Associative morphine tolerance in the rat : a meta-analytic review
by Amy Sue Johnson

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Applied Psychology
Montana State University

Abstract:
From repeated administration, morphine tolerance develops when higher morphine doses are required to produce the same effect as the initial dose. Tolerance arises from two different mechanisms. The non-associative tolerance mechanism is tolerance due to compensatory responses elicited from morphine itself. The associative mechanism is tolerance due to compensatory responses elicited by distinctive cues associated with the morphine effect. Researchers usually use a paired-unpaired design with rats and morphine when studying associative tolerance. During tolerance acquisition, the DC group of rats is given morphine injections paired with distinctive cues during training and the HC group of rats is given the same number of morphine injections in their home cage. During the tolerance testing phase, both groups are given a morphine injection in the presence of the distinctive cue, and tested for analgesic tolerance. Since both groups received the same amount and number of morphine injections, the two groups should acquire the same amount of non-associative tolerance. Only the DC group, however, may acquire greater overall tolerance due to associative tolerance. According to previous studies, associative tolerance may be explained in terms of classical conditioning. The purpose of this meta-analysis was to determine if associative tolerance exists and to identify which variables may significantly moderate the acquisition of associative tolerance. Effect sizes (d) were calculated for 27 studies and study variables were coded. The DC group acquired greater tolerance (associative tolerance) than the HC group. The inter-dose interval or hours between each conditioning trial, the ratio of dose and inter-dose interval, the number of morphine injections, type of distinctive cue (interoceptive or exteroceptive), and the duration of time the rat was exposed to the distinctive cue after an injection of morphine were significant moderators of the acquisition of associative tolerance. The explanation of the moderator results are explored.

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Amy Sue Johnson

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

From repeated administration, morphine tolerance develops when higher morphine doses are required to produce the same effect as the initial dose. Tolerance arises from two different mechanisms. The non-associative tolerance mechanism is tolerance due to compensatory responses elicited from morphine itself. The associative mechanism is tolerance due to compensatory responses elicited by distinctive cues associated with the morphine effect. Researchers usually use a paired-unpaired design with rats and morphine when studying associative tolerance. During tolerance acquisition, the DC group of rats is given morphine injections paired with distinctive cues during training and the HC group of rats is given the same number of morphine injections in their home cage. During the tolerance testing phase, both groups are given a morphine injection in the presence of the distinctive cue, and tested for analgesic tolerance. Since both groups received the same amount and number of morphine injections, the two groups should acquire the same amount of non-associative tolerance. Only the DC group, however, may acquire greater overall tolerance due to associative tolerance. According to previous studies, associative tolerance may be explained in terms of classical conditioning. The purpose of this meta-analysis was to determine if associative tolerance exists and to identify which variables may significantly moderate the acquisition of associative tolerance. Effect sizes (*d*) were calculated for 27 studies and study variables were coded. The DC group acquired greater tolerance (associative tolerance) than the HC group. The inter-dose interval or hours between each conditioning trial, the ratio of dose and inter-dose interval, the number of morphine injections, type of distinctive cue (interoceptive or exteroceptive), and the duration of time the rat was exposed to the distinctive cue after an injection of morphine were significant moderators of the acquisition of associative tolerance. The explanation of the moderator results are explored.

INTRODUCTION

Morphine, one of the active ingredients in opium, is in the opiate class of drugs and produces a variety of effects including analgesia, decreased respiration, drowsiness, and nausea. Following a morphine injection, the drug distributes evenly in tissue throughout the body. Morphine has a profound effect on the central nervous system. In the CNS, there are receptors for opiates at almost every level involved in pain perception. Morphine molecules bind to these specific opiate receptors to produce analgesia (Diaz, 1997). Morphine acts mostly on Mu receptors in the CNS but also binds to Sigma, Kappa, Delta, and Epsilon receptors to mediate analgesia (Gilman, Goodman, Rall, & Murad, 1985).

From repeated administration, morphine tolerance develops when higher morphine doses are required to produce the same effect as the initial dose. For instance, an initial injection of morphine produces a certain level of analgesia. A second injection of the same dose of morphine produces less analgesia than the first injection. Therefore, reduced analgesia with the second and subsequent injections suggests that a larger dose of morphine would be needed to obtain the same level of analgesia that was initially experienced from the first injection (Reynolds & Randall, 1959; Diaz, 1997). The decrease in analgesic effect is therefore, not a direct indication of tolerance but only an indirect indication that higher morphine doses would be needed to produce the original effect of morphine. For instance, Siegel (1975) assessed analgesic morphine tolerance in the rat. He repeatedly placed rats on a hot plate and measured the time for them to escape

from the hot plate. Shorter escape latency with repeated injections indicated decreased analgesia and, indirectly, indicated increased tolerance. In experimental designs when a researcher is discussing tolerance, he or she is typically referring to this type of indirect indication of tolerance.

Tolerance (resulting from repeated injections of morphine) is a consequence of two different mechanisms: non-associative and associative tolerance. Non-associative tolerance is a result of compensatory responses elicited by morphine that counteracts the initial effect of the drug (Diaz, 1997). For instance, morphine may initially produce analgesia but also elicits a compensatory response to the analgesic effect, hyperalgesia, an increased sensitivity to pain (Poulos & Cappell, 1991). In contrast to non-associative tolerance, associative tolerance may be due to the pairing of a distinctive cue with morphine effect. If morphine administration is always conducted in the presence of the same distinctive cue, then the compensatory responses elicited by morphine itself may become associated with and later elicited by the distinctive cue. Thus, associative tolerance is a decrease in the effect of morphine due to compensatory responses elicited by the distinctive cue (associated with morphine) and non-associative tolerance is a decrease in the morphine effect due to compensatory responses elicited by the drug itself.

Associative tolerance and non-associative tolerance are typically additive. This can be demonstrated experimentally by comparing the tolerance levels of two different groups of subjects that receive a series of morphine injections either paired or unpaired with the distinctive cue. The group that receives morphine paired with the distinctive cue will typically acquire more overall tolerance in the presence of those cues than the group

receiving identical repeated morphine treatment not paired with the distinctive cue (Adams, Yeh, Mitchell & Woods, 1969, Poulos & Cappell, 1991). These results suggest that tolerance resulting from a series of morphine injections paired with the distinctive cue is derived from both non-associative and associative mechanisms.

Pavlovian Explanation of Associative Tolerance

Pavlov (1927) conducted some of the earliest studies using morphine as an unconditioned stimulus. Pavlov injected dogs with a small dose of morphine. After a few minutes, a high pitch tone was presented for 10 minutes. While the noise was still sounding, the dog secreted saliva and became restless from the morphine injection. After repeated pairings of the tone with injections of morphine, the tone without the injection of morphine elicited salivation and restlessness in the dog. A morphine injection in the presence of a tone can be considered a conditioning trial. The unconditioned stimulus (US) was the morphine and the conditioned stimulus (CS) was the tone. The unconditioned response (UR) and the conditioned response (CR) included both restlessness and salivation. The morphine (US) produced restlessness and salivation (UR) and after repeated pairings of the tone (CS) and morphine (US), the tone (CS) produced restlessness and salivation (CR) (Pavlov, 1927). Thus, the tone (CS) is a distinctive cue, which predicts the presentation of the morphine (US).

Like conditioned restlessness and salivation, associative tolerance is also presumed to be based on Pavlovian conditioning. The unconditioned stimulus consists of the morphine effect. The unconditioned response is the compensatory response to this

morphine effect. The morphine effect (US) produces a compensatory response to the morphine effect (UR). The conditioned stimulus is a distinctive cue that predicts the morphine effect. After repeated pairing of the distinctive cue (CS) with the morphine effect (US), the distinctive cue will come to elicit the compensatory response (CR) (Siegel et al., 2000). The conditioned response will be similar to the unconditioned compensatory response to morphine (Pavlov, 1927).

If associative tolerance can be explained in terms of classical conditioning, then various learning phenomena based on classical conditioning should be demonstrated with associative tolerance. Thus, extinction (MacRae & Siegel, 1987; Siegel, Hinson & Krank, 1979; Siegel, Sherman & Mitchell, 1980), inhibition (Poulose, et al., 1988), and overshadowing (Walter & Riccio, 1983) have all been successfully demonstrated using the associative morphine tolerance paradigm. Another parallel exists between classical conditioning and associative morphine tolerance. Researchers have suggested that injections of glucose after conditioning trials facilitate learning in rats (Okaichi & Okaichi, 1997). If tolerance is due to an association between the distinctive cue and the effect of the drug, then glucose should enhance this association. Siegel (1999) conducted a study using glucose and associative morphine tolerance in which glucose was injected immediately after injections of morphine paired with the distinctive cue. As predicted, the association between the distinctive cue and the morphine effect was strengthened, as indicated by enhanced tolerance.

