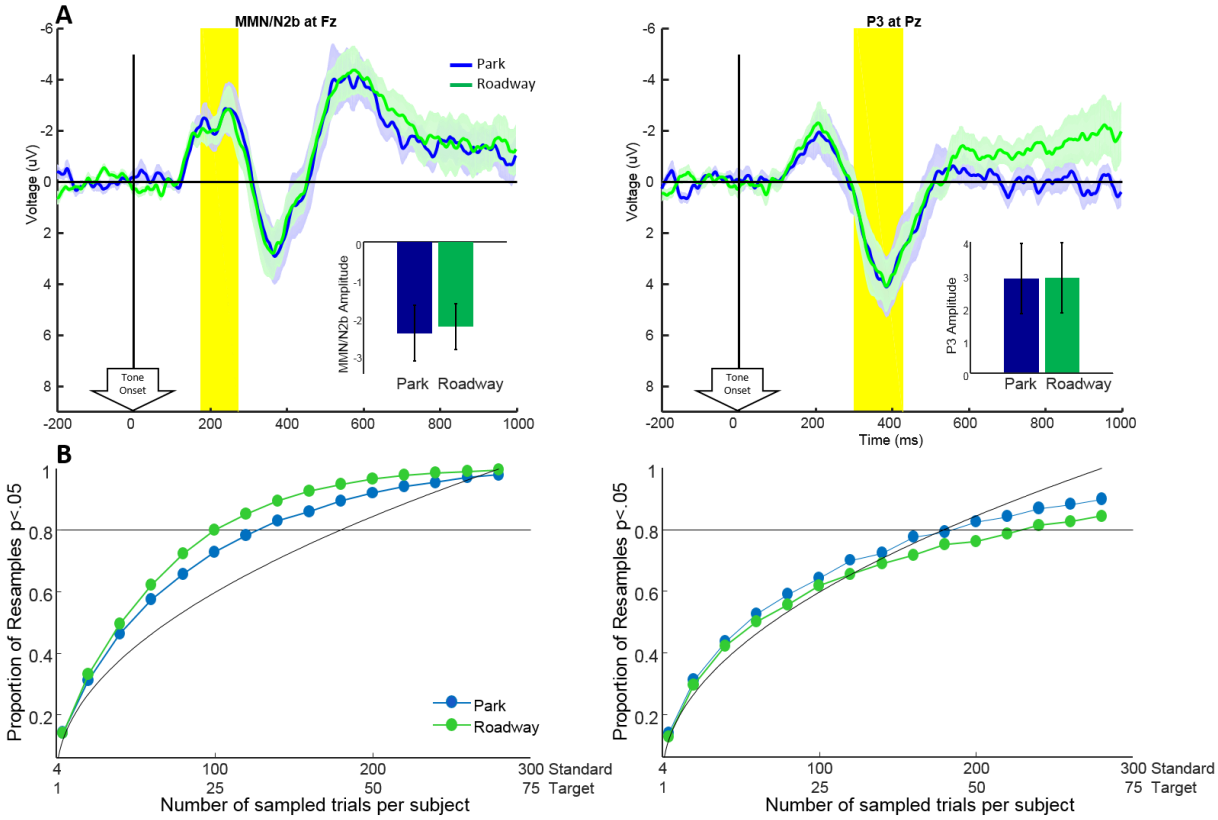

Figure 5. Difference waves and ERP power analysis.

