

## BRIEF COMMUNICATIONS

# Examination of Bilateral Eyeblink Conditioning in Rats

Matthew M. Campolattaro and John H. Freeman  
University of Iowa

This experiment monitored eyelid responses bilaterally during delay eyeblink conditioning in rats. Rats were given paired or unpaired training with a tone or light conditioned stimulus (CS) and a unilateral periorbital shock unconditioned stimulus (US). Rats given paired training acquired high levels of conditioned responses (CRs), which occurred in both eyelids. However, acquisition was faster, and the overall percentage of CRs was greater in the eyelid that was ipsilateral to the US. CRs in the eyelid ipsilateral to the US also had shorter onset latencies and larger amplitudes than CRs in the contralateral eyelid. Both eyelids consistently showed high percentages of unconditioned responses (UR) to the US, and the UR amplitude decreased across training sessions in the paired group. The present study demonstrated that CRs occur robustly in both eyelids of rats given eyeblink conditioning, which is similar to previous findings in humans and monkeys. The results also showed that conditioning occurs more prominently in the eyelid that is ipsilateral to the US, which is similar to previous findings in humans, monkeys, dogs, and rabbits.

**Keywords:** learning, Pavlovian conditioning, cerebellum, timing

Pavlovian eyeblink conditioning procedures have been used extensively to investigate the behavioral and neural mechanisms of associative learning (e.g., Christian & Thompson, 2003; Gormezano, Kehoe, & Marshall, 1983; Gormezano, Schneiderman, Deaux, & Fuentes, 1962; Schneiderman, Fuentes, & Gormezano, 1962; Spence, 1953). Eyeblink conditioning is established using presentations of a conditioned stimulus (CS), such as a tone, that is paired with an unconditioned stimulus (US), such as a periorbital shock or an air-puff directed at one eye. Before training only the US elicits the blink reflex, but after sufficient CS–US pairings conditioned blink responses (CRs) are elicited by the CS, which precede the onset of the US.

Eyeblink and/or nictitating membrane (NM) response conditioning has been established in various mammalian species including rabbits (e.g., Gormezano et al., 1983; Gormezano et al., 1962; Schneiderman et al., 1962; Thomas & Wagner, 1964), rats (e.g., Hughes & Schlosberg, 1938; Skelton, 1988; Stanton, Freeman, & Skelton, 1992), humans (e.g., Hilgard & Campbell, 1936; Hilgard & Marquis, 1936; Spence & Trapold, 1961; Woodruff-Pak & Steinmetz, 2000), monkeys (e.g., R. E. Clark & Zola, 1998; Hilgard & Campbell, 1936; Marquis & Hilgard, 1937), ferrets

(e.g., Ivarsson & Hesslow, 1993; Ivarsson, Svensson, & Hesslow, 1997; Jirenhed, Bengtsson, & Hesslow, 2007), cats (e.g., Gruart, Blázquez, & Delgado-García, 1995; Harrison & Buchwald, 1983; Jiménez-Díaz, Navarro-López, Gruart, & Delgado-García, 2004), dogs (e.g., Hilgard & Marquis, 1935, 1936; Vardaris & Fitzgerald, 1969), and mice (e.g., Bao, Chen, & Thompson, 1998; Kishimoto, Suzuki, Kawahara, & Kirino, 2001; Park, Onodera, Nishimura, Thompson, & Itohara, 2006). Acquisition and expression of the eyeblink CR is similar across species. For example, CR percentage and amplitude both increase as a function of training, and in well-trained subjects eyelid and/or nictitating membrane closure reaches maximal amplitude immediately preceding the onset of the US.

A difference in eyeblink conditioning that has been found among species involves the expression of the CR in the reinforced and nonreinforced eyes. Eyeblink conditioning is typically established with an US that is directed at one eye, but unilateral US presentations produce URs in both eyelids (Brandon, Betts, & Wagner, 1994; Hilgard & Campbell, 1936; Hilgard & Marquis, 1936; Ivarsson, & Hesslow, 1993; McCormick, Lavond, & Thompson, 1982). However, the percentage and amplitude of bilateral CRs that develop during training differs across species. For example, early studies of eyeblink conditioning found that humans and monkeys express CRs in both eyes throughout training, whereas dogs exhibit relatively few CRs in the nonreinforced eye (Hilgard & Campbell, 1936; Hilgard & Marquis, 1936). Subsequent research has shown that rabbits also express significantly fewer CRs in the nonreinforced eye relative to the reinforced eye (Brandon et al., 1994; Disterhoft, Kwan, & Lo, 1977; Lee, Kim, & Wagner, 2008; McCormick et al., 1982). A consistent finding in most species is that CRs in the nonreinforced eye have smaller amplitudes and longer onset latencies than CRs in the reinforced eye (Disterhoft et al., 1977; Hilgard & Marquis, 1936; McCormick

---

*Editor's Note.* Paul Gold served as the action editor for this article.—MSB

---

Matthew M. Campolattaro and John H. Freeman, Department of Psychology, University of Iowa.

This research was supported by National Institute for Mental Health Grant 080005 to John H. Freeman.

Correspondence concerning this article should be addressed to Matthew M. Campolattaro, Department of Psychology, University of Iowa, E11 Seashore Hall, Iowa City, IA 52242. E-mail: matthew-campolattaro@uiowa.edu

et al., 1982). These findings demonstrate bilateral CR expression varies across species, but that conditioning is consistently stronger in the reinforced eye.