Experimental Design of Associative Tolerance Studies

Some of the earliest studies demonstrating associative tolerance used morphine and rats (Adams et al., 1969; Siegel, 1975) and most subsequent studies have followed this tradition. However, research on associative tolerance has also demonstrated the phenomenon with other types of drugs including ethanol (Duncan, Alici & Woodward, 2000; Siegel & Sdao-Jarvie, 1986; Weise-Kelly & Siegel, 2001), nicotine (Epstein et al., 1991), and cocaine (Partridge & Schenk, 1999; Smith, 1990). The phenomenon has also been demonstrated with different types of subjects including humans (Epstein et al., 1991) and pigeons (Poling et al., 1996). However, few studies have used drugs other than morphine or organisms other than rats (Baker & Tiffany, 1985; Siegel et al., 2001).

The experimental design used to study associative tolerance is the so-called paired-unpaired design (Siegel, Hinson & Krank, 1978). Studies using this design consist of two phases, a tolerance acquisition phase and an analgesic tolerance testing phase. The tolerance acquisition phase includes a series of morphine injections. During this phase there are two groups of rats, one group given a series of morphine injections in the presence of the distinctive cue (DC group) and another group given the same series of morphine injections in their home cage (HC group). Rats in each group receive an equal dose and number of morphine and saline injections. The groups differ in terms of the cues associated with each type of injection. Rats in the DC group receive morphine only paired with the distinctive cue. In addition, these rats receive an equal number of saline injections in their home cage. Rats in the HC group receive morphine in their home

cages and saline injections in the presence of the distinctive cue. Saline injections are given to rats in both groups so that the injection itself, or the handling cues associated with the injection, do not become the distinctive cue for the morphine effect. The testing phase is the same for all rats. Rats in both groups receive a single morphine injection while exposed to the distinctive cue. The testing phase is the first time rats in the HC group receive a morphine injection in the presence of the "distinctive cue". In the presence of morphine and the distinctive cue, all rats are tested for analgesic tolerance. The group with the shorter latency reaction time to the analgesic testing device is assumed to have a greater level of overall tolerance.

Analgesic levels are assessed by providing a nociceptive stimulus and measuring the latency of an unconditioned response. Nociceptive stimuli have included shock (Tilson, Rech, & Stolman, 1973), heat (Dafters & Odber, 1989), or pressure (Cepeda-Benito & Tiffany, 1999). After the onset of these nociceptive stimuli, a researcher measures the latency of a predetermined response like tail flick (Krank, 1987), vocalization (Cepeda-Benito & Tiffany, 1999), escape response (Cepeda-Benito & Tiffany, 1999), and paw lick (Siegel, 1977). The group of rats that responds to the stimuli with a shorter latency is presumed to be experiencing less analgesia (more tolerance).

Two different types of distinctive cues have been manipulated in associative tolerance studies, exteroceptive cues that are external to the organism and interoceptive cues that are internal (Siegel et al., 2000). Most research on associative tolerance has manipulated exteroceptive cues. In the paired unpaired experimental design, the distinct

exteroceptive cues have included a cage distinct from the home cage environment (Cepeda-Benito & Tiffany, 1996), sound (Krank, 1987), light (Tiffany, Maude-Griffin & Drobes, 1991), odor (Tiffany & Maude-Griffin, 1988), the analgesic testing device itself (Adams et al., 1969), or some combination of these (Krank, 1987; Tiffany & Maude-Griffin, 1988; Tiffany, Maude-Griffin & Drobes, 1991). Interoceptive cues are of three different types: administration cues, inter-drug cues, and intra-drug cues. An administration cue is a cue that is associated with the main drug effect and is derived from the actual administration of the drug. Some administrative cues have included injection ritual cues like handling and the actual injection (Dafter & Bach, 1985) and self-administration cues(Weise-Kelly & Siegel, 2001). An inter-drug cue is a cue arising from another drug besides the main drug. For example, Siegel (1988) administered a low dose of pentobarbital prior to morphine administration during the tolerance acquisition phase. The pentobarbital acted as a distinct interoceptive cue associated with the morphine effect. The last type of interoceptive cue is an intra-drug cue resulting from the main drug cueing the main effect of the drug. Some examples of intra-drug cues are a small dose of morphine as a cue for a larger effect of morphine (Cepeda-Benito & Tiffany, 1993) and a slow, long duration infusion of morphine as a cue for the larger morphine effect (Kim, Siegel, & Patenall, 1999).

PURPOSE AND HYPOTHESES

The purpose of the present meta-analysis is to determine whether rats given a series of morphine injections and tested in the presence of the distinctive cue (DC group) acquire greater tolerance than rats given the same morphine treatment in their home cage and tested in the presence of the distinctive cue (HC group). Rats in the DC group may acquire associative tolerance in addition to non-associative tolerance (Poulos & Cappell, 1991). In contrast, rats in the HC group are expected to acquire only non-associative tolerance. Therefore, due to the additive nature of tolerance, the DC group may develop greater overall tolerance (less analgesia) than the HC group. Thus, the primary hypothesis of this meta-analysis is that rats in the DC group will acquire greater tolerance than rats in the HC group.

Another purpose of this meta-analysis is to determine which of several independent variables moderate the acquisition of associative tolerance. The variables that are believed to moderate associative tolerance are: (a) morphine dose; (b) inter-dose interval or inter-trial interval; (c) the number of morphine injections; (d) the duration of distinctive cue exposure; and (e) the type of distinctive cue, interoceptive or exteroceptive. Each of these theoretically relevant moderators is discussed next.

Morphine Dose

Morphine dose can be considered a moderator of associative tolerance. In the classical conditioning literature (Annau & Kamin, 1961), it is well known that as the

intensity of the unconditioned stimulus increases, the rate of acquisition and the strength of the conditioned response both increase. With morphine, larger doses result in larger drug effects (Diaz, 1997). Thus, a larger dose can be considered a more intense unconditioned stimulus. Kesner and Cook (1983) explored the differences in morphine tolerance between a group of rats that received a series of 5 mg/kg injections of morphine and another group that received 15 mg/kg injections of morphine. Nested within each of the two dose groups were two subgroups, a DC group and a HC group. After analgesic tolerance testing in the presence of the distinctive cue, the rats in the DC group that received a series of 15 mg/kg injections of morphine displayed more tolerance (less analgesia) than all other groups including the rats in the DC group that received 5 mg/kg injections. Cox and Tiffany (1997) reported similar results using 20 mg/kg and 5 mg/kg doses of morphine. Thus, another hypothesis of the present meta-analysis is that rats in the DC group given larger doses of morphine will display greater associative tolerance than rats in the DC group given smaller doses of the drug.

Inter-dose Interval

Another moderator that can influence the acquisition of associative morphine tolerance is inter-dose interval (IDI). IDI is the number of hours between subsequent administrations of morphine paired with the distinctive cue during the tolerance acquisition phase. Each injection of morphine in the presence of the distinctive cue can be considered to define a conditioning trial (Pavlov, 1927). After an injection of morphine, the effect of the drug does not begin immediately (Diaz, 1997). Thus, the

conditioned stimulus, the distinctive cue, is typically presented before the unconditioned stimulus, the morphine effect. If the distinctive cue (CS) remains present when the morphine effect begins, the distinctive cue becomes a reliable predictor of the morphine effect (Rescorla, 1966). In some instances, however, when the inter-dose interval is short, the rat may still be experiencing the effect of the drug (US), when the next injection is given in the presence of the distinctive cue (CS). In this case, the distinctive cue may be an unreliable predictor of the morphine effect (Rescorla, 1966). In order to provide adequate time for morphine to "run its course" during a single conditioning trial, the inter-dose interval must be relatively long. Many researchers have tested the relationship between inter-dose interval and magnitude of the associative tolerance effect. A 96-hour inter-dose interval was more effective at developing associative tolerance than a 24-hour inter-dose interval (Tiffany & Maude-Griffin, 1988). In addition, a 96-hour IDI was also found to be more effective than either a 12-hour (Tiffany, Maude-Griffin & Drobes, 1991) or 6-hour IDI (Cox & Tiffany, 1999; Tiffany, Drobes & Cepeda-Benito, 1992). Thus, another hypothesis of this meta-analysis is that rats in the DC group will display greater associative tolerance with longer inter-dose intervals than shorter inter-dose intervals.

Number of Morphine Injections

During the tolerance acquisition phase, rats in the DC group are given morphine injections in the presence of the distinctive cue (CS). After the injection of morphine, the morphine effect (US) begins in the presence of the distinctive cue (CS). This pairing of

the CS and US may be considered a conditioning trial (Pavlov, 1927). Several pairings of the CS and the US are necessary for the CS to elicit conditioned compensatory responses (CR). In classical conditioning, as the number of conditioning trials, pairing of the CS with the US, increases, the conditioned responding gradually increases in strength (Tradpold & Spence, 1960). The same may be true for associative tolerance. Therefore, the greater the number of CS-US pairings in the tolerance acquisition phase, the greater the associative tolerance that should be observed during testing. Cepeda-Benito and Tiffany (1992) conducted a study determining the influence of the number of conditioning trails on the development of associative tolerance to morphine in the rat. These researchers had two main groups of rats, a DC group and a HC group. Each main group was divided into seven subgroups differing in the number of conditioning trials during the tolerance acquisition phase. Each subgroup received either 1, 3, 5, 8, or 12 administrations of morphine (trials). The difference in tolerance levels between DC and HC groups given 1 or 3 administrations of morphine was not significant. The DC group given 5 administrations of morphine, however, showed significantly greater tolerance than the HC group given the same number of morphine administrations. In addition, this study showed that with each additional conditioning trial, tolerance increased slightly more for rats in the DC group than the HC group. Therefore, another hypothesis for this meta-analysis is that as the number of injection trials increases for the DC group, more associative tolerance will develop.

