COMPARISON OF BACKWARD AND DELAYED GROUPS IN PAVLOVIAN DRUG-DRUG CONDITIONING PROCEDURES: IMPLICATIONS FOR CONTROL OF NONASSOCIATIVE FACTORS

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by

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A thesis submitted to the School of Graduate Studies
in partial fulfillment of the requirements
for the degree of
Master of Science

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May, 1990

ISBN 0-315-68270-1

Canada

ABSTRACT

Rats receiving paired injections of sodium pentobarbital followed 30 minutes later by d-amphetamine sulfate have been reported to show an effect of pairings over trials in the form of an increase in heart rate in response to pentobarbital relative to rats receiving the two drugs 24 hours apart (delayed control; e.g., Revusky, Davey, & Zagorski, 1989). This Pavlovian conditional response (CR) has been obtained only if rats are placed in a heart rate recording apparatus during acquisition. However, home cage conditioning was assessed relative to rats that received the two drugs in reverse order (backward control), which without direct evidence assumes that delayed and backward groups are equivalent. The unconditional response to pentobarbital (UR) in drug-naive rats is similar to the pentobarbital CR: A nonassociative drug interaction could maintain the pentobarbital UR, which otherwise diminishes over trials in delayed controls. In two experiments reported here, equivalent increases in heart rate in forward and backward groups were found relative to a delayed control whether training or testing was carried out in the recording apparatus or in the home cage. This finding suggests that a

drug interaction present in forward and backward groups and absent in the delayed control has yet to be eliminated in accounting for the heart rate effect. Comparison of backward and delayed controls in a drug-drug conditioning procedure using a taste aversion test revealed that both forward and delayed pairings can produce attenuated aversions relative to a backward group whether the US is amphetamine (Experiment 2) or lithium chloride (Experiment 3). This finding was discussed in terms of the role of number and intensity of US preexposures in attenuating subsequent taste aversion conditioning.

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CHAPTER 1:

HISTORICAL PERSPECTIVE

Rats learn an aversion to a novel taste if they are made sick by drug injection, or by some other means, within hours of consumption. Virtually all drugs are capable of producing such taste aversions (e.g., Gamzu, 1977; but cf., Hunt & Amit, 1987). Low doses of commonly abused psychoactive drugs such as pentobarbital can be used but are not very effective. According to Pavlovian conditioning principles, it should be possible to increase pentobarbital's effectiveness by first pairing it with a high dose of a more effective drug such as lithium chloride. Rats should learn an aversion to pentobarbital, much as they might learn an aversion to a taste paired with lithium, and this should make the pentobarbital more effective in a subsequent taste aversion procedure than would otherwise be expected.

Different rationales have been offered for initiating the investigation of this sort of procedure. Revusky, Taukulis, Parker, and Coombes (1979) set out to improve chemical aversion therapy (CAT) for alcoholism. CAT pairs alcoholic beverages with drug-induced sickness in a Pavlovian procedure in order to produce an aversion to the beverage. CAT may produce an aversion to the taste of the

beverage and not to the state of alcohol intoxication: A confirmed drinker will "force booze down for the pleasure of intoxication" (Revusky, 1985, p. 251) and this will extinguish the taste aversion. Perhaps the alcohol state fails to become aversive because the taste competes with and overshadows the alcohol state for association with induced sickness. By this reasoning, eliminating the taste cue in a modification of the CAT procedure might be an effective strategy for improving CAT by producing an aversion to the alcohol state. Moreover, the modified CAT procedure might be used to treat drug dependencies not involving tastes. Using an animal model, these investigators induced an equivalent to the alcohol state by injecting rats with a low dose of pentobarbital. A high dose of lithium was used to induce sickness. Whether pairings of pentobarbital and lithium produce an aversion to the pentobarbital state was assessed by testing for a change in pentobarbital's ability to produce an aversion to a sodium saccharin taste in a subsequent procedure.

The rationale offered by Cunningham and Linakis (1980) was very different. These investigators set out to show that intraperitoneal injection of ethanol produces a taste. Humans report a sweet taste following intravenous injection of saccharin (Fishberg, Hitzig, & King, 1933), and saccharin injected intravenously or intraperitoneally is effective as a cue in a taste aversion procedure (Burešova & Bureš, 1977;

Bradley & Mistretta, 1971). Substances other than saccharin may have similar properties. Cunningham (1978) had earlier found that whether ethanol injection retarded, enhanced or had no effect on extinction of a lithium-induced aversion to an orally ingested taste solution depended on the particular taste of the solution. Perhaps this interaction between injected ethanol and ingested taste is mediated by an ethanol taste. Cunningham and Linakis (1980) later confirmed the existence of such a taste by demonstrating an aversion to the taste of oral ethanol following paired injections of ethanol and lithium. In order to assess whether properties of ethanol injection other than its taste were entering into association with the lithium, these researchers also tested for a change in ethanol's ability to produce a saccharin aversion.

Revusky et al. (1979) and Cunningham and Linakis (1980) viewed this sort of procedure, in which a low dose of a psychoactive drug is first paired with more severe toxicosis and is then tested for a change in its ability to produce a taste aversion, as a higher-order conditioning procedure. In Pavlovian terminology, pentobarbital or ethanol serves as a first-order conditional stimulus (CS1) and is expected to acquire some of the unconditional stimulus (US) properties of the lithium through association. A property of lithium is its effectiveness as a reinforcer in a taste aversion procedure in which the US is commonly supposed to be the

nausea or sickness produced by lithium injection or by some other means (e.g., Garcia, Lasiter, Bermudez-Rattoni, & Deems, 1985; cf., Grant, 1987; Hunt & Amit, 1987). Pairings of drug CS and lithium US are expected to increase the ability of the CS drug to reinforce an aversion to a novel taste serving as CS2 in a higher-order test.

The expected higher-order conditioning does not occur. On the contrary, the surprising finding is that pairings of drug CS and lithium US appear to eliminate or reduce the ability of the CS drug to reinforce a subsequent taste aversion. The procedure typical of the more extensive work of the Memorial University laboratory is outlined in Table 1 (see Revusky, 1985, for a review). A forward pairings group receives pentobarbital followed by lithium with an interinjection interval of 30 minutes. Rats with a history of forward drug pairings given saccharin solution followed by pentobarbital as the reinforcer typically do not differ from rats with forward pairings or other sorts of drug histories (i.e., backward, CS-, and US-only treatments) given saccharin alone or saccharin followed by saline injection. Both groups show increasing consumption over repeated exposures as they recover from the intense neophobia produced by the strong-tasting saccharin solution typically used. Backward controls receive pentobarbital and lithium in reverse order. When rats with a history of backward pairings, or rats with histories of CS- or US-only

drug exposure, are given saccharin followed by pentobarbital, they show at least a relative failure to increase consumption over exposures. This diminished recovery from neophobia is taken as evidence of a mild saccharin aversion. Backward, CS- and US-only controls do not differ, and a backward control is the single most commonly used control. The effect has been called avfail (aversion failure; Revusky et al., 1979, p. 186). Using ethanol as the CS drug in a somewhat different procedure, Cunningham and Linakis (1980) show a similar effect relative to a delayed control that receives the two drugs one day apart.

Avfail is obtained by comparison to Pavlovian controls usually considered appropriate in traditional procedures for ruling out most other sorts of explanation. Such controls are not obviously adequate for ruling out the possibility that avfail is due to some sort of pharmacological drug interaction not involving learning. To control for this possibility, a number of different drug combinations have been used in the avfail procedure (Revusky, Taukulis, Parker, & Coombes, 1979; Revusky, Coombes, & Pohl, 1982). With lithium as the reinforcer, low doses of ethanol, chlordiazepoxide, morphine and d-amphetamine have been substituted for pentobarbital with at least partial success. Atropine and apomorphine are ineffective. With pentobarbital as the cue drug, a sublethal dose of

amphetamine has been substituted for lithium with partial success: Lithium and amphetamine produce intense sickness at effective doses. Thus, avfail is not due to a pharmacological interaction between specific drugs. However, it is not obtained using different doses of amphetamine as CS and US (cf., Greeley, Lē, Poulos, & Cappell, 1984). It is not obtained with atropine, a drug that is highly discriminable but is not self-administered: Within an associative framework, it is puzzling that discriminability of the CS drug state may not be sufficient to determine the effectiveness of avfail CS drugs.

Several attempts have been made to account for avfail in terms of known conditioning principles. Perhaps the avfail procedure endows saccharin with conditional inhibitory properties (Pavlov, 1927): Saccharin may come to signal the omission of expected sickness. However, alternating drug pairings and taste aversion conditioning trials in order to facilitate inhibitory conditioning does not affect saccharin consumption in the expected manner (Revusky, Taukulis, & Peddle, 1979). Perhaps avfail is due to associative blocking (Kamin, 1968). Blocking is typically said to occur when prior conditioning to a stimulus prevents conditioning to a second stimulus that is presented in compound with the first and paired with the original US. Avfail is not obviously a blocking procedure because the original lithium US is omitted on test (but cf.,

Randich & Ross, 1985; Klein, Mikulka, & Lucci, 1986). Cunningham and Linakis (1980) nevertheless suggested that conditioning of an aversion to the intraperitoneally mediated taste of injected ethanol during the drug pairings phase could block subsequent conditioning of an ethanol-induced aversion to an orally ingested saccharin taste. Although they failed to substantiate this hypothesis, they did find evidence suggesting that handling cues might serve a similar role. However, Martin (1982) was unable to demonstrate extinction of the postulated association between handling cues and the forward pairings drug state in a procedure typical of the Memorial laboratory.

Lett (1983) proposed a conditioned antisickness interpretation of avfail. The dose of lithium required to produce avfail is much higher than that required to produce a taste aversion and is very toxic. Such a high dose might trigger substantial physiological homeostatic adjustments that could then become conditioned to an appropriate cue preceding their occurrence. These putative homeostatic responses are supposed to serve as a Pavlovian unconditional response (UR): They are collectively labelled "antisickness." When pentobarbital is paired with lithium, it may come to elicit a conditional response (CR) that is similar to the lithium antisickness UR. Taste aversion learning subsequently fails to occur because the

antisickness CR triggered by pentobarbital attenuates the sickness that the pentobarbital would otherwise produce.

How is it that tastes and certain drug states can be supposed to promote such different outcomes when one or the other precedes sickness? Conditioned antisickness theory has sometimes been considered an extension of Siegel's (e.g., 1983) Pavlovian model of drug tolerance in which drug compensatory responses are conditioned to exteroceptive cues such as those provided by the injection ritual. The two theories are actually quite different. The direction of the CR elicited by exteroceptive cues which accompany drug administration is determined by the US drug and can be accounted for within a stimulus substitution framework if the effective site of action of the US drug is correctly specified (Eikelboom & Stewart, 1983). Within conditioned antisickness theory, the direction of the CR is additionally determined by the nature of the CS and involves a new kind of selective association (Revusky, 1984). The theory assumes that drug states model naturally-occurring internal states. Just as tastes are readily associated with sickness and poorly associated with pain, interoceptive cues are readily associated with homeostatic responses to sickness and poorly associated with the sickress itself. Any propensity for selective association is presumed to have evolved because it was biologically adaptive. Tastes can themselves be avoided and are selectively associated with

sickness because avoidance of the taste enables avoidance of the sickness. Naturally-occurring interoceptive cues are selectively associated with homeostatic antisickness because such cues cannot themselves be avoided. Conditioned antisickness enables the animal to cope with unavoidable sickness (Lett, 1983). By extension, drug-drug conditioning may provide a general model for the involvement of Pavlovian mechanisms in homeostatic regulation (Revusky, 1985).

Evidence consistent with a conditioned antisickness interpretation of avfail has been found by interpolating conditioned pentobarbital between saccharin consumption and lithium injection during the taste aversion phase of the avfail procedure (Lett, 1983). An antisickness response conditioned with a high dose of lithium should be able to attenuate not only mild sickness produced by pentobarbital but also more intense lithium sickness. The conditioned antisickness procedure yields an effect similar to avfail. Because the conditioned antisickness effect is obtained whether the US used during the drug pairings phase is the same as or different from the US used to condition a taste aversion, it may not depend on amelioration of the particular physiological effects of a toxin, but rather on amelioration of the distress that might be produced in common by a variety of toxins. If this is true, and by analogy to conditional analgesia mediated by endorphins in anticipation of pain, perhaps conditioned antisickness is

mediated by an endogenously occurring antiemetic substance in anticipation of sickness (Revusky & Harding, 1986).

The theory is elegant and compelling. The conditioned antisickness procedure is a type of associative blocking procedure, however, and the conditioned antisickness effect is consistent with an alternative blocking interpretation (Lett, 1983; Revusky & Harding, 1986). Whereas conditioned antisickness theory maintains that pentobarbital injection furnishes an interoceptive drug state cue that selectively enters into association with the lithium antisickness UR, a straightforward blocking account would maintain that such a cue competes with other conditionable features of pentobarbital injection for association with the lithium sickness US. Taste aversion conditioning subsequently fails to occur because saccharin fails to enter into association with the sickness US when that US is predicted by pre-conditioned pentobarbital (e.g., Rescorla & Wagner, 1972).

Indirect support for a blocking interpretation of the conditioned antisickness effect is provided by evidence that drug pairings endow features of the CS treatment with conditional aversive properties. The presence of such cues during subsequent taste aversion conditioning could then block an association between saccharin consumption and lithium toxicosis. In addition to the findings of

Cunningham and Linakis (1980) mentioned earlier, Revusky, Taukulis, and Peddle (1979) demonstrated suppression of saccharin drinking following injection of lithium-conditioned pentobarbital. This conditioned sickness effect was not found with substitution of amphetamine or chlordiazepoxide for the pentobarbital and was therefore attributed to a pharmacological drug interaction. In separate experiments, Lett (1986) paired pentobarbital, morphine or place cues with lithium and demonstrated on test that CS exposure enhanced the slowing of stomach emptying induced by the lithium. Delayed stomach emptying indexes activation of emetic mechanisms in animals that cannot vomit, and its enhancement suggests that the various CSs acquired conditional aversive properties. A pharmacological drug interaction obviously cannot account for these findings.

Direct support for a blocking interpretation of the conditioned antisickness effect might be found by substituting place cues for pentobarbital in the conditioned antisickness procedure, thereby eliminating the CS drug state as a necessary condition. Successful substitution would weaken the empirical basis of conditioned antisickness theory but would not disprove it. An association between features of the CS drug injection other than the drug state itself and lithium toxicosis would not be expected to interfere with an association between the CS drug state and

the postulated lithium antisickness UR. Conditioned antisickness and blocking accounts of the conditioned antisickness effect are therefore not readily dissociable. Martin (1982) offers some independent support for conditioned antisickness theory. He modified the typical avfail procedure by presenting novel vinegar solution and pentobarbital injection as a compound paired with lithium during the drug pairings phase. Pairings endowed the vinegar taste with conditional aversive properties but did not weaken the ability of conditioned pentobarbital to attenuate a subsequent saccharin taste aversion. A weakened avfail effect is expected if avfail is based on an association between pentobarbital and lithium toxicosis because the vinegar should compete with and overshadow such an association.

