Title
Taste aversion learning despite long delays: How best explained?

Journal
International Journal of Comparative Psychology, 28(1)

ISSN
0889-3675

Authors
Kwok, Dorothy W.S.
Boakes, Robert Alan

Publication Date
2015

DOI
10.46867/ijcp.2015.28.01.04

INTERNATIONAL JOURNAL OF COMPARATIVE PSYCHOLOGY

2015, 28
José E. Burgos
Special Issue Editor
Peer-reviewed

Taste Aversion Learning Despite Long Delays: How Best Explained?

Dorothy W. S. Kwok and Robert A. Boakes

University of Sydney, Australia

Taste aversion learning (i.e., conditioned taste aversions or CTA) can occur even when there is delay of some hours between experience of the taste and the subsequent onset of illness. This property of CTA is quite distinct from other forms of associative learning, where typically no association between two events is acquired if they are separated by more than a minute. This paper provides an overview of a series of recent experiments based on the assumption that long-delay CTA is possible only when no potentially overshadowing – or *concurrently interfering* (Revusky, 1971) – events occur during the delay. The general method is one in which in a single conditioning session the rats are first given 8% sucrose, providing the sweet target taste, and 65 min later are injected with lithium chloride. What vary across experiments are the potentially interfering events occurring during the 65-min delay period. When the interfering event is a second, and quite different, taste, namely sour-tasting hydrochloric acid solution (HCl), this produces 1-trial overshadowing of the sucrose aversion, to a degree that is greater when HCl is given late in the delay period, greater when HCl is given in the same context as sucrose and greater when HCl has not been pre-exposed. Other intervening events can also overshadow sucrose aversion learning. These include placement into a novel context, as long as this occurs immediately before injection, and even stimuli that evoke memories of food-related experiences. These results can be accounted for by adding to the Rescorla-Wagner model (Rescorla & Wagner, 1972) the assumption that a sickness episode consists of a succession of bouts and the assumption that context-event associations are important in long-delay CTA.

If an animal eats or drinks a novel tasting food or liquid and later experiences some form of sickness, subsequently it is likely to display an aversion to the taste. In a typical experiment in our laboratory on such taste aversion learning (aka conditioned taste aversion, CTA), a mildly thirsty rat is transferred from its home cage to a drinking chamber in which it has previously consumed water, and for the first time is given a sweet solution. Subsequently, it is given an intra-peritoneal (i.p.) injection of lithium chloride at a dose that produces mild and transient malaise. When given the sweet solution for a second time some days later, the rat will drink very little compared to control rats that have not had the sweet taste paired with the lithium injection.

Please send correspondence to Dr. R.A. Boakes, School of Psychology (A18), University of Sydney, NSW 2006, Australia (Email: bob.boakes@sydney.edu.au). https://doi.org/10.46867/ijcp.2015.28.01.04

Of special interest here is that such learning can take place even when there is a long delay, possibly several hours, between first experiencing the sweet taste and the subsequent lithium injection. Following his group's initial discovery of CTA (Garcia, Kimeldorf, & Koelling, 1955) and examination of learning despite these long delays (Garcia, Ervin, & Koelling, 1966), Garcia suggested that this was a special form of learning – a feature of the 'gut defense system' (Garcia, 1989) – that was quite different from the types of learning that were studied in the research traditions initiated by Thorndike (1898) or Pavlov (1927).

It was soon pointed out that many of the properties deemed by Garcia to be unique to taste aversion learning are in fact displayed by many other forms of learning (Domjan, 1980; Logue, 1979; Revusky, 1977; see also Bouton, 2007; pp.186-200). One such property, latent inhibition, is discussed later in this paper: The more surprising features of taste aversion learning are most pronounced when the taste is entirely novel. As already noted, one of those surprising features is its tolerance of delays much longer than those tolerated by other forms of learning. Another is the high degree of stimulus selectivity displayed by taste aversion learning: Tastes are readily associated with subsequent malaise but not with electric shocks, whereas audiovisual stimuli are readily associated with shocks but not with malaise (Garcia & Koelling, 1966). The latter property provides the keystone for the explanation of long delay learning that is the focus of the present paper, Revusky's (1971) *concurrent interference* theory. However, before describing his theory we need to look briefly at attempts to 'explain away' long delay taste aversion learning.

One suggestion involves the supposed persistence of aftertastes long after ingesting some food or drink. This account proposes that the overlap between an aftertaste and sickness produces a conditioned aversion to the aftertaste that then generalizes to the original taste (e.g., Bitterman, 1975). Some problems with this account are detailed by Domjan (1980). What seems to us the most compelling counter-evidence is the finding that an aversion can be established to the high or low temperature of a drink if followed by delayed sickness, even though the fluid must have reverted to body temperature a short time after being ingested (Nachman, 1970). Another suggestion appeals to interruption with the normal attenuation of neophobia as time passes (Mitchell, Scott, & Mitchell, 1977). However, this proposal applies only when the target taste evokes strong neophobia when first presented (Domjan, 1980) and in the majority of CTA experiments the target solutions are ones that rats readily drink from the start, as with 0.15% saccharin solution or the 8% sucrose solutions that provide the standard target taste in most of our experiments. Dismissing such denials of the possibility of associative learning over long delays leaves just two kinds of theory: One appeals to slow 'trace decay' of taste memories, whereas the other is concurrent interference theory.

Concurrent Interference Theory

Revusky's (1971) most basic assumption is that the extent to which a target taste can become associated with subsequent lithium-induced sickness does not depend on the *time* separating these two events but rather depends only on *concurrent*

interference, meaning competition from other events for association with either the target taste or with the sickness. A second assumption is that interference from other events depends both on their intrinsic properties, or salience – for example, other things being equal, a strong taste will produce more interference than a weak taste – and on their *relevance* to sickness. A third assumption is that interference can arise from events preceding the target taste or following the bout of sickness but, because of what Revusky (1971) termed the *proximity corollary*, such interference is less effective than that arising from events occurring within the interval separating the target taste and sickness. Revusky (1971) used the term *relevance* to refer to the cue-to-consequence effect: As already noted, taste cues – or, more generally, food-related cues – are more readily associated with illness than are visual or auditory cues (Domjan & Wilson, 1972; Garcia & Koelling, 1966).

In summary, Revusky's (1971) explanation for why animals can form associations over unusually long delays in sickness-based learning is that, unlike, for example, fear conditioning involving electric shock, where other audio and visual cues are likely to occur, normally there are no sources of strong interference – no *relevant* events – occurring during the interval between the target taste and subsequent sickness; see Figure 1.

