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

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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.

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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.

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

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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 (Villarreal 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 (DelBello 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, Knuttinen 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