Revusky, Davey, and Zagorski (1989) report a preliminary attempt to cross-validate conditioned antisickness theory by establishing heart rate as a physiological index of drug-drug conditioning. Heart rate can be measured over the course of conditioning and this obviates a blocking interpretation. We paired a pentobarbital CS with an amphetamine US and found higher heart rates in response to pentobarbital relative to a delayed control. The conditional response was opposite in direction to the effect of the amphetamine. Other reports of drug-drug conditioning are available. Taukulis (1982,

1986a, 1986b) found conditional hyperthermia in response to a CS drug paired with a hypothermia-inducing US drug. Wilkin, Cunningham, and Fitzgerald (1982) paired ethanol or saline CSs with a lithium US in a differential heart rate conditioning procedure and found conditional responses to the different CSs that were in the same direction as the observed effect of the lithium US.

Heart rate and taste aversion measures cannot of course be presupposed to involve related response systems. Indeed, there is no evidence that heart rate is a direct measure of what is learned. That is, an effect of pairings relative to appropriate controls points to a Pavlovian interpretation of the heart rate effect but does not establish its physiological basis. Perhaps the conditioning of amphetamine-induced arousal translates into higher heart rates, for example. Whether or not heart rate and taste aversion measures are somehow related, the different procedures may both model homeostatic conditioning (Revusky, 1985). Demonstrating a CR that is directionally opposite to the observed effect of the US drug has a certain face validity in terms of the proposed model. Of course it does not establish whether the CR compensates in some way for a departure from homeostatic equilibrium induced by the US drug. Furthermore, compensatory conditioning does not itself establish selective association between an interoceptive drug state CS and some sort of homeostatic

aftereffect of the US treatment. Demonstrating selective association using a physiological measure is complicated by the fact that the CS drug signals not the US drug alone but the interaction between CS and US drugs. This could make it difficult to dissociate the different sorts of associations postulated for interoceptive and exteroceptive cues paired with the same nominal US treatment. The present investigation raises another complication. It questions whether controls for nonassociative factors have been fully addressed in these procedures.

CHAPTER 2:

HOME ENCLOSURE HEART RATE EFFECT

2.1 EXPERIMENT 1A

2.1.1 Introduction

Revusky (1985) proposed that drug-drug conditioning models conditioning between naturally-occurring internal states. According to this proposal, the direction of the CR is partly determined by the nature of the CS. Within a taste aversion framework, for example, a taste paired with sickness acquires a sickness response whereas a drug state paired with sickness acquires a homeostatic antisickness response. Selective association between an interoceptive CS and a homeostatic aftereffect of the sickness US confers adaptive advantage because it enables the animal to cope with unavoidable sickness.

Conditioned antisickness may be an instance of general Pavlovian involvement in homeostatic regulation. Revusky, Davey, and Zagorski (1989) report a preliminary attempt to validate this homeostatic conditioning model using a heart rate procedure. We failed to substantiate a crucial condition of the model, namely, that the interoceptive drug state paired with the US treatment serves as the effective

CS. We nevertheless argued that drug state conditioning occurs but could not eliminate the possibility that conditioning to exteroceptive cues occurs and is state-dependent. Specifically, we found that pairing a pentobarbital CS and an amphetamine US on three or more occasions produced a change in the effect of pentobarbital on heart rate. Rats received pentobarbital injection while in a heart rate recording chamber and amphetamine immediately after removal from the chamber. Cues made available by the injection and recording procedures did not produce a change in heart rate in the absence of pentobarbital sedation. But home cage drug pairings conducted in the absence of recording cues did not yield evidence of conditioning on a postconditioning transfer test. The novelty of the recording chamber may have produced external inhibition which prevented transfer. We favoured this sort of failure-of-transfer interpretation despite failure to demonstrate facilitation of transfer with habituation to the testing environment. State-dependent conditioning to exteroceptive cues seemed implausible because several precautions were taken to minimize the possibility of conditioning to exteroceptive cues, and because conditioning was not found in rats trained and tested in the non-drugged state, that is, with saline substituted for the pentobarbital.

The present investigation addresses an artifact in the

experiment that was designed to show home cage conditioning (Revusky et al., 1989, Experiment 2). In that experiment, two groups received either forward or 24-hour delayed pairings of pentobarbital and amphetamine with rats receiving pentobarbital injection while in the recording chamber. These groups replicated the original finding. An additional two groups received either forward or backward pairings in the home cage. The postconditioning test used to assess home cage conditioning permits a backward control. Presupposing that backward and delayed controls would yield equivalent results, we chose a backward control because backward pairings may be safer than delayed pairings: Pentobarbital may serve as an antidote to the occasionally lethal effects of the amphetamine. Home cage groups did not differ.

I propose that our earlier conclusions were based on a faulty premise. Backward and delayed groups are not equivalent. Rather, forward and backward pairings produce equivalent results relative to a delayed control. The most parsimonious interpretation of such a finding would be that the heart rate effect is due to a pharmacological interaction between pentobarbital and amphetamine not involving learning. The present proposal is warranted because the evidence that conditioning occurs in this procedure is not yet persuasive for the following three reasons:

1. An effect of forward pairings relative to a delayed control is not convincingly associative in the absence of additional evidence ruling out a nonassociative drug interaction of some sort: (a) Suitable drug substitutions may be used to provide converging evidence consistent with a conditioning interpretation (cf., Revusky, Coombes, & Pohl, 1982). The more sophisticated strategies required to demonstrate whether a particular drug combination is uniquely associable (Revusky, 1985) are not available. The heart rate effect appears to lack such generality. Of the limited number of US drugs tested, only amphetamine has so far proven effective. Substitutions for the pentobarbital CS have not been attempted. It is noteworthy that lithium is ineffective as a US: Avfail would be expected on taste aversion post-test (Revusky, Davey, & Reilly, 1987; cf., Wilkin, Cunningham, & Fitzgerald, 1982). (b) A backward control may be used to rule out drug interactions of the sort conjectured to depend on temporal proximity but not on order of drug injections. Of course drug interactions need not be symmetrical in this sense. The heart rate effect has not been demonstrated relative to a backward control.

2. For a particular target measure, a convincing argument that conditioning occurs can be made with less rigour if the unconditional effect of the CS drug is either minimal or at least directionally opposite to its conditional effect. Questionable assumptions may otherwise

be required in order to infer the existence of isodirectional unconditional and conditional effects of the CS drug. Traditional Pavlovian CSs are restricted to stimuli that are initially neutral with respect to the target system because this ensures that the stimulus antecedents for the CR can be correctly specified (Gormezano & Kehoe, 1978). Pentobarbital has an unconditional effect on heart rate in naive rats that is similar in magnitude and duration to its putative conditional effect (Revusky et al., 1989; Figure 8). This makes it difficult to infer that pentobarbital acquires a property of amphetamine as required by a Pavlovian account of the heart rate effect. Although the pentobarbital UR diminishes over trials in rats receiving delayed drug injections, it could be maintained in forward and backward groups. This could occur because a drug interaction present in forward and backward groups and absent in the delayed control produces apparent differential tolerance to pentobarbital's heart rate effect. A straightforward Pavlovian account must suppose that conditioned pentobarbital loses its intrinsic effect on heart rate at about the time it acquires a new similar effect through association with the amphetamine. Although associative mechanisms may be postulated in maintenance of the initial pentobarbital UR, an effect of pairings consistent with this reasoning is nonassociative by the usual definitions.

3. Latency characteristics of the heart rate effect suggest Pavlovian delay conditioning, and this has been taken as the single most specific indicator that conditioning has occurred (Revusky et al., 1989). For example, when the amphetamine injection is omitted on test, group differences have been found to emerge or intensify at about the time the amphetamine would normally have taken effect. The pentobarbital CR has also been found to antedate the amphetamine injection during training, thus making it appear that rats in the forward group anticipate the amphetamine. This argument is not entirely convincing because these latency characteristics do not contradict the possibility that a drug interaction serves to maintain the pentobarbital UR: The heart rate effect emerges as the magnitude and duration of the pentobarbital UR diminish over trials in the delayed group. Moreover, pentobarbital is known to produce "paradoxical" excitement both during induction of and recovery from sedation (e.g., Harvey, 1985). Perhaps the pentobarbital UR indexes such excitation. Temporal parameters of the heart rate procedure are such that maintenance of an excitatory pentobarbital UR could account for the latency characteristics of the heart rate effect, which might be supposed to parallel induction of and the beginning of recovery from sedation in the forward group.

The present experiment was designed to permit direct

comparison between backward and delayed controls in a home cage conditioning procedure. Three groups of rats received six training trials consisting of forward, backward, or delayed drug pairings. Heart rate was not recorded during the drug pairings phase. This eliminates a potential confound because heart rate cannot be recorded under identical conditions across all groups. It also serves to eliminate any participation of apparatus cues, which include restraint by the recording leads, in conditioning per se. All groups received pentobarbital alone on the single test trial. Testing was designed to minimize any disruption produced by the novelty and stress of the recording procedure. Such disruption could compromise assessment of an effect of pairings by producing external inhibition on test or by interacting with the pharmacological effect of pentobarbital in such a way as to mask the development of tolerance (cf., Cunningham & Bischof, 1987). Thus, rats remained in the home enclosure on test. Advantage was taken of pentobarbital's sedative effect by delaying heart rate recording until 20 minutes after injection. Rats were expected to be sufficiently sedated by this time so as to be little disturbed by the recording procedure. The purpose of the experiment was to determine whether the heart rate effect reported by Revusky, Davey, and Zagorski (1989) depends on the order of drug injections. A heart rate effect in forward and backward groups would explain our earlier failure to obtain home cage conditioning; more

important, it would also call a conditioning account of the heart rate effect into question.

2.1.2 Method

2.1.2.1 Subjects

Forty-five naive male Sprague Dawley rats served as subjects. They were obtained from Canadian Breeding Farms (Halifax, NS) at a weight range of 190-200 g and had attained a weight range of 295-310 g at the start of the experiment. They were housed individually in translucent polypropylene enclosures (Hazleton, HP301) lined with wood-chip bedding, and had free access to Purina Rat Chow at all times. A water deprivation cycle in effect thoughout the experiment provided free access to water for one day in three. Water deprivation commenced on the day prior to the first experimental day such that rats were approximately 20-24 hr deprived at the beginning of any given trial and did not again have access to water until at least 1 hr after the completion of all procedures for that trial. Restricting water intake made it unlikely that ingestion would occur in conjunction with drug treatment, and ensured that all testing occurred in a deprived state. Rats were weighed as necessary for assignment to groups and every third day just before initiation of the deprivation cycle. Safety pin heart rate recording electrodes were inserted subcutaneously, one each on the right shoulder and left

flank, on the day prior to the first experimental day. Continuous lighting conditions were in effect in the colony room. The experiment took place in the colony room with rats removed from their enclosures only for weighing and injection.

2.1.2.2 Apparatus

Revusky et al. (1989) provide a detailed description of the heart rate recording apparatus. Briefly, heart rate was recorded by clipping the rats' electrodes to leads feeding into a system which in turn amplified, filtered, and digitized the signal in preparation for computer processing. The processing algorithm operated on heart rate samples several seconds in duration that had been taken sequentially for each of four rats at 2-min measurement intervals. Determination of a characteristic peak-to-peak interval for four subsamples of five successive r-waves formed the basis for obtaining a single duration that was converted to heart beats per minute. In this experiment, rats remained in their home enclosures during heart rate recording. The electrodes of sedated rats were clipped to leads feeding directly into the recording system. Obtaining heart rate readings required little or no handling.

2.1.2.3 Drugs

Sodium pentobarbital (Somnotol) served as the CS drug. It was diluted with normal saline to a concentration of 36

mg/ml and was injected intraperitoneally (ip) at a dose of 36 mg/kg. D-amphetamine sulfate served as the US drug. It was dissolved in saline to a concentration of 18 mg/ml and injected intramuscularly (im) at a dose of 18 mg/kg. Because rats in the present experiment were somewhat heavier, the amphetamine dose was lower than the 24 mg/kg successfully used by Revusky et al. (1989). It nevertheless proved excessive and was reduced to 14 mg/kg on the first trial after 5 rats in each group had been run.

2.1.2.4 Procedure

Rats were assigned to three groups of 15 each such that group mean weights were equated. There were six drug-drug training trials. On the conditioning day, all groups received paired injections spaced 30 min apart. A forward pairings group received injections of pentobarbital followed by amphetamine. A backward pairings group received the two drugs in reverse order. A delayed pairings group received injections of pentobarbital followed by saline on the conditioning day, and amphetamine on the following day: For this group, the total amphetamine dose was given in three volumetrically equal injections spaced four hours apart. The technique of spacing the amphetamine dose in delayed controls has been used in previous work because pentobarbital does not serve as an antidote to the occasionally lethal effects of the amphetamine in this group (Revusky et al., 1989). Heart rate was not recorded during

this phase of the experiment. Three rats in the forward group, seven rats in the backward group, and two rats in the delayed group died prior to test and their data were discarded.

The heart rate effect is most readily demonstrated using a probe procedure, that is, by delaying or omitting the second injection on test. All groups received pentobarbital alone on the single test trial. Heart rate readings were taken over a 40 min period beginning 20 min after pentobarbital injection. Baseline readings were not feasible because unsedated rats tested in a large enclosure vigorously resist novel restraint by the recording leads, and were not considered necessary because group differences on these readings have not been found in this procedure (e.g., Revusky et al., 1989).

2.1.2.5 Statistical analysis

Because heart rate readings are taken at 2-min intervals, they are statistically intercorrelated and therefore may not conform to the requirements for a conventional repeated measures analysis (see Keselman, Mendoza, Rogan, & Breen, 1980). Conservative tests such as those used in Experiment 2 are not appropriate when sample sizes are not equal. Heart rates for each rat were averaged across each of five successive 8-min sample periods. These averages served as the data, and separate analyses were undertaken for each sample period. Using the mean of the

sample period as the datum did not justify a repeated measures analysis, but did offer some protection against the sorts of spurious conclusions that can occur when separate analyses are undertaken for each of a large number of measurement intervals. Preliminary testing of the equality of within-group variances for each sample period did not yield significant results. In the event of a significant omnibus F for the sample period, differences between pairs of group means were evaluated using a per comparison error rate equal to alpha. In the absence of a significant F, the alpha level applies to the set of comparisons (Dunn's procedure). Pairwise comparisons were made using F tests based on the error term of the overall ANOVA. An alpha level of .05 was adopted.