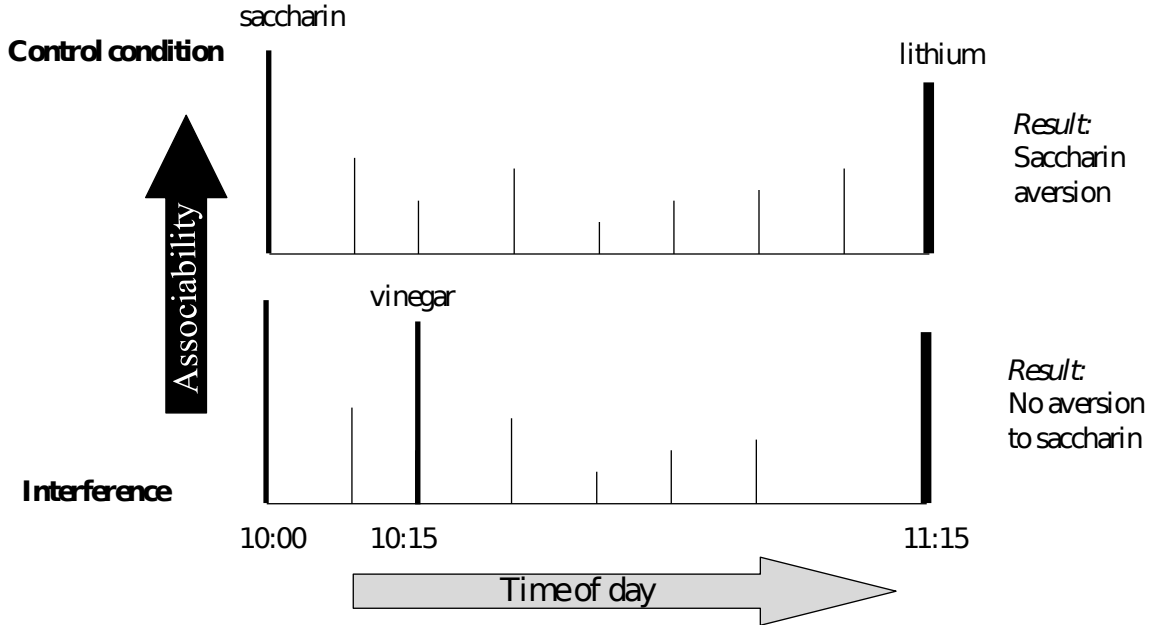


Figure 1. Schematic representation of Revusky's Concurrent Interference theory of 1971. This proposes that long-delay learning between two events is possible in any domain to the extent that other events do not interfere with the reference association. As Revusky (1971) clearly demonstrated in an experiment with a design illustrated here, giving a rat an additional taste during this interval can strongly interfere with - and under some conditions completely prevent - acquisition of an aversion to the target taste. The thin vertical lines represent incidental events that become only weakly associated with lithium-induced sickness.

Serial Overshadowing in Long Delay Taste Aversion Learning

Revusky's (1971) theory appeared at the time when modern theories of associative learning were being developed, notably that by Rescorla and Wagner (1972; Wagner & Rescorla, 1972). The central concern of such theories is competition between stimuli for associative strength and a major example of such competition is the phenomenon of overshadowing (Pavlov, 1927). Following the revival of research on this phenomenon that occurred in the early 1970s (Mackintosh, 1973) most overshadowing experiments have used *simultaneous* compounds. Thus, across a variety of conditioning procedures a target stimulus, A, is presented contemporaneously with a potentially overshadowing stimulus, X, and this compound (AX) is followed by some unconditioned stimulus (US). In a subsequent test the magnitude of a conditioned response to A is compared with that to a control stimulus, B, that has previously been presented on its own when paired with the US. Overshadowing by X is demonstrated to the extent that the response to A is weaker than the response to B.

Far fewer experiments have examined *serial* overshadowing in which, during conditioning, the potential overshadowing stimulus, X, occurs at a different time from the target stimulus, A, and in most experiments during the *trace* or *delay* period following A (A→X) and preceding the US. Revusky (1971) recognized that what he termed *interference* effects were examples of serial overshadowing. Since this is the more generally recognized term, henceforth we will use the term 'serial overshadowing' to refer to empirical results and use the term *interference* only in the context of Revusky's theory of long delay learning.

Revusky's (1971) chapter includes a number of brief reports of pioneering experiments on serial overshadowing in long delay CTA. In a key experiment that is illustrated in Figure 1 rats were first given 2ml of 0.2% saccharin as the target taste, were injected with relatively strong (20 ml/kg of .15*M*) lithium chloride 75 min later, and in the delay interval between saccharin and the injection they were given either a vinegar solution, as the potentially overshadowing taste, or water. Following two days of access to water only, the rats were given a choice between the saccharin and a coffee solution. The degree to which they avoided the saccharin solution indicated the extent to which they had become averted to its taste. The results indicated that serial overshadowing of a saccharin aversion by vinegar could be obtained in a single trial and that stronger concentrations of vinegar produced more overshadowing than weak solutions (Revusky, 1971, pp. 189-192). Revusky (1971, pp. 192-197) also reported some evidence that overshadowing by saccharin of a vinegar or coffee aversion could be obtained even when access to saccharin *preceded* access to one or other of the target tastes.

The possibility of overshadowing by a stimulus that precedes the target taste (X→A) has been examined in two subsequent studies. Both Bond (1983) and Kwok, Livesey and Boakes (2012) failed to find good evidence of such an effect following a single conditioning trial, whereas following two conditioning trials Kwok et al. (2012) detected overshadowing of a sucrose aversion by both exposure to saline and placement in a novel context given prior to the target sucrose solution. On the other hand, as in Revusky (1971), both studies obtained overshadowing of a target taste by a taste that followed the target after a single conditioning trial; see Figure 2.