Duration of Exposure to the Distinctive Cue

After an injection of morphine, rats in the DC group are placed in the presence of the distinctive cue (CS) for a certain number of minutes. The effect of morphine (US) does not start immediately after the injection (Diaz, 1997). If the duration of distinctive cue exposure is short, the morphine effect (US) may start after the termination of exposure to the distinctive cue (CS). If the duration of the exposure to the distinctive cue is sufficiently long, the morphine effect (US) will start and continue while the rat is still exposed to the distinctive cue (CS). The CS will be paired with the US. Thus, the distinctive cue (CS) may become a more effective predictor of the morphine effect (US) (Rescorla, 1966). Schwarz-Stevens and Cunningham (1993) studied the influence of the duration of the exposure to the distinctive cue on associative morphine tolerance. They examined the difference between rats in the DC group and the HC group that were exposed 5, 15, and 60 minutes to the distinctive cue following a morphine injection. After 10 trials of 5 minutes of distinctive cue exposure, no difference was found in the tolerance levels between the DC group and the HC group. In contrast, after 10 trials of 15 minutes of distinctive cue exposure, significant associative tolerance was apparent. Even greater associative tolerance developed in 10 trials of 60 minutes of distinctive cue exposure for rats in the DC group. Similar results were found in a study by Hinson et al. (1993). Therefore, another hypothesis of this meta-analysis is that longer duration exposure to the distinctive cue in the tolerance acquisition phase will produce greater associative tolerance.

Type of Distinctive Cue

Interoceptive cues are sometimes more salient (apparently) than exteroceptive cues. As stated previously, as the intensity of the US increases, the acquisition rate and strength of the conditioned response increases (Annau & Kamin, 1961). In addition to the strength of the US, a more intense or salient CS (distinctive cue) may produce stronger conditioning (Trapold & Spence, 1960). Kim, Siegel, and Patenall (1999) paired exteroceptive cues and interoceptive cues with morphine. The exteroceptive cue was light-sound compound stimulus and the interoceptive cue was an intra-drug cue, a long duration injection of morphine. During tolerance acquisition, a series of 6 morphine injections was paired with both types of distinctive cues. The rats were divided into four different assessment groups differing in terms of the type of distinctive cue present during tolerance testing: one with both cues present, another with only the interoceptive cue, another with only the exteroceptive cue, and the last with no distinctive cues. When both cues were present during tolerance testing, relatively strong associative tolerance was measured. When only interoceptive cues were present, a small amount of associative tolerance was measured. No associative tolerance was measured with no cues present or with only the exteroceptive cue. These results seem to suggest that the interoceptive cue may be more salient than the exteroceptive cue. Thus, when interoceptive cues are presented together with exteroceptive cues, interoceptive cues may appear to overshadow exteroceptive cues. Thus, the final hypothesis of this meta-analysis is that more associative tolerance will develop in rats that are exposed to distinctive interoceptive cues

than those exposed to distinctive exteroceptive cues during associative tolerance acquisition and testing.

METHOD

Sample of Studies

Articles were located in several ways. On-line database searches were conducted in PsycInfo and PubMed using various keywords: *associative tolerance*, *conditioned tolerance*, *tolerance*, *learning*, and *Pavlov*. In addition to using keywords, database searches were conducted for publications by authors who are prominent in the associative tolerance literature: Baker, Cepeda-Benito, Cox, Drobes, Hinson, Krank, Siegel, and Tiffany. An archival search method was also used by searching the reference pages of reviews on associative tolerance (Ehrman & Ternes, 1984; Eikelboom & Stewart, 1982; Goddard, 1999; Goudie & Demellweek, 1986; Goudie & Griffiths, 1984; Hayes & Mayer, 1978; Hinson & Siegel, 1982; Macrae, Scoles & Siegel, 1987; O'Brien, Ehrman & Ternes, 1984; O'Brien, Ternes, Grabowski & Ehrman, 1981; Pearce & Hall, 1980; Poulos, Hinson & Siegel, 1981; Siegel, 1978; Siegel, 1998; Siegel 1999; Siegel et al., 2000; Siegel & Kim, 2000; Siegel & MacRae, 1984).

The general design for studies included in the present meta-analysis was the following: (a) The study had to have a tolerance acquisition phase in which two groups of rats received the same number of morphine administrations; (b) The study also had to include an analgesic tolerance testing phase; (c) At least two groups of rats had to have been experimentally manipulated during the tolerance acquisition phase with one group receiving morphine in the presence of the distinctive cue (DC group) and the other group receiving morphine in the presence the home cage (HC group); and (d) The tolerance

testing phase had to include a morphine injection given in the presence of the distinctive cue for both groups followed by analgesic testing.

Studies were eliminated for the following reasons: (a) if the researchers did not report the relevant statistics (Bardo & Hughes, 1978; Cepeda-Benito & Tiffany, 1992; Dafters & Bach, 1985; Grisel, Wiertelak, Watkins & Maier, 1994; Mucha, Kalant & Birbaumer, 1996; Sherman, Lewis & Liebeskind, 1979; Siegel, 1976; Siegel, Hinson & Krank, 1981; Tiffany et al., 1993;); (b) if only nonparametric statistics were reported (Siegel, 1975; Siegel, Hinson & Krank, 1978; Dafters, Hetherington & McCartney, 1983; see Glass, McGaw & Smith, 1981, for a discussion of the issues); (c) if the rats were given one or more morphine injections before the tolerance acquisition phase (Advocat, 1989; Dafters & Odber, 1989; Fanselow & German, 1982; Foo, 1998; Kim, Siegel & Patenall, 1999; Krank, Hinson & Siegel, 1984; Oliveto, Picker & Dykstra, 1991; Sherman, Proctor & Strub, 1982; Shippenberg, Emmett-Oglesby & Herz, 1989; Tye & Iversen, 1975); (d) if the paired-unpaired design with a tolerance acquisition phase and a testing phase was not used (Jacobs, Zellner, LoLordo & Riley, 1981; Hinson et al., 1993; Hinson et al., 1986; Raffa & Porreca, 1986; Siegel, 1977); or (e) if saline injections were not given to the rats either before the tolerance acquisition phase, during the tolerance acquisition phase, or both before and during the tolerance acquisition phase, to control for the actual injection acting as the distinctive cue (Advokat, 1980; Kesner & Cook, 1983; Krank, Hinson & Siegel, 1981; Sherman, 1979).

Coded Variables

Several variables were coded in each study to determine which variables moderated the acquisition of associative tolerance. The variables thought to be responsible for associative tolerance development are those present during the tolerance acquisition phase. The following variables were coded: (a) dose of morphine in milligrams per kilogram of body weight; (b) inter-dose interval in hours; (c) number of morphine injections (trials) during tolerance acquisition; (d) minutes each rat was exposed to the distinctive cue after each morphine injection during the tolerance acquisition phase; (e) the type of cue used in the study (exteroceptive or interoceptive); (f) date article was published; (g) number of rats in each group used in the experiment; (h) average weight of rats in grams; (i) exposure or no exposure to testing device during the tolerance acquisition phase; (j) hours between tolerance acquisition phase and testing phase; (k) the specific type of distinctive exteroceptive cue (novel cage environment, sound, odor, light, or a combination); (l) analgesic test stimuli (heat, shock, or pressure); and (m) type of analgesic response observed after test stimuli (tail flick, vocalization, paw lick, or escape response).

Computation and Analysis of Effect Sizes

Two researchers independently coded potential moderator variables and calculated effect sizes (*d*) for the selected articles. After this, both sets of results were compared. Any disagreement was resolved by discussion. Both coders reread the

method section until agreement was reached. If the statistics reported allowed the researchers to calculate the effect size in different ways, an average effect size was calculated for the study.

The effect size calculated was *d*, the difference between the mean indicated analgesic tolerance levels of the HC group minus the mean analgesic tolerance levels of the DC group divided by the pooled standard deviation (Hedges & Olkin, 1985). A positive *d* indicated that the DC group displayed greater tolerance (less analgesia) than the HC group. A negative *d* indicated that the HC group displayed greater tolerance. The effect size *d* is commonly used in experimental studies (Johnson & Eagly, 2001). The software used to calculate the effect size *d* and to manage the effect sizes was DSTAT (Johnson, 1993).