2.1.3 Results

Group heart rates for successive 8-min sample periods are shown in Figure 1. The general impression afforded by inspection of this figure is that either forward or backward pairings increase heart rate relative to a delayed control. Group differences emerge or increase late in the testing period, which suggests that they are contingent upon the physiological effects of pentobarbital injection. More specifically, heart rates for all three groups are roughly similar over approximately the first half of the testing period, relative to the orderly divergence between forward

and backward groups on the one hand, and the delayed group on the other hand, seen within the second half. This general impression is consistent with the results of statistical tests for individual sample periods. These results are summarized in Table 2. For the first three sample periods, no test yielded significant results. For the last two sample periods, forward and backward groups each differed from the delayed control. Forward and backward groups did not differ.

2.1.4 Discussion

Replication of the heart rate effect in rats receiving drug pairings in the home enclosure indicates that apparatus cues need not be present during procedural conditioning trials in order to obtain the effect. Such cues can therefore have no necessary role in conditioning per se. Similar effects of forward and backward pairings suggest that a nonassociative drug interaction has yet to be ruled out. Because both forward and backward groups show intensification of the heart rate effect more than 30 minutes after pentobarbital injection, a probable basis for a delay conditioning effect is also eliminated: Rats in the backward group presumably cannot time the occurrence of the US, for example by discriminating early and late effects of pentobarbital relative to the effects of amphetamine, because the usual relation for these rats is essentially

reversed on test.

A nonassociative drug interaction could serve to maintain the pentobarbital UR. This speculation is based on the premise that an effect of pairings relative to a backward control is useful in ruling out certain sorts of drug interactions. Amphetamine decreases heart rate, and a straightforward homeostatic conditioning interpretation suggests that the pentobarbital CR compensates for amphetamine's heart rate effect. Revusky et al. (1989, Figure 5) failed to show such compensation. Failure to compensate does not militate against a homeostatic conditioning interpretation but does permit alternative interpretations. Pentobarbital is commonly reported to have no effect on heart rate other than a decrease secondary to sedation (e.g., Harvey, 1985). We were therefore surprised to find that the drug produced an increase in heart rate in naive rats of about 40 beats per minute which was sustained throughout the 30-minute measurement period in our procedure. Pentobarbital is known to produce "paradoxical" excitement under certain conditions, and this may serve as the basis of pentobarbital's unconditional and putative conditional effects. Amphetamine and pentobarbital show mutual potentiation on measures of behavioral activation (e.g., Rushton & Steinberg, 1963). The site and mechanism of this synergistic interaction are unknown, and the possibility that a drug interaction affects the development

of tolerance to pentobarbital in such a way as to maintain the pentobarbital UR cannot be ruled out a priori. By this reasoning, timing of the heart rate effect may be relatively independent of the order of drug injections, and its magnitude may actually increase in novel or stressful testing situations. Drug substitutions might be found which provide converging evidence favoring an associative interpretation. Perhaps the most convincing single substitution would replace pentobarbital with a CS drug that has little or no intrinsic effect on heart rate.

A qualifier to conclusions involving the backward group is that many rats died and number of deaths was related to experimental treatment. More rats in the backward group died presumably because they received the full dose of amphetamine unprotected by pentobarbital. This was surprising because the dose was lower than that used successfully by Revusky et al. (1989). However, the results of Experiments 1B and 2 suggest that differential attrition does not account for the present results.

2.2 EXPERIMENT 1B

This study replicated Experiment 1A with minor changes intended to reduce differential subject loss.

2.2.1 Method

2.2.1.1 Subjects

Fifty-four naive male Sprague Dawley rats served. They were obtained from Charles River (Canada) at a weight range of 190-200 g and had attained a weight range of 255-275 g at the start of the experiment.

2.2.1.2 Drugs

The amphetamine dose was increased from an initial 8 mg/kg to 12 mg/kg in increments of 2 mg/kg/trial. It was reduced to 10 mg/kg on the third trial after 6 rats per group had been run.

2.2.1.3 Procedure

Rats were weight-assigned to three groups of 18 each. There were 12 trials in all. Trials 1-6 and 8-11 were drug-drug training trials. Trials 7 and 12 were test trials. On the first test trial, all groups received forward-paired injections of pentobarbital and amphetamine with an interinjection interval of 90 min. Occasional forward-pairings test trials have been used in previous work, and are not contraindicated because demonstrating a heart rate effect has required more than one or two pairings (Revusky et al., 1989). On the second test trial, all groups received pentobarbital alone. Heart rate readings were taken over a 40-min period. In unspecified respects

the procedure is identical to Experiment 1A. Two rats in the forward group, seven rats in the backward group, and three rats in the delayed group died prior to the first test trial. An additional two rats in the backward group died prior to the second test trial.

2.2.2 Results and Discussion

The results of the first test trial are shown in Figure 2. By inspection, these results suggest that both forward and backward pairings increase heart rate relative to a delayed control until such time as the delayed group recovers from pentobarbital sedation. Specifically, heart rates for forward and backward groups are similar, and higher than the rate for the delayed control over a large portion of the testing period, although the rate for the backward group is higher initially and tends to decrease whereas that for the forward group remains relatively stable. This general impression is consistent with the results of statistical tests for individual sample periods. These results are summarized in Table 3. The forward group differed from the delayed control during sample periods 3-6. The backward group differed from the delayed control during sample periods 1-5. Forward and backward groups did not differ on any test.

Group differences did not emerge in a way that would

offer some protection against the absence of baseline readings, and rats were therefore given additional conditioning trials followed by a second test trial. The results of the second test trial are shown in Figure 3. Differences between forward and backward groups are minimal. Heart rates for these groups are relatively stable over the testing period, and higher than the progressively decreasing rate for the delayed control. The results of statistical tests are summarized in Table 4. For every sample period, forward and backward groups each differed from the delayed control. Forward and backward groups did not differ on any test.

The apparent robustness of the heart rate effect on the second test trial permitted the following check on whether an effect of backward pairings might not have been found had number of deaths been unrelated to treatment. Data for the six rats in the delayed group with the lowest average heart rates were cast out and the data reanalyzed. The reanalysis is based on 16 rats in the forward group and 9 rats each in the backward and delayed groups. Casting out increased heart rate in the delayed group by about 14-17 beats per min during each sample period (cf., Figure 3). The results of statistical tests are summarized in Table 5. Group differences are lost during the first two sample periods. However, the pattern of significant results for sample periods 3-5 is not changed. This implies that backward

pairings do not produce higher heart rates simply because amphetamine is lethal for those rats which had they survived would have shown lower heart rates on test: Backward and delayed pairings produce different effects. It seems unlikely that backward and forward pairings could produce such similar effects as a consequence of differential subject loss.

CHAPTER 3:

HOME CAGE AND RECORDING CHAMBER HEART RATE CONDITIONING PROCEDURE WITH TASTE AVERSION POST-TEST

3.1 EXPERIMENT 2

3.1.1 Introduction

The present experiment replicates Experiment 2 of Revusky, Davey, and Zagorski (1989) with the addition of a delayed home cage control. If forward and backward home cage drug pairings produce equivalent effects without the differential subject loss of Experiment 1, this would conclusively establish the relevance of the present findings to the earlier body of work. The replication includes a taste aversion post-test. Avfail is obtained with this drug combination relative to a backward control (Revusky, Coombes, & Pohl, 1982). Heart rate and avfail measures are not necessarily related, and avfail is expected relative to a backward control in this experiment. Backward and delayed controls have not been directly compared in the drug-drug conditioning literature. A difference between these groups using a taste aversion measure would implicate the involvement of factors that have not previously received explicit examination.

All groups received two pretraining trials with saline injected in the recording chamber: One or two pretraining trials have been typical of past work (Revusky et al., 1989). During the training phase, two groups received forward or delayed pairings of pentobarbital and amphetamine with rats receiving pentobarbital injection while in the recording chamber. Three additional groups received forward, backward, or delayed drug pairings in the home cage. After eight training trials, all groups received the same treatment on each of three heart rate test trials. The first test was a forward pairing probe trial with pentobarbital injected in the recording chamber. The additional tests were designed to rule out participation of cues associated with the injection and heart rate recording procedures in recording chamber and home cage groups. They were identical to the first test except that (a) for the second test, saline was substituted for the drugs, and (b) for the third test, amphetamine was omitted and heart rate was recorded in the home cage beginning 20 minutes after pentobarbital injection. Recording chamber groups were discarded after the final heart rate test trial.

For the taste aversion post-test, a fourth group was formed from subsamples of the three home cage groups. The parent groups were injected with pentobarbital immediately after consumption of saccharin solution on each of four taste aversion conditioning trials. For the fourth group,

which served as a no-aversion baseline, saline was substituted for the pentobarbital. Based on findings obtained with a lithium US, the heart rate test trials were considered unlikely to affect the results of the taste aversion post-test. Avfail is not obtained after one or two forward drug pairings (Revusky, Taukulis, Parker, & Coombes, 1979), and injections of pentobarbital or saline have been interpolated between the drug pairings and taste aversion conditioning phases of the avfail procedure with minimal effect (see Martin, 1982).

3.1.2 Method

3.1.2.1 Subjects

Eighty naive male Sprague Dawley rats served as subjects. They were obtained from Charles River at a weight range of 170-180 g and had attained a weight range of 193-223 g at the start of the experiment. They were housed individually in rack-mounted stainless steel wire mesh cages under continuous lighting conditions and had free access to Purina Rat Chow at all times. The water deprivation schedule in effect for the first part of the experiment, prior to the taste aversion conditioning phase, consisted of alternating 48-hr deprivation and 24-hr free access, with the following modification: Rats were additionally allowed 15-min access 28 hr after the water bottles were removed and each repetition of the deprivation cycle initiated. They

were approximately 12-16 hr deprived for all pretraining and training injections, and 8-20 hr deprived for test injections. The deprivation schedule in effect for the taste aversion conditioning phase consisted of 15 min access per day.

3.1.2.2 Apparatus

An appropriately lined cylindrical metal container (diameter 19.1 cm, height 12.2 cm) with a cover and swivel device served as the heart rate recording chamber. Rats could be placed in the chamber and their electrodes clipped to leads that made contact through the swivel with the signal processing system. Heart rate could also be recorded from the home cage.

3.1.2.3 Drugs

The amphetamine concentration was 16 mg/ml. The initial 7 mg/kg dose was incremented by 1 mg/kg/trial to 12 mg/kg and held constant thereafter.

3.1.2.4 Procedure

Rats were weight-assigned to five groups of 16 each. The experiment was conducted in four consecutive phases.

Pretraining phase. All groups received the same treatment on each of two pretraining trials. The procedure for these trials was the same as for the training trials of the drug pairings phase except that (a) an equal volume of

physiological saline was substituted for the drugs, and (b) home cage as well as recording chamber groups were placed in the chamber.

Drug pairings phase. There were eight drug-drug training trials. A trial consisted of paired injections spaced 30 min apart on each of two consecutive days. The two pairs of injections were spaced approximately 28 hr apart. Recording chamber groups were placed in the chamber 20 min prior to the first of the paired injections on the first or conditioning day. They were removed from the chamber as necessary for injections, spent the interval between injections in the chamber, and were returned to the home cage after the second injection. They were not placed in the chamber for the second pair of injections. Home cage groups were removed from the home cage only as necessary for injections and spent the interval between injections in the home cage.

On the conditioning day, forward pairings groups received pentobarbital followed by amphetamine. The backward pairings group received the two drugs in reverse order. Delayed pairings groups received pentobarbital as the first injection of the first pair on the conditioning day and amphetamine as the second injection of the second pair on the day after the conditioning day: The total amphetamine dose was delivered in a single injection. All remaining injections were equivalent-by-volume saline injections such that the two pairs of injections were

identical for all groups except for the contents of the syringe.

Recording chamber groups received either forward or delayed pairings of pentobarbital and amphetamine. Home cage groups received either forward, backward, or delayed pairings. Heart rate was recorded in recording chamber and not in home cage groups during this phase of the experiment.

Heart rate testing phase. There were three heart rate test trials. All groups received the same treatment on each of these trials. The interinjection interval was 50 rather than 30 min. For the first two trials, all groups were placed in the recording chamber 20 min prior to the first injection and spent the interval between injections in the chamber. On the first trial, all groups received pentobarbital followed by amphetamine. On the second trial, saline was substituted for both drugs. For the third trial, all groups received pentobarbital followed by saline, and heart rate was recorded in the home cage beginning 20 min after pentobarbital injection. Recording chamber groups were discarded after the final test trial.

Taste aversion conditioning phase. Subsamples of four rats were removed from each of the home cage groups such that the mean weight of the subsample was equal to the mean weight of the parent group. The subsamples were pooled to form a fourth group that served as a no-aversion baseline. On the day following the third heart rate test trial and 24 hr drinking period, rats were placed on a schedule of 15 min

access to room-temperature tap water per day. On the 8th, 11th, 14th and 17th day of this schedule, the water was flavored with sodium saccharin (0.75 % w/v). The parent groups were injected with pentobarbital as the saccharin bottle was removed. The baseline group was injected with an equivalent volume of saline.

3.1.2.5 Statistical analysis

Heart rate measure. The present repeated measures analysis assumes that the validity conditions underlying conventional F tests involving the within-subjects factor(s) are violated. Although conventional degrees of freedom are reported, the obtained F ratios were evaluated using a corrected degrees of freedom test which conservatively assumes maximum violation of the required pattern of variances and covariances both within and across groups (Geisser & Greenhouse, 1958). With n subjects per group, the test reduces the degrees of freedom with which the F table is entered to 1 and (n -1). Error terms for all within-subjects tests were based on data entering into the particular analysis.

Taste aversion measure. Saccharin consumption scores were converted to preference scores in the form of suppression ratios. The ratio was S/(S+W), where S is the amount of saccharin consumed on any training day and W is the amount of water consumed on the day prior to the training day. A ratio below 0.50 indicates lower saccharin

consumption on the training day than water consumption on the previous day. Preference scores on the first training day served as the covariate, and the mean of the scores on the remaining days served as the datum, in a covariance analysis (ANCOVA). F tests based on the error term and adjusted means of the overall ANCOVA were used for pairwise comparisons.

3.1.3 Results

3.1.3.1 Heart Rate Measure

Baseline heart rate readings for recording chamber groups and for pairs of home cage groups were entered by trials into a series of two-way ANOVAs (Groups X Intervals). No tests involving the Groups factor were significant.

