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Pharmacological dissociation of trace and long-delay fear conditioning in young rats

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Abstract

In most studies comparing trace and delay conditioning, CS duration is kept constant across training conditions but the interstimulus interval (ISI), the time from CS onset to US onset, is confounded. In the infrequently used long-delay condition, however, ISI is kept constant across the trace and delay conditions but CS duration varies. A recent study reported that trace and long-delay fear conditioning have the same developmental trajectory, with both emerging later in development than standard-delay conditioning (Barnet & Hunt, 2005). Past studies have shown that trace conditioning is mediated by the cholinergic system; given the parallel developmental emergence of trace and long-delay conditioning, the present study examined whether the cholinergic system also mediates long-delay conditioning. Two experiments, both involving Sprague–Dawley-derived rats and using freezing as a measure of learned fear, showed that the cholinergic system is critically involved in trace conditioning but is not involved in long-delay conditioning. Specifically, pre-training injections of the muscarinic receptor antagonist scopolamine impaired acquisition of a CS–US association in 32-day-old rats trained with a trace procedure but had no effect on rats this age trained with a long-delay procedure (Experiment 1). Similarly, pre-training injections of physostigmine, a cholinesterase inhibitor, enhanced acquisition of trace conditioning in 25-day-old rats but had no effect on long-delay conditioning in rats this age (Experiment 2). Taken together, the results indicate that despite the similarities between trace and long-delay conditioning in terms of developmental emergence and level of conditioned responding, they are mediated by different physiological systems.

Keywords: Fear conditioning; Trace conditioning; Long-delay conditioning; Freezing; Cholinergic; Physostigmine; Scopolamine; Development

1. Introduction

In Pavlovian fear conditioning an animal forms an association between an initially neutral conditioned stimulus (CS) and an aversive unconditioned stimulus (US). It is generally assumed that to achieve the strongest learning the two stimuli must occur contiguously, such that the onset of the CS precedes the occurrence of the US. In some such cases, the CS and US overlap (with the US usually occurring at the end of the CS) while in others there is a gap between the two (i.e., the CS terminates prior to the US). The former situation is referred to as *delay* conditioning while the latter case is referred to as *trace* conditioning. Although both procedures can result in robust conditioned responding, trace conditioning typically results in weaker responding than does delay conditioning. Further, the magnitude of trace conditioning is inversely related to the duration of the trace interval; conditioned responding typically declines as the trace interval is lengthened (Ellison, 1964; Moye & Rudy, 1987a).

A great deal of evidence suggests that different neural processes are involved in delay and trace conditioning. For example, numerous studies using both fear and eyeblink conditioning procedures have shown that lesions of the hippocampus impair trace conditioning while having little, if

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any, effect on delay conditioning (e.g., McEchron, Bouwmeester, Tseng, Weiss, & Disterhoft, 1998; McEchron, Tseng, & Disterhoft, 2000; Quinn, Oommen, Morrison, & Fanselow, 2002; Solomon, Solomon, Vander Schaaf, & Perry, 1983; Solomon, Vander Schaaf, Thompson, & Weisz, 1986). In addition, manipulations of central cholinergic systems have been shown to affect trace but not delay conditioning (Disterhoft & Oh, 2003; Kaneko & Thompson, 1997; Moye & Rudy, 1987b; Seager, Asaka, & Berry, 1999). Finally, trace and delay conditioning have been shown to emerge at different ages. For example, Moye and Rudy (1987a) reported that rats as young as 15 days of age could associate an auditory CS (a 15-s tone) and a shock US when a delay fear conditioning procedure was used. Animals this age, however, showed no evidence of learning when a 10-s trace interval separated the offset of the CS and the onset of the US. It was not until 17 days of age that rats were able to exhibit conditioning with a 10-s trace interval, and not until 21 days of age when the trace interval was lengthened to 30 s. A similar pattern of results was reported with a visual CS, with the exception that the function was shifted to the right; i.e., learning with delay and trace procedures occurred a few days later in development with the visual CS compared to the auditory CS.

