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Unimpaired trace classical eyeblink conditioning in Purkinje cell degeneration (*pcd*) mutant mice

Kevin L. Brown, Alexis Agelan, Diana S. Woodruff-Pak *

Temple University, 1701 North 13th Street/Weiss Hall, Philadelphia, PA 19122, United States

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ABSTRACT

Young adult Purkinje cell degeneration (*pcd*) mutant mice, with complete loss of cerebellar cortical Purkinje cells, are impaired in delay eyeblink classical conditioning. In the delay paradigm, the conditioned stimulus (CS) overlaps and coterminates with the unconditioned stimulus (US), and the cerebellar cortex supports normal acquisition. The ability of *pcd* mutant mice to acquire trace eyeblink conditioning in which the CS and US do not overlap has not been explored. Recent evidence suggests that cerebellar cortex may not be necessary for trace eyeblink classical conditioning. Using a 500 ms trace paradigm for which forebrain structures are essential in mice, we assessed the performance of homozygous male *pcd* mutant mice and their littermates in acquisition and extinction. In contrast to results with delay conditioning, acquisition of trace conditioning was unimpaired in *pcd* mutant mice. Extinction to the CS alone did not differ between *pcd* and littermate control mice, and timing of the conditioned response was not altered by the absence of Purkinje cells during acquisition or extinction. The ability of *pcd* mutant mice to acquire and extinguish trace eyeblink conditioning at levels comparable to controls suggests that the cerebellar cortex is not a critical component of the neural circuitry underlying trace conditioning. Results indicate that the essential neural circuitry for trace eyeblink conditioning involves connectivity that bypasses cerebellar cortex.

1. Introduction

Eyeblink classical conditioning is of demonstrated utility as a model system for the study of neurobiological mechanisms underlying associative learning and memory. A substantial body of data has demonstrated that the cerebellar interpositus nucleus ipsilateral to the conditioned eye is essential for the acquisition and maintenance of eyeblink conditioning (see Christian and Thompson (2003) for a review). In eyeblink conditioning, conditioned stimulus (CS) and unconditioned stimulus (US) information are transmitted to the cerebellum via mossy fibers originating in the pontine nuclei and climbing fibers originating in the inferior olive, respectively (Mauk, Steinmetz, & Thompson, 1986; Steinmetz, Lavond, & Thompson, 1989; Steinmetz, Rosen, Chapman, Lavond, & Thompson, 1986; Steinmetz et al., 1987). This CS and US information converge upon (1) Purkinje cells in the cerebellar cortex and (2) the cerebellar interpositus nucleus (Gould, Sears, & Steinmetz, 1993; Steinmetz & Sengelaub, 1992; Thompson, 1986; Tracy, Thompson, Krupa, & Thompson, 1998). Repeated pairings of this convergent information are hypothesized to yield robust synaptic plasticity (e.g., long-term depression – LTD; long-term potentiation – LTP) within each cerebellar region, resulting in learning of the contingent CS–US relationship (Hansel, Linden, & D'Angelo, 2001; Linden & Connor, 1995; Nores, Medina, Steele, & Mauk, 2000; Pugh & Raman, 2006). Whereas it is widely-accepted that the cerebellar interpositus nucleus is essential for all forms of eyeblink classical conditioning, there is debate about the role of cerebellar cortical integrity in normal acquisition.

Lesions of the cerebellar cortex have produced dramatically different results, ranging from mild impairments to complete abolition of the eyeblink conditioned response (CR, Lavond & Steinmetz, 1989; Lavond, Steinmetz, Yokaitis, & Thompson, 1987; Yeo, Hardimann, & Glickstein, 1985). A mutant mouse model – the Purkinje cell degeneration (*pcd*) mouse – has provided valuable data for addressing this debate. Mice homozygous for the *pcd* mutation are born with Purkinje cells, but by the fourth postnatal week all Purkinje cells have been eliminated (Mullen, Eicher, & Sidman, 1976). Importantly, the integrity of the interpositus nucleus is maintained in these mice (Chen, Bao, Lockard, Kim, & Thompson, 1996). Since Purkinje cells represent the sole output of the cerebellar cortex, these mice exhibit a "functional lesion" of the entire cerebellar cortex that obviates potential methodological pitfalls inherent with traditional lesion methods. Delay eyeblink conditioning – a paradigm in which the CS overlaps and coterminates with the US – is impaired in young adult *pcd* mice relative to controls, though *pcd* mice produce low levels of conditioning (Chen et al., 1996). CR levels during delay eyeblink conditioning in *pcd*

* Corresponding author.
E-mail address: pak@temple.edu (D.S. Woodruff-Pak).