Some researchers manipulated different levels of an independent variable (e.g., different IDIs for example) within an experiment. If adequate information was supplied, including the statistics comparing the DC group with the HC group at a particular level of a variable, an effect size was calculated for each level of that variable. These effect sizes were used in moderator analyses. If separate effect sizes were not able to be calculated for a moderator analysis, then that particular variable was coded as *unknown*. All separately calculated effect sizes were averaged to provide a single effect size estimate for each experiment for the purpose of calculating the overall effect size.

Two different types of variables were recorded in this meta-analysis, continuous and categorical. Continuous moderator variable analysis was conducted using SPSS and DSTAT (Johnson, 1993). In SPSS a weighted-least squares regression model was used

and the SPSS results were entered into DSTAT to make the appropriate corrections. Categorical moderator variables were calculated directly using DSTAT (Johnson, 1993).

The homogeneity was indexed by the Q statistic and associated p-value, calculated using DSTAT (Johnson, 1993). Homogeneity of the overall effect size was calculated to determine if the effect sizes in this study would adequately be described by a single effect size (Q_T). For categorical moderator variables, a between class effect (Q_B) was calculated to determine whether study outcomes vary across levels of the variable. In addition, for categorical variables, a test of the homogeneity of the effect sizes within each class (Q_W) was calculated to determine the variability within each category of the variable. This statistic determines the completeness with which a particular moderator explains the variation among study outcomes (Johnson & Turco, 1992). One or more significant Q_W values indicates significant heterogeneity within the category. In other words, the variable did not provide a complete explanation or model of the effect sizes suggesting that there may be other significant moderator variables. For continuous moderator variables, a test of the significance of each predictor (Q_R) was calculated. A homogeneity test was conducted to evaluate whether significant systematic variation remains unexplained (Q_E). Again, this statistic determines the completeness with which a particular moderator explains the variation among study outcomes. A significant Q_E indicates heterogeneity of effect sizes; in other words, the variable does not provide a complete model of the effects within the literature (Johnson & Turco, 1992).

RESULTS AND DISCUSSION

Overall Effect Size

A total of 27 experiments from 19 articles were used in this meta-analytic review (see Figure 1). The mean publication year was 1990. A total of 957 rats were in the DC groups, and 962 rats were in the HC groups. A total of 17 effect sizes was estimated from *F*-values, 1 from *M*s and *SD*s, 1 from a *t*-value, 1 from a related *F*-value, 1 by assuming that $d = 0$ (the effect was reported as non-significant and we were unable to determine the direction of the effect), and 6 by assuming an exact *p*-value ($p = .01$) for a reported inexact *p*-value ($p < .01$). The weighted mean effect size $d_+ = 0.72$, 95% confidence interval (*CI*) = 0.63 to 0.81, indicated that significant associative tolerance (less analgesia) developed in the DC group during analgesic tolerance testing ($p < .00001$). Sequential deletion of outliers did not significantly change the overall weighted mean (d_+). The homogeneity statistic indicated that effect sizes were heterogeneous, $Q_T (26) = 63.8$, $p < .00001$. Therefore, the coded variables were used to further explain the sources of variability in effect sizes.

According to the overall effect size, rats in the DC group acquired more tolerance than rats in the HC group. These results supported the primary hypothesis. Both groups of rats received the same number of morphine injections and the same total amount of morphine. Both groups therefore presumably acquired the same amount of non-associative tolerance due to physiological compensation of the effect of morphine. Thus,

the difference in the tolerance levels between the two groups of rats appears to be due to the associative tolerance acquired by the DC group.

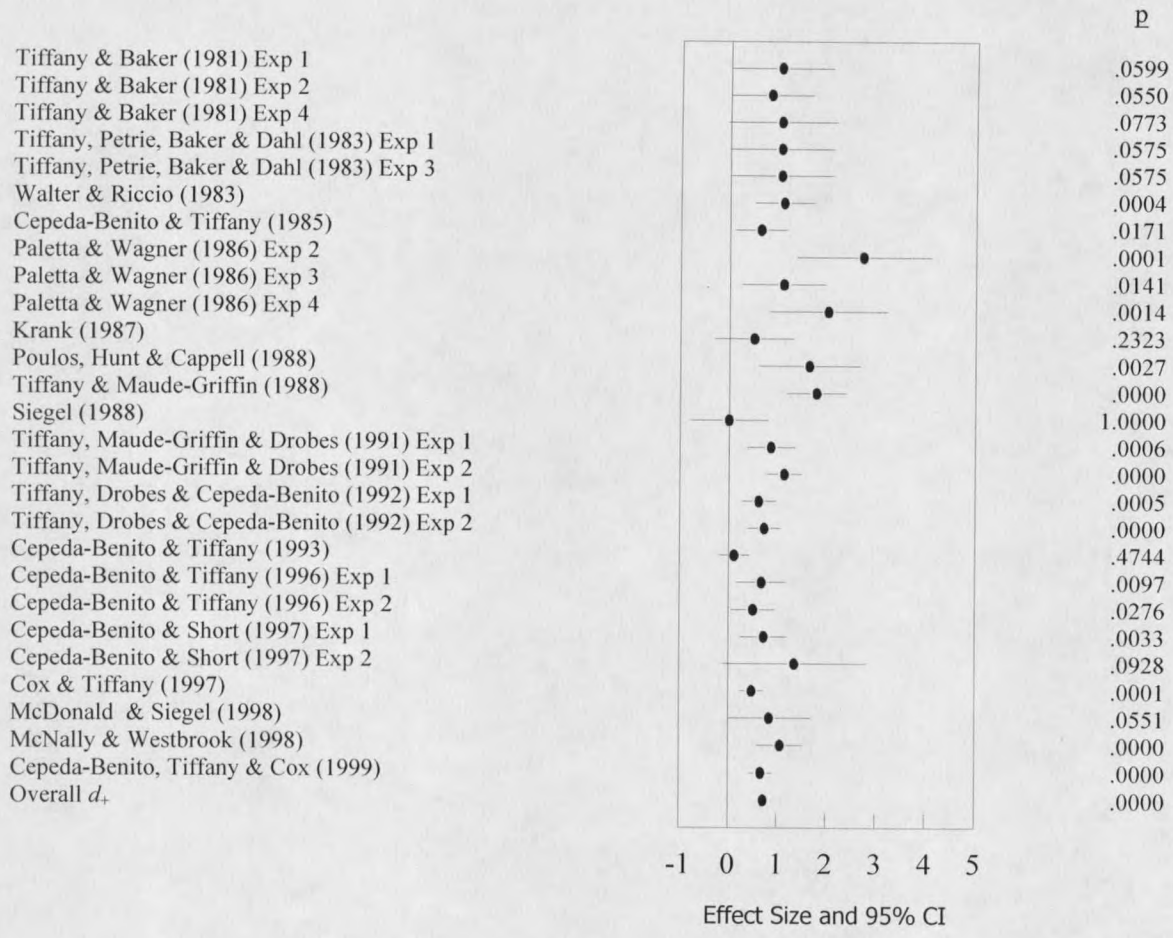

Figure 1. The effect size (*d*) and 95% *CI* for each experiment included in this meta-analysis.

Moderator Analysis

For some variables, a moderator analysis was not conducted. The specific type of distinctive exteroceptive cue, nociceptive testing stimulus, and the type of behavior

measured in response to nociceptive stimulus were not included in the moderator analysis. For these categorical variables, moderator analysis was not conducted because some categories had two or fewer effect sizes. The predominate level for each of these variables were the following: type of distinctive exteroceptive cue was usually a cage environment, nociceptive testing stimulus was usually heat, and the type of behavior in response to the nociceptive stimulus was usually a tail flick response.

Inter-Dose Interval

The mean hours between subsequent morphine injections (IDI) was 48.8 hours. IDI significantly moderated the overall effect size, $Q_R(1) = 4.6, p = 0.03$. Given that there was significant heterogeneity, this variable does not provide a complete model, $Q_E(25) = 54.2, p < .001$. The correlation between effect size and the inter-dose interval was significant but small, $r(25) = .30, p < .05$ (see Figure 2).

The hours between subsequent injections of morphine paired with the distinctive cue (inter-dose interval) is a significant moderator of the acquisition of associative tolerance as predicted from previous literature (Tiffany & Maude-Griffin, 1988; Tiffany, Maude-Griffin & Drobes, 1991; Tiffany, Drobes & Cepeda-Benito, 1992; Cox & Tiffany, 1999). The results of the meta-analysis indicate that as the time increases between subsequent injections of morphine in the tolerance acquisition phase, the acquisition of associative tolerance increases (see Figure 2). The effect of morphine (US) lasts for a certain amount of time (Diaz, 1997). Long inter-dose intervals apparently provide enough time between conditioning trials [the injection of morphine in the presence of with the distinctive cue (CS)] so the effect of morphine dissipates before the next trial

begins. Thus, the distinctive cue (CS) becomes a more reliable predictor of the morphine effect (US) after repeated pairing (Rescorla, 1966). Rats in the DC group are therefore, more likely to make a conditioned compensatory response to the morphine effect (CR). Thus, the overall analgesic morphine effect decreases and rats in the DC group display greater tolerance.