The expression of bilateral CRs and URs during eyeblink conditioning has not been investigated in rats, although evidence suggests that the cerebellar processing important for eyeblink conditioning in rats is lateralized. For example, unilateral lesions and reversible inactivation in the ipsilateral cerebellar hemisphere to the trained eye have been shown to prevent acquisition and expression of delay eyeblink conditioning in rats (Campolattaro & Freeman, 2008, 2009; Freeman, Carter, & Stanton, 1995; Freeman, Halverson, & Poremba, 2005). A recent metabolic mapping investigation of eyeblink conditioning in rats also has shown that increased glucose uptake is greater in the cerebellar cortical hemisphere ipsilateral to the trained eye relative to contralateral eye (Plakke, Freeman, & Poremba, 2007). It is, therefore, possible that expression of eyeblink CRs is strongly lateralized to the reinforced eye in rats, as previously observed with rabbits and dogs (Brandon et al., 1994; Disterhoft et al., 1977; Hilgard & Campbell, 1936; Hilgard & Marquis, 1936; McCormick et al., 1982). Alternatively, CR expression may occur robustly in both eyelids, which would be more consistent with the findings in humans and monkeys (Hilgard & Campbell, 1936; Hilgard & Marquis, 1936). The present experiment was designed to examine the extent of bilateral UR and CR expression during eyeblink conditioning with rats. Here, activity from both eyelids of rats was monitored simultaneously during 10 sessions of paired or unpaired training with a CS (tone or light) and a unilateral US.

## Method

### *Subjects*

Subjects were 24 male Long Evans rats (200 to 250 g), approximately 150 days old at the beginning of the experiment. The rats were housed in Spence Laboratories of Psychology at the University of Iowa with a 12-hr light–dark cycle, with light onset at 7 a.m.

### *Surgery*

One week prior to training, rats were removed from their home cage and anesthetized by isoflurane. The rats were fitted with differential electromyograph (EMG) electrodes that were implanted in the upper left and right eyelid muscles (orbicularis oculi) and ground electrodes were attached to a stainless steel skull screw. The EMG electrode leads terminated in gold pins held in a plastic connector, which were secured to the skull with bone cement. Bipolar stimulating electrodes (for delivering the shock US) were implanted subdermally, immediately caudal to each eye. The bipolar electrodes terminated in a plastic connector and were secured to the skull with bone cement.

### *Conditioning Apparatus*

The conditioning apparatus and eyelid EMG recording procedures used in the present study have been previously described in other reports (e.g., Campolattaro, Schnitker, & Freeman, 2008; Freeman et al., 2005; Nicholson & Freeman, 2002; Plakke et al., 2007).

### *Conditioning Procedure*

All rats in this experiment received 10 sessions of training. On each day of training they were given a 100-trial session of either paired ( $n = 16$ ) or unpaired ( $n = 8$ ) presentations of a 400-ms CS (2 kHz, 85d tone or 6W light; counterbalanced) and a 25-ms periorbital unconditioned shock stimulus (US; 1–2 mA, DC constant current). Every 10th trial in the paired training was a CS-alone probe trial (10 probe trials per 100-trial session). These trials were necessary to obtain behavioral eyeblink responses without contamination by the presence of the US. Half of the rats in each group were given US presentations to the left periorbital region. The other half of rats in each group were given US presentations to the right periorbital region. For rats given paired training, the offset of the CS coincided with the onset of the US yielding a nonoverlapping 400-ms interstimulus interval. For rats given paired training, trials were separated by a variable intertrial interval (ITI) that averaged 30 s. For rats given unpaired training, CS and US presentations were separated by a variable ITI that averaged 15 s.

### *Responses*

CRs were defined as EMG activity that exceeded a threshold of 0.4 units (amplified and integrated units in volts) above the baseline mean during the CS period after 80 ms. CRs during CS–US trials were defined as responses obtained after the baseline period, but before the onset of the US. Measurements of CR amplitude, onset latency and peak latency were taken from CS-alone probe trials. Measurements of UR amplitude and peak latency were taken from trials when a US occurred. Onset latency of a CR was defined as the initial EMG activity that exceeded threshold after the baseline period. Peak latency of a CR and UR were defined as the moment when the response reached maximum amplitude.

## Results

Rats that received paired CS–US training acquired high CR percentages in both eyelids relative to the rats given unpaired training (Figure 1A). Paired training resulted in a high percentage of CRs (~85%) by the 10th session of training in both eyelids, whereas unpaired training only produced baseline responding (~10%). This difference was confirmed with an analysis of variance (ANOVA) that showed a significant interaction between the group, session and eye (ipsilateral vs. contralateral) variables,  $F(9, 198) = 3.69, p < .01$ . Follow-up tests (Tukey's honestly significant difference, HSD) showed that the rats in the paired group had significantly more CRs on Sessions 2 to 10 than unpaired control rats (all comparisons,  $p < .05$ ).

