

COMMENTARIES

The Importance of Awareness for Eyeblink Conditioning Is Conditional: Theoretical Comment on Bellebaum and Daum (2004)

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Eyeblink conditioning entails a variety of paradigms that differ in terms of the brain systems that support conditioning and the importance of awareness. In this issue, C. Bellebaum and I. Daum (2004) report that conditional discrimination learning depends on awareness. Their findings, along with other recent work, suggest a framework whereby the temporal features of the conditioning paradigm are critical in determining the ability of the cerebellum to support conditioning and, as a result, the role of awareness in conditioning.

One of the best-studied examples of mammalian memory is classical conditioning of the eyeblink response (Christian & Thompson, 2003). In its simplest form (single-cue delay conditioning), a single neutral conditioned stimulus (CS), such as a tone, is followed by the presentation of an unconditioned stimulus (US), such as a mild puff of air to the eye. The CS and US overlap and coterminate. With repeated presentations of the CS and US, the CS comes to elicit an eyeblink in advance of the US. Differential delay conditioning involves two CSs. One CS reliably predicts the US whenever it is presented (CS+), and the other CS is never followed by the US (CS−). Differential conditioning occurs when more conditioned responses (CRs) are elicited by the CS+ than by the CS−. Conditional discrimination is similar to differential conditioning. One of two different stimuli (for example, a red or green light) is presented on each trial. A brief interval is often interposed (e.g., 1–3 s), and then the CS is presented. One of the lights signals that the US will follow the CS (S+), and the other light signals that the US will not follow the CS (S−). Conditional discrimination occurs when more CRs are elicited on S+ trials than on S− trials.

In recent years, there has been renewed interest in the nature of conditioning and the role of cognitive factors. For example, what is the relationship between awareness of the stimulus contingencies and conditioning? How is conditioning performance related to normal aging? What are the brain systems that support different forms of eyeblink conditioning?

In this issue, Bellebaum and Daum (2004) explore these questions in the case of conditional discrimination. In earlier work, conditional discrimination learning was found to be deficient in patients who had undergone unilateral temporal lobectomy, whereas single-cue delay conditioning was intact (Daum, Channon, & Gray, 1992; Daum, Channon, Polkey, & Gray, 1991). In the new work presented in this issue, Bellebaum and Daum report that older individuals (aged 51–80 years) failed to develop conditional responding. Further, among the younger participants (20–50 years), only those who were designated as aware of the stimulus contingencies exhibited successful conditioning. Lastly, some measures of declarative memory (e.g., delayed story recall) correlated with conditioning performance.

These findings, together with earlier observations by this group, identify a set of characteristics that illuminate the nature of conditional discrimination learning (impaired by temporal lobe lesions, impaired in aging, dependent on awareness). It is striking that assessments of awareness have proven useful for understanding the nature of conditioning tasks and that the importance of awareness for conditioning is related to which brain systems are important.

In the most familiar paradigm (single-cue delay conditioning), awareness of the stimulus contingencies is irrelevant to successful conditioning (Frcka, Beyts, Levey, & Martin, 1983; Manns, Clark, & Squire, 2001; Papka, Ivry, & Woodruff-Pak, 1997). This finding should not be surprising, given that delay eyeblink conditioning is completely intact in decerebrate rabbits (Mauk & Thompson, 1987). Indeed, successful delay conditioning is dependent specifically on the cerebellum and associated brainstem structures. The findings are markedly different when a brief interval separates the CS offset and the US onset. In this variant of single-cue conditioning, known as *trace conditioning*, successful conditioning occurs in close association with the development of awareness of the stimulus contingencies (Clark & Squire, 1998; Manns, Clark, & Squire, 2000a, 2000b). Trace conditioning resembles delay conditioning in that it depends on the cerebellum (Takehara, Kawahara, & Kirino, 2003; Woodruff-Pak, Lavond, & Thompson, 1985), but

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trace conditioning is additionally dependent on the hippocampus and neocortex (for a review, see Clark, Manns, & Squire, 2002).