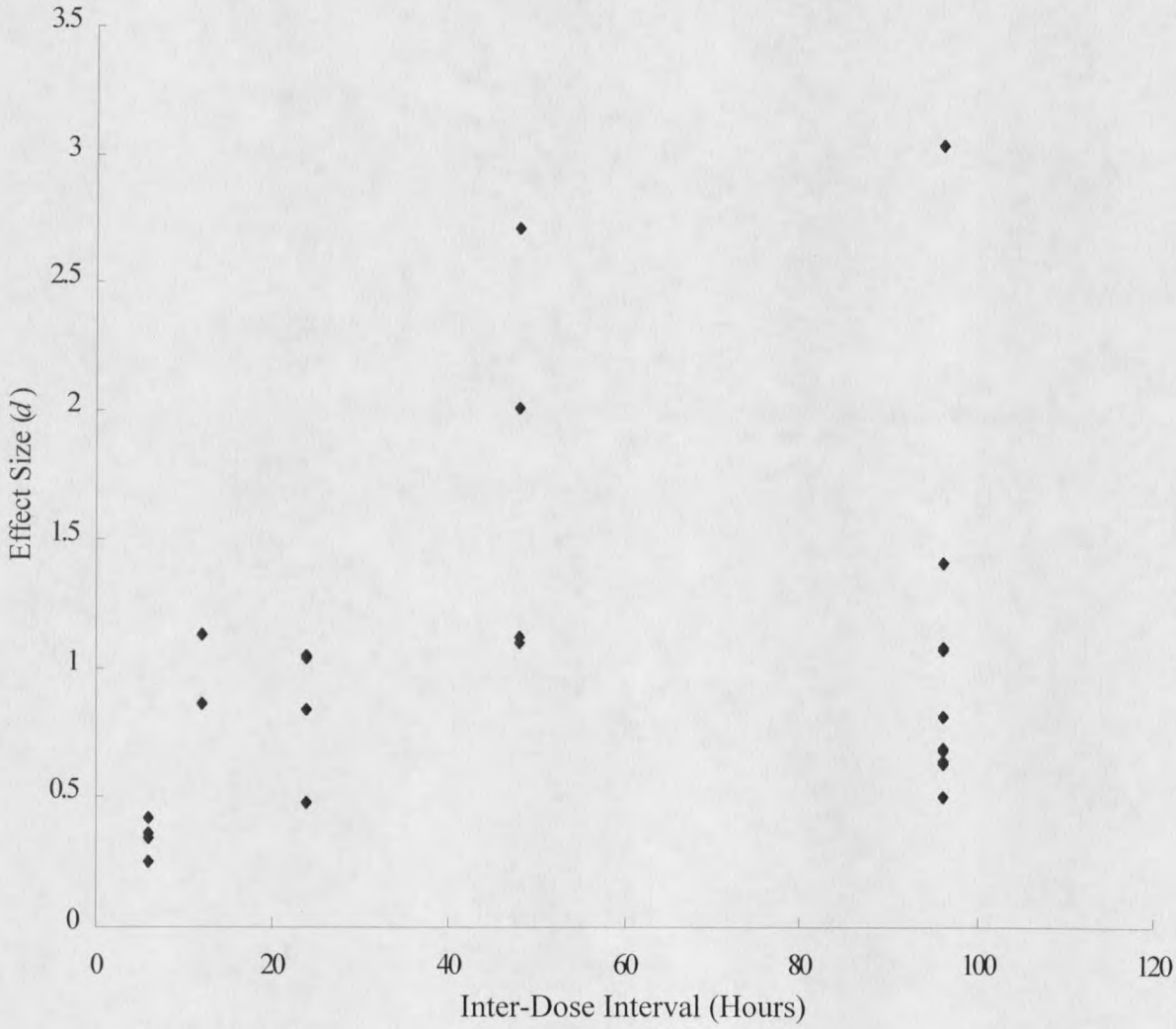

Figure 2. Effect size (*d*) as a function of inter-dose interval for each study included in this meta-analysis.

Duration of Exposure to the Distinctive Cue

The mean number of minutes both groups (HC and DC groups) were exposed to the distinctive cue after an injection in the tolerance acquisition phase was 33.3 minutes. This variable significantly moderated the overall effect sizes $Q_R(1) = 10.8, p < .01$. Considering that there was significant heterogeneity, this variable does not provide a complete model, $Q_E(22) = 45.5, p < .01$. The correlation between effect size and minutes paired with the distinctive cue was highly significant, $r(22) = .44, p < .001$.

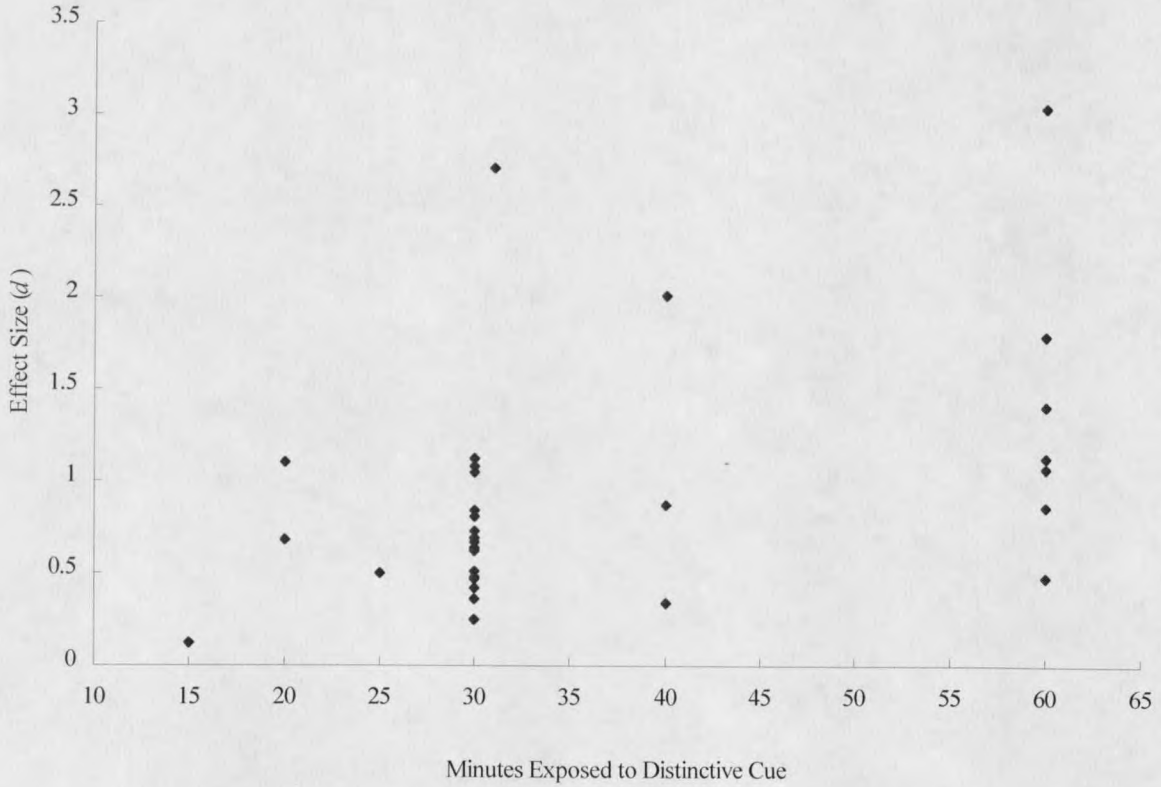

Figure 3. Effect size (*d*) as a function of duration of exposure (in minutes) to distinctive cue for each study included in this meta-analysis.

The results of this moderator analysis support the hypothesis that more associative tolerance develops with longer duration exposure to the distinctive cue (see Figure 3). After an injection of morphine, the effect of the drug does not begin immediately. A long distinctive cue exposure provides adequate time for the morphine effect to begin while the rat remains in the presence of the distinctive cue (Diaz, 1997). Thus, a longer distinctive cue exposure may provide adequate time for the development of an association between the distinctive cue (CS) and the morphine effect (US). Therefore, the distinctive cue (CS) may become a more effective predictor of the morphine effect (Rescorla, 1966).

Number of Morphine Injections

The mean number of injections of morphine in the tolerance acquisition phase was 6.9 injections. The number of injections significantly moderated the overall effect size, $Q_R(1) = 4.3, p < .05$. Because there was significant heterogeneity, this variable does not provide a complete model, $Q_E(29) = 62.6, p < .001$. The correlation between effect size and the number of morphine injections in the tolerance acquisition phase was significant, $r(32) = -.26, p < .05$ (see Figure 4).