Differences in percentage and topography of eyeblink CRs were found between the eyelids that were ipsilateral and contralateral to the US for rats in the paired group. Measurements of CR amplitude, onset latency, and peak latency were obtained from CS-alone probe trials when a CR was produced. Topographical measurements of CRs in the unpaired group were not analyzed because these rats produced very few responses (~10%). For rats in the paired group, acquisition was faster (Figure 1A), response amplitudes were greater (Figure 1B), and onset latencies were shorter (Figure 1C) in the eyelid that was ipsilateral to the US. These findings were confirmed with separate ANOVAs that revealed

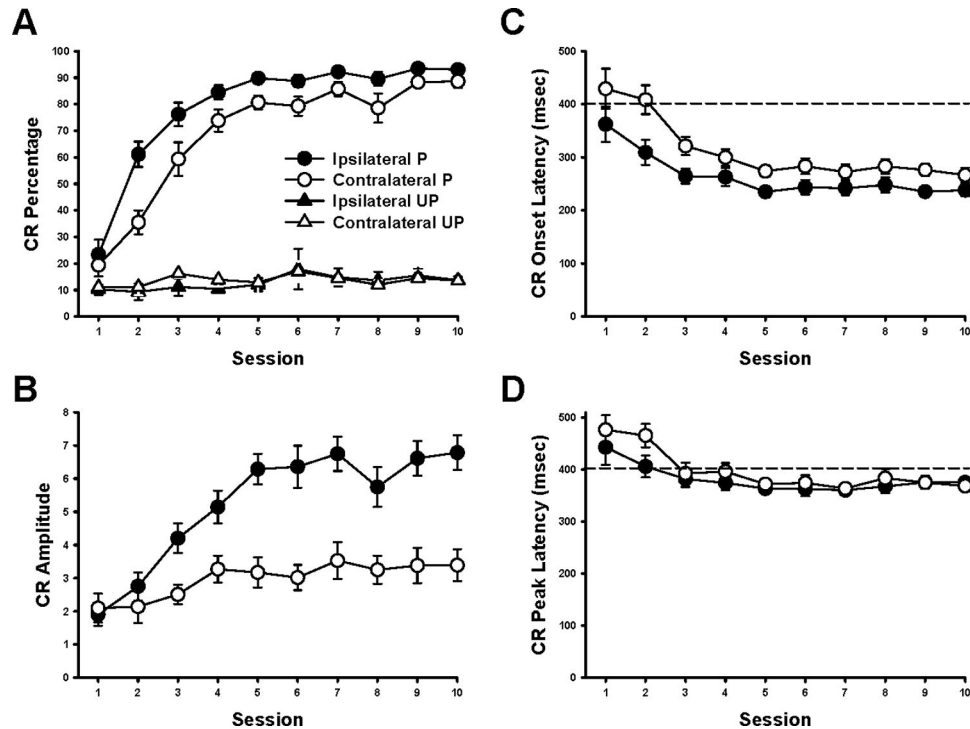


Figure 1. Mean ( $\pm$ SEM) conditioned response (CR) percentage (A), amplitude (B), onset latency (C), and peak latency (D) recorded in the eyelids that were ipsilateral (black plots) and contralateral (white plots) to the location of the unconditioned stimulus (US). Rats were given either paired (P; circles) or unpaired (UP; triangles) training with a tone or light CS and the US. The timescale for the CR onset and peak latency data is relative to the onset of the conditioned stimulus. The horizontal dashed line shows the onset of the US.

significant interactions between the eye and session factors for CR percentage,  $F(9, 135) = 6.68$ ,  $p < .01$ , CR amplitude,  $F(9, 81) = 2.31$ ,  $p < .03$ , and CR onset latency,  $F(9, 81) = 2.24$ ,  $p < .03$ . However, CR peak latencies did not differ significantly indicating that terminal closure of the eyelids was highly synchronized (Figure 1D). Follow-up tests (Tukey's HSD) showed that CR percentage in the ipsilateral eyelid was greater than the contralateral eyelid on Sessions 2 to 3,  $p < .05$ . CR amplitudes were also significantly greater in the ipsilateral eyelid than the contralateral eyelid during Sessions 3 to 10, and CR onset latencies were shorter in the ipsilateral eyelid relative to the contralateral eyelid for all 10 training sessions (all comparisons,  $p < .05$ ).

Composite eyelid traces recorded from the ipsilateral and contralateral eyelids from CS-alone probe trials during Sessions 1, 5, and 10 are shown in Figure 2A. The eyelid traces in Figure 2A were derived from the averaged eyelid activity recorded during the CS-alone probe trials for all rats that received paired training. Comparison between the eyelid traces revealed an increase in CR amplitude across training sessions in both eyelids, but eyeblink CR amplitudes observed in the ipsilateral eyelid were much larger, and response latencies were shorter, than the responses observed in the contralateral eyelid.

The US was effective for eliciting the UR in both eyelids. EMG amplification was gated during the US to avoid the electrical artifact, which resulted in a gap of 25 ms with no response data. It is therefore likely that the UR was predominately the R2 component of the EMG response to the US (Pellegrini, Horn, & Evinger,

1995). Overall the percentage of URs was greater in the eyelid ipsilateral to the US relative to the contralateral eyelid. An ANOVA using the group, session, and eyelid factors confirmed this observation,  $F(1, 22) = 15.6$ ,  $p < .01$ . For rats given paired training the percentage of URs in the eyelid ipsilateral to the US was 99.2, and the percentage of URs in the contralateral eyelid was 92.5. Similar UR percentages were observed between the ipsilateral and contralateral eyelids for the rats given unpaired training (99.2 and 93.4, respectively). UR peak latencies for both eyelids were the same in both groups, again demonstrating that terminal closure of the eyelids was highly synchronized. The UR amplitudes in the ipsilateral eyelid to the US were slightly larger than those observed in the contralateral eyelid for rats given paired training. However, the difference was only marginally significant,  $F(1, 15) = 4.2$ ,  $p = .059$ . There was no significant difference in UR amplitude between the eyelids during unpaired training. There was no main effect for the eye factor for UR peak latencies. It was not possible to analyze UR onset data precisely because EMG activity was not recorded during the US. Figure 2 shows composite eyelid traces to the US presentation for the ipsilateral and contralateral eyelids during paired (B) and unpaired (C) training during Sessions 1, 5, and 10. An ANOVA with the eyelid and session factors revealed that a decrease in UR amplitude occurred during paired training,  $F(9, 135) = 3.874$ ,  $p < .01$ . Follow-up tests (Tukey's HSD) showed that UR amplitudes were significantly greater on Session 1 than Sessions 5 and 10 ( $p < .05$ ). No significant changes in UR amplitude were observed throughout unpaired training.