Drug pairings phase. Heart rate was not recorded in home cage groups during this phase. Heart rate readings taken after pentobarbital injection for the two recording chamber groups were entered by trials into two-way ANOVAs. Statistically reliable differences between Groups emerged on the third and eighth training trials, $F(1,30) = 4.25$ and 5.67, respectively. No other tests involving the Groups factor were significant. Figures 4 and 5 illustrate group heart rates during successive 2-min measurement intervals on the third and eighth training trials. Rats exposed to forward drug pairings in the recording chamber showed higher

heart rates in response to pentobarbital injection than did delayed controls. Basing the analyses on heart rates taken more than 18 min after pentobarbital injection, Groups effects for the third, seventh and eighth trials were indicated by the criterion of Revusky et al. (1989). Results for recording chamber groups replicate those of Revusky et al. (1989), but in previous work at least four or five trials have been required for demonstrating an effect of pairings. The unreliability of the Groups effect across trials after its initial appearance on the third trial may be related to the amphetamine dose, which was lower than that used by Revusky et al. (1989).

The heart rate effect is superimposed on changes in rate due to other factors (Revusky et al., 1989). One such factor is the pharmacological effect of pentobarbital; additional factors are handling and injection, and the novelty of the recording chamber--all of these factors increase heart rate. An effect of pairings emerges as the combined effect of the remaining factors diminishes within and across trials and is seen as maintenance of a high rate which otherwise decreases in delayed controls. In order to provide a descriptive summary of changes in heart rate during the drug pairings phase of this experiment, readings taken before and after pentobarbital injection were entered into separate three-way ANOVAs (Groups X Intervals X Trials). Analysis of baseline rates yielded significant

main effects of Intervals, F (8,240) = 85.03, and Trials, F (7,210) = 27.99, indicating differences among overall interval and trial means. Analysis of post-injection rates yielded a similar pattern of results, with significant main effects of Intervals, F (13,390) = 97.91, and Trials, F (7,210) = 14.69. No other tests yielded significant results. Of course it is not surprising that no tests involving the Groups factor were significant because the drug pairings phase of the experiment ended once pairings produced an effect of reasonable reliability. Figures 6 and 7 show mean heart rates as a function of measurement intervals and trials, respectively. By inspection, heart rate decreases over successive measurement intervals and trials. The rate of decrease slows at higher values of the independent variables. These observations were supported by significant linear and quadratic components of the global trends.

Heart rate testing phase. The first test was a forward pairings probe trial with rats receiving pentobarbital injection while in the recording chamber. Figure 8 shows heart rates for home cage groups on the first trial. By inspection, forward or backward pairings produce similar increases in heart rate in response to pentobarbital injection relative to a delayed control. Post-injection rates for the three groups were entered by pairs into separate two-way ANOVAs in order to confirm the statistical

reliability of these observations. Comparisons of each of the forward and backward groups with the delayed control yielded significant main effects of Groups, $F(1,30) = 12.89$ and 13.75, respectively. Forward and backward groups did not differ ($F < 1$). The Groups X Intervals interactions were not significant. Two-tailed t tests for differences between forward and backward groups at each measurement interval confirmed that these groups are statistically indistinguishable (all $ps > .20$). The presence of apparatus cues during the drug pairings phase is not necessary in order to demonstrate an effect of pairings on test. Moreover, when the interinjection interval is 30 min, the heart rate effect does not depend on the order of drug injections. Figure 9 shows heart rates for recording chamber groups on the first test trial. ANOVAs confirmed an effect of pairings as seen on the third and eighth training trials. For the post-injection period, the main effect of Groups was significant, $F(1,30) = 9.79$. The Groups X Intervals interaction was not significant.

On test, recording chamber and home cage groups have differing amounts of prior experience in the chamber. The effect of differential habituation to the chamber was examined by entering baseline and post-injection heart rate readings for comparable recording chamber and home cage groups into three-way mixed ANOVAs (Groups X Intervals X Training Contexts). For the baseline period, the analysis

yielded significant main effects of Intervals, F (8,480) = 47.58, and Contexts, F (1,60) = 7.41, and a significant Intervals X Contexts interaction, F (8,480) = 4.27. The significant interaction indicates that the global trend among interval means differs as a function of training context. Its source is a difference in the quadratic component of the trend, F (1,60) = 13.48. Separate analyses under each level of the Contexts variable indicate significant quadratic curvature in recording chamber, F (1,30) = 29.44, but not in home cage groups. The linear component of the main effect of Intervals was also significant, F (1,60) = 123.84. The linear decrease in rate seen in all groups slows in recording chamber groups. For the post-injection period, the analysis yielded significant main effects of Groups, F (1,60) = 21.74, and Intervals, F (23,1380) = 21.67. No other tests yielded significant results. A series of *t* tests comparing pairs of forward and delayed groups across training contexts at each measurement interval confirmed that post-injection heart rates were not affected by whether training had taken place in the recording chamber or in the home cage (all *ps* > .10). Within-trial habituation is apparently sufficient so that the different amounts of prior exposure to the chamber in recording chamber and home cage groups affect baseline but not post-injection heart rates.

On the second trial, all groups received saline

injections in the recording chamber. The purpose of this trial was to determine whether the pairings effects shown in recording chamber and home cage groups on the previous trial could be obtained in the absence of pentobarbital sedation. Post-injection rates for pairs of home cage groups and for recording chamber groups were entered into separate two-way ANOVAs (Groups X Intervals). No tests involving the Groups factor were significant (all Fs < 1.19, ps > .20). A series of t tests comparing pairs of groups within training contexts at each measurement interval confirmed that groups did not differ in response to saline injection (all ps > .20).

On the third trial, all groups received pentobarbital alone. Heart rate was recorded in the home cage beginning 20 min after injection. Figure 10 shows heart rates for recording chamber and home cage groups on this trial. By inspection, heart rates are higher in rats with histories of paired drug injections than in delayed controls. Heart rates for recording chamber groups and for pairs of home cage groups were entered into separate two-way mixed ANOVAs (Groups X Intervals). For the home cage groups, comparisons of each of the forward and backward groups with the delayed control yielded significant main effects of Intervals, F (14,420) = 5.30 and 6.31, respectively, as well as significant Groups X Intervals interactions, F (14,420) = 4.91 and 7.06, respectively. The main effects of Groups

were not significant. The source of the significant interaction in each case is a difference in linear trend, F (1,30) = 10.31 and 19.06, respectively. By inspection, heart rate is a linear decreasing function of measurement interval in the delayed control. No similar trend is apparent in forward or backward groups. These observations are supported by followup analyses of the linear components of the simple main effects of Intervals at each level of the Groups variable. Heart rate is a linear function of measurement interval in the delayed control, F (14,210) = 69.64, but not in forward or backward groups (Fs < 1). Comparison of forward and backward groups did not yield significant results (all Fs < 1.89, ps > .20). A series of t tests comparing forward and backward groups at each measurement interval confirmed that these groups are statistically indistinguishable (all ps > .20). Thus, an effect of pairings was obtained in home cage groups on this trial. Heart rate decreases over intervals in the delayed group but not in forward or backward groups. For the recording chamber groups, the analysis yielded a significant main effect of Groups, F (1,30) = 4.23. Heart rate is higher in the forward group. No other test was significant.

3.1.3.2 Taste Aversion Measure

Saccharin consumption scores were converted to suppression ratios after it was determined by ANOVAs that groups did not differ in their water consumption. Figure 11

shows group suppression ratios over saccharin drinking days. Groups did not differ on the covariate ($F < 1$). Adjusted group means from the overall ANCOVA are displayed in Table 7. The omnibus ANCOVA yielded a significant Groups effect, $F(3,43) = 17.67$. Pairwise comparisons indicated that avfail effects were obtained in forward and delayed groups. That is, these groups each had higher suppression ratios (stronger saccharin preferences) than the backward control, $F(1,43) = 25.75$ and 19.79, respectively. The avfail effects were incomplete because these groups each had lower suppression ratios than the no-aversion baseline, $F(1,43) = 4.83$ and 7.97, respectively. The combination of pentobarbital and amphetamine is known to produce a partial rather than a complete avfail effect (Revusky, Coombes, & Pohl, 1982). Forward and delayed groups did not differ ($F < 1$).

3.1.4 Discussion

3.1.4.1 Heart Rate Measure

This experiment indicates that our earlier failure to demonstrate home cage heart rate conditioning was based on a methodological flaw in the procedure and not to failure of transfer to the recording chamber or to state-dependent conditioning to apparatus cues (Revusky et al., 1989). A forward pairings effect was originally demonstrated relative to a delayed control. Comparison of forward and delayed

home cage groups also yields an effect of pairings. Moreover, apparatus cues are of little consequence given the sort of limited preexposure that has been typical of past work. Such cues did not affect the response to pentobarbital in forward and delayed home cage groups on transfer test by comparison with recording chamber groups. In addition, recording chamber and home cage groups showed an effect of pairings in the presence of pentobarbital whether apparatus cues were present (first test trial) or absent (third test trial). No such effect was found in the absence of pentobarbital sedation (second test trial). The pentobarbital drug state may be a necessary and sufficient condition for demonstrating a heart rate effect in this procedure.

This experiment also confirmed equivalent effects of forward and backward pairings: These groups were statistically indistinguishable. Thus, the heart rate effect does not depend on the order of drug injections when the interinjection interval is 30 minutes. This finding militates against a conditioning account of the effect. A backward control may be useful for ruling out certain sorts of pharmacological drug interactions. Because a more parsimonious drug interaction account is not otherwise contraindicated, the finding implies that a nonassociative drug interaction has yet to be ruled out. The possibility that backward conditioning occurs in this procedure appears

remote and will be addressed in the General Discussion.

3.1.4.2 Taste aversion measure

If a partial avfail effect is defined in terms of two statistically significant differences (e.g., Revusky, Coombes, & Pohl, 1982), then partial avfail effects were obtained in forward and delayed groups in this experiment. Moreover, forward and delayed pairings produced effects of similar magnitude. A drug interaction of the sort conjectured to depend on temporal proximity but not on order of drug injections obviously cannot account for this pattern of results. In order to establish whether a delayed pairings avfail effect is unique to the combination of pentobarbital and amphetamine, Experiment 3 compares backward and delayed groups in an avfail procedure using a lithium US. A similar pattern of results whether the US is amphetamine or lithium would not eliminate the possibility that a drug interaction of some sort participates in the avfail effect, but would invite a more general interpretation than one based on some sort of order effect unique to the combination of pentobarbital and amphetamine.

CHAPTER 4:

COMPARISON OF BACKWARD AND DELAYED CONTROLS IN AN AVFAIL PROCEDURE--LITHIUM US

4.1 EXPERIMENT 3A

4.1.1 Introduction

Experiment 2 indicated that delayed pairings of pentobarbital and amphetamine can attenuate a subsequent taste aversion relative to a backward control. The present experiment compares backward and delayed controls in an avfail procedure using a lithium US. An avfail effect in the delayed group relative to a backward control whether the US is amphetamine or lithium would invite a more general interpretation than one based on some sort of nonassociative order effect unique to the combination of pentobarbital and amphetamine.

4.1.2 Method

4.1.2.1 Subjects

Forty-eight naive male Sprague Dawley rats served as subjects. They were obtained from Charles River at a weight range of 190-200 g and had attained a weight range of 318-397 g at the start of the experiment. The water

deprivation schedule in effect during the drug pairings phase of the present experiment was the same modified three-day schedule specified for the first part of Experiment 2. Rats were approximately 12-16 hr deprived at the time of drug injections. During the taste aversion conditioning phase, rats were placed on a schedule of 15 min access per day. They were removed from their home cages only as necessary for weighing and injection.

4.1.2.2 Drugs

Pentobarbital served as the CS drug. It was diluted with saline to a concentration of 10 mg/ml and injected ip at a dose of 20 mg/kg. Lithium chloride served as the US drug. It was prepared as a 2 % (w/v) solution in distilled water and was injected ip at a dose of 160 mg/kg.

4.1.2.3 Procedure

Rats were weight-assigned to four groups of 12 each. There were five drug-drug training trials. A forward group received injections of pentobarbital followed by lithium with an interinjection interval of 30 min. A backward group received the two drugs in reverse order. A delayed group received pentobarbital on the conditioning day and lithium on the day following the conditioning day. A no-aversion baseline group was further subdivided into three weight-equated subgroups during this phase of the experiment, with four rats each receiving either forward,

backward, or delayed pairings of pentobarbital and lithium.

On the day following the fifth drug pairings trial and 24 hr drinking period, rats were placed on a schedule of 15 min access to room-temperature tap water per day. On the 8th, 11th, 14th, and 17th day of this schedule, the water was flavored with saccharin (0.75 % w/v). Forward, backward, and delayed groups were injected with pentobarbital as the saccharin bottle was removed. The baseline group was injected with an equivalent volume of saline.

4.1.3 Results and Discussion

Groups did not differ in their water consumption, and saccharin consumption scores were converted to suppression ratios for analysis. Figure 12 shows group suppression ratios over saccharin drinking days. Groups did not differ on the covariate ($F < 1$). Adjusted group means from the overall ANCOVA are displayed in Table 6 . The overall ANCOVA yielded a significant Groups effect, $F(3,43) = 43.65$. Pairwise comparisons indicated that avfail effects were obtained in forward and delayed groups. These groups each had higher suppression ratios than the backward control, $F(1,43) = 69.53$ and 22.40, respectively. The avfail effects were incomplete because these groups each had lower suppression ratios than the no-aversion baseline, $F(1,43) = 5.74$ and 36.03, respectively. Saccharin

preferences were higher in the forward group than in the delayed group, $F(1,43) = 13.00$.

Delayed drug pairings can attenuate a subsequent taste aversion relative to a backward control whether the US is amphetamine (Experiment 2) or lithium (Experiment 3). One group of explanations that might be considered in attempting to account for these findings involves the known attenuating effect of preconditioning drug exposure on subsequent taste aversion conditioning. Several accounts of such US preexposure effects are available. Cunningham and Linakis (1980) made use of one such account in proposing an associative blocking interpretation of avfail. Revusky, Taukulis, Parker, and Coombes (1979) made use of an alternative account couched in terms of US habituation. According to this account, prior drug exposure in backward, CS-, and US-only groups, and probably in the forward group as well (e.g., Martin, 1982), attenuates taste aversion conditioning relative to a drug-naive control by producing habituation to the sickness US (see also Revusky & Coombes, 1982). Avfail is not due to such habituation because avfail is found in rats with a history of forward drug pairings and not in rats with other sorts of histories.