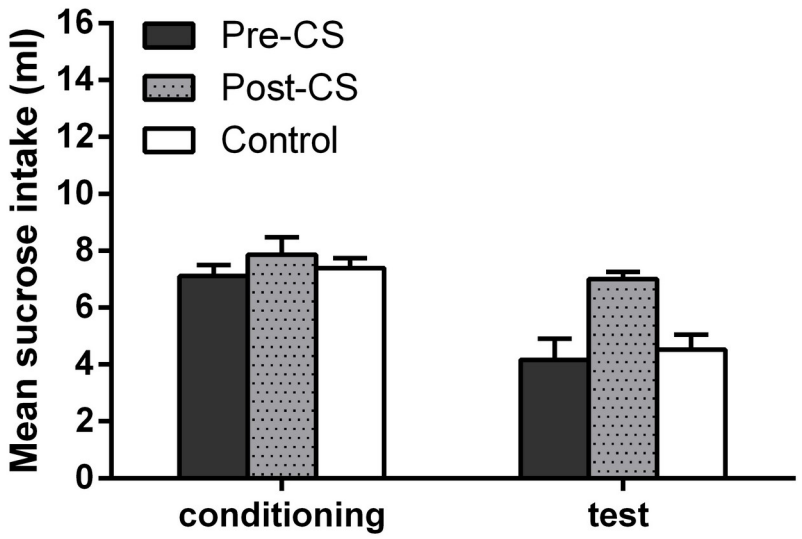


Figure 2. Sucrose intakes (and SEMs) during the conditioning and test session. Rats in the Pre-CS group were given saline prior to sucrose (CS), while rats in the Post-CS group were given saline after sucrose. Rats in the Control group were given water. Lithium injections were given 30-min following access to sucrose. Overshadowing by a subsequent taste (Post-CS) could be detected after a single conditioning trial, whereas the single trial failed to reveal overshadowing by the Pre-CS taste (Kwok et al., 2012).

The question of whether or not overshadowing can be detected after a single conditioning trial is important, in that – as discussed later in more detail – several influential associative learning theories predict that at least two conditioning trials are needed for overshadowing to occur. The failure in the two studies above to detect overshadowing by a pre-target stimulus after a single trial does not, of course, rule out the possibility that such an effect could be detected under some other set of conditions. For example, a strong pre-target taste may be able to overshadow a weak target taste after a single conditioning trial. Such an experiment has yet to be performed.

In Revusky's key experiment of 1971, vinegar solution was given only 15 min after rats had drunk the target saccharin solution and thus 60 min before the lithium injection. A theoretically interesting question is whether a larger or smaller overshadowing effect would have been obtained if the vinegar had been presented later in the saccharin-lithium interval. Of theories that can account for 1-trial overshadowing both Wagner's (1981) SOP theory and the disruption-of-consolidation account suggested by Kwok et al. (2012) predict that greater overshadowing occurs when a stimulus is presented soon after the target taste. This prediction is contradicted by three separate studies involving a long-delay CTA procedure that found an overshadowing taste to be more effective when it was presented long after the target taste, and thus shortly before the lithium injection (Cannon, Best, Batson, Rubenstein, & Carrell, 1985; Kaye, Gambini, & Mackintosh, 1988; Kwok, Harris, & Boakes, unpublished). Furthermore, we found that even placement in a novel context – an event that normally produces little overshadowing of a CTA – produced 1-trial overshadowing of a sucrose aversion when exposure to the new context occurred immediately before the lithium injection. How to explain this finding – and other features of serial overshadowing – is discussed later in this paper.

Another of Revusky's early CTA experiments confirmed that a novel event is likely to produce greater overshadowing than a familiar event, i.e. a type of latent inhibition effect (Revusky, 1971; see also Revusky & Garcia, 1970). As far as we are aware, a recent experiment of ours is the only one to have extended this finding. In this experiment sucrose solution served as the target taste that was followed 65 min later by lithium injection, with 5-min access to either water or a dilute hydrochloric acid (HCl) solution given 50 min after the sucrose (and thus there was a subsequent delay of 10 min before the injection). The more interesting groups of rats were those that were previously exposed to the HCl for two successive days either immediately before the conditioning day (*Proximal* condition) or 10 days before the conditioning day (*Distal* condition); during each of these ten days the latter group received water in the drinking chambers. In confirmation of Revusky's original finding, and shown in Figure 3, overshadowing of the sucrose aversion by the Proximal group was weaker than in the group whose first encounter with HCl was in the conditioning session (*Novel* group). The new result was that overshadowing in the Distal group was as great as in the Novel group; in other words, due to the passage of time – plus, no doubt, repeated access to water in the drinking chambers – these rats responded to the taste of HCl in the conditioning session as if it were completely new, in that no difference in the sucrose test was detected between the Novel and Distal groups (Kwok & Boakes, in press). This finding resembles the results from other kinds of conditioning preparations indicating that the longer the interval between pre-exposure of a stimulus and its involvement in a conditioning episode, the weaker is the latent inhibition effect (e.g., Holmes & Harris, 2010).

***Situational Relevance*: Context-dependency of Overshadowing Effects**

In a T-maze experiment rats were given one trial each day in which to choose one arm or the other. They were then returned to their home cages where they remained until the next day, when they were given food only if on the previous day they had chosen the correct arm. Despite the 24-h response-reinforcement delay, the rats appeared to learn which arm to choose (Lett, 1973). In considering Lett's pre-publication data, together with other research indicating long-delay learning in paradigms other than CTA, Revusky (1971, pp. 184-187) suggested that whether intervening events interfere with acquisition of an association between two events separated by a long delay depends on the context – or *situation* – in which the various events occurred. Thus, if the target event, e.g., a left turn, and its outcome, food reward, took place in one situation and all intervening events occurred elsewhere, the latter would produce little interference. This proposal was not accompanied by relevant data from CTA experiments or, for that matter, by any kind of evidence in support of this proposal. Particularly since the reliability of Lett's data has long been challenged (e.g., Lieberman, McIntosh, & Thomas, 1979; Roberts, 1976), we became interested in testing for 'situational relevance' within what has become our standard long-delay procedure. Our first experiment on this topic simply compared a group (Context-Same) in which the overshadowing taste – 5-min access to HCl once again – was presented in the

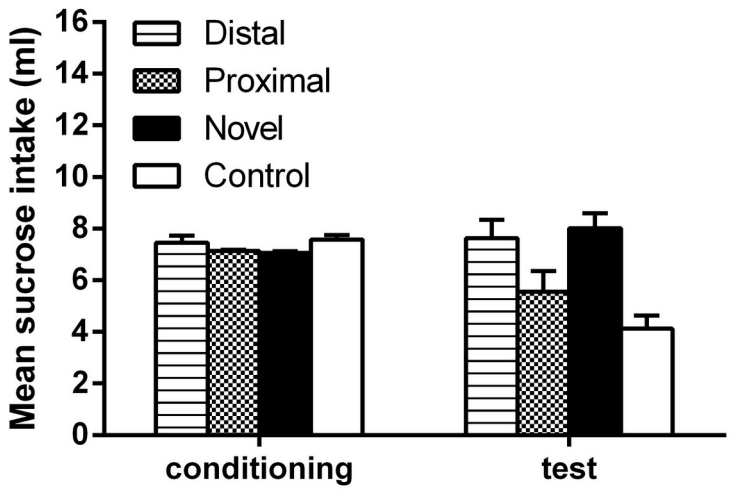


Figure 3. Sucrose intakes (and SEMs) in the conditioning and test sessions. Proximal exposure to HCl produced a strong LI effect, in that HCl was less effective in overshadowing acquisition of the sucrose aversion in rats that had recently been pre-exposed to HCl than in rats for which HCl was novel in the conditioning session. The new result was that introducing an extended delay between pre-exposure and conditioning weakened this latent inhibition effect: For Distal rats, the interfering stimulus conditioned well and sucrose was overshadowed, as if HCl had not been pre-exposed at all (Kwok & Boakes, in press).