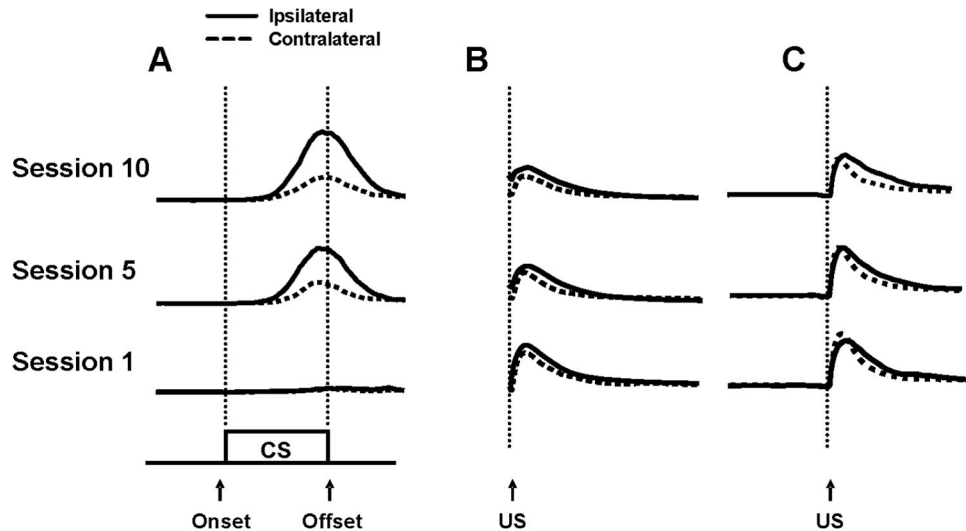


Figure 2. Composite electromyograph activity for the eyelids that were ipsilateral (solid line) and contralateral (dashed line) to the location of the unconditioned stimulus (US) during Sessions 1, 5, and 10. Eyelid responses were elicited by the conditioned stimulus (CS) on CS-alone probe trials (A) and to the US on paired trials (B) for rats given paired training. Eyelid responses were elicited to US-alone presentations (C) for rats given unpaired training.

### Discussion

The present study demonstrated that eyeblink conditioning in rats with a unilateral US produces bilateral CRs and URs. The rate of acquisition was faster and overall percentage of CRs was greater in the eyelid that was ipsilateral to the US. These findings show that expression of bilateral eyeblink CRs in rats is more similar to humans and monkeys than rabbits and dogs. CRs in the eyelid ipsilateral to the US had shorter onset latencies and larger amplitudes than CRs in the contralateral eyelid. These results are consistent with previous findings with humans, monkeys, and rabbits (Disterhoft et al., 1977; Hilgard & Marquis, 1936; McCormick et al., 1982). Both eyelids also consistently showed high percentages of URs to the US, and the UR amplitude decreased across training sessions. The reduction in UR amplitude observed in the current experiment, also referred to as conditioned diminution of the UR, is consistent with findings previously reported in rabbits (Canli, Detmer, Donegan, 1992; Donegan, 1981; Kimble & Ost, 1961). Specifically, increased levels of associative responding to the CS have been shown to produce a reduction in the amplitude of the eyeblink UR.

Rats acquired high percentages of CRs in both eyes during eyeblink conditioning with a unilateral US, which has been found in both humans and monkeys. Rabbits and dogs, however, express substantially fewer CRs in the nonreinforced eye compared to the reinforced eye. Numerous studies with rabbits have shown that the percentage of eyeblink responses in the contralateral eyelid rarely exceed frequencies much greater than spontaneous blinking (Brandon et al., 1994; Disterhoft et al., 1977; Kettlewell, O'Connell, & Berger, 1974; Pearce, Montgomery, & Dickinson, 1981). However, a recent study showed that rabbits can acquire higher percentages of contralateral eyeblink CRs above spontaneous blinking rates, although they still occurred much less frequently than in the ipsilateral eye (Lee et al., 2008). Nevertheless, a consistent finding

in most species is that CR amplitudes are smaller and onset latencies are longer in the contralateral eye.

It has been previously shown that elicitation of the reflexive blink response in the contralateral eyelid requires approximately three times the stimulation threshold that is sufficient to elicit a UR in the ipsilateral eyelid (Pellegrini et al., 1995). Although not tested in the present study, it is possible that a lower intensity US would produce weaker conditioning in the contralateral eyelid. A previous study showed that conditioning of the ipsilateral eye with a weak US produces significant savings in the contralateral eye when the US (of a greater intensity) is subsequently used on that side (Kettlewell et al., 1974). This result implies that a memory of the eyeblink CR may be formed in the contralateral cerebellum even when training is given with a relatively weak US on the opposite side. As previously suggested by Lee et al. (2008), additional studies will be necessary to determine the precise neural mechanisms for the lateralization of eyeblink conditioning.

