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A magnetic resonance imaging-safe method for the study of human eyeblink conditioning

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Abstract

Eyeblink conditioning (EBC) is a widely used translational probe of cerebellar function in both humans and non-human animals. Decades of animal research have identified the cerebellum as critical for EBC. While there is evidence for the involvement of the cerebellum in human EBC, the neural circuitry of EBC in healthy humans has yet to be fully elucidated. The purpose of this study was to design and validate a highly customisable system for EBC stimulus presentation and response recording using infrared (IR) reflectance suitable for use in magnetic resonance imaging (MRI) environments; in this way, the neural activity of EBC could be investigated using fMRI in humans. Four participants underwent delay EBC and simultaneous fMRI. The results indicate (1) a high signal-to-noise ratio in the IR reflectance data that effectively quantifies the eyeblink morphology and timing and (2) evidence of conditioning in the fMRI environment. The quality of the data, the feasibility of conducting EBC experiments in the fMRI environment, and the customisability of the current system to fit a variety of EBC experimental design parameters are discussed.

Keywords

Eyeblink conditioning; Cerebellum; Infrared; fMRI; Translational

1. Introduction

Eyeblink conditioning (EBC) is a form of associative learning that is known to depend on the integrity of the cerebellum and related brain circuits and, therefore, is widely used to probe cerebellar function. Although there are a number of variants of the EBC paradigm, the most common is the delay EBC procedure. Delay EBC is composed of repeated presentations of a conditioned stimulus (CS) that is paired with a co-terminating unconditioned stimulus (US). The US, typically a corneal airpuff in humans, naturally elicits an eyeblink, which is the unconditioned response (UR). The CS, usually a brief tone or visual stimulus, does not typically elicit a blink response. However, upon repeated pairing with the co-terminating US, the CS reliably elicits an eyeblink response prior to US

presentation, which is the conditioned response (CR). The application of this classic, highly versatile procedure to pressing questions in contemporary human cognitive neuroscience is limited, however, by the lack of instrumentation suitable for functional magnetic resonance neuroimaging.

Decades of non-human animal research have demonstrated unequivocal involvement of the cerebellum in delay EBC (Steinmetz, 2000; Christian and Thompson, 2003; Thompson and Steinmetz, 2009). Particularly striking evidence comes from studies that report preserved conditioning in decerebrate animals (Mauk and Thompson, 1987; Kotani et al., 2002). In addition, the neural circuitry that underlies delay EBC in animals has been well characterised. Specifically, the neural substrate of delay EBC has been localised to the anterior interpositus nucleus (Steinmetz et al., 1991, 1992; Wikgren and Korhonen, 2001; Lincoln et al., 1982), and areas of the cerebellar cortex involved in the timing and execution of CRs have been identified (Villarréal and Steinmetz, 2005; Perrett et al., 1993; Green and Steinmetz, 2005). There has also been evidence of CR learning-related plasticity in other areas of the brain, such as the hippocampus, neostriatum, and thalamus (Berger and Thompson, 1978; White et al., 1994; Sears et al., 1996).

Such extra-cerebellar neural activity is believed to be a consequence of associative learning. Such activity might comprise an additional, distributed EBC system and be possibly involved in integrating information related to the context under which delay EBC occurs and other aspects of the delay EBC paradigm that are not critical to the formation of the CR (see Steinmetz, 2000, for a discussion of these issues).

The highly translational nature of delay EBC makes this paradigm a potentially excellent candidate model for assessing cerebellar function in humans, and interest in studying EBC in humans has gained traction due to accumulating evidence in two major research areas. First, interest in the cerebellum has piqued in recent years due to the increasing body of evidence indicating a role for the cerebellum in higher-order cognitive functions (Leiner et al., 1989, 1991; Schmahmann and Pandya, 1997; Strick et al., 2009; Le et al., 1998; Kim et al., 1994; Paulesu et al., 1993; Desmond et al., 2005). Second, evidence of cerebellar dysfunction has been documented in many clinical populations, including schizophrenia (Andreasen and Pierson, 2008; Picard et al., 2008), bipolar disorder (DeBello et al., 1999; Strakowski et al., 2005), autism (Courchesne, 1997; Fatemi et al., 2001), dyslexia (Eckert et al., 2003; Fawcett et al., 1996), and attention-deficit/hyperactivity disorder (Valera et al., 2005; Berquin et al., 1998; Mostofsky et al., 1998).