According to the classical conditioning literature and the hypothesis for this moderator, as the number of injections or conditioning trials increases, conditioned responding gradually increases in strength (Trapold & Spencer, 1960). Thus, more associative tolerance develops. The results of this moderator analysis suggest that associative tolerance decreases with an increased number of morphine administrations (see Figure 4). As predicted, number of injections moderates the acquisition of

associative tolerance but it does not moderate associative tolerance in the direction predicted. However, the negative correlation between effect size and number of injections is small.

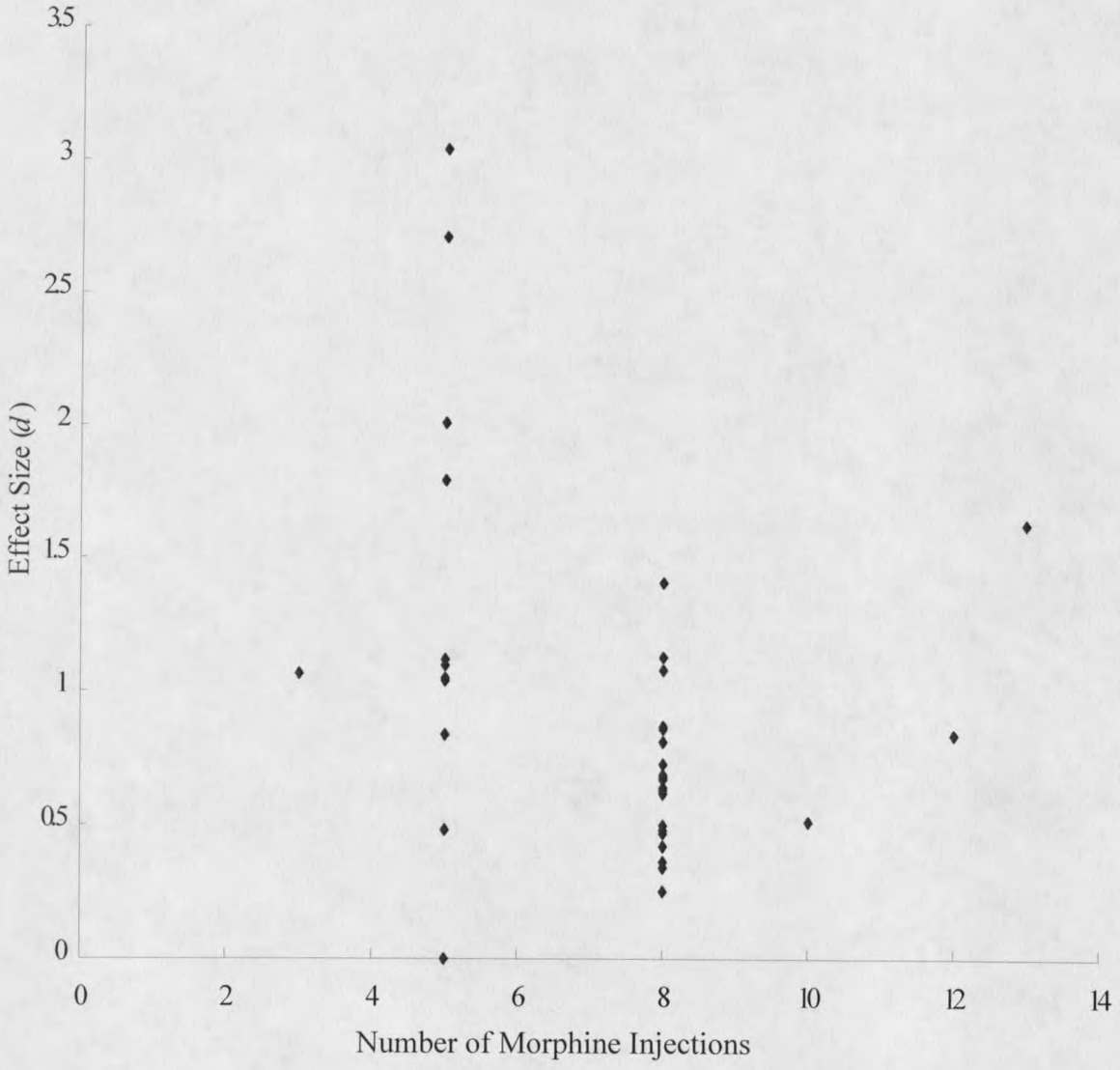

Figure 4. Effect size (*d*) as a function of number of morphine injections for each study included in this meta-analysis.

The results of this moderator analysis may be due to the small range of injections (3 to 13 injections) used in the studies included in this analysis. Would number of injections remain negatively correlated to associative tolerance if a researcher gave rats a number of injections outside the range in studies included in this meta-analysis? A future study should be conducted to determine if associative tolerance decreases with more injections. HC and DC groups should be included in this study, with several different subgroups each with a different number of morphine injections ranging from say, 1 to 50 injections. In this proposed study, it would be possible to compare the associative tolerance development of subgroups receiving different numbers of injections.

Date of Publication

The mean publication date was 1990. The publication date of the study significantly moderated the effect size, $Q_R(1) = 9.5, p < .01$. Given that there was significant heterogeneity, this variable does not provide a complete model, $Q_E(25) = 53.8, p < .01$. The correlation between effect size and date was significant, $r(25) = -.39$, $p < .05$. These results suggest that more recent studies have produced smaller effect sizes than older studies. Perhaps the methods (and potential moderators) for studying associative tolerance have changed over the years.

Number of Subjects

The mean number of rats used in each experiment was 71.1. The number of rats in the experiment significantly moderated the effect sizes, $Q_R(1) = 9.0, p < .01$. Since there was significant heterogeneity, this variable does not provide a complete model, Q_E

(31) = 54.2, $p < .001$. The correlation between effect size and number of subjects was small but significantly negative, r (31) = −.38, $p < .05$ (see Figure 5). These results suggest that when researchers used more rats in an experiment less associative tolerance developed. The number of rats used in the study was also significantly correlated with the date of publication (r (27) = .56, $p = .001$).

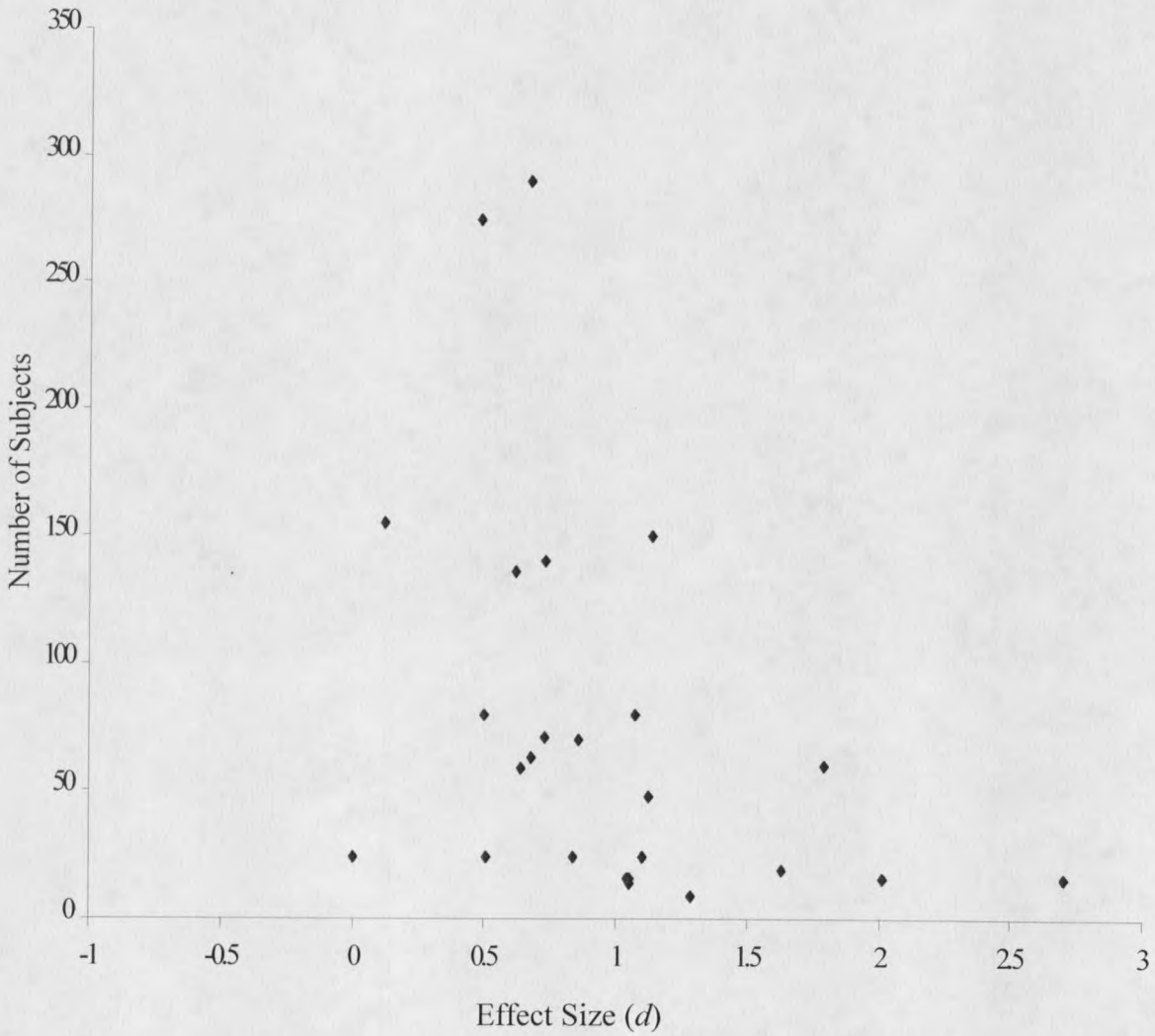

Figure 5. Number of subjects as a function of effect size (*d*) for each study included in this meta-analysis.

