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Hippocampal and cerebellar single-unit activity during delay and trace eyeblink conditioning in the rat

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Abstract

In delay eyeblink conditioning, the CS overlaps with the US and only a brainstem–cerebellar circuit is necessary for learning. In trace eyeblink conditioning, the CS ends before the US is delivered and several forebrain structures, including the hippocampus, are required for learning, in addition to a brainstem–cerebellar circuit. The interstimulus interval (ISI) between CS onset and US onset is perhaps the most important factor in classical conditioning, but studies comparing delay and trace conditioning have typically not matched these procedures in this crucial factor, so it is often difficult to determine whether results are due to differences between delay and trace or to differences in ISI. In the current study, we employed a 580-ms CS–US interval for both delay and trace conditioning and compared hippocampal CA1 activity and cerebellar interpositus nucleus activity in order to determine whether a unique signature of trace conditioning exists in patterns of single-unit activity in either structure. Long–Evans rats were chronically implanted in either CA1 or interpositus with microwire electrodes and underwent either delay eyeblink conditioning, or trace eyeblink conditioning with a 300-ms trace period between CS offset and US onset. On trials with a CR in delay conditioning, CA1 pyramidal cells showed increases in activation (relative to a pre-CS baseline) during the CS–US period in sessions 1–4 that was attenuated by sessions 5–6. In contrast, on trials with a CR in trace conditioning, CA1 pyramidal cells did not show increases in activation during the CS–US period until sessions 5–6. In sessions 5–6, increases in activation were present only to the CS and not during the trace period. For rats with interpositus electrodes, activation of interpositus neurons on CR trials was present in all sessions in both delay and trace conditioning. However, activation was greater in trace compared to delay conditioning in the first half of the CS–US interval (during the trace CS) during early sessions of conditioning and, in later sessions of conditioning, activation was greater in the second half of the CS–US interval (during the trace interval). These results suggest that the pattern of hippocampal activation that differentiates trace from delay eyeblink conditioning is a slow buildup of activation to the CS, possibly representing encoding of CS duration or discrimination of the CS from the background context. Interpositus nucleus neurons show strong modeling of the eyeblink CR regardless of paradigm but show a changing pattern across conditioning that may be due to the necessary contributions of forebrain processing to trace conditioning.

Keywords: Eyeblink classical conditioning; Hippocampus; Interpositus nucleus; Trace conditioning; Delay conditioning; Interstimulus interval

1. Introduction

Previous research has shown that all forms of eyeblink conditioning require a discrete brainstem–cerebellar circuit for acquisition and retention of conditioned eyeblink responses (see Christian & Thompson, 2003 for a review). In delay eyeblink conditioning, a conditioned stimulus (CS; most often a tone or a light) consistently precedes (by less than 1-s) and overlaps with an unconditioned stimulus (US; a corneal airpuff or periorbital stimulation). Initially, only the US elicits an eyeblink response (the unconditioned response; UR). Eventually, the CS also elicits an eyeblink response (the conditioned response; CR). Delay eyeblink conditioning requires only a brainstem–cerebellar circuit for acquisition. The CA1 field of the hippocampus shows nearly immediate increases in pyramidal cell activity to the

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US during delay conditioning with a 250-ms CS–US interval and rapidly develops increased activity to the CS that models the amplitude–time course of the behavioral CR (Berger, Rinaldi, Weisz, & Thompson, 1983; Berger & Thompson, 1978a). However, lesions of the hippocampus do not affect (and may sometimes even enhance) delay eyeblink conditioning (Akase, Alkon, & Disterhoft, 1989; Beylin et al., 2001; Lee & Kim, 2004; Port, Mikhail, & Patterson, 1985; Schmaltz & Theios, 1972; Solomon & Moore, 1975), although they may have effects on retention of delay conditioning (Akase et al., 1989) or acquisition with non-optimal CS–US intervals (Beylin et al., 2001; Port et al., 1985).