drinking chambers where these rats had been given the target sucrose solution 50 min earlier, with a group (Context-Different) for which HCl was given in distinctly different chambers where previously only water had been available. A third group (Control) was given water 50 min after access to sucrose in either the sucrose or the other context. In the single conditioning session all rats were injected with lithium chloride 65 min after they had been given sucrose. The Context-Same group replicated the serial overshadowing effect, in that in a subsequent 1-bottle test they drank more sucrose, i.e. showed a weaker aversion, than the Control group. The new result was that this overshadowing effect was weaker in the Context-Different group. Thus, in Revusky's terms for this group the HCl had less *situational relevance*.

We followed the above experiment with one using a more complex design that involved pre-exposure to the potentially overshadowing taste of HCl. The main aim was to assess the context-dependency of the latent inhibition effect shown by the Proximal group in the experiment described in the previous section. All rats were given a single conditioning session in which 5-min access to sucrose was followed 65 min later by lithium injection. As shown in Table 1, what varied across a total of six groups (all given sucrose in context A) was *where* they were pre-exposed to HCl (A or B *vs* Water) and *where* in the conditioning session they were given HCl 50 min after receiving sucrose (A *vs* B). Thus, rats in two groups, AAA and BAA, were pre-exposed to HCL over two days immediately prior to the conditioning day in either the sucrose (A) or another (B) context, in the conditioning session they were presented with HCl in the sucrose context (A) and subsequently tested in this context (A). Differences between the two would indicate the well-documented context-dependency of an LI effect. Two control groups, BAB and AAB tested for a possible effect of consistency between the context in which HCl was first presented and the context in which it was given during conditioning. The

final two groups, WaterAA and WaterAB, provided controls for latent inhibition in that HCl was novel on the conditioning day and presented either in A or B. Thus, these last two groups also repeated the comparison examined previously.

Intakes of sucrose in the 1-bottle tests carried out in the sucrose context (A) showed that animals that had not been pre-exposed to HCl (WaterAA and WaterAB) consumed more sucrose than those that had been pre-exposed to HCl. In addition, rats that experienced HCl in two different contexts (BAA and AAB, where HCl consumption during conditioning occurred a different context to which it had been pre-exposed) showed greater overshadowing than rats that consistently experienced HCl in the same context (AAA, BAB), suggesting that when a taste is encountered in a different context from the one in which it was previously encountered its associability is greater than when both encounters occurred in the same context. Finally, these results confirmed the previous finding that presenting the interfering HCl in the same context as sucrose produced greater overshadowing than when HCl was presented in a different context to the sucrose. This change of context during the conditioning session was found to have a greater impact on sucrose intake on test than whether or not HCl was consumed in a consistent or inconsistent context.

In summary, the experiments reported in this section confirmed that the context in which a taste is experienced influences the degree to which it overshadows acquisition of an aversion to a target taste.

Table 1
Design of experiment examining the effects of context shifts between pre-exposure and conditioning

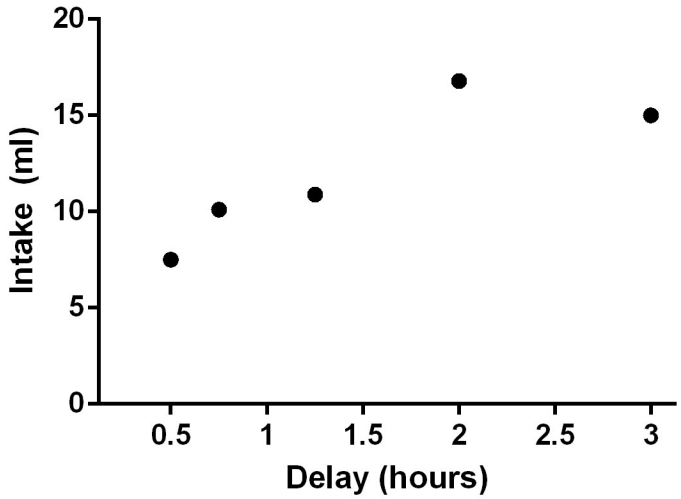
Group	Pre-exposure	Conditioning			Test
		Sucrose	HCl	LiCl	
Consistent-context same (AAA)	A: HCl	A: Sucrose	A: HCl	LiCl	A: Sucrose
Consistent-context shift (BAB)	B: HCl	A: Sucrose	B: HCl	LiCl	A: Sucrose
Inconsistent-context same (BAA)	B: HCl	A: Sucrose	A: HCl	LiCl	A: Sucrose
Inconsistent-context shift (AAB)	A: HCl	A: Sucrose	B: HCl	LiCl	A: Sucrose
Novel-context same (WaterAA)	A/B: Water	A: Sucrose	A: HCl	LiCl	A: Sucrose
Novel-context shift (WaterAB)	A/B: Water	A: Sucrose	B: HCl	LiCl	A: Sucrose

Note. HCl = hydrochloric acid; LiCl = lithium injection. A 3 x 2 design in which all of the groups experienced an interfering taste (a hydrochloric acid solution) presented between the target solution (sucrose) and a lithium injection. What differed between these groups was firstly, whether the target (sucrose) and interfering (HCL) tastes were experienced in the same context (context-same vs. context-shift), and secondly, whether the location in which rats experienced HCL and whether this location was the same as, or differed from, their previous exposures to HCL (consistent vs. inconsistent). Two groups of control rats received no pre-exposure to the interfering taste, but instead given water to equate for consumption (novel). This inclusion was to ensure that the mere act of pre-exposure itself was not a factor for differences in consumption at test.

Why Does Delay CTA Display a Gradient?