Sickness habituation may be governed by the number and intensity of US preexposures (cf., Groves & Thompson, 1970). Differential habituation in backward and delayed groups

could occur because backward and delayed pairings differ in effective intensity or because delayed controls receive two discrete sickness presentations on each trial. Experiment 3B examines the effect of manipulating lithium dose in delayed and backward procedures.

4.2 EXPERIMENT 3B

4.2.1 Introduction

The combination of pentobarbital and lithium typically yields a complete avfail effect. Experiment 3A yielded a partial effect in the forward group, perhaps due to failure to adjust the lithium dose sufficiently to account for the fact that rats in that experiment were heavier than has been typical of past work. A higher lithium dose would be expected to yield a complete avfail effect in the forward group and to have no effect on saccharin preference in backward controls (Revusky, Coombes, & Pohl, 1982). At issue is whether an increase in lithium dose would increase, decrease, or have no effect on saccharin preferences in a delayed pairings procedure. The present experiment addresses this issue by manipulating lithium dose in backward and delayed groups.

4.2.2 Method

4.2.2.1 Subjects

Thirty-six naive male Sprague Dawley rats served as subjects. They were obtained from Charles River at a weight range of 190-200 g and had attained a weight range of 214-263 g at the start of the experiment.

4.2.2.2 Procedure

Rats were weight-assigned to four groups of nine each. Three groups received delayed pairings of pentobarbital and lithium. The fourth group received backward pairings. Rats in the backward group were weight-assigned to three subgroups of three rats each. Each of the delayed groups, and each of the backward pairings subgroups, received a different dose of lithium. The lithium dose was 80, 160, or 240 mg/kg. The procedure was otherwise the same as that specified for Experiment 3A.

4.2.3 Results and Discussion

Groups did not differ in their water consumption, and saccharin consumption scores were converted to suppression ratios. Backward groups differed on the first saccharin drinking day, $F(2,6) = 10.75$, but not on subsequent days ($Fs < 1$). Because differences on the first saccharin day have not been found in previous work (cf., Revusky, Coombes,

& Pohl, 1982, Experiment 6), the finding was considered a sampling error and backward groups were pooled for subsequent analyses. Adjusted group means from the overall ANCOVA are 0.30, 0.28, 0.26 and 0.19 for low-, medium-, and high-dose delayed groups and for the pooled backward group, respectively. Groups did not differ on the covariate. The overall ANCOVA yielded a significant Groups effect, $F(3,31) = 5.16$. Pairwise comparisons indicated that each of the delayed groups had a higher suppression ratio than the backward group, $F(1,31) = 14.21$, 9.10, and 5.40 for low-, medium-, and high-dose groups, respectively. Delayed groups did not differ among themselves ($ps > .10$).

The results of this experiment imply that attenuation of taste aversion conditioning in backward and delayed groups does not depend on the lithium dose. This suggests that as an account of the results of Experiments 2 and 3, differential habituation to the sickness US depends on the number of sickness preexposures and not on differences in effective US intensity.

CHAPTER 5: GENERAL DISCUSSION

5.1 Heart rate.

In three experiments reported here, forward and backward pairings of pentobarbital and amphetamine produced similar increases in heart rate relative to a 24-hour delayed control. When the interinjection interval is 30 minutes, the heart rate effect does not depend on the order of drug injections, and the finding therefore militates against a Pavlovian account of the heart rate effect. Available evidence does not warrant the speculation that some sort of backward conditioning occurs in this procedure, and the a priori expectation is that a backward group is appropriate as a control for nonassociative effects of drug pairings.

Forward conditioning in backward pairings groups has occasionally been reported in conditioned taste aversion procedures using a sickness US and a nominal 30-minute US-CS interval (e.g., Barker & Smith, 1974). Such conditioning is attributable to delayed onset or recruitment of the US-induced sickness relative to US administration, which produces effective forward pairings in the backward group; it is unlikely in this procedure because amphetamine acts quickly and this makes it improbable that pentobarbital will

serve a signalling function in the backward group. Because the effective events underlying conditioning have not been specified, some sort of delayed response to the direct or immediate effects of the amphetamine might be supposed as the US-related event that supports conditioning in forward and backward groups. Alternatively, pentobarbital and other drug state CSs might be supposed to have distinct characteristics that facilitate conditioning to amphetamine's immediate effects in a backward procedure. However, there is no a priori reason to suppose that amphetamine's immediate effects would not be conditionable to pentobarbital as to any conventional CS. A backward group is considered appropriate as a control for nonassociative effects in drug-drug conditioning and in most sorts of more conventional procedures. Furthermore, there is no persuasive reason to expect true backward conditioning (e.g., Champion & Jones, 1961) in this procedure.

A conditioning account of the heart rate effect must suppose that pentobarbital loses its intrinsic effect on heart rate at about the time it acquires a similar effect through association with the amphetamine. A more parsimonious account is that a drug interaction maintains pentobarbital's intrinsic effect in forward and backward groups. Tolerance to pentobarbital and other barbiturates is primarily pharmacodynamic (neuronal), and the mechanisms of such tolerance are largely unknown, as are the mechanisms

involved in reported interactions between pentobarbital and amphetamine (e.g., Harvey, 1985). A nonassociative interaction between pentobarbital and amphetamine, or between pentobarbital and other potential US drugs that can be classed as having actions similar to amphetamine, could account for the heart rate effect. Temporal parameters of the heart rate effect are consistent with this hypothesis and are otherwise difficult to explain. The most convincing single substitution that would exclude the sort of drug interaction account proposed here would replace pentobarbital with a drug that has little or no intrinsic effect on heart rate.

5.2 Taste aversion.

Heart rate and taste aversion measures are not necessarily related, and a drug interaction account of the heart rate effect does not imply that such an interaction is responsible for avfail obtained with the same drug combination. Moreover, avfail is obtained with the combination of pentobarbital and amphetamine relative to a backward control. A drug interaction may be involved in avfail, although an interaction of the sort conjectured to depend on temporal proximity but not on order of drug injections, such as proposed to account for the heart rate effect, obviously cannot account for avfail. Backward and delayed controls have not been directly compared in the

drug-drug conditioning literature, and such comparison could implicate factors that have not received explicit examination. For these reasons, backward and delayed controls were compared in an avfail post-test.

Comparison of backward and delayed groups using an amphetamine US and a taste aversion measure revealed equivalent effects of forward and delayed drug pairings relative to a backward control. Because long-delay conditioning in the delayed group is unlikely, this finding might be taken to imply that some sort of nonassociative order effect unique to the combination of pentobarbital and amphetamine is responsible for avfail with this drug combination. Because a similar effect of delayed pairings is found using a lithium US, however, other sorts of explanations appear warranted. One such explanation is that backward and delayed pairings differ in effective intensity or in number of sickness preexposures and this accounts for a difference between these groups. Absence of a dose effect in backward and delayed groups seems to recommend differential numbers of sickness preexposures as a possible explanation of avfail effects using delayed pairings. The delayed group receives two sickness preexposures on each trial, and this could enhance US habituation. Differential habituation would then be expected to produce greater attenuation of a subsequent taste aversion in that group.

Failure to obtain an effect of lithium dose in delayed and backward groups does not eliminate consideration of drug dose or intensity effects as contributors to the attenuating effect of drug preexposure on subsequent taste aversion conditioning in the avfail procedure. In particular, perhaps forward or backward pairings modulate the effect of pentobarbital preexposure on subsequent attenuation of a pentobarbital-induced taste aversion in a nonassociative fashion.

Table 1

Design of Avfail Procedure

Groups	Group Names	Treatment: Drug Pairings Phase	Treatment: Taste Aversion Conditioning Phase
experimental	forward	(CS1 → US) pentobarb → lithium	(CS2 → CS1) saccharin → pentobarb
control (avfail)	backward	(CS1 ↛ US) lithium → pentobarb	(CS2 → CS1) saccharin → pentobarb
	CS-only	pentobarb alone	
	US-only	lithium alone	
control (baseline)	no aversion	(CS1 → US)[a] pentobarb → lithium	(CS2 ↛ CS1) saccharin alone

[a]Backward, CS-only and US-only treatments have also been used. The different treatments do not affect results.

Table 2

Statistical Summary for Experiment 1A

F statistic	Sample Period 1	2	3	4	5
omnibus[a]	2.43	1.69	2.00	7.91**	13.77**
comparison[b]					
PA and AP	0.35	1.33	0.00	0.29	3.67
PA and PdA	4.66	3.25	3.20	14.08**	27.27**
AP and PdA	1.74	0.18	2.47	7.81**	7.32*

Note. In the absence of a significant omnibus F, pairwise comparisons were evaluated using Dunn's procedure.

[a]df = 2,30. [b]df = 1,30.

*p<.05. **p<.01.

Table 3

Statistical Summary for the First Test Trial of Experiment 1B

	Sample Period							
F statistic	1	2	3	4	5	6	7	8
omnibus[a]	4.11*	4.28*	4.56*	6.46**	4.97*	3.41*	1.44	0.19
comparison[b]								
PA and AP	2.48	1.27	0.15	1.08	0.03	0.19	0.03	0.29
PA and PdA	2.09	3.67	6.28*	6.86*	7.47*	6.26*	2.56	0.27
AP and PdA	8.21**	8.12**	7.08*	11.57**	7.12*	3.34	1.58	0.00

[a] $\underline{df} = 2,39$. [b] $\underline{df} = 1,39$.

*$p<.05$. **$p<.01$.

Table 4

Statistical Summary for the Second Test Trial of Experiment 1B

	Sample Period				
F statistic	1	2	3	4	5
omnibus[a]	4.04*	6.48**	11.23**	12.38**	13.35**
comparison[b]					
PA and AP	0.13	0.07	0.51	0.59	0.08
PA and PdA	5.80*	11.41**	15.66**	17.19**	20.86**
AP and PdA	5.83*	6.78*	16.68**	18.46**	17.49**

[a] $\underline{df}$ = 2,37. [b] $\underline{df}$ = 1,37.

*p<.05. **p<.01.

Table 5

Statistical Summary for the Second Test Trial of Experiment 1B
(adjusting for unequal n)

F statistic	Sample Period 1	2	3	4	5
omnibus[a]	0.76	1.57	4.26*	4.83*	4.79*
comparison[b]					
PA and AP	0.13	0.08	0.49	0.56	0.08
PA and PdA	0.94	3.01	5.71**	6.47**	7.67**
AP and PdA	1.40	1.65	7.47**	8.49**	7.32**

[a] df = 2,31. [b] df = 1,31.

*p<.05. **p<.01.

Table 6

Adjusted Group Saccharin Suppression Ratios for Experiments 2 and 3

	Saccharin Suppression Ratios	
Group	Experiment 2	Experiment 3
baseline	0.43	0.42
forward	0.36	0.36
delay	0.34	0.28
backward	0.20	0.17

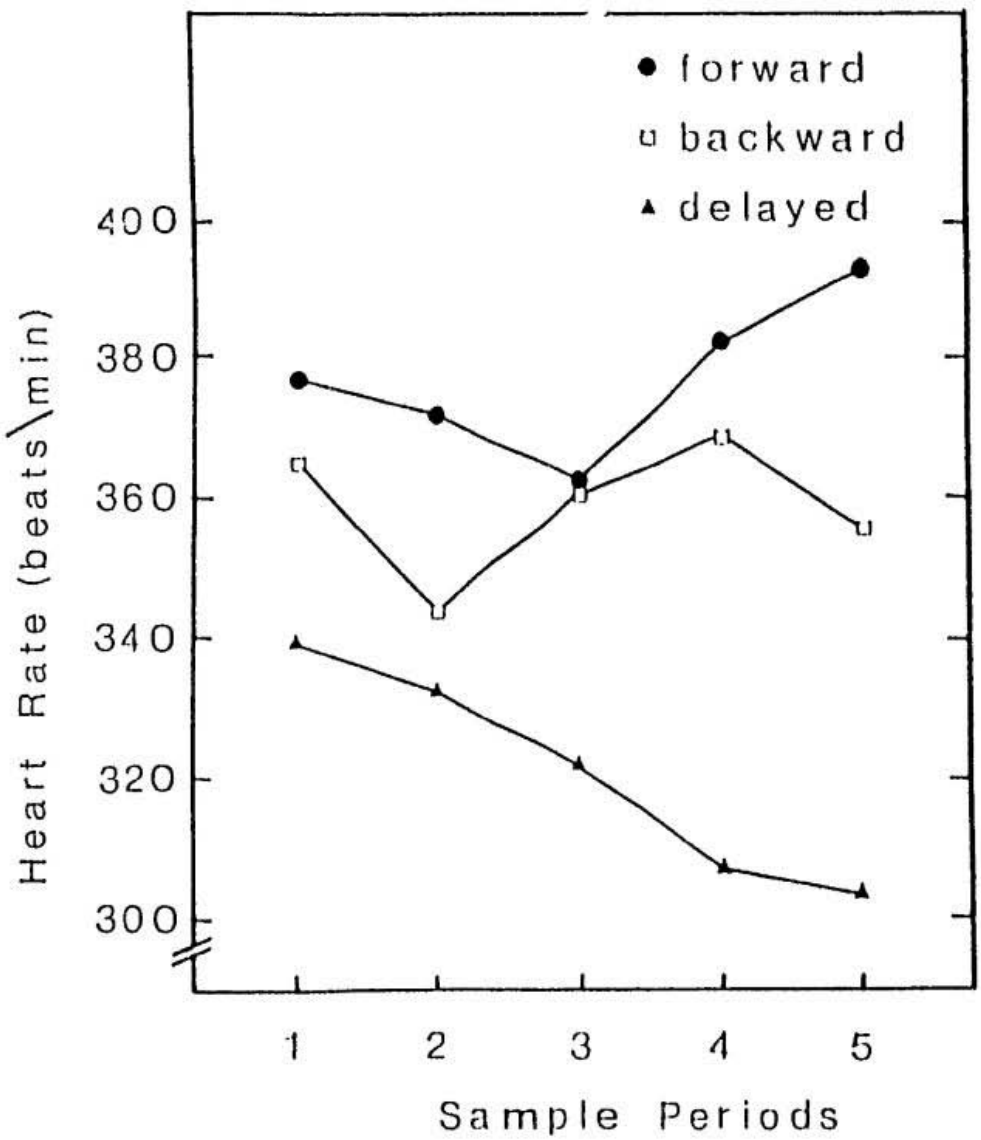

Figure 1. Group heart rates as a function of sample periods (Experiment 1A).