A: Difference waves indicating the average difference between standard and target trials for the two conditions are plotted for electrode locations Fz and Pz. Yellow highlighted regions depict the main time windows compared, particularly the MMN/N2b at Fz (left) and the P3 at Pz (right). B: The result of a permutation test in which a number of trials selected within 10,000 permutations varied between 5 and 280, while keeping the 4:1 ratio of standards to targets. Within each permutation, trials are selected randomly and averaged to compute subject ERPs. Differences in the MMN/N2b and P3 time windows between target and standard trials is computed and compared using an across-subjects (paired samples) one-tailed t test (a = 0.05), before grand average statistics are computed. For each number of trials and each condition, the

graph plots the proportions of the 10,000 permutations in which an uncorrected significant difference was obtained. The dashed horizontal line at 0.8 indicates the threshold to achieve 80% power to find an existing effect. The gray line represents a square root of the number of standard trials, scaled to a range between 0 and 1 on the vertical axis by dividing by the square root of the maximum number of trials.

3.7 N1 and P2 Amplitudes

In order to observe the effects of two different environments on general stimulus processing, grand averaged ERPs with a comparison of park vs. roadway at the Fz and Pz electrode locations are plotted in Figure 6A and B, respectively. A visual inspection of these plots shows increased amplitude in the N1 component (100-175 ms) in the roadway condition, both in targets and standards and at electrodes Fz and Pz. Two-tailed paired t-tests of these differences indicated significantly larger N1 amplitude during the roadway compared to the park condition at Fz (standards: M_{diff}=0.92109; SD_{diff}=1.1408; $t(9)$ =2.5533; p=0.031024; targets: M_{diff}=1.0752; SD_{diff}=1.433; $t(9)$ =2.3727; p=0.041723) and Pz (standards: M_{diff}=0.87623; SD_{diff}=1.0001; $t(9)$ =2.7706; p=0.021733; targets: M_{diff}=1.0437; SD_{diff}=1.1443; t(9) =2.8844; p=0.018049). Figure 6C plots topographies of these differences, indicating a frontocentral distribution.

Additionally, as previous studies have shown increased background noise to decrease the amplitude of the P2 component between 175 and 275 ms, one may expect to observe a similar effect in which the roadway condition shows a lower P2 amplitude. However, evident from the plots in Figure 6A and B, no difference in P2 amplitude exists between the two conditions. Two-tailed paired samples t-tests revealed no significant P2 amplitude differences between conditions

within both targets and standards at electrodes Fz (standards: M_{diff}=-0.024896; SD_{diff}=0.90966; $t(9)$ =-0.086546; p=0.93293; targets: M_{diff}=-0.1931; SD_{diff}=1.7528; $t(9)$ =-0.34838; p=0.73556) and Pz (standards: M_{diff}=0.075133; SD_{diff}=0.83215; $t(9)$ =0.28552; p=0.78171; targets: M_{diff}=0.33211; SD_{diff}=1.8257; $t(9)$ =0.57523; p=0.57923).