One possible interpretation of the work described above is that the cholinergic system, and in particular the intrahippocampal cholinergic system, is critically involved in forming associations between temporally discontiguous events (Wallenstein, Eichenbaum, & Hasselmo, 1998). That is, the hippocampus could be responsible for maintaining a mental representation of the CS during the temporal gap between CS offset and US onset in the trace conditioning procedure. It has been suggested that lesions of the hippocampus, or manipulations of central cholinergic activity, severely affect trace conditioning by impairing the animal's ability to maintain the CS representation in short-term memory (McEchron, Tseng, & Disterhoft, 2003; Rodriguez & Levy, 2001). From this perspective, young rats are deficient in acquiring a CS–US association in the trace procedure because of a relatively undeveloped hippocampus (Altman & Bayer, 1975) and/or septohippocampal cholinergic system (Coyle & Yamamura, 1976; Gould, Woolf, & Butcher, 1991).

Although the notion that the hippocampus, and cholinergic activity within that structure, is important for maintaining a representation of the CS in short-term memory during the trace interval is certainly plausible (McEchron et al., 2003; Rodriguez & Levy, 2001), it must be noted that delay and trace conditioning procedures differ in at least one other important way, in addition to the presence of a temporal gap between CS offset and US onset in the trace procedure. Delay and trace procedures also typically differ in terms of the interstimulus interval (ISI) – the time from CS onset to US onset. The duration of the CS is typically held constant in studies comparing delay and trace conditioning, and this necessarily confounds ISI. That is, standard-delay procedures involve shorter ISIs than comparison trace procedures. To examine the importance of this difference, several researchers have instead, or in addition to, begun using a long-delay procedure that equates ISI across delay and trace conditions, but confounds CS duration (e.g., Barnet & Hunt, 2005; Ivkovich, Paczkowski, & Stanton, 2000; Quinn et al., 2002; Solomon & Groccia-Ellison, 1996). Some interesting findings have been reported from comparisons of trace and long-delay procedures, especially in developmental studies.

Barnet and Hunt (2005), using CS-elicited freezing as a measure of fear conditioning, replicated the finding that delay conditioning emerges earlier in development than does trace conditioning (Moye & Rudy, 1987a). This finding involved comparing trace conditioning to the standard-delay procedure (i.e., CS duration was equated, but ISI differed for the trace and delay groups). Interestingly, Barnet and Hunt (2005) also found that the developmental emergence of long-delay conditioning paralleled that of trace conditioning. This was found for both auditory and visual CSs (see Fig. 1). These results are quite surprising from the commonly held view that the critical difference between trace and delay conditioning procedures is the presence/absence of a temporal gap between the offset of the CS and the onset of the US. From this perspective, because rats trained in the long-delay condition did not experience this temporal gap they should have performed similarly to the rats in the standard-delay condition. The fact that their performance was virtually identical to that of the trace-conditioned groups suggests that the ISI plays a critical role in these different Pavlovian conditioning procedures (see also Claflin, Garrett, & Buffington, 2005; Ivkovich et al., 2000; Kehoe & Napier, 1991; Quinn et al., 2002; Smith, 1968). The identical developmental trajectory of trace and long-delay conditioning suggests that either they are mediated by the same neural system or by different systems that exhibit a similar, protracted rate of functional maturation. As mentioned earlier, the cholinergic system is critically involved in trace conditioning and

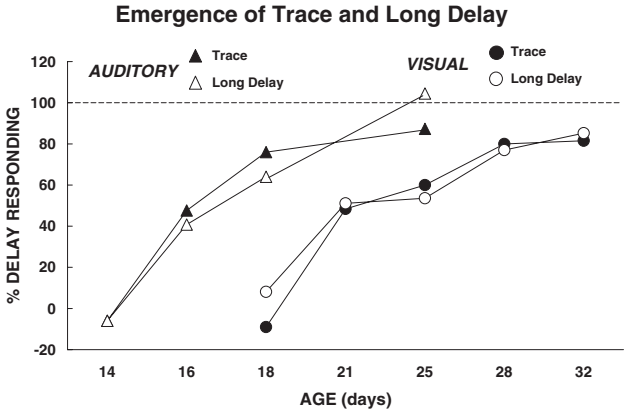

Fig. 1. Summary of the findings from Barnet and Hunt (2005) showing the parallel development of trace and long-delay conditioned responding. The performance of these groups was converted into a *percentage of short-delay responding* for comparison (from Barnet and Hunt, 2005; Copyright 2005 Psychonomic Society Inc., reproduced with permission).