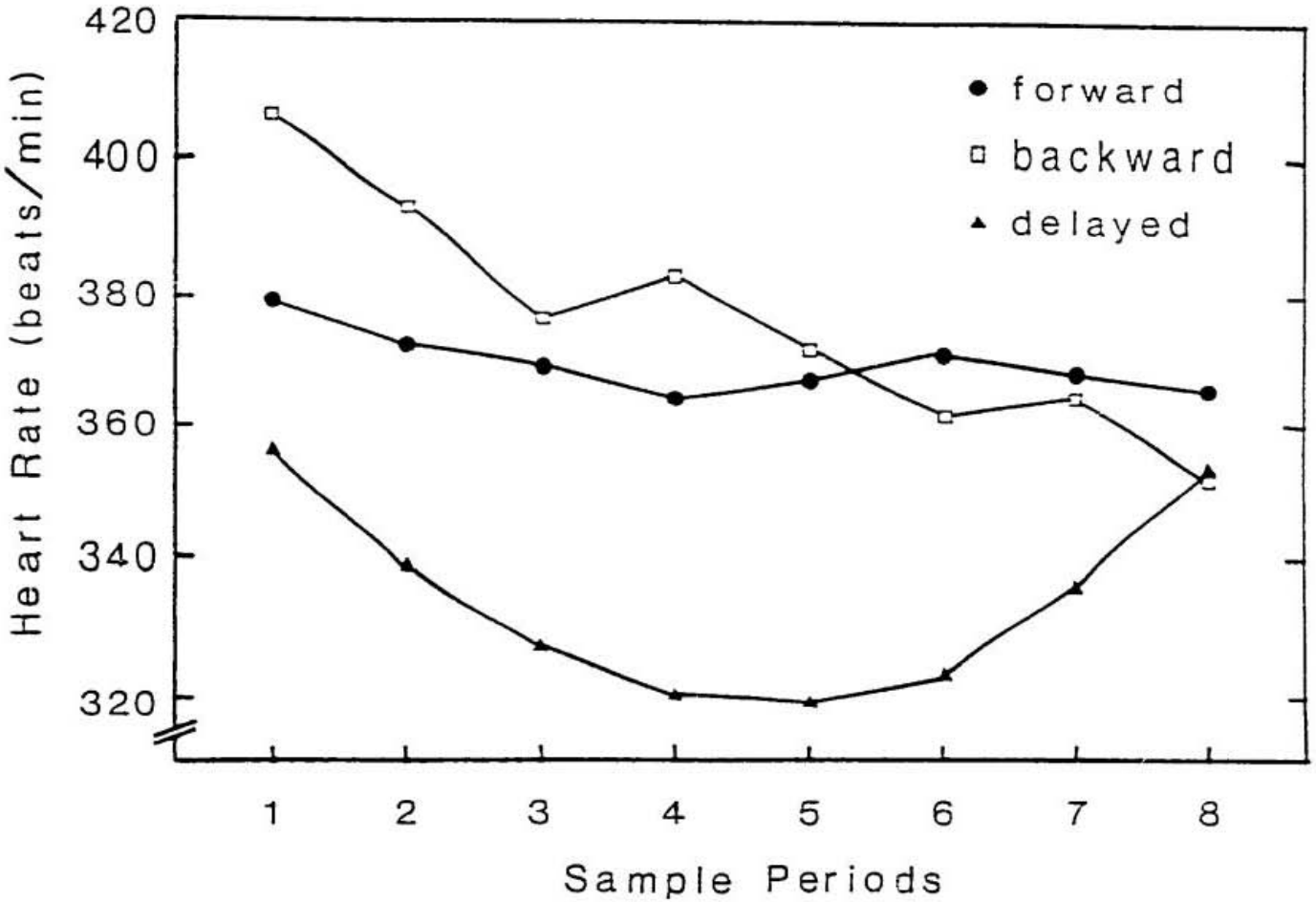

Figure 2. Group heart rates as a function of sample periods on the first test trial of Experiment 1B.

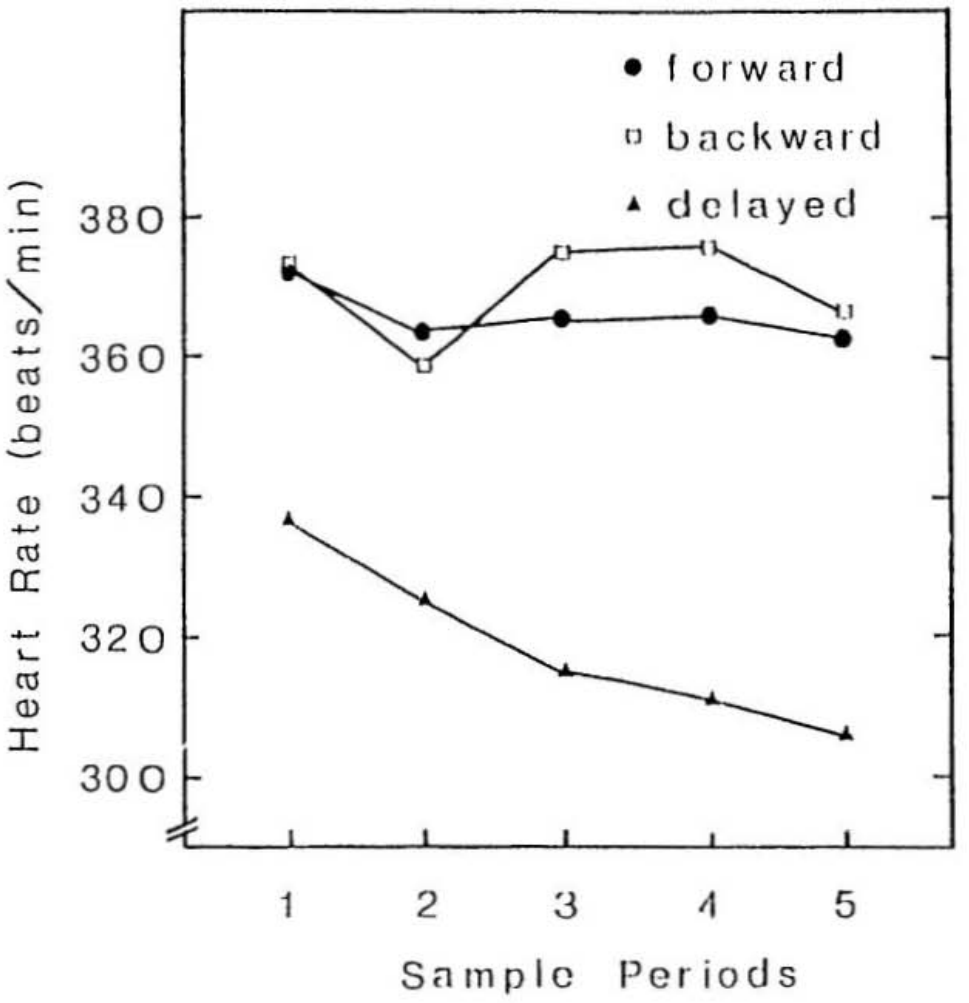

Figure 3. Group heart rates as a function of sample periods on the second test trial of Experiment 1B.

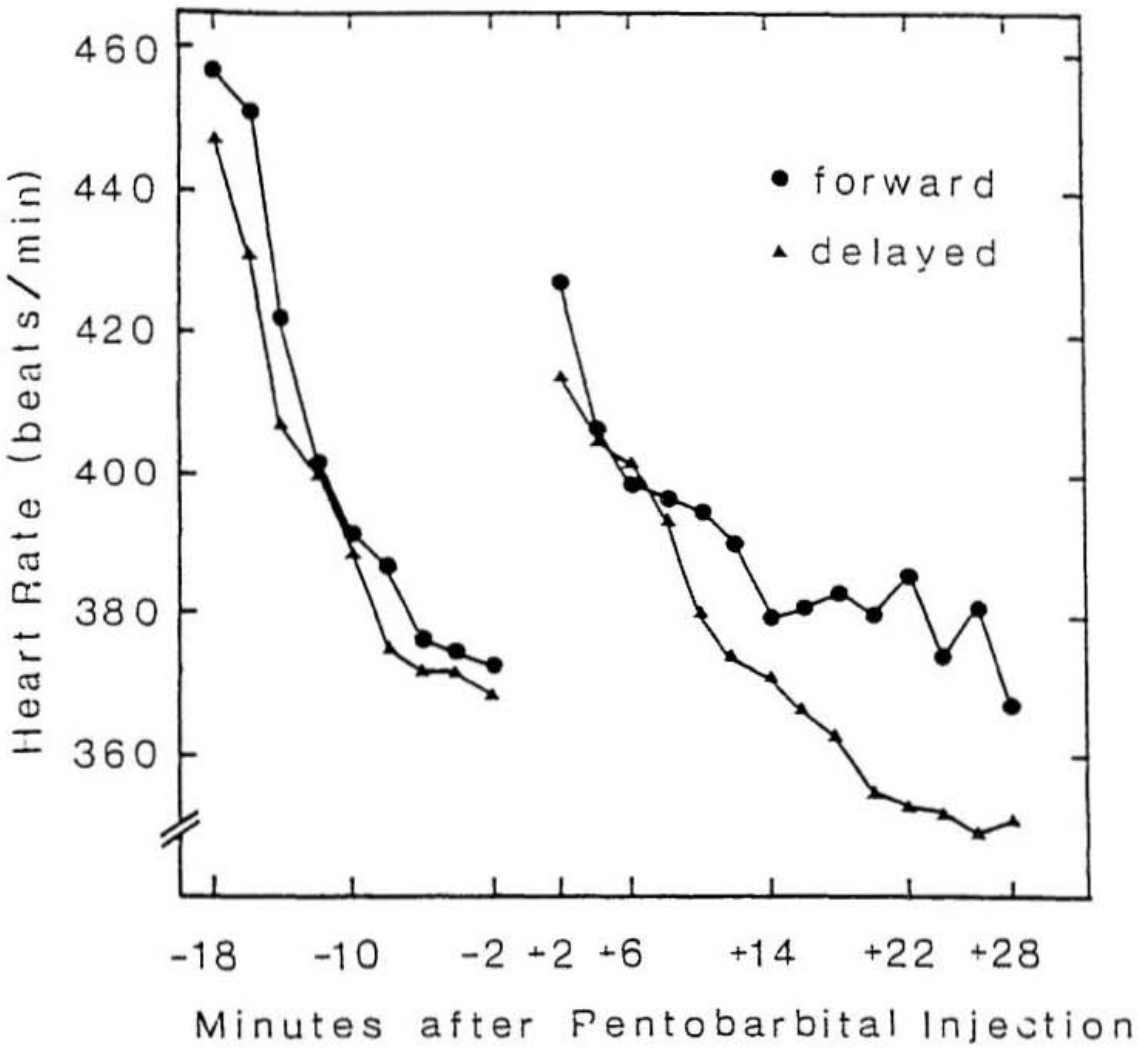

Figure 4. Heart rates for recording chamber groups on the third training trial of Experiment 2.

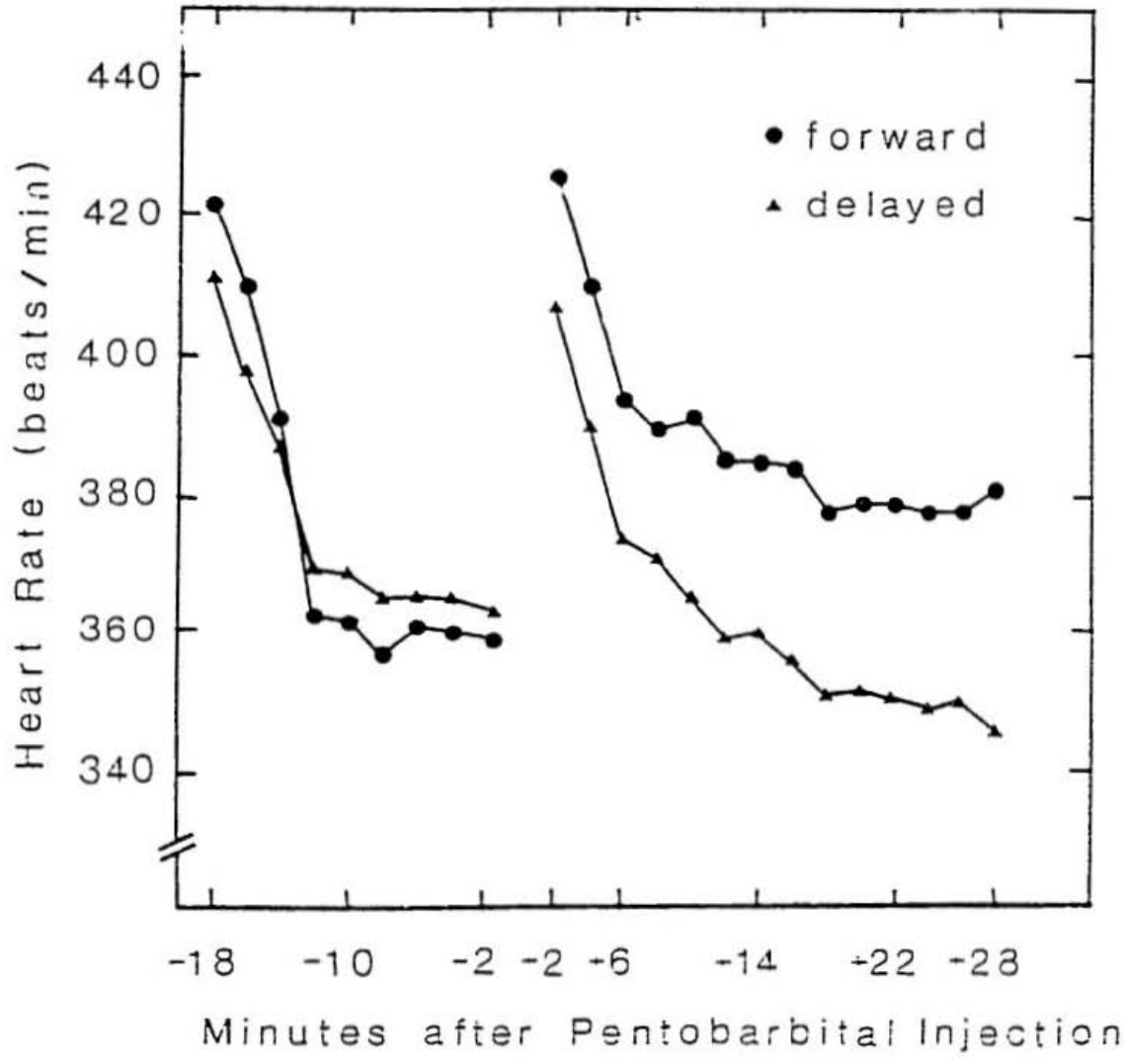

Figure 5. Heart rates for recording chamber groups on the eighth training trial of Experiment 2.