However, it is currently unclear to what extent the neural circuitry of EBC in non-human animals overlaps with the circuitry that is engaged during human EBC. There is evidence for a critical role of the cerebellum in human delay EBC, which first emerged from studies reporting impaired delay EBC performance in individuals with cerebellar lesions (Lye et al., 1988; Solomon et al., 1989; Daum et al., 1993; Topka et al., 1993; Woodruff-Pak et al., 1996; Timmann et al., 2005). There have been a number of brain imaging studies using positron emission tomography (PET) that have reported changes in cerebellar activation following delay EBC (Molchan et al., 1994; Logan and Grafton, 1995; Blaxton et al., 1996; Schreurs et al., 1997; Parker et al., 2012). However, various changes in extracerebellar activation were also reported in these studies. Thus, while the cerebellum appears to be heavily involved in human delay EBC, it is important to identify the underlying neural circuitry and determine the extent to which it parallels the well-characterised circuitry in non-human animals to completely realise the translational potential of EBC. In addition to broadening opportunities for translational research, elucidating the neural circuitry that underlies human delay EBC would be highly useful to the burgeoning lines of human research described above because comprehensive assessment of cerebellar function will be a

crucial aspect of research investigating the relationship between cerebellar function and cognition and psychopathology. There are few techniques that allow for the study of brain involvement in human EBC. The fine-grained spatial resolution of fMRI has the potential to serve as a powerful tool in the investigation of the neural activity that instantiates delay EBC in humans, but relatively few studies have utilised this technique. Lemieux and Woodruff-Pak (2002) further outline the suitability of fMRI for elucidating the neural mechanisms of EBC and discuss the many parametric and analytic considerations germane to imaging this task. The impetus for the present paper is that the necessary equipment for simultaneous delay EBC and fMRI is not readily available. To date, three in-house techniques for simultaneous delay EBC data collection and fMRI scanning have been presented. First, Ramnani and colleagues (2000) used an optomechanical low-torque transducer to quantify eyeblink responses in an MRI environment. Second, Knutinen and colleagues (2002) used electrodes designed to monitor the electrocardiogram signal in an MRI environment to record the electromyographic signal of eyeblink movement. Finally, Cheng and colleagues (2008) used infrared (IR) reflectance off of the eyelid to collect EBC data during fMRI recording. The current study describes the development and initial validation of a highly customisable IR instrument for the stimulus presentation and recording of human EBC in an MRI environment.

2. Materials and methods

2.1. Device development

2.1.1. Design objectives—The goal was to develop and validate a highly customisable infrared (IR)-based instrument for stimulus presentation and recording of human EBC in an MRI environment. This system allows for CS stimuli of different modalities (auditory and visual) and durations and is amenable to both the delay (co-terminating CS and US) and trace (CS and US do not overlap) EBC paradigms. This instrument manages both stimulus presentation and data collection.

2.1.2. Stimulus control features

2.1.2.1. Software user interface: An Eye Blink Detector (EBD) program was written in Visual Basic C++ to control stimulus presentation and data collection. The EBD program was designed such that the many parameters of EBC stimulus presentation could be easily manipulated (see Fig. 1a–n) for either delay or trace forms of conditioning. The CS options include a choice between a 1000 or 2000 Hz tone (Fig. 1a) as well as volume control (Fig. 1b). There is also an option to use a visual CS (white light) (Fig. 1c). The CS and US can be presented together as a paired trial or separately in a number of pseudorandom orders (Fig. 1d). A maximum number of sequential presentations of the same stimulus can be set (Fig. 1e). A recurrent pseudorandom order, in which stimuli are presented in a random order consistently for each subject, can be predetermined by a settings file and is referenced by the EBD program using a random seed number (Fig. 1f). In addition, there is a parameter that specifies the length of the program's data acquisition window (Fig. 1g). The maximum and minimum inter-trial interval (ITI) values (i.e., the period of time between data acquisition windows) can be established (Fig. 1h), and there is an option to specify recurrent pseudorandom ITIs in which the same order of randomly generated ITI values that fall between minimum and maximum ITI values will be used for the stimulus presentation every time data are collected by the program (Fig. 1i). The inter-stimulus interval (ISI) can be easily adjusted by parameters that denote the latency of each stimulus from the start of the data acquisition window (Fig. 1j and k). Furthermore, a variety of settings files can be created and loaded such that entire sets of parameters are established upon opening the program (Fig. 1l). Finally, the operating system in which the EBD software runs has been optimised for scientific stimulus presentation and data collection through the removal of

unnecessary background programs and disabling hardware and software that is not required for the EBD program.