The results of this moderator analysis may be due to the correlation between publication date and number of subjects. The correlation of date and number of subjects suggests that recent studies may have used more rats. Recent studies have also tended to yield less associative tolerance. Thus because there was a interaction between date and the number of subjects, studies that yielded smaller effect sizes used more rats in the study. Figure 5 is a funnel plot of sample size as a function of effect size (*d*). Since some of the effect sizes are approximately zero; a publication bias is not apparent.

Exposure to Tolerance Testing Device

In some studies, rats were exposed to a non-functional nonciceptive device during the tolerance acquisition phase as part of the distinctive cue exposure. Rats in the DC group were injected with morphine, exposed to the distinctive cue, and placed on the nonciceptive device. In other studies rats were not placed on the nonciceptive device. Exposure to the nociceptive device during the tolerance acquisition phase was a significant moderator of associative tolerance overall effect size, $Q_B(1) = 9.0, p < .01$. The 95% confidence interval (*CI*), which was from + 0.62 to + 0.81, did not include zero. For rats exposed to the nociceptive device during the tolerance acquisition phase, the weighted mean effect size was $d_+ = 0.67$, and the 95% *CI* was from + 0.58 to + 0.77. Heterogeneity within this category was significant, $Q_W(18) = 44.3, p < .001$. For rats not exposed to the nociceptive device in the tolerance acquisition phase, the weighted mean effect size was $d_+ = 1.14$, and the 95% *CI* was from + 0.85 to + 1.44. Heterogeneity within this category was also significant, $Q_W(5) = 9.7, p < .05$. Exposure to testing device (1 was coded as exposure to testing device and 2 was coded as no exposure to

testing device) was significantly correlated with date of publication, r (30) = −.48, p < .05.

These results reveal that previous exposure to the testing device significantly moderates associative tolerance. More associative tolerance develops when rats are not exposed to the testing device than when the rats are exposed. These results may be due to a correlation between exposure to testing device and date of publication. The correlation with publication date suggests that more recent studies tended to expose rats to the testing device more often than was true in older studies. And as stated previously, recent studies have yielded less associative tolerance. Therefore, studies that exposed rats to the testing device may have yielded less associative tolerance.

Dose of Morphine

The mean dose of morphine was 12.5 mg/kg. The dose of morphine during the tolerance acquisition phase did not moderate the effect size, $Q_R(1) = 0.05, p = .83$. The results suggest that morphine dose by itself does not moderate the acquisition of associative tolerance. This does not support the hypothesis that more associative tolerance will develop with the use of larger doses. These findings may be due to a more complicated relationship between associative tolerance and dose.

Dose effectiveness may be dependent on the inter-dose interval (IDI). Dafters and Odber (1989) studied the difference between the DC group and the HC group given different doses of morphine at a 48-hour inter-dose interval. The doses of morphine were 2.5, 5, 7.5, 10, 15, and 20 mg/kg. Rats developed associative tolerance with 2.5, 5, and 7.5 mg/kg doses of morphine. No associative tolerance developed with the larger doses.

In contrast, Cox and Tiffany (1997) showed associative tolerance in rats with 20 mg/kg doses of morphine (a large dose of morphine). But these researchers used a 96-hour inter-dose interval instead of a 48-hour inter-dose interval.

To determine if dose is dependent on inter-dose interval (IDI), a dose-IDI ratio was calculated for each study included in this meta-analysis. For instance, calculated from the previous examples from Dafters and Odber (1989) and Cox and Tiffany (1997), small ratios are a 5 mg/kg dose of morphine divided by 48 hour IDI (.10 dose-IDI ratio) and a 20 mg/kg dose of morphine divided by 96 hour IDI (.21 dose-IDI ratio) respectively. These small ratios produced associative tolerance. An example of a larger ratio from Dafters and Obder (1989) is 20 mg/kg dose of morphine divided by 48 hour IDI (.42 dose-IDI ratio). This larger ratio did not produce associative tolerance. The dose-IDI ratio was a significant moderator of associative tolerance, $Q_R(1) = 10.5, p < .01$. Given that there was significant heterogeneity, this ratio does not provide a complete model, $Q_E(25) = 48.3, p < .01$. The correlation between effect size and dose-IDI ratio was significant, $r(25) = -.42, p < .05$. This analysis suggest that smaller dose-IDI ratios produce greater associative tolerance. This result supports the possibility that dose effectiveness may be dependent on inter-dose interval.

Classical conditioning may explain this relationship between dose of morphine and inter-dose interval. When a large dose of morphine is injected into a rat, the effect lasts a longer duration than a small dose (Diaz, 1997). If the large dose is followed by a short IDI, then the effect of morphine (US) may overlap with the next trial (injection of morphine and presentation of the distinctive cue (CS)). Thus, there may be less

correlation between the distinctive cue (CS) and the morphine effect (US). The distinctive cue may be an unreliable predictor of the morphine effect, thus leading to weak conditioning of associative tolerance (Rescorla, 1966). Thus, a researcher may have to allow a greater time between trials (IDI) as the size of the morphine dose increases (Cox & Tiffany, 1999). Long inter-dose intervals are needed for large doses of morphine while short inter-dose intervals may be adequate with small doses of morphine. Therefore due to the possible confounding of inter-dose interval and dose, the results of this meta-analysis may not have shown dose as a moderator of associative tolerance.

Type of Distinctive Cue

The type of distinctive cue was a significant moderator of the overall associative tolerance effect size, $Q_B(1) = 9.5, p < .01$. The 95% confidence interval (*CI*), which was from + 0.62 to + 0.81, did not include zero. For exteroceptive cues, the weighted mean effect size was $d_+ = 0.78$, and the 95% *CI* was from + 0.68 to + 0.88. Heterogeneity within the exteroceptive cue category was significant $Q_w(21) = 41.4, p < .01$. For interoceptive cues, the weighted mean effect size was $d_+ = 0.37$, and the 95% *CI* was from + 0.13 to + 0.82. Heterogeneity within the interoceptive cue category was also significant $Q_w(4) = 12.4, p < .05$. To reveal potentially different moderators for studies with exteroceptive cues and studies with interoceptive cues, a separate moderator analysis was conducted for each type of cue.

A total of 22 experiments (with 819 rats in the DC group and 822 rats in the HC group) contributed to effect size estimates for studies with exteroceptive cues. The weighted mean effect size $d_+ = 0.78$, 95% confidence interval (*CI*) = 0.68 to 0.88,

indicated more analgesia was displayed by the HC group than the DC group during tolerance testing ($p < .00001$). Thus, the DC group displayed more tolerance suggesting acquisition of associative tolerance. The homogeneity statistic indicated that effect sizes were heterogeneous, $Q_T(21) = 41.4$, $p < .01$. Therefore, the coded variables were used to explain the variability in effect sizes. Inter-dose interval, the number of morphine injections, duration of distinctive cue exposure, date, number of rats used in the study, and exposure to testing device were all significant moderators of associative tolerance development in studies using exteroceptive cues. These are the same moderators seen to be significant for the overall effect.

A total of 5 experiments (with 138 rats in the DC group and 140 rats in the HC group) contributed to effect size estimates for studies with interoceptive cues. The weighted mean effect size, $d_+ = 0.37$, 95% confidence interval (CI) = 0.13 to 0.61, indicated more tolerance was experience by the DC group during tolerance testing ($p < .01$). The homogeneity statistic indicated that effect sizes were heterogeneous, $Q_T(4) = 13.0$, $p < .05$. Therefore, the coded variables were used to explain the variability in effect sizes. After a moderator analysis, none of the variables was found to be a significant moderator of the development of associative tolerance in studies that used interoceptive cues.

The type of distinctive cue may moderate the acquisition of associative tolerance. The results suggest that rats given an injection and placed in the presence of distinctive exteroceptive cues acquire greater associative tolerance than rats given an injection and placed in the presence of distinctive interoceptive cues. These results do not support the

hypothesis of this meta-analysis, which stated that rats that experienced distinctive interoceptive cues during tolerance acquisition will acquire more associative tolerance.