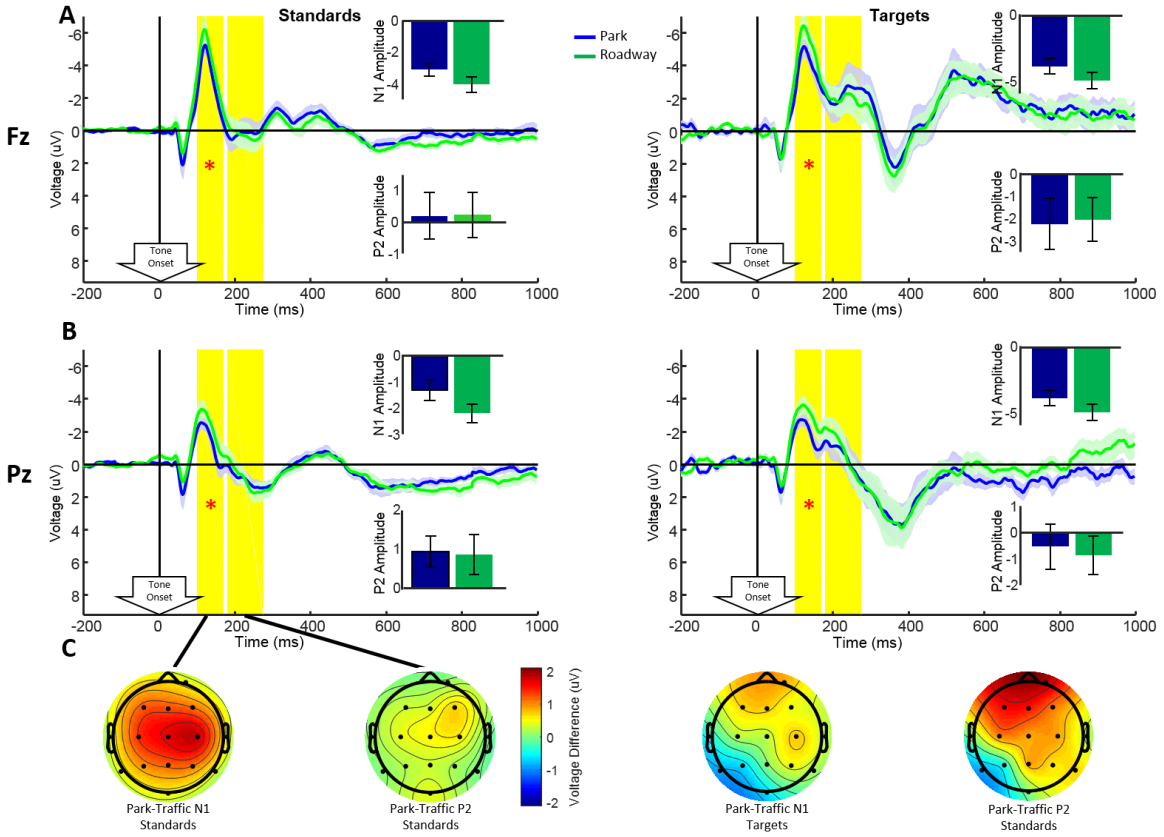

Figure 6. Grand average ERPs

A: Grand average ERPs collected at the Fz electrode location, plotted separately to compare standards and targets between conditions. Shaded regions indicate the standard error of the mean. Inset bar graphs show the mean and standard error across participants in the N1 and P2 time windows. B: Grand average ERPs for the Pz electrode location, plotted separately to compare standards and targets between conditions. Inset bar graphs depict mean and standard error across

participants in the N1 and P2 time windows. C. Topographies of the N1 and P2 time window plotted for the park-roadway condition differences.

3.8 Combined experimental analyses

Figure 7A, B and C shows three bar graphs of N1 amplitude, P2 amplitude and alpha power, respectively, compared across 5 experiments which all used the oddball task (Scanlon et al., 2017a; Scanlon et al., 2017b; Scanlon et al., *under review*; Scanlon et al., *In preparation*; Current study). Four of these experiments used the same auditory oddball task, while the fifth used a visual oddball task, and is therefore only included on the plot of alpha power. All of these experiments were performed on different participants. Experiment 1 is a previous study in which participants performed an auditory oddball task on a stationary bicycle (Scanlon et al., 2017a). While performing the oddball task, participants were asked to sit still (pre), pedal sub-aerobically (bike) and sit still again (post). Experiment 2 is a previous study in which participants performed the oddball task both while sitting inside a laboratory faraday cage (inside) and cycling outside the lab (outside; Scanlon et al., 2017b). In experiment 3, participants performed the auditory oddball task with four different background noise and volume conditions (Scanlon et al., *under review*). The task was performed with the presence of background outdoor sounds recorded near a roadway (sound), with the presence of background white noise (white), with a silent background and the tones played at reduced volume (silent low), and with a silent background and regular tone volume (silent). In experiment 4 participants performed a visual oddball task with two visual backgrounds. The backgrounds were a static or ‘snowstorm’ screen and a point-of-view video of someone cycling through traffic (Scanlon et al., *In preparation*). Experiment 5 is the current study, with the oddball task performed in the quiet park (park) and next to the noisy