In contrast to delay conditioning, a number of forms of eyeblink conditioning require the hippocampus (and other forebrain areas such as the medial prefrontal cortex) in addition to the brainstem–cerebellar areas required for delay conditioning (Christian & Thompson, 2003; Green & Woodruff-Pak, 2000). The simplest form of eyeblink conditioning that requires the hippocampus for acquisition and short-term retention is trace conditioning. In trace conditioning, the CS consistently precedes, but does not overlap with, the US. In trace eyeblink conditioning, the CS ends 250–500 ms prior to delivery of the US. Trace eyeblink conditioning appears to require the same brainstem–cerebellar circuit for acquisition and retention as delay eyeblink conditioning (Takehara, Kawahara, & Kirino, 2003; Woodruff-Pak, Lavond, & Thompson, 1985) but, when the stimulus-free trace period is long enough (250-ms for rodents; Tseng, Guan, Disterhoft, & Weiss, 2004; Weiss et al., 1999; 500-ms for rabbits, Moyer, Deyo, & Disterhoft, 1990) the hippocampus is required as well. Lesion studies have indicated that the hippocampal formation is necessary for normal acquisition and/or proper timing of trace eyeblink CRs (Beylin et al., 2001; Ivkovich & Stanton, 2001; James, Hardiman, & Yeo, 1987; Kishimoto, Nakazawa, Tonegawa, Kirino, & Kano, 2006; Moyer et al., 1990; Port, Romano, Steinmetz, Mikhail, & Patterson, 1986; Solomon, VanderSchaaf, Thompson, & Weisz, 1986; Takehara et al., 2003; Tseng et al., 2004; Weiss et al., 1999) and for short-term retention (perhaps up to several weeks; Kim, Clark, & Thompson, 1995; Takehara et al., 2003; Takehara, Kawahara, Takatsuki, & Kirino, 2002) of trace eyeblink conditioning. However, recording studies of CA1 unit activity during trace eyeblink conditioning have yielded somewhat inconsistent results. For example, it has been reported that, relative to a control group that received explicitly unpaired stimulus presentations, CA1 pyramidal cell activity in rabbits that underwent trace conditioning with a 600-ms CS–US interval (100-ms CS followed by a 500-ms trace period prior to US delivery) showed increases in activity during and immediately after the CS and after the US only during the initial blocks of trials of sessions when CRs begin to emerge (McEchron & Disterhoft, 1997). These increases in activity rapidly became smaller both within and between sessions as the CR emerged. As CRs became asymptotic, CA1 pyramidal cells showed little responsiveness to the CS and showed a decrease in firing after the US relative to unpaired stimulus presentations (McEchron & Disterhoft, 1997; Weiss, Kronforst-Collins, & Disterhoft, 1996). However, Delgado-Garcia and colleagues have reported that CA1 pyramidal cells in a group of cats that underwent trace eyeblink conditioning with a 520-ms CS–US interval (20-ms CS followed by a 500-ms trace period prior to US delivery) showed increases to CS and US onset from the beginning of CS–US presentations. Similar increases in pyramidal cell activity to CS and US onset were evident in a 500-ms delay procedure (Munera, Gruart, Munoz, Fernandez-Mas, & Delgado-Garcia, 2001), suggesting that the hippocampus may not differentiate between delay and trace conditioning at the level of single-unit activity. The amount of pyramidal cell activation to CS onset was reported to increase as delay or trace conditioning progressed. However, it is difficult to directly compare these results with those of Disterhoft and colleagues since the CS used in trace conditioning in Munera et al. (2001) was very short (20-ms) and conditioning was preceeded by extensive (240 presentations) exposure to the CS, which tends to dampen the CR-related modeling of hippocampal units during delay conditioning (Katz, Rogers, & Steinmetz, 2002).

In the current study, we sought to determine the extent to which the timing and pattern of CA1 pyramidal cell activation that develops during trace eyeblink conditioning is different from the activation that develops during a delay eyeblink conditioning procedure which differed only in the lack of a trace period between the CS and US. Trace conditioning represents perhaps the simplest form of learning that requires the hippocampus. A number of proposals have been advanced regarding why the brief gap between the CS and the US should engage the hippocampus, including filling the trace period gap (Rodriguez & Levy, 2001; Sutton & Barto, 1981), timing the relation between the CS and US (McEchron & Disterhoft, 1999), configuring CS onset and offset into a single CS (Kehoe & Weidemann, 1999), discriminating the trace period from the intertrial interval (Bolles, Collier, Bouton, & Marlin, 1978; Kaplan & Hearst, 1982; Marchand, Luck, & DiScala, 2004), and subserving the awareness that the CS predicts the US (Clark & Squire, 1998, 1999; Clark, Manns, & Squire, 2001; Manns, Clark, & Squire, 2000a, Manns, Clark, & Squire, 2000b, 2002). Comparison of hippocampal single-unit activity during trace conditioning with a procedure (delay conditioning) that does not require the hippocampus and that is identical to trace conditioning except for the lack of a trace period, would be helpful in beginning to discriminate among these alternatives.

In addition, we compared hippocampal activation patterns to those of interpositus nucleus neurons in rats undergoing delay or trace eyeblink conditioning. While it is clear that the interpositus nucleus is necessary (but not sufficient) for trace eyeblink conditioning (Woodruff-Pak et al., 1985), recent studies have raised the question of whether the cerebellum processes trace eyeblink conditioning somewhat differently than delay eyeblink conditioning. Specifically,