Trace conditioning likely requires the participation of the hippocampus and neocortex, in addition to the cerebellum, because the trace interval makes it difficult for the cerebellum to associate the CS and US and to form a memory. Electrophysiological studies have not detected activity in the cerebellum for longer than 100 ms following the termination of a single input pulse (Eccles, Ito, & Szentagothai, 1967). In trace conditioning the US follows the offset of the CS by as much as 1,000 ms, and rarely by less than 250 ms. Accordingly, the cerebellum is most likely unable to maintain a representation of the CS across the trace interval. If, however, the hippocampus and neocortex have represented the stimuli, then information about the CS may be transmitted to the cerebellum at a time during each trial that is optimal for cerebellar plasticity (i.e., just before and during the US). The reason that awareness is associated with successful trace conditioning is not that awareness drives the acquisition of CRs in the cerebellum. Rather, awareness is best viewed as a byproduct of the fact that the hippocampus and structures in the neocortex are effectively engaged by the task and working with the cerebellum so that conditioning can occur.

Can the findings reported by Bellebaum and Daum be understood within this framework? First, consider the results for younger participants (20–50 years). The overall frequency of CRs in response to the CS increased in these individuals regardless of whether they were designated as aware or unaware and regardless of whether they discriminated between the S+ and S-. Thus, the frequency of CRs increased even for participants who were designated as unaware and who did not discriminate between the S+ and S-. This finding is what might be expected given that the CS and US overlapped, as they do in delay conditioning. Accordingly, the cerebellum would have access to both CS and US information concurrently and would be able to form an association between the CS and US, even if no association between the S+, CS, and US were formed.

Next, consider the results for older participants (51–80 years). Bellebaum and Daum found that conditional discrimination failed to develop and that, in addition, there was little or no increase in the overall frequency of CRs to either the S+ or the S-. As discussed by Bellebaum and Daum, older individuals have been found to condition more poorly than younger individuals in a number of conditioning paradigms. Moreover, both the number of cerebellar Purkinje cells (in animal studies) and cerebellar volume (in human studies) have been correlated with conditioning performance (Woodruff-Pak, Cronholm, & Sheffield, 1990; Woodruff-Pak et al., 2001). Given that all true classically conditioned eyeblink responses (in both delay and trace paradigms) are thought to be formed and stored in the cerebellum, significant cerebellar pathology should disrupt conditioning regardless of whether participants are aware or unaware of the stimulus contingencies.

Finally, the finding that successful conditional discrimination learning occurred only in individuals designated aware is what might be expected, given that the S+ and S- were terminated 3 s before the presentation of the paired CS and US. Because the cerebellum is unable to maintain the representation of a single stimulus for longer than 100 ms, an association cannot be made between the S+, CS, and US. If, however, the hippocampus and neocortex represented these contingencies, then these structures

could work with the cerebellum to achieve conditional discrimination performance.

An alternative possibility is that it was not the 3-s gap itself that caused awareness to be important for conditional discrimination (and made the task hippocampus-dependent). In a recent study, patients with medial temporal lobe lesions were impaired in a similar version of the conditional discrimination task in which no gap was present and the S+ and S- preceded and overlapped an 800-ms CS (Fortier et al., 2003). Thus, perhaps the complexity of the task (for example, the use of compound stimuli), rather than the presence or absence of a gap, is also an important factor. In any case, it is interesting that some studies, like the new one by Bellebaum and Daum, have focused on the role of awareness in eyeblink conditioning, and that other studies have focused on the role of the hippocampus. Yet, a principle that appears to hold across studies is that those conditioning tasks that require the integrity of the hippocampus are the same tasks that aware participants can acquire and unaware participants cannot (see Table 1).