roadway (roadway). Figure 7A shows the N1 amplitudes to standard tones in 11 conditions from four experiments using the same auditory oddball task. Evident from the graph is that experimental conditions in which the participant performed the task in non-ideal auditory conditions (e.g. with background noise or low volume; shades of green and yellow) show generally larger N1 amplitudes than when the task was performed in ideal situations (silent background, normal volume; shades of grey). Figure 7B depicts the P2 amplitudes to standard tones in 11 conditions from four experiments using the same auditory oddball task. The graph shows that experimental conditions in which the participant performed the task with background noise (shades of green) have generally lower P2 amplitude than those in which the task was performed in silence (shades of grey and yellow). Figure 7C demonstrates the alpha power for standard tones in 13 conditions within five experiments using either auditory or visual oddball tasks. Evident from the plot is that experimental conditions in which the participant viewed visually rich stimuli had generally lower alpha power than those conditions in which the participant viewed a blank screen during the experiment.

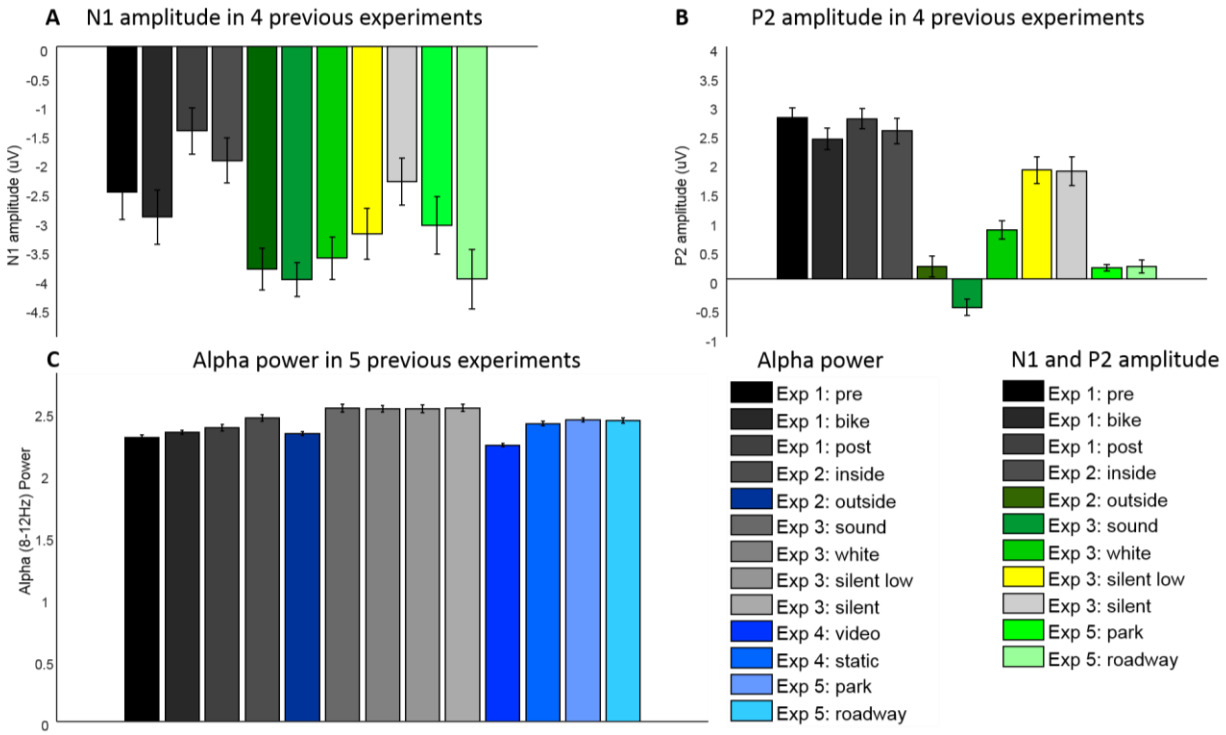

Figure 7. Combined experimental comparisons of the N1, P2 and alpha power.