The longer the delay between exposure to a novel taste and subsequent lithium chloride injection, the weaker the resultant taste aversion. The results from the first published experiment that manipulated the length of delay in a CTA experiment are shown in Figure 4. One account of such gradients is to appeal to memory decay: Taste memories may exhibit unusually slow decay. A problem with this suggestion comes from experiments involving sensory preconditioning. In the first stage of such a CTA experiment Taste 1 is followed after some delay by Taste 2; in the following stage a conditioned aversion to Taste 2 is produced by pairing it with lithium injection; and in the final test stage the strength of an aversion to Taste 1, relative to appropriate controls, indicates the degree to which Taste 1 and Taste 2 became associated in Stage 1; i.e., the extent of sensory preconditioning. The memory decay theory suggests that a sensory preconditioning effect should be obtained even when there is a long delay between Taste 1 and Taste 2 in Stage 1. This has not been found. Rather, sensory preconditioning effects involving two tastes have been found only when the delay is no more than a few seconds (Lavin, 1976; Lyn & Capaldi, 1994). The contrast between such results and those indicating long delay CTA suggest that the decay of sensory memories of tastes may occur almost as rapidly as sensory memories for other kinds of events.

According to concurrent interference theory, the decay function results from low-level interference – weak overshadowing by stimuli that either have low *relevance* or are highly familiar – that accumulates during the decay. Domjan (1985) noted: "What



these intervening stimuli are in the typical long-delay taste aversion

Figure 4. Strength of conditioned aversion to saccharin as a function of the length of delay before injection of apomorphine, from data reported by Garcia et al.'s (1966) showing that the lengthening of inter-stimulus intervals produces a gradient of conditioning. While Garcia et al.'s (1966) study was the first to systematically vary the interval between the CS and US, subsequent studies have shown that when the CS-US interval was shorter than 30 min - for example, presented simultaneously or in a backward procedure - conditioning was also less effective (Baker & Smith, 1974).

experiment is a matter of speculation. This makes the concurrent-interference explanation of the delay gradient *post hoc* in most cases." (p. 63). Very recently we have become involved in determining what these intervening stimuli might be.

As described earlier, our standard procedure for long-delay CTA experiments is one in which group-housed and water-restricted rats are removed from their home cage and transferred to their drinking cages where they have already been trained to drink water. After drinking the 8% sucrose target solution that is available for the first time, they are returned to their home cage and remain there – with possibly a brief interruption for exposure to a potentially overshadowing event – until it is time for injection with lithium chloride in some already familiar context. The important thing to note here is that the rats' normal chow is just as available during the delay period as during the rest of the day. It seems that having chow remain available during the delay period is also common practice in other laboratories where the procedures are performed on single-housed rats and taste solutions are presented in the home cages (Batsell, personal communication, January, 2015). Although the taste of chow for our rats is as familiar a taste as any and is located in a different place from the target solution, it may nonetheless provide a low level source of interference, assuming that latent inhibition is never so strong as to reduce associability to zero. Consequently, we are currently testing – apparently for the first time (Riley, personal communication, January, 2015) – whether rats that have access to chow during the delay period acquire a weaker sucrose aversion than rats that have had their chow removed during this time.

A more complicated idea about a possible source of interference arises from experiments on mediated overshadowing. In what appears to be the only published report of such an effect, Holland (1983) described two experiments of the following form. In the first stage thirsty rats in the overshadowing group were trained to associate a tone with the delivery of a saline solution. In the second stage the tone was presented when the rats were drinking a sucrose solution – the target taste – and later they were injected with lithium chloride. In subsequent test these rats showed a reduced aversion to sucrose compared to various control groups. Holland (1983) concluded that in the second stage the tone elicited a representation of saline and this served to overshadow acquisition of an aversion to the sucrose.

Table 2

Design of experiment to test for mediated serial overshadowing by almond that has become associated with HCl

Group	Pre-test	Training	Almond Test	Conditioning	Test
Paired 2	Almond vs. Water	2 Almond-HCl	Almond vs. Water	Sucrose → Almond → LiCl	Sucrose
Unpaired 2		2 Almond/ 2 HCl			
Paired 6		6 Almond-HCl			
Unpaired 6		6 Almond/ 6 HCl			

Note. 2 x 2 design of the mediated overshadowing experiment, where the factors were the number of exposures to almond and HCl (two vs. six) and whether these tastes were presented separately (Unpaired, presented in blocked sessions) or as a simultaneous compound (Paired). A 2-bottle test involving a choice between the almond and water was given both before and after the training stage. During the single

conditioning session, all rats received sucrose, followed by almond, and were subsequently injected with lithium. Sucrose was measured at test.

Whereas Holland (1983) used a simultaneous overshadowing procedure with effectively immediate lithium chloride injection following the sucrose, we have recently examined whether a mediated effect might be obtained in our long-delay procedure (Kwok, Sun, & Boakes, unpublished data). The basic design of one of our three experiments on this topic is shown in Table 2. In an initial stage, designed to train the two Paired groups to associate a almond flavor with the sour taste of a dilute HCl solution, thirsty rats were given the HCl to which the flavor was added in either two or six 10-min drinking sessions. The Unpaired groups were given HCl and almond-flavored water in separate sessions. Number of training sessions was included as a factor in this 2 x 2 design in the light of Holland's (1990) finding that this can be critical in determining whether representation-mediated effects are detectable; in particular, to obtain a mediated effect few pairings can be better than many. In the 1-trial delay conditioning session that followed sucrose again served as the target taste and a lithium chloride injection was given 55 min later. During this delay period, and 35 min after the rats' 5-min access to sucrose, they were returned to the drinking cages where they had access to almond-flavored water for 10 min before being returned to their home cages. After two intervening days of receiving water in the drinking cages, all rats were tested for consumption of sucrose in two successive sessions.

These test sessions revealed greater consumption of sucrose in the Paired 2 group than in the Unpaired 2 group, thus suggesting that the almond evoked a representation of the HCl that overshadowed to some extent acquisition of the sucrose aversion. Interestingly the opposite pattern was found in the groups that previously had more extensive exposure to almond and HCl; the Paired 6 group drank less sucrose on test than the Unpaired 6 group. One interpretation is that in the Paired 6 group the almond flavor evoked an aversive response rather than memory of the sour taste of HCl and that this aversive response added to that produced by the sucrose-lithium chloride pairing. We were able to replicate the above results in an experiment with a similar design that used vanilla instead of almond flavor and also in one in which rats were trained to associate HCl with a distinctive context.

Returning now to the question of potential interfering events during long-delay CTA, our demonstrations of mediated serial overshadowing suggests a further source. While rats are waiting in their home cages following consumption of the target solution, they may be exposed to visual stimuli, sounds or odors that have become associated with foods. These stimuli *per se* may be of little relevance in Revusky's terms but, if they evoke representations of food-related stimuli, they may serve as sources of interference that contribute towards the CTA delay function.

To What Extent Can Current Associative Learning Theories Account for Long-Delay CTA?