On the basis of the new study by Bellebaum and Daum, the conditional discrimination paradigm can join trace conditioning paradigms as an example of classical eyeblink conditioning that depends on the development of awareness. Until recently, there has been some uncertainty about differential delay conditioning (which involves both a CS+ and a CS-) and its relation to awareness. In the case of standard paradigms (for example, a tone and white noise or a low tone and a high tone), differential delay conditioning has been found to be independent of awareness and fully intact in amnesic patients (Carrillo et al., 2001; Clark & Squire, 1998). Yet, in an early, frequently cited study in which the CS+ and CS- were two tones (Nelson & Ross, 1974), young participants who were aware of the stimulus contingencies conditioned more successfully than unaware participants. Further, Knutinen, Power, Preston, and Disterhoft (2001) reported that differential delay conditioning in both younger and older participants

Table 1
Relationship Between Awareness and the Effects of Hippocampal Lesions on Eyeblink Conditioning

Conditioning task	Is awareness important?	Hippocampal impairment?	
		Humans	Animals
Single-cue delay	No	No	No
Differential delay ^a	No	No	No
Single-cue trace	Yes	Yes	Yes
Differential trace	Yes	Yes	—
Conditional discrimination	Yes	Yes	—

Note. Dashes indicate that these studies were not performed in animals. ^aA recent reexamination of differential delay conditioning found in four separate experiments that conditioning was independent of awareness (Smith et al., in press). Nevertheless, not all studies have reached this conclusion (see text). In this regard, it is useful to note that rabbits with hippocampal lesions exhibited normal acquisition of differential delay conditioning (Berger & Orr, 1983). We suggest that this finding should carry some weight when considering the importance of awareness in differential conditioning, because (a) the findings for experimental animals and humans are congruent, and (b) across the various tasks that have been studied, there is a clear relationship between the role of the hippocampus and the importance of awareness.

was successful only for those who were aware of the stimulus contingencies (the CS+ and CS− were a tone and white noise).

We have recently completed a reexamination of the relationship between differential delay conditioning and awareness using a tone and white noise as CS+ and CS− and also two tones as CS+ and CS−. In addition, we tested both younger and older participants, and we used several different methods to identify participants as aware or unaware (Smith, Clark, Manns, & Squire, in press). In four experiments, differential delay conditioning was found to be identical in aware and unaware participants. We considered possible reasons why this result has not always been found. These findings conform to the framework outlined here; namely, when the CS and US overlap (as they do in differential delay conditioning), the cerebellum can support conditioning independently of any contribution from the forebrain. However, when even a brief interval is interposed between a signal relevant to conditioning and the US, as in trace conditioning or in the conditional discrimination paradigm studied by Bellebaum and Daum, then the forebrain must work together with the cerebellum to achieve successful conditioning. In this case, awareness is related to successful conditioning and unaware participants cannot condition. In contrast, when conditioning does not require the forebrain, awareness is irrelevant.