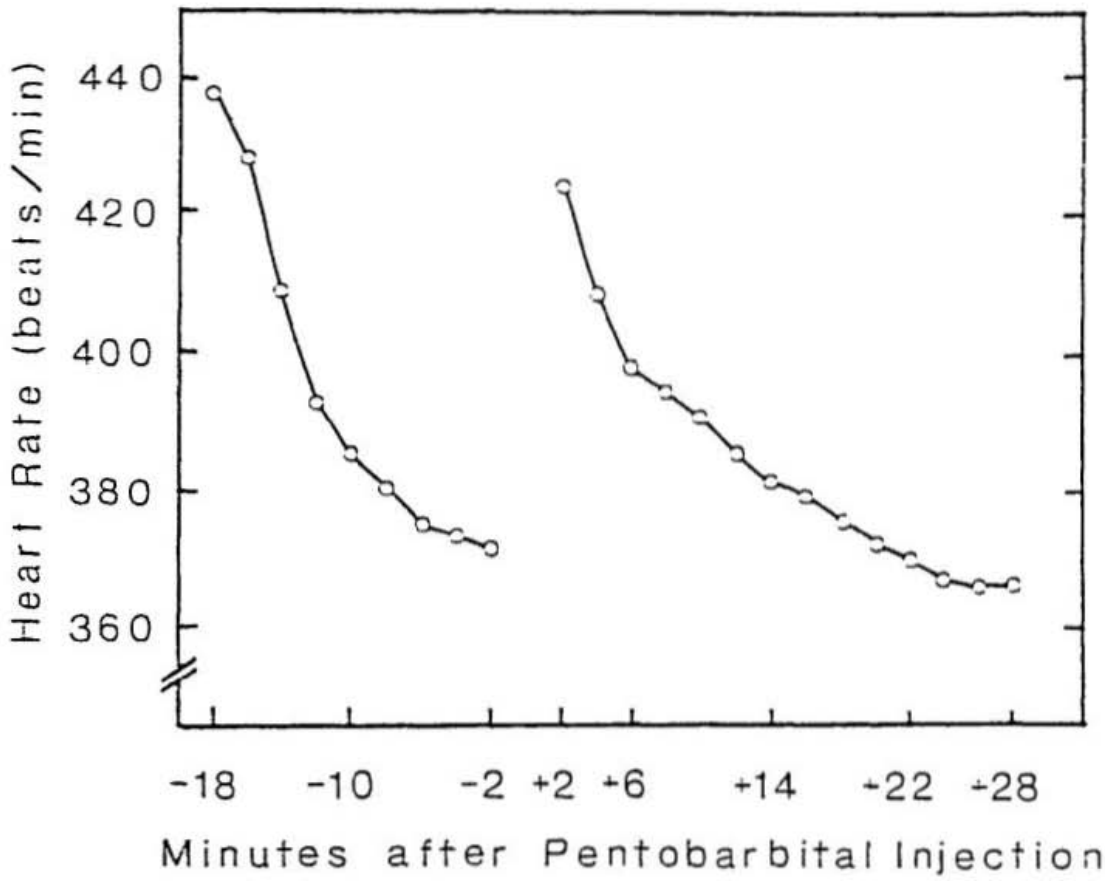

Figure 6. Heart rate as a function of measurement intervals during training (Experiment 2).

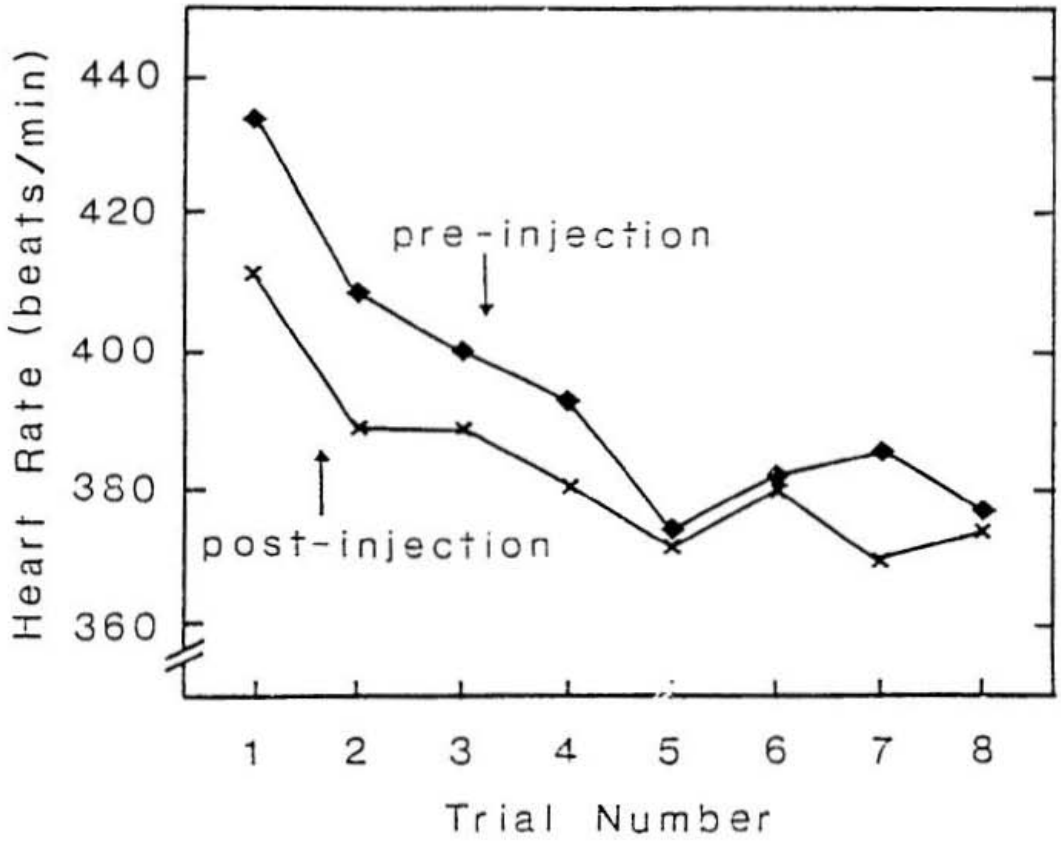

Figure 7. Heart rate as a function of trials during training (Experiment 2).

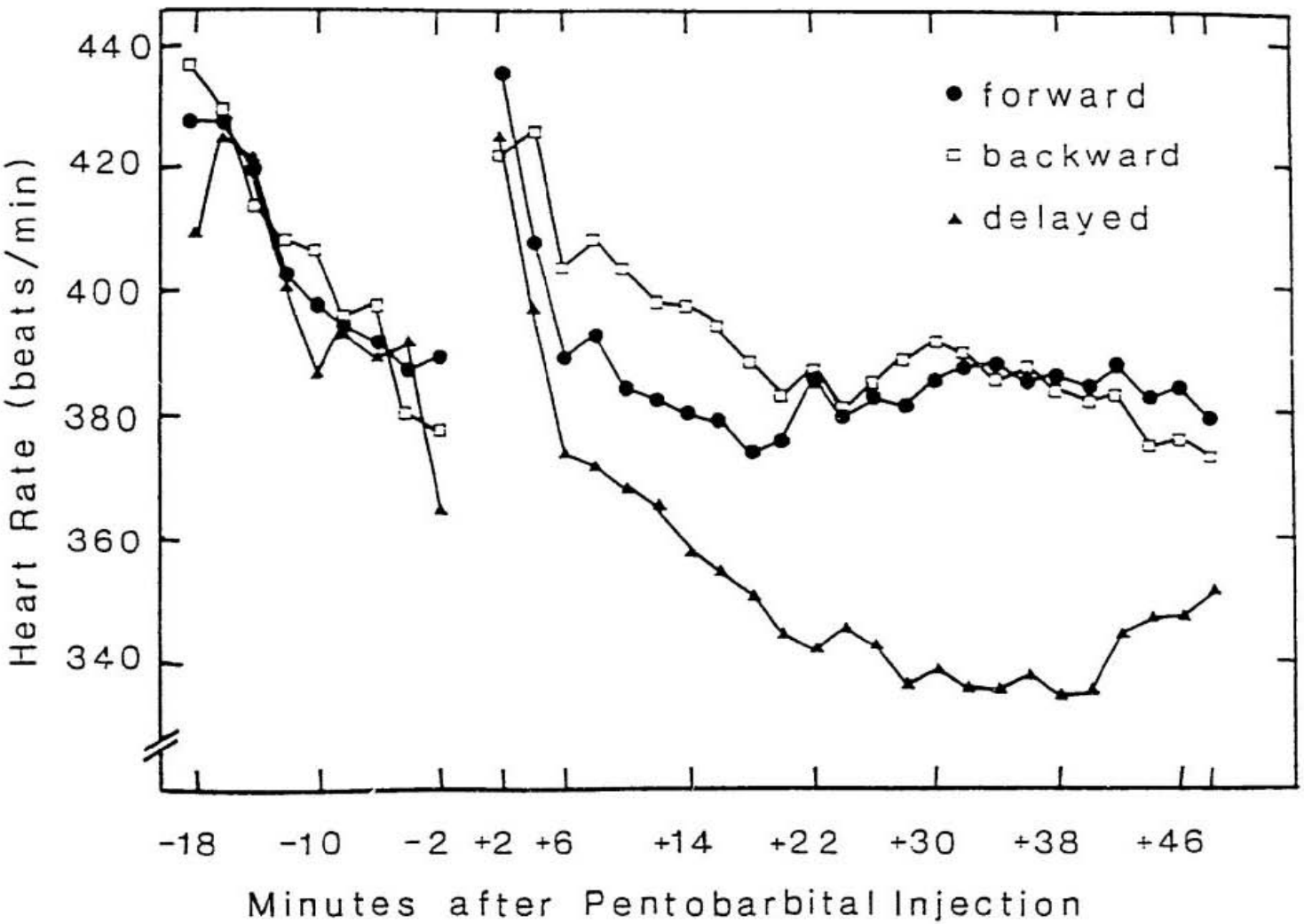

Figure 8. Heart rates for home cage groups on the first test trial of Experiment 2.

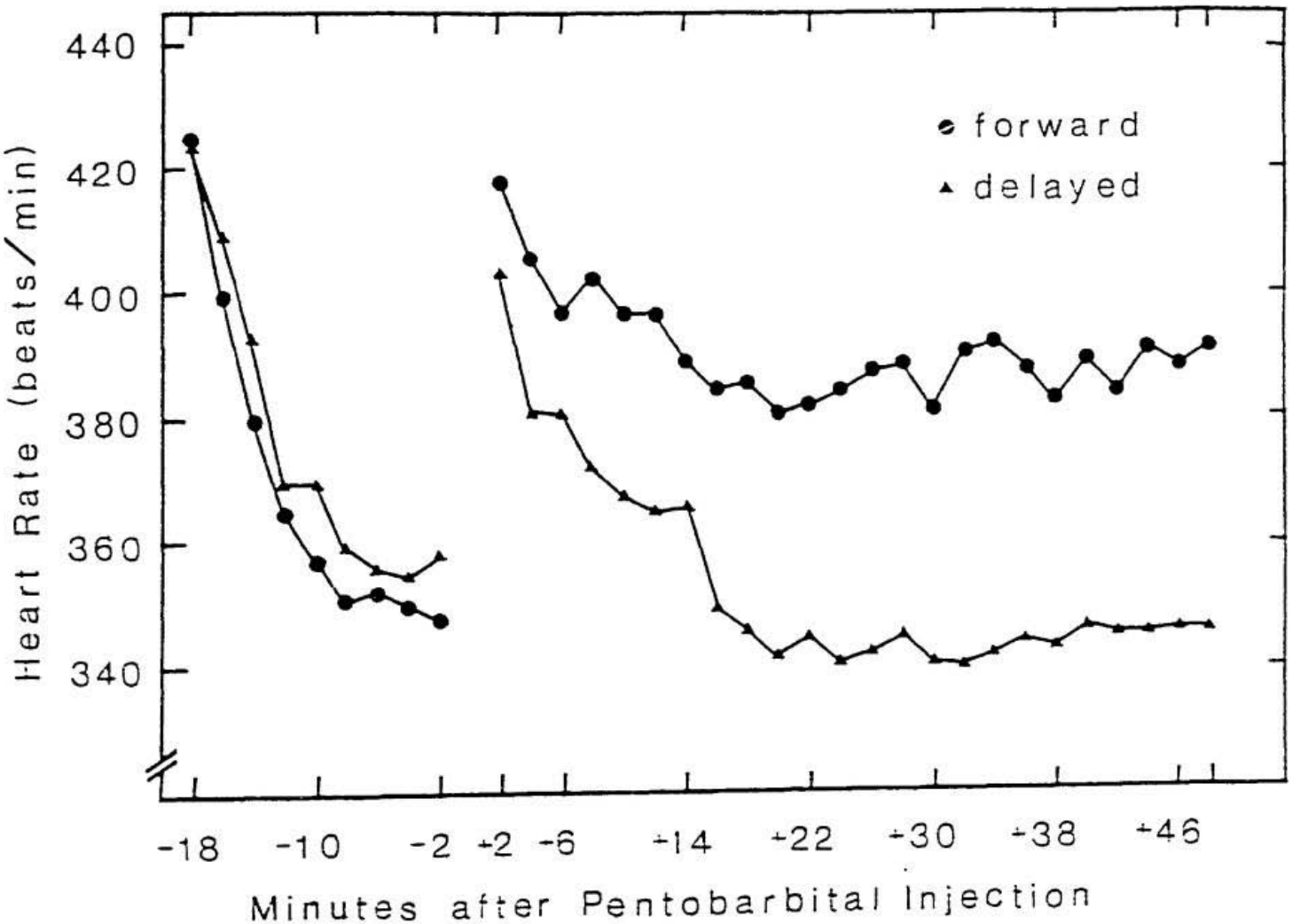

Figure 9. Heart rates for recording chamber groups on the first test trial of Experiment 2.