By revisiting the Kim, Siegel, and Patenall (1999) study, these results may be explained. Their study is the only experiment to date that intentionally manipulated both interoceptive and exteroceptive cues within a single study. This study also attempted to compare the two types of cues. Thus, the hypothesis for this meta-analysis was based largely on Kim, Siegel, and Patenall's (1999) work. By revisiting their study and seeking a new interpretation of their results we may be able to explain the results of this current meta-analysis.

During the tolerance acquisition phase, Kim, Siegel, and Patenall (1999) paired both interoceptive and exteroceptive cues with 6 morphine injections for one group of rats and the other group of rats received the morphine injections paired with no cues. For the tolerance testing phase, rats were divided into four different testing groups: one with both cues present, another with just the interoceptive cue, one with just the exteroceptive cue, and the last one with no cues present. No associative tolerance was measured when exteroceptive cues were present or when no cues were present. The researchers measured significant associative tolerance when both cues were present and a significant, small amount of associative tolerance when only the interoceptive cue was present.

The results of the Kim, Siegel, and Patenall (1999) experiment may be due to "occasion setting" rather than conditioned stimulus strength. An occasion setter is a stimulus that may determine whether or not a rat will respond to another conditioned stimulus. Ross and Holland (1981) conducted an experiment with two types of

conditioning trials. On half of the trials, a light was presented for a few seconds, followed by a tone, followed by food. The other half of the trials no light was present but a tone was presented not followed by food. Thus, the presence of light predicted that the tone would be followed by food. If the light was not present, the tone predicted no food. The results showed that when the light was presented followed by a tone, rats looked for food and when no light was presented followed by a tone, rats did not look for food. In Kim, Siegel, and Patenall's (1999) study the interoceptive cues may have acted as an "occasion setter". During the tolerance acquisition phase, the interoceptive cue was presented together with the exteroceptive cue followed by the effect of morphine. Thus, when the interoceptive cue was presented together with the exteroceptive cue, rats may have expected the morphine effect based on the exteroceptive cue. If the interoceptive cues was not present, then the rat may not have expected the morphine effect. According to the results the study, when the occasion setting interoceptive cues were present, associative tolerance developed. In contrast, when the interoceptive (occasion setting) cues were not present, no associative tolerance developed.

Based on the results of the present moderator analysis, we cannot assume that distinctive interoceptive cues are stronger conditioned stimuli than exteroceptive cues based on previous research. The results of the moderator analysis of the type of distinctive cue may be in part a result of the small number of studies using interoceptive cues. A future study to determine the strength of interoceptive versus exteroceptive cues might be done as a variation of Kim, Siegel, and Patenall's (1999) study. Instead of conditioning all the rats with interoceptive and exteroceptive cues, this new study might

include two more groups of rats one trained with only interoceptive cues and another with only exteroceptive cues. In this case it would be possible to directly compare the associative tolerance development of rats that had the same morphine treatment exclusively with interoceptive or exteroceptive cues. The intensity of the interoceptive cue and the exteroceptive cue need to be similar in this type of study because one type of cue can overshadow the other, based on relative intensity. If both cues have relatively similar intensity, then it would be possible to determine which cue produces stronger conditioning. It may be a problem to determine the relative intensity of an interoceptive cue and an exteroceptive cue since they are different types of cues. Thus, comparing interoceptive and exteroceptive cues may be difficult.

In addition to studying the difference between interoceptive and exteroceptive cues, it may be interesting to study the difference between the sub-categories of interoceptive cues: administration cues (injection and handling cues), intra-drug cues (cue arising from another drug besides the main drug), and inter-drug cues (a cue from the main drug cueing the main effect of the drug). In this meta-analysis, all studies that manipulated interoceptive cues used either intra-drug cues or inter-drug cues. Thus, a moderator analysis of the sub-categories of interoceptive cues was not conducted. Intra-drug cues and inter-drug cues may be closer in time to onset of the morphine effect while administration cues last for a shorter duration and may be further removed in time from the onset of the morphine effect. Intra-drug cues and inter-drug cues may therefore be more reliable predictors of the morphine effect thus, producing more associative tolerance. A future study to determine the difference in associative tolerance

development with different types of interoceptive cues would include two groups of rats receiving both administration cues and inter-drug cues in the tolerance acquisition phase. All rats would have a catheter to administer a long duration morphine infusion (the inter-drug cue). Prior to the long duration infusion, rats would receive a saline injection (administration cue). This would be the only time rats would receive a saline injection. After several pairings of the long duration infusion and the injection of saline, analgesic tolerance would be assessed in four different groups: one group with both types of cues, another group with only the administration cue, another group with only the inter-drug cue, and the final group with no cues present. In this proposed study, it would be possible to directly compare the associative tolerance development between two different types of interoceptive cues.

Overall Moderator Analysis

Three variables were not significant moderators of associative tolerance: dose of morphine, average weight of rats, and hours from tolerance acquisition to tolerance testing phase. The reason why dose of morphine was not a significant moderator may be due to the confounding relationship between dose and inter-dose interval. The weight of the rats and hours to tolerance acquisition phase were not correlated with any other variables.

Seven variables were significant moderators of the overall effect size; date of study, number of rats used in the study, inter-dose interval, number of morphine injections, duration of exposure to distinctive cue, exposure to testing device, and type of cue. The four theoretically important and significant moderators (type of cue, inter-dose

interval, number of morphine injections, and duration of exposure to distinctive cue) were entered into a step-wise multiple regression model as predictors and *d* as the dependent variable. Publication date was excluded from the step-wise multiple regression because it was not theoretically important. In addition, the number of rats used in the study, and exposure to testing device, were excluded from the step-wise multiple regression as these variables were correlated to date. The best fitting regression model contained only two significant moderator variables ($R = 0.60$, $Q_R(2) = 19.6$, $p < .01$): inter-dose interval (standardized regression weight $\beta = 0.43$, $p < .001$) and duration of distinctive cue exposure ($\beta = 0.48$, $p < .001$). The overall homogeneity was not significant; thus, these variables provide a complete model, $Q_E(19) = 35.4$, $p = .076$. The fit was not as good when type of distinctive cue and number of morphine injections was added to the multiple regression. Therefore, as inter-dose interval and duration of distinctive cue exposure increased, associative tolerance increased.

CONCLUSION

Rats given a series of morphine injections and tested in the presence of the distinctive cue (DC group) acquire greater tolerance than rats given the same morphine treatment in their home cage and tested in the presence of the distinctive cue (HC group). Both groups acquire non-associative tolerance. The DC group also acquires associative tolerance. Due to the additive nature of tolerance, the DC group will have greater overall tolerance. No additional studies on associative tolerance are needed to simply test for the effect. Additional studies on associative tolerance are needed to investigate potential moderator variables.

As predicted and according to the results of this meta-analysis, inter-dose interval and duration of distinctive cue exposure were both moderators of the acquisition of associative tolerance. The type of distinctive cue (interoceptive or exteroceptive) and the number of morphine injections were also moderators, as hypothesized, but it did not moderate associative tolerance in the way previous research predicted. This study found exteroceptive cues produced more associative tolerance and fewer morphine injections produced more associative tolerance. Lastly, the dose of morphine was not found to be a significant moderator. Other research (Cox & Tiffany, 1997; Dafters & Odber, 1989) suggests that this may be due to the fact that dose and inter-dose interval are often confounded. In fact, the ratio of dose and inter-dose interval was a significant moderator of associative tolerance suggesting smaller dose effectiveness may be dependent on the inter-dose interval.

This meta-analysis helped identify the most important variables that moderate associative tolerance. For example, it suggested that longer inter-dose intervals and longer duration of exposure to the distinctive cue lead to greater associative tolerance. This information may provide an experimental framework for future researchers. Future researchers should use long IDIs (or small dose-IDI ratios) and long duration of distinctive cue exposure when conducting research on associative morphine tolerance. This experimental framework may help researchers isolate other variables like dose of morphine and type of distinctive cue, about which many questions remain to be explored.

The results of this meta-analysis are restricted to the range of variables used in the studies included in this analysis. It is possible that if variables outside this range were included in this meta-analysis that a different results would be found. For example in this meta-analysis, the data shows longer exposures to the distinctive cue produces greater associative tolerance. It is important to remember that the results of this moderator analysis are restricted to the studies that have been included in this meta-analysis which range from 15 minute to 60 minute distinctive cue exposures. As the minutes of exposure increase outside this range, this variable may not continue to have a linear relationship with associative tolerance. Do even longer exposures produce greater associative tolerance? Is there an optimum amount of time a rat should be exposed to the distinctive cue after an injection? The range included in this meta-analysis may not have been broad enough to expose the complete relationship between associative tolerance and duration of distinctive cue exposure. A future study to determine the relationship between associative tolerance and duration of distinctive cue exposure would include a HC and

DC group with several different subgroups each with a different duration of distinctive cue exposure (15, 30, 60, 120, 240, 480, and 960 minutes). In this case, it would be possible to directly compare the associative tolerance development of groups of rats that had the same morphine treatment but with different durations of distinctive cue exposure.

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