It is important to note that the variety of customisable stimulus presentation settings is extremely conducive to event-related fMRI experimental designs. Maximum and minimum ITI values (Fig. 1h) can be defined as sufficiently large to allow for the analysis of neural activity on a trial-by-trial basis. Using the recurrent pseudorandom ITI option allows for within-subject jittered presentation of the EBC stimuli such that paradigm predictability can be avoided while maintaining this jittered pattern of stimulus timing between subjects (Fig. 1i). While this EBC stimulus presentation system is optimised for event-related fMRI, it should be noted that block design fMRI experiments can also be conducted using the current system. To conduct blocked conditioning experiments, the ITIs (Fig. 1h) would be short and consistent across all trials and would, thereby, maintain the neural activation necessary for delay EBC across multiple trials. The total number of trials (Fig. 1m) would be reduced in such an experimental set-up. Finally, blocked periods of rest, against which active conditioning blocks could be compared, could be introduced before a conditioning run by specifying the number of MRI triggers that the program must wait through before initiating the EBC stimulus presentation (Fig. 1n).

2.1.2.2. Hardware: The EBD software program manipulates the parameters of EBC and then communicates with a custom-built EBD control box to regulate stimulus presentation. The control box houses a National Instruments USB-6210 Data Acquisition (DAQ) device. The control box system is connected through a USB pathway to a computer (e.g., a Dell Latitude 820 laptop), where the EBD control software is installed. According to the settings and parameters established with the EBD software, the DAQ sends signals to an electronics board in the chassis with information regarding stimulus presentation (i.e., under what circumstances and at what time to present which type of stimulus). A number of parameter settings can also be adjusted using the EBD control. The only setting that is accessible exclusively through the EBD control box is the US duration, which can be set to either 50 or 100 ms. In addition, buttons on the EBD control box and in the EBD program can be used to deliver a stimulus manually. During data collection, the EBD software program settings override the settings indicated on the EBD control box in the event of inconsistencies between the EBD control box and the software program. The US is delivered to the eye via a tube attached to modified swimming goggles, which have a low profile for fitting within the head coil. The US airpuff travels 13 feet from an air tank (regulator set to 30 psi) to an air solenoid regulator (set to 20 psi) and then 25.5 feet further to the modified goggles for delivery into the inner corner of the participant's eye (i.e., between the iris and the caruncula lacrimalis). Under these conditions, the total travel time of the airpuff to the eye is 33 ms, and this delay can be accounted for in the airpuff onset parameter built into the EBD program. The CS tone is also produced in the EBD control box with an Exar XR-2206 Monolithic Function Generator integrated circuit, which produces a sine waveform that is delivered through MR-safe headphones.

2.1.3. Response recording

2.1.3.1. Software: To maximise the quality of the IR signal and to streamline the process of positioning the IR emitter/sensor, the EBD software program has a "Monitor Eyeblink" feedback mode for use during experiment set-up. When "Monitor Eyeblink" mode is on, the voltage of the IR reflectance is collected and displayed by the EBD software program in successive 1 s data display windows (see Fig. 2). Such a display function gives the experimenter vital visual feedback regarding the amplitude, morphology, and signal-to-noise ratio of the IR signal. The effects of physical adjustments of the position of the goggles and IR emitter/sensor can be readily observed to optimise placement. During data collection, the

EBD software program records event-related data from four channels: (1) the IR reflectance; (2) the state of the auditory stimulus generator (i.e., on/off); (3) the state of the airpuff regulator (i.e., closed/open); and (4) the MRI trigger pulses (see Fig. 3). The duration of the recording epochs can be customised to be between 1 and 10 s (a 5 s epoch is illustrated in Fig. 3). Data from all four channels are sampled at 1000 Hz and are saved in a text file for each individual trial. These text files can be exported via an external hard drive for subsequent processing and analysis.