References

- Bellebaum, C., & Daum, I. (2004). Effects of age and awareness on eyeblink conditional discrimination learning. *Behavioral Neuroscience, 118*, 6, 1157–1165.
- Berger, T., & Orr, W.B. (1983). Hippampectomy selectively disrupts discrimination reversal conditioning in the rabbit nictitating membrane response. *Behavioural Brain Research, 8*, 49–68.
- Carrillo, M. C., Gabrieli, J. D., Hopkins, R. O., McGlinchey-Berroth, R., Fortier, C. B., Kesner, R. P., & Disterhoft, J. F. (2001). Spared discrimination and impaired reversal eyeblink conditioning in patients with temporal lobe amnesia. *Behavioral Neuroscience, 115*, 1171–1179.
- Christian, K. M., & Thompson, R. F. (2003). Neural substrates of eyeblink conditioning: Acquisition and retention. *Learning & Memory, 11*, 427–555.
- Clark, R. E., Manns, J. R., & Squire, L. R. (2002). Classical conditioning, awareness, and brain systems. *Trends in Cognitive Sciences, 6*, 524–531.
- Clark, R. E., & Squire, L. R. (1998, April 3). Classical conditioning and brain systems: A key role for awareness. *Science, 280*, 77–81.
- Daum, I., Channon, S., & Gray, J. A. (1992). Classical conditioning after temporal lobe lesions in man: Sparing of simple discrimination and extinction. *Behavioural Brain Research, 52*, 159–165.
- Daum, I., Channon, S., Polkey, C. E., & Gray, J. A. (1991). Classical conditioning after temporal lobe lesions in man: Impairment in conditional discrimination. *Behavioral Neuroscience, 105*, 396–408.
- Eccles, J. C., Ito, M., & Szentagothai, J. (1967). *The cerebellum as a neuronal machine*. New York: Springer-Verlag.
- Fortier, C. B., Disterhoft, J. F., Capozzi, S., Kilduff, P., Cronin-Golomb, A., & McGlinchey, R. E. (2003). Conditional discrimination learning in patients with bilateral medial temporal lobe amnesia. *Behavioral Neuroscience, 117*, 1181–1195.
- Frcka, G., Beyts, J., Levey, A. B., & Martin, I. (1983). The role of awareness in human conditioning. *Pavlovian Journal of Biological Sciences, 18*, 69–76.
- Knuttinen, M. G., Power, J. M., Preston, A. R., & Disterhoft, J. F. (2001). Awareness in classical differential eyeblink conditioning in young and aging humans. *Behavioral Neuroscience, 115*, 747–757.
- Manns, J. R., Clark, R. E., & Squire, L. R. (2000a). Awareness predicts the magnitude of single-cue trace eyeblink conditioning. *Hippocampus, 10*, 181–186.
- Manns, J. R., Clark, R. E., & Squire, L. R. (2000b). Parallel acquisition of awareness and trace eyeblink classical conditioning. *Learning & Memory, 7*, 267–272.
- Manns, J. R., Clark, R. E., & Squire, L. R. (2001). Single-cue delay eyeblink conditioning is unrelated to awareness. *Cognitive, Affective, and Behavioral Neuroscience, 2*, 192–198.
- Mauk, M. D., & Thompson, R. F. (1987). Retention of classically conditioned eyelid responses following acute decerebration. *Brain Research, 403*, 89–95.
- Nelson, M. N., & Ross, L. E. (1974). Effects of masking tasks on differential eyelid conditioning: A distinction between knowledge of stimulus contingencies and attentional or cognitive activities involving them. *Journal of Experimental Psychology, 102*, 1–9.
- Papka, M., Ivry, R. B., & Woodruff-Pak, D. S. (1997). Eyeblink classical conditioning and awareness revisited. *Psychological Science, 8*, 404–408.
- Smith, C. N., Clark, R. E., Manns, R. E., & Squire, L. R. (in press). Acquisition of differential delay eyeblink classical conditioning is independent of awareness. *Behavioral Neuroscience*.
- Takehara, K., Kawahara, S., & Kirino, Y. (2003). Time-dependent reorganization of the brain components underlying memory retention in trace eyeblink conditioning. *Journal of Neuroscience, 23*, 9897–9905.
- Woodruff-Pak, D. S., Cronholm, J. F., & Sheffield, J. B. (1990). Purkinje cell number related to rate of classical conditioning. *NeuroReport, 1*, 165–168.
- Woodruff-Pak, D. S., Lavond, D. G., & Thompson, R. F. (1985). Trace conditioning: Abolished by cerebellar nuclear lesions but not lateral cerebellar cortex aspirations. *Brain Research, 348*, 249–260.
- Woodruff-Pak, D. S., Vogel, R. W., Ewers, M., Coffey, J., Boyko, O. B., & Lemieux, S. K. (2001). MRI-assessed volume of cerebellum correlates with associative learning. *Neurobiology of Learning and Memory, 76*, 342–357.

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