The brain areas necessary for acquiring delay eyeblink conditioning are located within the cerebellum and its interconnected brainstem nuclei (Christian & Thompson, 2003; Hesslow, Svensson, & Ivarsson, 1999; Mauk, Steinmetz, & Thompson, 1986; Steinmetz, Lavond, & Thompson, 1989; Steinmetz et al., 1987; Steinmetz, Rosen, Chapman, Lavond, & Thompson, 1986). Functional lateralization has been observed within the cerebellar circuitry that is necessary for eyeblink conditioning in many species. For example, permanent or reversible lesions in one cerebellar hemisphere (i.e., interpositus nucleus) have been shown to prevent eyeblink conditioning of the ipsilateral eye in rabbits (Krupa, Thompson, & Thompson, 1993; Krupa & Thompson, 1997; McCormick, Clark, Lavond, & Thompson, 1982) and also in rats (Campolattaro & Freeman, 2008, 2009; Freeman et al., 2005). Studies with rabbits also have shown that unilateral lesions to the interpositus nucleus have no effect on conditioning of the con-



tralateral eye (G. A. Clark, McCormick, Lavond, & Thompson, 1984; Lincoln, McCormick, & Thompson, 1982; Polenchar, Patterson, Lavond, & Thompson, 1985; Steinmetz, Logue, & Steinmetz, 1992).

The memory trace of the eyeblink CR may form in both cerebellar hemispheres during training, although it tends to be dominant on the side ipsilateral to the US. For example, learning-related neuronal activity occurs within both cerebellar hemispheres during eyeblink conditioning with rabbits, but inactivation of the interpositus nucleus ipsilateral to the US abolishes learning-related activity in the contralateral cerebellar hemisphere (R. E. Clark, Zhang, & Lavond, 1997). Similarly, functional imaging studies have shown that metabolic changes occur within both cerebellar cortical hemispheres during eyeblink conditioning in rabbits (Miller et al., 2003) and also humans (Molchan, Sunderland, McIntosh, Herscovitch, & Schreurs, 1994; Schreurs et al., 1997), but that the changes are larger in the hemisphere that is ipsilateral to the US. The Miller et al. (2003) study specifically showed that the bilateral metabolic changes in deep cerebellar nuclei (i.e., interpositus nuclei) occurred early during training, but became more lateralized to the side that was ipsilateral to the US later in training (Miller et al., 2003). Together, these results support the hypothesis that processing of eyeblink conditioning is lateralized to the cerebellar hemisphere that is ipsilateral to the US, but that learning related changes can occur in both hemispheres as a result of unilateral training.

Learning in the contralateral cerebellar hemisphere may play an important role in establishing savings in the contralateral eye (R. E. Clark et al., 1997; Gruart & Yeo, 1995; Ivarsson et al., 1997; Lee et al., 2008; Pearce et al., 1981). That is, relatively few training trials are needed to acquire conditioning when training is subsequently switched to the contralateral eye because some plasticity had already occurred in the contralateral cerebellum during initial training. Consistent with this hypothesis is the finding that posttraining lesions of the ipsilateral cerebellar interpositus nucleus do not affect savings of eyeblink conditioning when training is switched to the contralateral eye (Lavond, Kanzawa, Ivkovich, & Clark, 1994; McCormick et al., 1981). However, savings in the contralateral eye does not occur if lesions are made in the ipsilateral cerebellar hemisphere to the US before initial training, which suggests that plasticity must be established in the ipsilateral hemisphere before it is induced within the contralateral hemisphere (Lincoln et al., 1982).

The typical eyeblink conditioning experiment is conducted with a US directed at one eye and a CS that is presented in the ambient environment. CS information is relayed to the cerebellum as mossy fiber projections from the pontine nuclei (Hesslow et al., 1999; Steinmetz et al., 1986, 1987, 1989), and US information reaches the cerebellum via climbing fibers projections from the inferior olive (IO; Mauk et al., 1986). Converging activation of the ipsilateral mossy fiber and climbing fiber pathways in the cerebellum induces the plasticity for acquiring and storing the eyeblink CR memory for the trained eye (Gould, Sears, & Steinmetz, 1993; Mauk & Donegan, 1997; Steinmetz et al., 1989). Recently, a similar mechanism has been proposed to account for formation of the eyeblink CR memory in the contralateral eyelid (Lee et al., 2008). Specifically, the Lee et al. (2008) study proposed that CS and US inputs also converge in the contralateral cerebellar hemisphere to induce a memory of the eyeblink CR.

Neuroanatomical studies have shown that the climbing fiber projections from the IO to the contralateral deep cerebellar nuclei (ipsilateral to the US location) are more prominent than projections to the ipsilateral deep cerebellar nuclei (contralateral to the US location) in both rats (Ruigrok & Voogd, 1990; Sugihara, Wu, & Shinoda, 1999) and rabbits (Tracy, Thompson, Krupa, & Thompson, 1998). A sparse projection from the IO to the ipsilateral cerebellar cortex (lobule HVI) has been found in the rat (Sugihara et al., 1998), but not in the rabbit (Rosenfield & Moore, 1995; Yeo, Hardiman, & Glickstein, 1985). A possible mechanism underlying the difference in the magnitude of learning in the contralateral eyelid found between rats and rabbits is the different distribution of climbing fiber projections to the ipsilateral cerebellum in these species.

In conclusion, the present experiment demonstrated that CRs and URs are expressed in both eyelids during unilateral eyeblink conditioning in rats, but conditioning is more prominent in the eyelid that is ipsilateral to the US. The overall lateralization of eyeblink conditioning observed in rats is consistent with previous findings with rabbits, humans, monkeys, and dogs. Additional research is needed to investigate the neural mechanisms responsible for the lateralization of eyeblink conditioning, which may provide insights about the mechanisms that underlie the facilitated learning that occurs during transfer of training from one eyelid to the other.