A: Bar graph of mean N1 amplitude to standard tones in 11 conditions across four auditory experiments using the same auditory oddball task. Experiment 3 is not included to avoid confusion between auditory and visual ERP components. Shades of green are used to represent experimental conditions with background sounds, and shades of grey are used to represent conditions with silent backgrounds. Yellow is used to depict the low-volume tones condition of experiment 2. Error bars depict the standard error of the mean. B: Bar graph of mean P2 amplitude to the standard tone in 11 conditions across four auditory experiments using the same auditory oddball task. Experiment 3 is not included to avoid confusion between auditory and visual ERP components. Shades of green are used to represent experimental conditions with background sounds, and shades of grey are used to represent conditions with silent backgrounds. Yellow is used to depict the low-volume tones condition of experiment 2. Error bars depict the standard error of the mean. C: Bar graphs of mean alpha power for standard tones in 13

conditions across five experiments all using an auditory or visual oddball task. Shades of blue represent experimental conditions in which the participant viewed rich visual stimuli, and shades of grey represent experimental conditions in which the participant viewed a blank screen during the experiment. Error bars depict the standard error of the mean. Experiment references: 3 stage bike (Scanlon et al., 2017a), Exp 1 (Scanlon et al., 2017b), Exp 2 (Scanlon et al., *under review-a*), Exp 3 (Scanlon et al., *In preparation*), Exp 4 (Current study).

4. Discussion

The present study directly examined the effectiveness of recording ERPs during mobile cycling in two different outdoor environments, using a backpack to contain and carry the EEG system outside of the lab. We implemented new compact methods for stimulus presentation (Kuziek et al., 2017) through the use of a Raspberry Pi computer to present the auditory oddball task through headphones and to mark stimulus events within the EEG data for analysis. We also used a tablet computer to store the marked EEG data via Brain Products v-AMP 16-channel amplifier as the EEG system. We used active electrodes based on previous findings which showed favorable results for active electrodes compared to other electrode types in mobile EEG (Oliviera et al., 2016) and high impedance levels (Lazlo et al. 2014; see also Mathewson et al. 2017). Active electrodes have also been used to effectively record mobile EEG in previous studies within our lab (Scanlon et al. 2017a; Scanlon et al., 2017b). During the experiment participants were asked to avoid moving their heads from side to side or looking around the scenery to mitigate data noise due to movement. However in future studies we will aim to reduce the need for movement constraints to allow for free observation of the environment while cycling. This has been shown to be possible in previous ERP studies during video game play

(Maclin et al., 2011; Mathewson et al., 2012). We hope to combine this mobile EEG recording with first person video recordings in the future.

4.1 Behavioural differences

As in previous studies (Scanlon et al., 2017a; Scanlon et al., 2017b; Scanlon et al., *under review*; Scanlon et al., *In preparation*), the ERP differences between conditions did not appear to contribute to behavioural differences in the oddball task, as there were no significant differences in reaction time between conditions. This indicates that the N1 may reflect a compensatory process, allowing the mind to put greater focus on a target when distractions are present. However, a marginal increase in response rate in the roadway condition may indicate an increase in attention to the task tones in that condition, which can also be related to the increased N1 component amplitude in that condition.

4.2 Data noise

As expected, we were able to overcome noise created by mobile cycling and collect laboratory quality ERPs (Figure 3A), however it appears that the park condition had a larger amount of baseline noise than the roadway condition (Figure 3), with higher RMS in the baseline EEG period for both trial averaged ERP and single-trial data. This may have possibly been due to slightly higher levels of elevation change within the park. While we were careful to find two environments that were similar in elevation, real world studies often have the constraint of being unable to change what the current study can access in the real world. However, the increases in single-trial noise were less than half the magnitude of the increases in noise observed between sitting still and cycling outside in a previous study (Scanlon et al., 2017b), in which the ERPs recorded appeared largely unaffected by this difference. Additionally, the ERP trial averaged RMS was increased in the park condition to a much smaller degree than the single trial RMS.