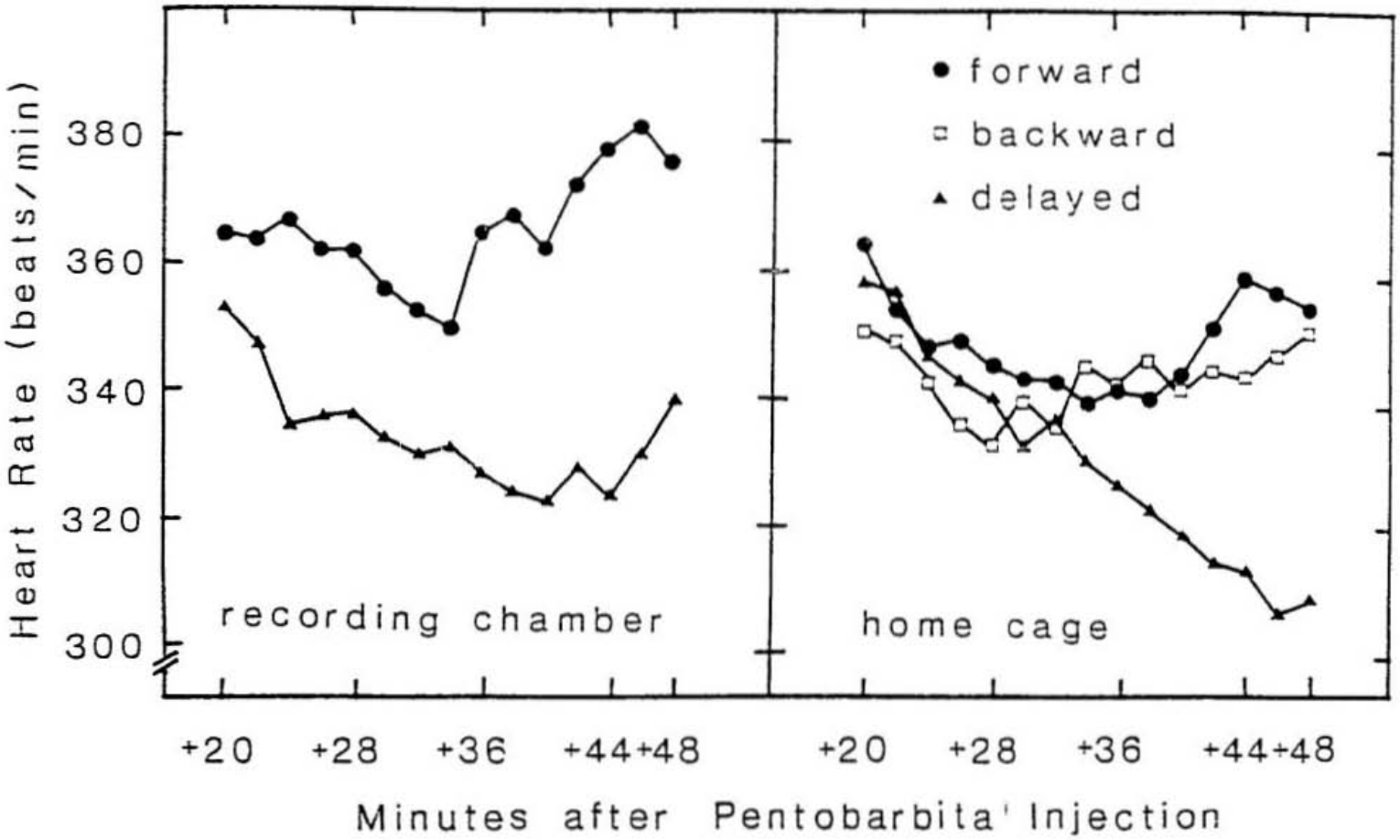

Figure 10. Heart rates for recording chamber and home cage groups on the third test trial of Experiment 2.

Figure 11. Group saccharin preferences as a function of saccharin drinking days--Amphetamine US (Experiment 2).

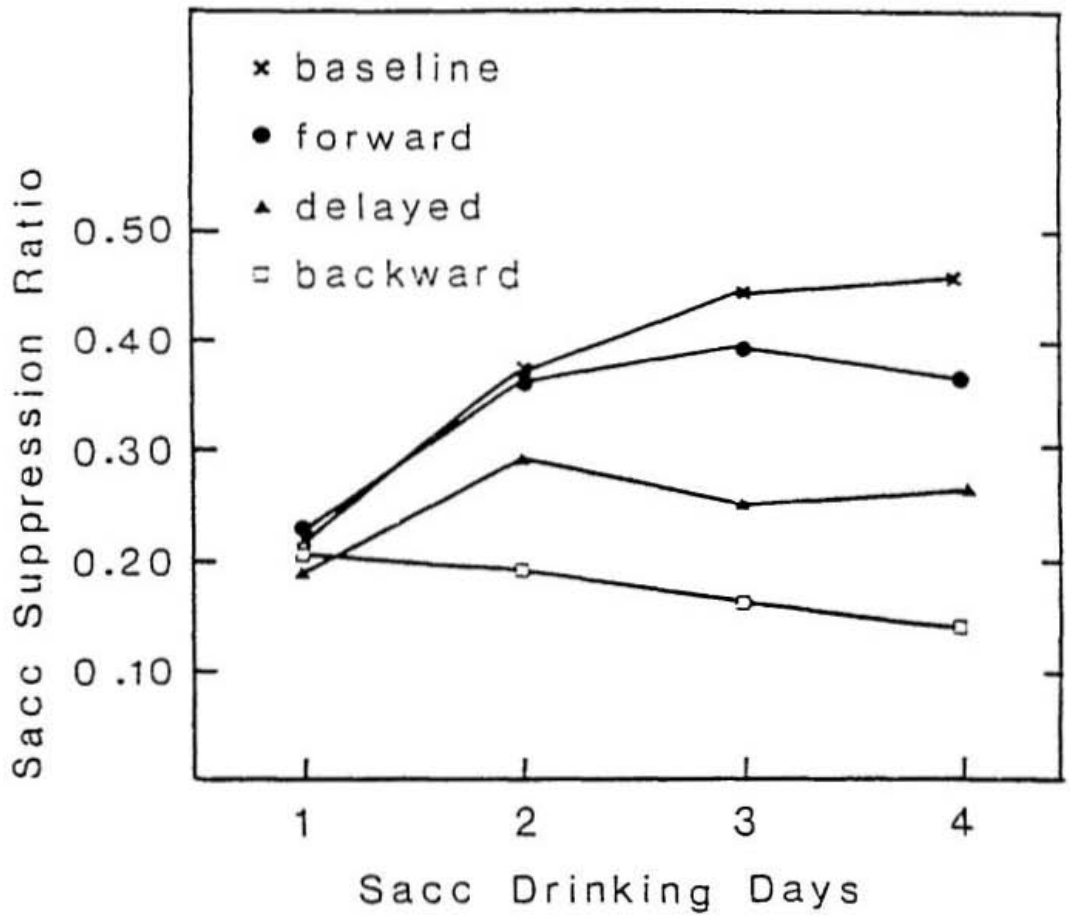

Figure 12. Group saccharin preferences as a function of saccharin drinking days--Lithium US (Experiment 3A).

REFERENCES

Barker, L. M., & Smith, J. C. (1974). A comparison of taste aversions induced by radiation and lithium chloride in CS-US and US-CS paradigms. Journal of Comparative and Physiological Psychology, 87, 644-654.

Bradley, R. M., & Mistretta, C. M. (1971). Intravascular taste in rats as demonstrated by conditioned aversion to sodium saccharin. Journal of Comparative and Physiological Psychology, 75, 186-189.

Buresova, O., & Bures, J. (1977). The effect of anesthesia on acquisition and extinction of conditioned taste aversions. Behavioral Biology, 20, 41-50.

Champion, R. A. & Jones, J. E. (1961). Forward, backward, and pseudoconditioning of the GSR. Journal of Experimental Psychology, 62, 58-61.

Cunningham, C. L. (1978). Alcohol interacts with flavor during extinction of conditioned taste aversion. Physiological Psychology, 6, 510-516.

Cunningham, C. L., & Bischof, L. L. (1987). Stress and

ethanol-induced hypothermia. Physiology and Behavior, 40, 377-382.

Cunningham, C. L., & Linakis, J. G. (1980). Paradoxical aversive conditioning with ethanol. Pharmacology, Biochemistry, and Behavior, 12, 337-341.

Eikelboom, R. & Stewart, J. (1982). Conditioning of drug-induced physiological responses. Psychological Review, 89, 507-528.

Fishberg, A., Hitzig, W. M., & King, R. H. (1933). Measurement of the circulation time with saccharin. Proceedings of the Society for Experimental Biology and Medicine, 30, 651-652.

Gamzu, E. (1977). The multifaceted nature of taste aversion inducing agents: Is there a single common factor? In L. M. Barker, M. R. Best, & M. Domjan (Eds.), Learning mechanisms in food selection. Waco, TX: Baylor University Press.

Garcia, J., Lasiter, P. S., Bermudez-Rattoni, F., & Deems, D. A. (1985). A general theory of aversion learning. Annals of the New York Academy of Sciences, 443, 8-21.

Geisser, S., & Greenhouse, S. W. (1958). An extension of

Box's results on the use of the E distribution in multivariate analysis. Annals of Mathematical Statistics, 29, 885-891.

Gormezano, I. & Kehoe, E. J. (1975). Classical conditioning: Some methodological-conceptual issues. In W. K. Estes (Ed.), Handbook of learning and cognitive processes (Vol. 2, pp. 143-179). Hillsdale, NJ: Erlbaum.

Grant, V. L. (1987). Do conditioned taste aversions result from activation of emetic mechanisms? Psychopharmacology, 93, 405-415.

Greeley, J., Le, D. A., Poulos, C. X. & Cappell, H. (1984). Alcohol is an effective cue in the conditional control of tolerance to alcohol. Psychopharmacology, 83, 159-162.

Groves, P. M., & Thompson, R. F. (1970). Habituation: A dual process theory. Psychological Review, 77, 419-450.

Harvey, S. C. (1985). Hypnotics and sedatives. In A. G. Goodman, L. S. Goodman, T. W. Rall, & F. Murad (Eds.), Goodman and Gilman's the pharmacological basis of therapeutics (7th ed., pp. 339-371). New York:

Macmillan.

Hunt, T. & Amit, Z. (1987). Conditioned taste aversion induced by self-administered drugs: Paradox revisited. Neuroscience and Biobehavioral Reviews, 11, 107-130.

Kamin, L. J. (1968). "Attention-like" processes in classical conditioning. In M. R. Jones (Ed.), Miami symposium on the prediction of behavior: Aversive stimulation (pp. 9-33). Coral Gables, FL: University of Miami Press.

Keselman, H. J., Mendoza, J. L., Rogan, J. C., & Breen, L. J. (1980). Testing the validity conditions of repeated measures F tests. Psychological Bulletin, 87, 479-481.

Klein, S. B., Mikulka, P. J., & Lucci, K. (1986). Influence of lithium chloride intensity on unconditioned stimulus-alone interference in a flavor aversion paradigm. Learning and Motivation, 17, 76-90.

Lett, B. T. (1983). Pavlovian drug-sickness pairings result in the conditioning of an antisickness response. Behavioral Neuroscience, 97, 779-784.

Lett, B. T. (1986). Conditioned slowing of stomach emptying

produced by Pavlovian pairings of a drug CS or a place with lithium chloride. Psychopharmacology, 90, 49-53.

Martin, G. M. (1982). Examination of factors which might disrupt a learned association between pentobarbital and LiCl. Learning and Motivation, 13, 185-199.

Pavlov, I. P. (1927). Conditioned reflexes (G. V. Anrep, Trans.). New York: Dover.

Randich, A. & Ross, R. T. (1985). Contextual stimuli mediate the effects of pre- and postexposure to the unconditioned stimulus on conditioned suppression. In P. D. Balsam & A. Tomie (Eds.). Context and Learning (pp. 105-132). Hillsdale, NJ: Erlbaum.

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. (1984). Associative predispositions. In P. Marler & H. S. Terrace (Eds.), Dahlem workshop on the biology of learning (pp. 447-460). Berlin: Springer-Verlag.

Revusky, S. (1985). Drug interactions measured through taste aversion procedures with an emphasis on medical implications. Annals of the New York Academy of Sciences, 443, 250-271.

Revusky, S. & Coombes, S. (1982). Long-delay associations produced in rats by injecting two drugs in sequence. Journal of Comparative and Physiological Psychology, 96, 549-556.

Revusky, S., Coombes, S., & Pohl, R. W. (1982). Pharmacological generality of the Avfail effect. Behavioral and Neural Biology, 34, 240-260.

Revusky, S., Davey, V., & Reilly, S. (1987). [Use of amphetamine, atropine, or lithium as USs in drug-drug heart rate conditioning.] Unpublished raw data.

Revusky, S., Davey, V., & Zagorski, M. (1989). Heart rate conditioning with pentobarbital as a CS and amphetamine as a US. Behavioral Neuroscience, 103, 296-307.

Revusky, S. & Harding, R. K. (1986). Pairing pentobarbital with one toxin causes it to attenuate taste aversions produced by a different toxin: Implications for conditioned antisickness theory. Behavioral

Neuroscience, 100, 685-694.

Revusky, S., Taukulis, H. K., Parker, L. A., & Coombes, S. (1979). Chemical aversion therapy: Rat data suggest it may be countertherapeutic to pair an addictive drug state with sickness. Behaviour Research and Therapy, 17, 177-188.

Revusky, S., Taukulis, H. K., & Peddle, C. (1979). Learned associations between drug states: Attempted analysis in Pavlovian terms. Physiological Psychology, 7, 352-363.

Rushton, R., & Steinberg, H. (1963). Mutual potentiation of amphetamine and amylobarbitone measured by activity in rats. British Journal of Pharmacology, 21, 295-305.

Schull, J. (1979). A conditioned opponent theory of Pavlovian conditioning and habituation. In G. H. Bower (Ed.), The psychology of learning and motivation (Vol. 13, pp. 57-90). New York: Academic Press.

Siegel, S. (1983). Classical conditioning, drug tolerance, and drug dependence. In Y. Israel, F. B. Glaser, H. Kalant, R. E. Popham, W. Schmidt, & R. G. Smart (Eds.), Research advances in alcohol and drug problems (Vol. 7, pp. 207-246). New York: Plenum.

Taukulis, H. K. (1982). Attenuation of pentobarbital-elicited hypothermia in rats with a history of pentobarbital-LiCl pairings. Pharmacology, Biochemistry, and Behavior, 17, 695-697.

Taukulis, H. K. (1986a). Conditional hyperthermia in response to atropine associated with a hypothermic drug. Psychopharmacology, 90, 327-331.

Taukulis, H. K. (1986b). Conditional shifts in thermic responses to sequentially paired drugs and the "conditional hyperactivity" hypothesis. Pharmacology, Biochemistry, and Behavior, 25, 83-87.

Wilkin, L. D., Cunningham, C. L., & Fitzgerald, R. D. (1982). Pavlovian conditioning with ethanol and lithium: Effects on heart rate and taste aversion in rats. Journal of Comparative and Physiological Psychology, 96, 781-790.