## References

- Bao, S., Chen, L., & Thompson, R. F. (1998). Classical eyeblink conditioning in two strains of mice: Conditioned responses, sensitization, and spontaneous eyeblinks. *Behavioral Neuroscience*, 112, 714–718.
- Brandon, S. E., Betts, S. L., & Wagner, A. R. (1994). Discriminated lateralized eyeblink conditioning in the rabbit: An experimental context for separating specific and general associative influences. *Journal of Experimental Psychology Animal Behavior Processes*, 20, 292–307.
- Campolattaro, M. M., & Freeman, J. H. (2008). Eyeblink conditioning in 12-day-old rats using pontine stimulation as the conditioned stimulus. *Proceedings of the National Academy of Sciences, USA*, 105, 8120–8123.
- Campolattaro, M. M., & Freeman, J. H. (2009). Cerebellar inactivation impairs cross modal savings of eyeblink conditioning. *Behavioral Neuroscience*, 123, 292–302.
- Campolattaro, M. M., Schnitker, K. M., & Freeman, J. H. (2008). Changes in inhibition during differential eyeblink conditioning with increased training. *Learning & Behavior*, 36, 159–165.
- Canli, T., Detmer, W. M., & Donegan, N. H. (1992). Potentiation or diminution of discrete motor unconditioned responses (rabbit eyeblink) to an aversive Pavlovian unconditioned stimulus by two associative processes: Conditioned fear and a conditioned diminution of unconditioned stimulus processing. *Behavioral Neuroscience*, 106, 498–508.
- Christian, K. M., & Thompson, R. F. (2003). Neural substrates of eyeblink conditioning: Acquisition and retention. *Learning & Memory*, 10, 427–455.
- Clark, G. A., McCormick, D. A., Lavond, D. G., & Thompson, R. F. (1984). Effects of lesions of cerebellar nuclei on conditioned behavioral and hippocampal neuronal responses. *Brain Research*, 291, 125–136.
- Clark, R. E., Zhang, A. A., & Lavond, D. G. (1997). The importance of cerebellar cortex and facial nucleus in acquisition and retention of eyeblink/NM conditioning: Evidence for critical unilateral regulation of the conditioned response. *Neurobiology of Learning and Memory*, 67, 96–111.
- Clark, R. E., & Zola, S. (1998). Trace eyeblink classical conditioning in the