2.1.3.2. Hardware: Eyeblink responses were recorded using the goggles and a detachable, plastic emitter/sensor arm that can be positioned over the eye (Fig. 4). This emitter/sensor arm can be adjusted to position two fibre optic cables (with a conducting diameter of 980 μm) over the eye. One fibre optic cable emits the IR signal (originating from an IR-emitting LED), and the other fibre optic cable is used to transmit the reflectance of the IR from the eyelid to the EBD control box. It should be noted that the IR wavelength (870 nm) used in the IR system described in the present paper is classified as part of the near infrared (NIR) portion of the infrared spectrum. IR light travels 20 feet through a fibre optic cable from an IR-emitting LED in the scanning room and presents a 12.7 mm circle of IR light to the eyelid surface at a distance of 19.05 mm from the plastic emitter/sensor arm. IR reflectance is then passively conducted 29.75 feet through the second fibre optic cable to a photodiode detector in the EBD control box. The emitter and sensor arm can be adjusted both horizontally and vertically. For maximum signal, the IR-emitting cable is placed directly above a participant's iris/pupil area and is oriented perpendicular to the apex of the curvature of the eye (see Fig. 5).

2.2. Initial validation

2.2.1. Participants—Four participants (2 male, mean age 28.75 years) were recruited for this experiment and completed the study protocol, which was approved by the Indiana University Human Subjects Internal Review Board.

2.2.2. Procedure—The participants were scanned with a Siemens Trio 3T MRI scanner using a 1.5 s TR (repetition time) while undergoing delay EBC. The experiment was composed of four blocks of 690 s duration. Subjects were at rest for the first and last 30 s (20 MRI triggers) of each block. The first block was a pseudo-conditioning run in which 26 tones and 26 airpuffs were randomly presented. The remaining three blocks were identically structured conditioning runs in which 52 paired trials were presented. The CS was a 1000 Hz 400 ms tone, and the US was a 50 ms, 20 psi airpuff at the source with a 328 ms post-tone onset latency (i.e., 328 ms ISI). A random ITI between 9.6 and 13.6 s was used (mean = 11.6 s). During the experiment, subjects were asked to keep their eyes comfortably open and to keep their eyes focused in roughly the same position when they were not blinking.

3. Results

Data from four participants who underwent simultaneous delay EBC and fMRI are presented in Fig. 6 (panels a–d), which depicts the eyeblink performance of each subject by block. There are several important aspects of the data, most notably that these subjects show evidence of conditioning as indicated by the shift in the blink onset latency to earlier in time (i.e., the blinks occur earlier and earlier with respect to the airpuff, or unconditioned stimulus) as a function of the block number. Specifically, this shift appears to occur as early as block 2 (the first block comprised of paired trials) for subject 1 (Fig. 6a). Conditioning appears later for the other subjects; subject 2 (Fig. 6b) shows earlier blink latencies starting in block 3, and subjects 3 and 4 (Fig. 6c and d, respectively) show evidence of conditioning beginning in block 4. It is also important to note the reduced UR amplitude over time, as

evidenced by smaller blink amplitudes in the latter 3 blocks compared to the first block (only US-alone trials are plotted in Fig. 6). Such UR amplitude modification has been shown to be associated with CR acquisition (e.g., Sears and Steinmetz, 1991; Schreurs et al., 2000). Moreover, such UR attenuation across trial blocks is consistent with previously published human EBC data (Brown et al., 2005; Forsyth et al., 2012; Bolbecker et al., 2009a,b). Finally, it appears that alpha responses to the CS can be seen in subject 2, as is sometimes observed early in learning (Schreurs et al., 1997; Sommer et al., 1999).