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mice do not appear to represent pseudoconditioning, as unpaired presentations of the CS and US in *pcd* mice yield significantly lower CR percentages (<20%) than counterparts given paired CS–US training (Chen, Bao, & Thompson, 1999). Further evidence that eyeblink conditioning impairments in *pcd* mice are associative in nature is provided by standard performance measures, as UR amplitudes (Chen et al., 1996) and tone-induced activity in cochlear nuclei (Chen et al., 1999) do not differ between *pcd* and control mice. Additionally, lesions of the interpositus nucleus in *pcd* mice abolish conditioned eyeblink responses (Chen et al., 1999). Impairments in delay eyeblink conditioning have also been shown in "waggler", a mutant mouse which lacks brain-derived neurotrophic factor (BDNF) in cerebellar granule cells but appears normal in morphological features of cerebellar deep nuclei (Bao, Chen, Qiao, Knusel, & Thompson, 1998). These findings suggest that the cerebellar cortex is normally involved in delay eyeblink conditioning but is not essential.

Similar findings of impaired (but not abolished) eyeblink conditioning in the delay paradigm have been shown in various mutant and transgenic mouse models exhibiting impaired cerebellar cortical LTD. Specifically, glial fibrillary acidic protein (GFAP; Shibuki et al., 1996), PTPMEG (a cytoplasmic protein–tyrosine phosphatase expressed in Purkinje cells; Kina et al., 2007), and δ2 glutamate receptor (GluRδ2; Kakegawa et al., 2008) knockout mice as well as phospholipase C β4 (PLCβ4; Kishimoto, Hirono et al., 2001) and metabotropic glutamate receptor 1 (mGluR1; Aiba et al., 1994) mutant mice all show impairments in cerebellar cortical LTD and delay eyeblink conditioning. Recently, Lee, Chatila, Ram, and Thompson (2009) demonstrated impaired retention but unimpaired acquisition in calcium/calmodulin-dependent protein kinase type IV (CaMKIV) knockout mice, behavioral effects that parallel findings of normal acquisition but impaired maintenance of LTD in mice deficient in CaMKIV (Ho et al., 2000). Furthermore, genetically modified mice that exhibit alterations in cerebellar cortical functioning without impairing LTD are unimpaired in delay eyeblink conditioning (see Endo et al., 2009; Tanaka et al., 2008). These findings provide substantial evidence that LTD at Purkinje cell synapses is the primary cerebellar cortical mechanism by which normal acquisition and retention of delay eyeblink conditioning is produced and maintained.

Trace eyeblink conditioning is a variant of eyeblink conditioning in which a stimulus free ("trace") period – usually 250–1000 ms (depending on the species that is tested) – occupies the interval between the offset of the CS and the onset of the US. When sufficiently long trace intervals are used, acquisition of trace eyeblink conditioning requires the integrity of forebrain areas such as the hippocampus (Kim, Clark, & Thompson, 1995; Moyer, Deyo, & Disterhoft, 1990; Solomon, Vander Schaaf, Thompson, & Weisz, 1986) and prefrontal cortex (Oswald, Knuckley, Mahan, Snaders, & Powell, 2006; Weible, McEchron, & Disterhoft, 2000) in addition to the cerebellum. Consistent with findings in delay eyeblink conditioning paradigms, lesions of the cerebellar interpositus nucleus abolish trace eyeblink conditioning in rabbits (Pakaprot, Kim, & Thompson, 2009; Woodruff-Pak, Lavond, & Thompson, 1985) whereas lesions of the cerebellar cortex produce only transient impairments in retention of trace eyeblink CRs (Woodruff-Pak et al., 1985). Recent findings in humans further suggest that trace eyeblink conditioning is not dependent on cerebellar cortical integrity, as patients with cerebellar cortical lesions displayed comparable levels of conditioning to normal controls in the acquisition of a forebrain-dependent trace eyeblink conditioning task (Gerwig et al., 2008).