This indicates that a great deal of the noise added was not stimulus locked and was mitigated by trial averaging. This effect can also be seen in increased beta power (15-30Hz) at Fz in the park condition, possibly also due to this increase in mechanical noise, similar to increased high-frequency oscillations in the beta range found in previous studies during stationary pedaling (Scanlon et al., 2017a) and outdoor biking (Scanlon et al., 2017b) compared to rest.

Following this observation of increased single-trial and trial-averaged noise in the park condition, one may expect to also see decreased statistical power in the park condition. However this was not the case, as when we analyzed ERP power as a function of the number of resampled trials required to achieve 80% significance, we observed that neither condition was better at both components we analyzed. While the MMN/N2b required 20 more standard and 5 more target trials to reach significance in the park condition, the P3 required 40 fewer standard and 10 fewer target trials in this condition. This pattern was opposite, with respect to noise, to what was previously observed in the comparison between laboratory and outdoor mobile environments, as the condition with more data noise observed more statistical power for the MMN/N2b and less for the P3. This implies that the statistical power may be more affected by ERP differences than RMS baseline noise.

4.3 MMN and P3 morphology

As expected there were no significant differences observed between the two conditions for the P3 and MMN/N2b components. As the participants were performing the same dual task in both conditions, it appears that the P3 component was equally reduced between the two conditions. This is evident because the peak amplitude for the P3 for both conditions was approximately 2 μV lower than those observed in previous studies done by this lab in which there was no concurrent task (Scanlon et al., 2017a; Scanlon et al., 2017b). Given the well-

established observation that performing a secondary task during an auditory oddball task will reduce cognitive resources available for the task, therefore decreasing target-standard P3 amplitude (Wicken et al., 1983; Polich, 1987; Kramer & Strayer, 1988; Polich & Kok, 1995), this observation was not unexpected.

4.4 N1 and P2 morphology

Previous studies indicated that ambient noise during an oddball task decreased the P2 and increase N1 amplitude for both standards and targets during the auditory oddball task due to a process of filtering out irrelevant sounds in order to perform the task (Scanlon et al., 2017b; Scanlon et al., Under review). We initially expected that we would find a similar difference in this study, with a reduced P2 and increased N1 while participants were cycling next to noisy roadway compared to the quiet park. However this was not the case, as no significant differences were found for the P2 component. This may indicate that the excess noise of the roadway did not sufficiently create the necessity to filter out ambient noise, or, that this noise filtering process was present during both conditions. If one compares the current study to Scanlon et al. (2017a) or Scanlon et al. (2017b), the P2 amplitude in both current conditions appears to be at least 2 μV smaller than the indoor conditions of the previous studies. This leads us to deduce that the lower amount of ambient noise in the park condition was sufficient for the P2 to be reduced to an equal degree as the roadway condition, and therefore this effect was observed equally in both conditions. In contrast to this however, there was a significant difference in the N1 between the two conditions with a larger N1 amplitude while participants performed the task near the roadway. This is consistent with the increase in ambient noise shown in previous studies. This deviation indicates that while the N1 and P2 had previously been assumed to be modulated simultaneously to represent the same process, this may not be the case. Here, it appears that the

level of ambient background noise had the ability to augment the N1, but any amount of ambient noise is sufficient to reduce the P2.