The *constraints of learning* problem is not one that any well-specified associative learning theory has attempted to solve; that is, to provide an account of stimulus selectivity, a property of sickness-based aversion learning that, as Revusky's (1971)

proposed, is the key for understanding why taste aversions can be acquired even when there is a long delay before sickness occurs. On the other hand, most other properties of CTA are very similar to those displayed by other forms of conditioning, as, for example, the *overshadowing* and *latent inhibition* effects described here (Domjan, 1980; Logue, 1979; Revusky, 1977). Given the varieties of associative theory dating from Rescorla and Wagner's highly influential model of 1972 (R-W model), the question then is what theory provides the best account of what we now know about long delay CTA?

The research described in this paper has demonstrated three major properties of long-delay CTA. First, serial overshadowing can be found following a single conditioning trial. Second, the closer in time is the interfering event to lithium chloride injection – and thus the longer the interval between the target taste and the interfering event – the greater the degree of overshadowing acquisition of an aversion to the target taste. Finally, overshadowing of the target taste aversion by an interfering taste is greatest when both tastes are experienced in the same context. Each of these properties is now examined in turn.

A major division between current associative learning theories is in their treatment of phenomena such as overshadowing that indicate competition between stimuli. The most influential theories have been those, such as that described by Rescorla and Wagner (1972; see also Wagner & Rescorla, 1972), proposing that stimuli compete for a limited amount of associative strength. Such theories provide *acquisition-deficit* accounts of overshadowing. An alternative approach is one proposing that overshadowing represents a failure to fully perform a response to an overshadowed stimulus. Miller's comparator theory provides the most fully explicit *performance-deficit* account of overshadowing (e.g. Denniston, Savastano, & Miller, 2001). This account predicts that extinction of an overshadowing stimulus should produce *release from overshadowing*. This effect is now explained in terms of an unpublished experiment we have performed to test whether the effect can be found following overshadowing in long-delay CTA.

As previously, sucrose was used as the target taste and HCl as the overshadowing taste in a 1-trial long-delay CTA experiment. Comparator theory proposes that the sucrose-illness and HCl-illness associations should be just as strong as if both pairings had taken place on separate occasions and that overshadowing occurs because in the sucrose test a rat's response is reduced by comparison with the HCl aversion. It follows that, if the HCl aversion is extinguished prior to the sucrose test, the sucrose aversion will be more strongly expressed; i.e. it will be *released from overshadowing*. Consequently, to test this account of our overshadowing results, the novel feature of the present experiment was to repeatedly expose the critical group of rats to HCl until there was no longer any indication of aversion to this taste and then test sucrose. As seen in Figure 5, we found no indication at all of release from overshadowing and therefore concluded that the types of overshadowing examined here represent acquisition of a weaker aversion to the target taste, sucrose.

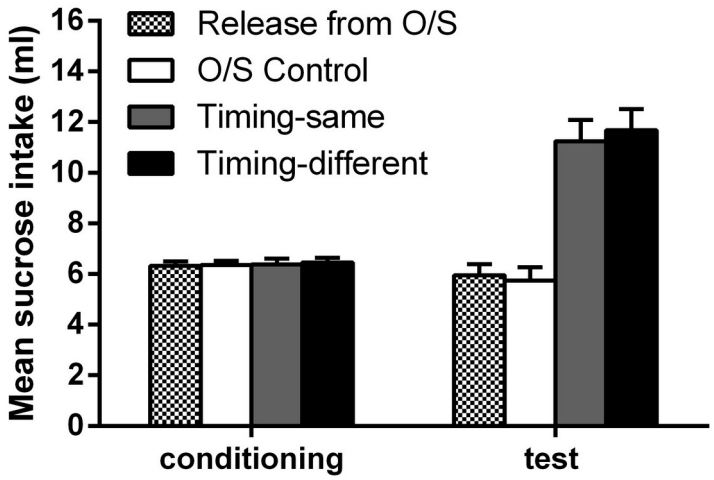


Figure 5. Sucrose intakes (and SEMs) in the conditioning and test sessions. No differences in sucrose aversion were observed between rats that had their HCl aversion extinguished before sucrose test (Group Release from O/S) and rats that were simply tested for their aversion to sucrose (O/S Control). Thus, there was no indication of release from overshadowing. Not discussed in the text was the inclusion of two further groups. These were included to test whether animals encode the temporal relationship between events – in the present case, sucrose followed by HCl – the very first time they are experienced, i.e. during the conditioning session. Prior to their final test on sucrose as shown in this figure, these rats were tested for their aversion to HCl following limited exposure to sucrose that took place either at exactly the same sucrose-HCl interval as during conditioning (Group Timing-same) or when at a longer interval than during conditioning (Group Timing-different). There was no evidence that rats had encoded the time relationship between Sucrose and HCl, in that both Timing groups rats showed a similar level of aversion to HCl. Sucrose intakes in Figure 5 for these groups are higher as a result of the brief exposure to sucrose during the preceding HCl test.

Returning now to acquisition-deficit accounts of overshadowing, it is not clear that any could have predicted what is now known about long-delay CTA. Neither the R-W model nor attention-based theories (Mackintosh, 1975; Pearce & Hall, 1980) can account for 1-trial overshadowing, since in different ways all three propose that an animal has to learn first that a compound – or succession – of two stimuli is no better than one of the stimuli alone in predicting the occurrence of a US before one stimulus comes to dominate. Pearce's configural theory (1987, 2002) predicts 1-trial overshadowing by proposing that animals encode an AX compound as a configuration that can start to become associated with a US on the first trial; when A is subsequently tested, there is a generalization decrement from AX to A. This may be a satisfactory account of some kinds of simultaneous overshadowing but seems quite implausible for serial overshadowing that is obtained when A and X are separated by 50 min or more, as in some of our experiments described earlier.

An acquisition-deficit theory of overshadowing that fits many of the properties of long-delay CTA described here is Wagner's (1981) SOP model. This predicts 1-trial overshadowing as a result of its central assumption of competition between the representations of stimuli in a limited-capacity short-term memory store (or A1 state). It also proposes that context-stimuli associations play an important role in any form of conditioning. Thus, when a stimulus is pre-exposed, i.e., experienced prior to a

conditioning trial, an association is formed between the stimulus and the context in which pre-exposure took place. Consequently, if conditioning takes place in a different context, the effects of pre-exposure – the latent inhibition effect – is smaller than if pre-exposure and conditioning take place in the same context. The results we have described that relate to Revusky's (1971) claim about *situational relevance* are consistent with Wagner (1981).