Mounting evidence indicates that the cerebellar cortex may be differentially engaged in delay relative to trace eyeblink conditioning. In a recent study, rats trained in a delay eyeblink conditioning task showed higher levels of metabolic activity in regions of the cerebellar cortex compared to rats trained in a trace eyeblink conditioning task (Plakke, Freeman, & Poremba, 2007). Perhaps the most compelling evidence for differential engagement of cerebellar cortical mechanisms between these tasks, however, comes from studies comparing delay and trace eyeblink conditioning in various mutant and transgenic mouse models. Specifically, the aforementioned GluRδ2 knockout and PLCβ4 mutant mice (with selective deficiencies of cerebellar cortical components critical for the induction of LTD at the parallel fiber-Purkinje cell synapse) showed robust impairments in delay eyeblink conditioning while trace eyeblink conditioning was unimpaired (Kishimoto, Hirono et al., 2001; Kishimoto, Kawahara, et al., 2001; Kishimoto, Kawahara, Fujimichi, et al., 2001). Similarly, a mouse strain with a selective knockout of Purkinje cell *Scn8a* sodium channels – a condition that disrupts normal firing patterns of Purkinje cells (Raman, Sprunger, Meisler, & Bean, 1997) – was impaired in delay but not in trace eyeblink conditioning (Woodruff-Pak, Green, Levin, & Meisler, 2006). Differences in performance of delay and trace eyeblink conditioning were evident when the tasks were matched for the interstimulus interval (ISI) between CS and US onset (Woodruff-Pak et al., 2006). Additionally, GluRδ2 knockout mice were impaired in delay eyeblink conditioning both when the CS–US interval was short (252 ms) and when it was long (852 ms), but they were not impaired when the CS–US interval was 852 ms long and included a 500 ms trace period (Kishimoto, Kawahara et al., 2001). These findings suggest that cerebellar cortical integrity is important for delay, but not for trace eyeblink conditioning (see Woodruff-Pak and Disterhoft (2008) for a review).

The present study used young adult homozygous male *pcd* mutant mice to assess whether a functional lesion of the entire cerebellar cortex, namely the complete absence of Purkinje cells, is capable of impairing hippocampus-dependent trace eyeblink conditioning (cf., Tseng, Guan, Disterhoft, & Weiss, 2004). Previous eyeblink conditioning studies with homozygous *pcd* mutant mice used the delay paradigm (Chen et al., 1996, 1999). Whereas aspects of cerebellar cortical functioning were compromised in transgenic mice previously shown to be unimpaired in trace eyeblink conditioning (Kishimoto, Hirono, et al., 2001; Kishimoto, Kawahara, Fujimichi, et al., 2001; Kishimoto, Kawahara, et al., 2001; Woodruff-Pak et al., 2006), the possibility exists that other, intact, cerebellar cortical functions (or regions) contributed to these high levels of performance. Demonstration of unimpaired trace eyeblink conditioning in *pcd* homozygous mice would provide considerable support for our contention that the cerebellar cortex is not important for the acquisition and maintenance of trace eyeblink conditioning.

2. Methods

2.1. Subjects

A total of 21 young adult male mice were tested. Nine mice were homozygous *pcd* mutant mice (Strain name: *B6.BR-Agtpbp1pcd/J*; complete loss of cerebellar Purkinje cells by the fourth postnatal week) and the remaining 12 mice were littermate wildtype controls of the C57BL/6J strain (Jackson Laboratories). All mice weighed between 16 and 40 g at the time of surgery, with the *pcd* mutant mice of notably smaller size and a mean weight of 19.8 (SD = 3.7) g. in comparison to the mean of 32.3 (SD = 6.9) g. of the wildtype littermates. At 4–5 months of age mice began eyeblink classical conditioning training. Mice were group-housed in standard polycarbonate cages and had ad libitum access to sterile food and water. Room lighting was timed for a 12:12 h light–dark cycle. All research methods were approved by Temple University's Institutional Animal Care and Use Committee.