- monkey: A nonsurgical method and behavioral analysis. *Behavioral Neuroscience*, 112, 1062–1068.
- Disterhoft, J. F., Kwan, H. H., & Lo, W. D. (1977). Nictitating membrane conditioning to tone in the immobilized albino rabbit. *Brain Research*, 137, 127–143.
- Donegan, N. H. (1981). Priming-produced facilitation or diminution of responding to a Pavlovian unconditioned stimulus. *Journal of Experimental Psychology Animal Behavior Processes*, 7, 295–312.
- Freeman, J. H., Jr., Carter, C. S., & Stanton, M. E. (1995). Early cerebellar lesions impair eyeblink conditioning in developing rats: Differential effects of unilateral lesions on postnatal day 10 or 20. *Behavioral Neuroscience*, 109, 893–902.
- Freeman, J. H., Jr., Halverson, H. E., & Poremba, A. (2005). Differential effects of cerebellar inactivation on eyeblink conditioned excitation and inhibition. *The Journal of Neuroscience*, 25, 889–895.
- Gormezano, I., Kehoe, E. J., & Marshall, B. S. (1983). 20 years of classical-conditioning research with the rabbit. *Progress in Psychobiology and Physiological Psychology*, 10, 197–275.
- Gormezano, I., Schneiderman, N., Deaux, E., & Fuentes, I. (1962). Nictitating membrane: Classical conditioning and extinction in the albino rabbit. *Science*, 138, 33–34.
- Gould, T. J., Sears, L. L., & Steinmetz, J. E. (1993). Possible CS and US pathways for rabbit classical eyelid conditioning: Electrophysiological evidence for projections from the pontine nuclei and inferior olive to cerebellar cortex and nuclei. *Behavioral and Neural Biology*, 60, 172–185.
- Gruart, A., Blázquez, P., & Delgado-García, J. M. (1995). Kinematics of spontaneous, reflex, and conditioned eyelid movements in the alert cat. *Journal of Neurophysiology*, 74, 226–248.
- Gruart, A., & Yeo, C. H. (1995). Cerebellar cortex and eyeblink conditioning: Bilateral regulation of conditioned responses. *Experimental Brain Research*, 104, 431–448.
- Harrison, J., & Buchwald, J. (1983). Eyeblink conditioning deficits in the old cat. *Neurobiology of Aging*, 4, 45–51.
- Hesslow, G., Svensson, P., & Ivarsson, M. (1999). Learned movements elicited by direct stimulation of cerebellar mossy fiber afferents. *Neuron*, 24, 179–185.
- Hilgard, E. R., & Campbell, A. A. (1936). The course of acquisition and retention of conditioned eyelid responses in man. *Journal of Experimental Psychology*, 19, 227–247.
- Hilgard, E. R., & Marquis, D. G. (1935). Acquisition, extinction, and retention of conditioned lid responses to light in dogs. *Journal of Comparative Psychology*, 19, 29–58.
- Hilgard, E. R., & Marquis, D. G. (1936). Conditioned eyelid responses in monkeys, with a comparison of dog, monkey, and man. *Psychological Monographs*, 47, 186–198.
- Hughes, B., & Schlosberg, H. (1938). Conditioning in the white rat. IV. The conditioned lid reflex. *Journal of Experimental Psychology*, 23, 641–650.
- Ivarsson, M., & Hesslow, G. (1993). Bilateral control of the orbicularis oculi muscle by one cerebellar hemisphere in the ferret. *NeuroReport*, 4, 1127–1130.
- Ivarsson, M., Svensson, P., & Hesslow, G. (1997). Bilateral disruption of conditioned responses after unilateral blockade of cerebellar output in the decerebrate ferret. *Journal of Physiology*, 502, 189–201.
- Jiménez-Díaz, L., Navarro-López, J. D., Gruart, A., & Delgado-García, J. M. (2004). Role of cerebellar interpositus nucleus in the genesis and control of reflex and conditioned eyelid responses. *The Journal of Neuroscience*, 24, 9138–9145.
- Jirnhed, D. A., Bengtsson, F., & Hesslow, G. (2007). Acquisition, extinction, and reacquisition of a cerebellar cortical memory trace. *The Journal of Neuroscience*, 27, 2493–2502.
- Kettlewell, N. M., O'Connell, M. F., & Berger, L. H. (1974). Bilateral nictitating membrane conditioning in rabbits under asymmetrical levels of cutaneous afferent activity. *Physiology & Behavior*, 13, 27–33.
- Kimble, G. A., & Ost, J. W. (1961). A conditioned inhibitory process in eyelid conditioning. *Journal of Experimental Psychology*, 61, 150–156.
- Kishimoto, Y., Suzuki, M., Kawahara, S., & Kirino, Y. (2001). Age-dependent impairment of delay and trace eyeblink conditioning in mice. *NeuroReport*, 12, 3349–3352.
- Krupa, D. J., Thompson, J. K., & Thompson, R. F. (1993). Localization of a memory trace in the mammalian brain. *Science*, 260, 989–991.
- Krupa, D. J., & Thompson, R. F. (1997). Reversible inactivation of the cerebellar interpositus nucleus completely prevents acquisition of the classically conditioned eye-blink response. *Learning & Memory*, 3, 545–556.
- Lavond, D. G., Kanzawa, S. A., Ivkovich, D., & Clark, R. E. (1994). Transfer of learning but not memory after unilateral cerebellar lesion in rabbits. *Behavioral Neuroscience*, 108, 284–293.
- Lee, T., Kim, J. J., & Wagner, A. R. (2008). Bilateral nature of the conditioned eyeblink response in the rabbit: Behavioral characteristics and potential mechanisms. *Behavioral Neuroscience*, 122, 1306–1317.
- Lincoln, J. S., McCormick, D. A., & Thompson, R. F. (1982). Ipsilateral cerebellar lesions prevent learning of the classically conditioned nictitating membrane/eyelid response. *Brain Research*, 242, 190–193.
- Marquis, D. G., & Hilgard, E. R. (1937). Conditioned responses to light in monkeys after removal of the occipital lobes. *Brain: A Journal of Neurology*, 60, 1–12.
- Mauk, M. D., & Donegan, N. H. (1997). A model of Pavlovian eyelid conditioning based on the synaptic organization of the cerebellum. *Learning & Memory*, 4, 130–158.
- Mauk, M. D., Steinmetz, J. E., & Thompson, R. F. (1986). Classical conditioning using stimulation of the inferior olive as the unconditioned stimulus. *Proceedings of the National Academy of Sciences, USA*, 83, 5349–5353.
- McCormick, D. A., Clark, G. A., Lavond, D. G., & Thompson, R. F. (1982). Initial localization of the memory trace for a basic form of learning. *Proceedings of the National Academy of Sciences of the United States of America*, 79, 2731–2735.
- McCormick, D. A., Lavond, D. G., Clark, G. A., Kettner, R. E., Rising, C. E., & Thompson, R. F. (1981). The engram found? Role of the cerebellum in classical conditioning of nictitating membrane and eyelid responses. *Bulletin of the Psychonomic Society*, 18, 103–105.
- McCormick, D. A., Lavond, D. G., & Thompson, R. F. (1982). Concomitant classical conditioning of the rabbit nictitating membrane and eyelid responses: Correlations and implications. *Physiology & Behavior*, 28, 769–775.
- Miller, M. J., Chen, N. K., Li, L., Tom, B., Weiss, C., Disterhoft, J. F., & Wywicz, A. M. (2003). fMRI of the conscious rabbit during unilateral classical eyeblink conditioning reveals bilateral cerebellar activation. *The Journal of Neuroscience*, 23, 11753–11758.
- Molchan, S. E., Sunderland, T., McIntosh, A. R., Herscovitch, P., & Schreurs, B. G. (1994). A functional anatomical study of associative learning in humans. *Proceedings of the National Academy of Sciences, USA*, 91, 8122–8126.
- Nicholson, D. A., & Freeman, J. H., Jr. (2002). Neuronal correlates of conditioned inhibition of the eyeblink response in the anterior interpositus nucleus. *Behavioral Neuroscience*, 116, 22–36.
- Park, J. S., Onodera, T., Nishimura, S., Thompson, R. F., & Itoharu, S. (2006). Molecular evidence for two-stage learning and partial laterality in eyeblink conditioning of mice. *Proceedings of the National Academy of Sciences, USA*, 103, 5549–5554.
- Pearce, J. M., Montgomery, A., & Dickinson, A. (1981). Contralateral transfer of inhibitory and excitatory eyelid conditioning in the rabbit. *Quarterly Journal of Experimental Psychology Section B—Comparative and Physiological Psychology*, 33, 45–61.
- Pellegrini, J. J., Horn, A. K. E., Evinger, C. (1995). The trigeminally