The N1-P2 complex is believed to be reflective of pre-attentive sound processing within the auditory cortex (Näätänen & Picton, 1987). The components of this complex, including the P1, N1 and P2 have been demonstrated to relate to several temporally overlapping processes which originate near or within the primary auditory cortex (Näätänen & Picton, 1987; Wolpaw and Penry, 1975; Wood & Wolpaw, 1982). P2 in particular has been shown to relate to cognitive functions such as working memory, memory performance and semantic processing during contextually based tasks (Dunn et al., 1998; Federmeier & Kutas, 2002; Lefebvre et al., 2005). The P2 is also thought to reflect a component of top-down cognition and perceptual processing, and may represent a process of inhibiting one's perception of unimportant or repetitive stimuli in order to perform a task (Luck & Hillyard, 1994; Freunberger et al., 2007). Additionally, the P2 has been hypothesized to reflect a process of suppressing the perception of irrelevant stimuli to allow stimulus discrimination within a primary task (Potts et al., 1996; Potts, 2004; Kim et al., 2008; Getzmann et al., 2016). The auditory P2 appears to relate to the subjective difficulty of stimulus discrimination, as both the auditory P2 and N1-P2 complex have been reliably found to have increased amplitude following discrimination training (Atienza et al., 2002; Hayes et al., 2003; Reinke et al., 2003; Trainor et al., 2003; Tremblay et al., 1997, 2002, 2001). More specifically to our task, one study showed a decrease in auditory P2 amplitude during a speech discrimination task while participants had to ignore irrelevant background speech in a 'cocktail party' scenario, compared to a condition with no background distractions (Getzmann et al., 2016). This could explain why the P2 appears to have been reduced in both conditions, as the

auditory P2 appears to be reduced any time stimulus discrimination in the task is more difficult, and therefore any amount of ambient noise could have this effect.

While the N1 and P2 are clearly related in a cluster of processes that take place within the N1-P2 complex, they appear here to serve similar but distinct functions. Previous research has proposed that the N1 itself appears to reflect several different auditory processes, with up to six distinct components (Näätänen & Picton, 1987). The most alterable of these being influenced by several contextual factors including a 'sensory acceptance-rejection' factor, which appears to attenuate all responses to uninteresting, irrelevant or unpleasant sensory inputs while enhancing responses to pleasant, interesting or important stimuli. The N1 tends to appear with shorter latency and larger amplitude when the attended and unattended sounds can be easily distinguished by physical cues such as pitch or location (Näätänen, 1982, 1992). For example, the N1 has been shown to increase when an auditory stimulus occurs in the location where an individual is attending, (Teder-Sälejärvi, et al. 1998) to allow an individual to enhance the auditory perception of this stimuli. This effect is increased in individuals who are blind (Roder et al., 1999). Overall, N1 amplitude is said to increase as a function of increased attentional allocation to a particular auditory input channel, which correlates to increased behavioural accuracy for targets in that channel (Hink, Voorhis, Hillyard, & Smith, 1977). Functionally, the amplitude and latency of the N1 is believed to represent the quantity of sensory information moving through an early auditory channel selection mechanism selection (Hillyard. Hink, Schwent, & Picton, 1973), greater processing of attended channel information (Okita, 1981; Näätänen, 1982), as well as how well-matched the eliciting stimulus and cue characteristics are within the attended auditory input channel (Näätänen, 1992). In this study, it appears that while participants had to filter out noise in both conditions, the roadway condition required a larger

amount of attention and processing of the task tones in order to perform the task, and therefore the N1 was increased and response rate was marginally increased in this condition.

4.5 Experimental set-up

In this study participants were asked to cycle as slow as they could comfortably go in order to minimize movement noise in both conditions. Therefore we believe that none of the effects on ERP's or data noise were due to the effect of exercise on the brain. The purpose of cycling in this study, similar to our previous work with stationary cycling (Scanlon et al., 2017a) and outdoor cycling (Scanlon et al., 2017b), was to allow a type of movement during tasks which minimizes data noise created by wire movements. For example, during walking and running, an individual's head will often move up and down with each step, allowing wire movements and artifacts to be created, while cycling allows one to move in a relatively smooth straight-forward motion. However cycling is commonly used activity which requires steering control, balance, situational awareness to be done properly, which makes this a unique and rich activity to be explored within cognitive neuroscience.