4. Discussion

The current study introduces and validates a new, highly customisable IR reflectance-based instrument for assessing eyeblink conditioning in humans in the fMRI environment. The quality of the IR reflectance at the single-trial and aggregate level is suitable for the assessment of conditioned eyeblink measurement. Furthermore, participants showed signs of learning in response to the delay EBC task that was administered in an fMRI environment. There are many advantages of the MRI-safe method for quantifying delay EBC performance presented in this paper. High data quality is particularly valuable for EBC data collection in the scanner given the high cost and large time investment incurred by fMRI studies. The large signal-to-noise ratio of the IR signal used to quantify the eyeblink response described here greatly increases the chances of collecting usable data. An additional notable aspect of the current method is the ability to explore the parameter space of delay EBC. Given the small number of studies that use fMRI to study delay EBC in humans, little is known about the optimal parameters for this paradigm in the MRI environment. Given that previously reported rates of learning are low, it appears to be plausible that the noisy environment of the scanner could distract participants from attending to the stimuli or otherwise interfere with delay EBC. Furthermore, it is possible that optimal ITI or ISI parameters are different in the MRI environment compared to normal laboratory conditions, or that using a visual CS could be more effective. The novel technique proposed in this paper allows for the exploration of a wide variety of delay EBC parameters, an issue that will no doubt grow in importance as functional neuroimaging is used increasingly frequently to investigate delay EBC in humans. Finally, the flexibility of the current system in presenting either blocked or event-related EBC stimuli is another advantage, particularly given that the only other published study describing an IR system to present and record EBC in humans utilises solely the blocked design (Cheng et al., 2008). There are several future directions inspired by the current study. Given the small number of studies published on this topic to date, examining fMRI activation in response to delay EBC-related learning will be a valuable contribution to the body of research that aims to elucidate the neural correlates of delay EBC in humans. As described above, an exploration of the parametric properties of delay EBC in the fMRI environment could also be of great value because very little is known about this issue, and subsequent years could see an increase in using fMRI to study delay EBC in humans. Finally, it would be worthwhile to explore the neural correlates of delay EBC using a visual CS because much less is known about the neural circuitry involved in this variant of delay EBC.

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HIGHLIGHTS

- We present a novel system for conducting simultaneous fMRI and EBC in humans.
- Blink morphology and timing is recorded using infrared (IR) reflectance.
- Subjects show evidence of conditioning in the fMRI environment.
- IR reflectance data are high quality and have a high signal-to-noise ratio.
- This system can be customised to accommodate various EBC parameters and modalities.

The screenshot displays a software interface for configuring experimental parameters. Key sections include:

- EB Display Gain:** A slider set to 07.
- Monitor Eye Blink Signal:** A checkbox.
- Threshold:** A text field set to 2.e-002 Volts.
- Session Information:** Includes 'Sequential Repeat Max' (4), 'Total # of Trials' (52), 'Seed Number' (1), and 'Recurrent / Random' options.
- Inter Trial Interval (ITI):** Fields for Minimum (5) and Maximum (9) seconds.
- Subject ID:** A text field with 'Enter Subject ID here' and gender selection (Male/Female).
- Audio Settings:** Includes 'Set Volume To' (1), 'A/V Duration' (0.4), and 'A/V Time' (2).
- Stimulus Modality:** Radio buttons for 'A/V Stimuli Only', 'A/V and Puff Stimuli', and 'Puff Stimuli Only'.
- Timing and Triggers:** Includes 'MRI Trigger Rate' (1500 ms), 'Puff Time' (2.295), and 'Start 1st Trial after # MRI Trigs' (20).
- Other Settings:** Includes 'Elapsed Time' (20321 ms), 'Puff' (0), 'Auditory' (0), 'Today's Date' (07/27/2011), and 'Phase #' (1).

Labels a through m point to specific settings: (a) Tone Frequency, (b) #2 Tone Frequency, (c) Test Audio, (d) Sequential Repeat Max, (e) Total # of Trials, (f) Seed Number, (g) Subject ID, (h) Trial Time, (i) Inter Trial Interval (ITI), (j) A/V Duration, (k) Puff Time, (l) Phase #, (m) Total # of Trials, and (n) Start 1st Trial after # MRI Trigs.

Fig. 1.

A screenshot of the parameter settings for: CS frequency selection (a), volume control (b), CS modality selection (c), stimulus presentation pairing options (d), maximum number of sequential repeat presentations for individually presented stimuli (e), randomisation options for stimulus presentation (f), data acquisition window time (g), maximum and minimum ITI values (h), options for randomising ITI presentation (i), stimulus latency values (j and k), settings file specification (l), total number of trials (m), and number of MRI triggers preceding initiation of the stimulus presentation procedures (n).

Fig. 2.
A screenshot of "Monitor Eyeblink" mode.

Fig. 3.
A screenshot of the 5 s data acquisition window. Note that the MRI trigger, tone signal, airpuff signal, and IR signal are recorded.

Fig. 4.
A model wears the modified goggles.

Fig. 5.
An example of the properly attached and positioned IR sensor block.

Fig. 6. Single-subject IR data across four experiment blocks for four subjects. Here, 2500 ms of the 5000 ms data acquisition window have been plotted, and the arrows indicate CS and US onset latencies.