- evoked blink reflex I. Neural circuits. *Experimental Brain Research*, 107, 166–180.
- Plakke, B., Freeman, J. H., & Poremba, A. (2007). Metabolic mapping of the rat cerebellum during delay and trace eyeblink conditioning. *Neurobiology of Learning and Memory*, 88, 11–18.
- Polenchar, B. E., Patterson, M. M., Lavond, D. G., & Thompson, R. F. (1985). Cerebellar lesions abolish an avoidance response in rabbit. *Behavioral and Neural Biology*, 44, 221–227.
- Rosenfield, M. E., & Moore, J. W. (1995). Connections to cerebellar cortex (Larsell's HVI) in the rabbit: A WGA–HRP study with implications for classical eyeblink conditioning. *Behavioral Neuroscience*, 109, 1106–1118.
- Ruigrok, T. J., & Voogd, J. (1990). Cerebellar nucleo-olivary projections in the rat: An anterograde tracing study with Phaseolus vulgaris-leucoagglutinin (PHA-L). *Journal of Comparative Neurology*, 298, 315–333.
- Schneiderman, N., Fuentes, I., & Gormezano, I. (1962). Acquisition and extinction of the classically conditioned eyelid response in the albino rabbit. *Science*, 136, 650–652.
- Schreurs, B. G., McIntosh, A. R., Bahro, M., Herscovitch, P., Sunderland, T., & Molchan, S. E. (1997). Lateralization and behavioral correlation of changes in regional cerebral blood flow with classical conditioning of the human eyeblink response. *Journal of Neurophysiology*, 77, 2153–2163.
- Skelton, R. W. (1988). Bilateral cerebellar lesions disrupt conditioned eyelid responses in unrestrained rats. *Behavioral Neuroscience*, 102, 586–590.
- Spence, K. W. (1953). Learning and performance in eyelid conditioning as a function of intensity of the UCS. *Journal of Experimental Psychology*, 45, 57–63.
- Spence, K. W., & Trapold, M. A. (1961). Performance in eyelid conditioning as a function of reinforcement schedules and changes in them. *Proceedings of the National Academy of Sciences, USA*, 47, 1860–1868.
- Stanton, M. E., Freeman, J. H. J., & Skelton, R. W. (1992). Eyeblink conditioning in the developing rat. *Behavioral Neuroscience*, 106, 657–665.
- Steinmetz, J. E., Lavond, D. G., & Thompson, R. F. (1989). Classical conditioning in rabbits using pontine nucleus stimulation as a conditioned stimulus and inferior olive stimulation as an unconditioned stimulus. *Synapse*, 3, 225–233.
- Steinmetz, J. E., Logan, C. G., Rosen, D. J., Thompson, J. K., Lavond, D. G., & Thompson, R. F. (1987). Initial localization of the acoustic conditioned stimulus projection system to the cerebellum essential for classical eyelid conditioning. *Proceedings of the National Academy of Sciences, USA*, 84, 3531–3535.
- Steinmetz, J. E., Logue, S. F., & Steinmetz, S. S. (1992). Rabbit classically conditioned eyelid responses do not reappear after interpositus nucleus lesion and extensive post-lesion training. *Behavioural Brain Research*, 51, 103–114.
- Steinmetz, J. E., Rosen, D. J., Chapman, P. F., Lavond, D. G., & Thompson, R. F. (1986). Classical conditioning of the rabbit eyelid response with a mossy-fiber stimulation CS: I. Pontine nuclei and middle cerebellar peduncle stimulation. *Behavioral Neuroscience*, 100, 878–887.
- Sugihara, I., Wu, H. S., & Shinoda, Y. (1999). Morphology of single olivocerebellar axons labeled with biotinylated dextran amine in the rat. *Journal of Comparative Neurology*, 414, 131–148.
- Thomas, E., & Wagner, A. R. (1964). Partial reinforcement of the classically conditioned eyelid response in the rabbit. *Journal of Physiological Psychology*, 58, 157–158.
- Tracy, J. A., Thompson, J. K., Krupa, D. J., & Thompson, R. F. (1998). Evidence of plasticity in the pontocerebellar conditioned stimulus pathway during classical conditioning of the eyeblink response in the rabbit. *Behavioral Neuroscience*, 112, 267–285.
- Vardaris, R. M., & Fitzgerald, R. D. (1969). Effects of partial reinforcement on a classically conditioned eyeblink response in dogs. *Journal of Comparative and Physiological Psychology*, 67, 531–534.
- Woodruff-Pak, D. S., & Steinmetz, J. E. (2000). Past, present, and future of human eyeblink classical conditioning. In D. S. Woodruff-Pak & J. E. Steinmetz (Eds.), *Eyeblink classical conditioning: Volume I: Applications in humans* (pp. 1–17). Boston, MA: Kluwer.
- Yeo, C. H., Hardiman, M. J., & Glickstein, M. (1985). Classical conditioning of the nictitating membrane response of the rabbit III. Connections of the cerebellar lobule HVI. *Experimental Brain Research*, 60, 114–126.

Received May 13, 2009

Revision received July 24, 2009

Accepted August 3, 2009 ■