On the other hand the feature of serial overshadowing in CTA that presents the greatest challenge to Wagner's SOP theory is that, when the overshadowing taste, X, occurs within the interval between the target taste, A, and lithium chloride injection, the later X occurs the more effective it is. In the first report of such a result the authors concluded that it was consistent with Wagner's (1981) SOP theory (Cannon et al., 1985). However, a recent simulation of the SOP model showed that their conclusion was incorrect; the model predicts that greater overshadowing should result when X occurs earlier rather than later in the A-US interval (Kwok et al., unpublished).

Speculative Conclusion

On the basis of the experiments we have outlined, we propose here a possible process whereby sickness-based aversions are acquired. When an unusual bout of nausea occurs, it activates a whole set of memories. A particular *constraint on learning* applies in that food-related memories are more strongly activated than other kinds of memories. Taste is a major component of such food-related memories but, as Domjan and Hanlon (1982) demonstrated, the texture of a food can also be an important component and, in the light of our evidence concerning context-dependency effects that was described earlier, the place where the event took place is another component of such memories. A factor affecting the degree to which a particular food memory is activated is whether it is of a novel event or one that has occurred before.

Strong activation of food-related memories and weak activation of other memories by the first bout of nausea produces associative links between all these events and nausea. The strength of a link between a particular memory and nausea depends – among other factors – on the degree to which the memory was activated. A further assumption is that nausea-produced activation of food-related memories persists longer, as well as being stronger, than activation of non-food memories. The associative links produced by the first bout of nausea mean that to some extent the second bout is predicted. As a result of the error-correction process described by the R-W model and other such theories, associative links between the persistent food-related memories and nausea are further strengthened, while there is little change in the relatively weak links between the non-food memories and nausea.

We can now sketch the process whereby serial overshadowing occurs under conditions similar to those first reported by Revusky (1971) and used in many of the experiments from our lab that have been outlined here. As already described, once rats have become fully familiar with the drinking cages and have learned to drink their very familiar tap water there, they are given the novel sucrose solution and then spend time in their home cages before returning to the drinking cages where now they are given the HCl solution. After another spell in their home cages, they are given a lithium

chloride injection in a familiar place. The resulting nausea activates a range of memories, most strongly and persistently those of drinking sucrose and of drinking HCl, that then become associated with the first bout of nausea. Other memories – eating familiar chow when in the home cage, being handled in a familiar way en route to the place where injected, and being injected – are also associated with the first bout but these links are relatively weak. As suggested above, this set of associative links mean that the second bout is predicted to some extent (Sigma V in the symbolism of the R-W model) and thus the strength of the taste-nausea associations is increased to a smaller extent than when the first bout occurred. Furthermore, the increment in strength to each taste-nausea association (delta V) is smaller when there have been two tastes than when only a target taste was presented; in a control group given a single taste Sigma V is smaller and hence the error term larger. As a result, the target taste-nausea association is weaker when another taste has been presented; i.e. overshadowing has occurred.

So, how to explain that such serial overshadowing is greater when the overshadowing taste occurs late in the target taste-injection interval? This follows from our earlier account of the delay gradient for CTA, namely, as the taste-injection interval gets longer, there are an increasing number of events with low associability that produce retroactive interference with the taste-nausea association. Consequently, when in the above experiments rats drank HCl shortly before the injection, the first bout of nausea produced a stronger HCl-nausea association than in the rats that drank HCl shortly after the sucrose and long before the injection. Following the R-W model, Sigma V is thus larger in the late condition and thus delta V is smaller when the second bout occurs and as a result the sucrose-nausea association is weaker than when HCl was given earlier in the sucrose-injection interval. The finding that even a novel context experienced immediately prior to injection can overshadow the sucrose-nausea association can be explained in the same way.

The final property of long-delay CTA that this informal theory needs to explain is Revusky's *situational relevance*. The results summarized above indicated, first, that tastes given in the same context as the target taste produce greater overshadowing than when given in a different context and, second, that a taste pre-exposed in the same context as the target taste produces weaker overshadowing than one pre-exposed in a different context. We propose that both of these context-dependency effects can be understood in terms of context-event associations. Let us take first, reduction of overshadowing by presenting the interfering taste in a different context from that of the target taste. On the conditioning day the Same Context group first learn to associate sucrose with Context A; when these rats later encounter HCl in the same context, HCl is more surprising and thus has higher associability than when the Different Context group encounter HCl in Context B, in which on previous days has contained only water. As for the context-dependent effects of pre-exposing rats to HCl, this follows from a straightforward application of Wagner's (1981) model and its assumption that context-event associations are established during a pre-exposure treatment: On conditioning day the presence of HCl in the same context as the one in which it was pre-exposed is less surprising – and therefore its associability less – than when it is present in a context different from that of pre-exposure.

Conclusion

The high degree of stimulus selectivity shown by illness-based learning is a clear *constraint on learning*. However, once that is allowed for, principles of associative learning developed in the context of very different conditioning paradigms provide a powerful way of understanding the development of aversions towards food-related stimuli

Acknowledgments

The research reported here was partly supported by grants to RAB from the Australian Research council and partly by grants to DWSK from the School of Psychology, University of Sydney. We are grateful to Justin Harris for his contributions to the theoretical ideas discussed here.

References

Bitterman, M. E. (1975). The comparative analysis of learning. *Science, 188,* 699-709.

Bond, N. W. (1983). Reciprocal overshadowing in flavor-aversion learning. *Quarterly Journal of Experimental Psychology, 35,* 265-274.

Bouton, M. E. (2007). *Learning and behavior: A contemporary synthesis.* Sunderland, MA: Sinauer Associates.

Cannon, D. S., Best, M. R., Batson, J. D., Brown, E. R., Rubenstein, J. A., and Carrell, L. E. (1985). Interfering with taste aversion learning in rats: the role of associative interference. *Appetite, 6,* 1-19.

Denniston, J. C., Savastano, H. C., & Miller, R. R. (2001). The extended comparator hypothesis: Learning by contiguity. In R. R. Mowrer & S. B. Klein (Eds.) *Handbook of contemporary learning theory* (pp.65-117). Mahwah, NJ: Erlbaum.

Domjan, M. (1980). Ingestional aversion learning: Unique and general processes. In J. S. Rosenblatt, R. A. Hinde, C. Beer, & M. Busnel. (Eds.) *Advances in the Study of Behavior, 11,* 275-336. New York, NY: Academic Press.

Domjan, M. (1985). Cue-consequence specificity and long-delay learning revisited. In N. S. Braveman & P. Bronstein (Eds.) *Experimental assessments and clinical applications of conditioned food aversions* (pp. 54-66). (*Annals of the New York Academy of Sciences, 443).* New York: New York Academy of Sciences.