This experimental setup also has some drawbacks in terms of the amount of equipment required to be carried around with the participant in the backpack. A large tablet computer was used for EEG data acquisition, while a small Raspberry Pi was used for stimulus presentation. We have recently started testing the use of miniature computer systems, such as the Latte Panda, and plan to use this in future studies. Further, the amplifier used was bulky and required long electrode wires to reach from the head to the backpack. Recent advances with EEG systems which are wireless and made specifically for mobile EEG (Debener et al., 2015; Zink et al., 2016; Hashemi et al., 2016; Krigolson et al., 2017) would greatly improve the portability of our system.

4.6 Combined Experimental comparisons

Figure 7 depicts the main measures (i.e. the N1, P2 and alpha power) from all four studies in this thesis as well as another previous study using the same task. Figure 7A demonstrates that experimental conditions in which the participant performed the task in non-ideal conditions (i.e., with background noise or low volume) had a higher N1 amplitude than conditions in which the task was performed in ideal conditions (i.e. silent background with normal volume). This demonstrates the effect of attention on N1 amplitude, as non-ideal conditions require the participant to 'tune in' to the stimulus more than they might have to in ideal conditions. Figure 7B shows that experimental conditions in which the participant performed the task in the presence of background sounds had generally lower P2 amplitude than conditions in which the task was performed in silence. This helps to demonstrate the way the P2 represents 'tuning out' distracting sounds in auditory tasks. The dichotomy between the functions of the N1 and P2 can especially be seen in the silent-low condition of experiment 2, as this condition had an enhanced N1 with a non-affected P2, demonstrating that the low-volume task required enhanced attention, with no requirement to tune out irrelevant information. The two conditions of experiment 4 also demonstrate this dichotomy, as both of these conditions have a larger N1 amplitude than any of the conditions performed in an ideal auditory situation, and the roadway condition has a significantly higher N1 than the park condition. However the P2 amplitude for both of these outdoor conditions is near zero and much lower than any condition in which the task was performed in silence, demonstrating that while there was no P2 difference within this experiment, the P2 was likely reduced in both conditions due to background sounds in the outdoor environment.

Figure 7C demonstrates that conditions in which the participants performed the task while viewing a visually rich stimulus had generally lower alpha power than those in which the task was performed while viewing a blank computer screen. This demonstrates the way alpha relates to visual attention and ecologically valid environments, as alpha has been shown to increase with decreasing attentional focus, with especially high power when an individual's eyes are closed (Berger, 1929; Adrian & Matthews, 1934; Mathewson et al., 2011). Here it appears that when participants are drawn to pay attention to the visual aspects of their environment, alpha is reduced. In comparison, when tasks are performed in visually impoverished environments, alpha appears to be high because there is nothing to draw their visual attention.

4.7 Future directions

This research program had the ultimate goal of measuring ERPs which naturally occur in the real world, in order to gain a deeper understanding of how the brain functions in during everyday life. We have recently begun testing EEG set-up which uses video capture and coding to identify moments in the EEG data when interesting events happen in an individual's environment. We have also started to test the use of virtual reality (VR) to create environments and allow stimulus presentation, which also opens up the possibility of testing the brain in any unlimited number of simulated environments.

4.8 Conclusion

In this study we showed that we were able to carry out a fully mobile cycling ERP study in which the environment itself is the independent variable. We also demonstrated that the environment in which one performs a task is enough to alter brain activity, and that the N1 and P2, while functionally related, clearly represent two separate processed in the early auditory system. It appears that the N1 increases when a situation requires increased attentiveness to a

stimulus, while the P2 is altered any time the auditory task is more difficult to discriminate. As shown in previous studies, this effect appears to be independent of movement, alpha power and behavioural measures.

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