Domjan, M., & Hanlon, M. J. (1982). Poison-avoidance learning to food-related tactile stimuli: Avoidance of texture cues by rats. *Animal Learning & Behavior, 10,* 293-300.

Domjan, M., & Wilson, N.E. (1972). Specificity of cue to consequence learning in the rat. *Psychonomic Science, 26,* 143-145.

Garcia, J. (1989). Food for Tolman: Cognition and cathexis in concert. In T. Archer & L-G. Nilsson (Eds.) *Aversion, avoidance, and anxiety: Perspectives on aversively-motivated behaviour* (pp. 45-85). Hillsdale, NJ: Lawrence Erlbaum Associates.

Garcia, J., Ervin, F. R., & Koelling, R.A. (1966). Learning with prolonged delay of reinforcement. *Psychonomic Science, 5,* 121-122.

Garcia, J., Kimmeldorf, D. J., & Koelling, R. A. (1955). Conditioned aversion to saccharin resulting from exposure to gamma rays. *Science, 122,* 318-321.

Garcia, J., & Koelling, R.A. (1966). Relation of cue to consequence in avoidance learning. *Psychonomic Science, 4,* 123-124.

Holland, P. C. (1983). Representation-mediated overshadowing and potentiation of conditioned food aversions. *Journal of Experimental Psychology: Animal Behavior Processes, 9,* 1-13.

Holland, P. C. (1990). Event representation in Pavlovian conditioning: Image and action. *Cognition, 37,* 105-131.
Holmes, N. M., & Harris, J. A. (2010). Latent inhibition. In C. J. Mitchell & M. E. Le Pelley (Eds.) *Attention and associative learning: From brain to behaviour* (pp. 99-130). Oxford. UK: Oxford University Press.
Kaye, H., Gambini, B., & Mackintosh, N. J. (1988). A dissociation between one-trial overshadowing and the effect of a distractor on habituation. *Quarterly Journal of Experimental Psychology, 40B,* 31-47.
Kwok, D. W. S., & Boakes, R. A. (in press). Proximal, but not distal, pre-exposure reduces serial overshadowing in one-trial taste aversion learning. *Behavioral Processes.*
Kwok, D. W. S., Livesey, E. J., & Boakes, R. A. (2012). Serial overshadowing of taste aversion learning by stimuli preceding the target taste. *Learning and Behaviour*, *40(4),* 427-438.
Lavin, M. J. (1976). The establishment of flavor-flavor associations using a sensory preconditioning training procedure. *Learning & Motivation, 7,* 173-183.
Lett, B. T. (1973). Delayed reward learning: Disproof of the traditional theory. *Learning and Motivation, 4,* 237-246.
Lieberman, D. A., McIntosh, D. C. & Thomas, G. V. (1979). Learning when reward is delayed: A marking hypothesis. *Journal of Experimental Psychology: Animal Behavior Processes, 5,* 224-242.
Logue, A. W. (1979). Taste aversion and the generality of the laws of learning. *Psychological Bulletin, 86,* 276-296.
Lyn, E. A., & Capaldi, E. D. (1994). Robust conditioned flavor preferences with a sensory preconditioning procedure. *Psychonomic Bulletin and Review, 1,* 491-493.
Mackintosh, H. J. (1973). Stimulus selection: Learning to ignore stimuli that predict no change in reinforcement. In R. A. Hinde & J. S. Hinde (Eds.) *Constraints on learning.* (pp. 75-96). London, UK: Academic Press.
Mackintosh, N. J. (1975). A theory of attention: Variations in the associability of stimuli with reinforcement. *Psychological Review, 82,* 276-298.
Mitchell, D., Scott, D. W., & Mitchell, L. K. (1977). Attenuated and enhanced neophobia in the taste aversion 'delay of reinforcement' effect. *Animal Learning & Behavior, 5,* 99-102.
Nachman, M. (1970). Learned taste and temperature aversions due to lithium chloride sickness after temporal delays. *Journal of Comparative and Physiological Psychology, 73,* 22-30.
Pavlov, I. P. (1927). *Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex.* Oxford, UK: Oxford University Press.
Pearce, J. M. (1987). A model for stimulus generalization in Pavlovian conditioning. *Psychological Review, 94*, 61-73.
Pearce, J. M. (2002). Evaluation and development of a configural theory of associative learning. *Animal Learning & Behavior, 30,* 73-95.
Pearce, J. M., & Hall, G. (1980). A model for Pavlovian learning: Variations in the effectiveness of conditioned but not of unconditioned stimuli. *Psychological Review, 87,* 532-552.
Rescorla, R. A., & Wagner, A. R. (1972). A theory of Pavlovian conditioning: Variations in the effectiveness of reinforcement and nonreinforcement. In A. H. Black & W. F. Prokasy (Eds.) *Classical conditioning II: Current research and theory* (pp. 64-99). New York: Appleton Century-Crofts.
Revusky, S. (1971). The role of interference in association over a delay. In W.K.Honig & P.H.R.James (Eds.) *Animal memory.* (pp.155-213).
Revusky, S. (1977). The concurrent interference approach to delay learning. In L. M. Barker, M. R. Best, & M. Domjan (Eds.) *Learning mechanisms in food selection.* (pp. 319-366). Baylor, TX: Baylor University Press.

Revusky, S., & Garcia, J. (1970). Learned associations over long delays. In G. H. Bower & J. T. Spence (Eds) *Psychology of learning and motivation, 4* (pp. 1-84). New York, NY: Academic Press.

Roberts, W. A. (1976). Failure to replicate visual discrimination learning with a 1-min delay of reward. *Learning and Motivation, 7,* 313-325.

Thorndike, E. L. (1898). Animal intelligence: An experimental study of the associative processes in animals. *Psychological Review, Monograph Supplement No.8,* 68-72.

Wagner, A. R. (1981). SOP: a model of automatic memory processing in animal behavior. In N. E. Spear & R. R. Miller (Eds.) *Informal processing in animals: Memory mechanisms* (pp. 5-47). Hillsdale, NH: Erlbaum Associates.

Wagner, A. R., & Rescorla, R. A. (1972). Inhibition in Pavlovian conditioning: Application of a theory. In R. A. Boakes & M. S. Halliday (Eds.) *Inhibition and learning* (pp. 302-336). London, England: Academic Press.

Financial Support: The research reported here was partly supported by grants to RAB from the Australian Research council and partly by grants to DWSK from the School of Psychology, University of Sydney.

Conflict of Interest: The author declares no conflict of interest.

Submitted: *March 1st, 2015*
Revision submitted: *July 21st, 2015*
Accepted: *July 22nd, 2015*