THE EFFECT OF ACUTE AGGREGATION ON CONDITIONED TASTE AVERSIONS INDUCED BY PSYCHOACTIVE DRUGS IN RATS

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ABSTRACT

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The effect of exposing individually housed rats to acute social aggregation immediately following drug administration was examined within a standard conditioned taste aversion paradigm. In the first experiment, water-deprived rats were allowed to consume a novel-tasting fluid immediately following which they were injected with one of three doses of morphine or Ringer's solution. After the injection, rats were either immediately returned to their respective home cages or were temporarily placed in groups of five in large plastic baskets for a period of approximately two hours. After this period of acute aggregation, each rat was returned to its individual home cage. This procedure was repeated on a second conditioning day. Results revealed that on presentations of the novel-tasting fluid subsequent to the initial conditioning day, rats given morphine at two of the three doses demonstrated significant reductions in fluid consumption, indicating formation of a conditioned taste aversion. Of these rats, animals exposed to the acute aggregation condition were found to demonstrate an enhanced taste aversion in comparison to those animals maintained in isolation. Although not reaching levels of statistical significance on any one occasion, this phenomenon has been observed across five separate replications and was found to be statistically significant there-

fore appearing to be a reliable effect. In the second experiment, the effect of acute aggregation was examined in regard to a conditioned taste aversion induced by lithium chloride, using the same procedure as in the first experiment. Following exposure to the novel-tasting fluid, rats were injected with either lithium chloride or with the drug vehicle. Results indicated no significant difference between isolated and acutely aggregated rats in magnitude of the lithium chloride-induced conditioned taste aversion, although a slight attenuation of the taste aversion was observed in the aggregated rats. The apparent divergence of acute aggregation effects in these two experiments is discussed in terms of the characteristic properties of the two drugs, morphine and lithium chloride. It would appear that investigation of effects of different social environments upon drug-induced conditioned taste aversions may lead to greater understanding of the important differences in motivational effects of such drugs as morphine and lithium chloride.

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The purpose of this thesis is to examine the possible influences of certain environmental factors on responses of animals to drug administration. A multitude of studies are available within the experimental literature which investigate the effects of drugs in animals. The majority of these studies assume the possible influence exerted upon the animal by that animal's immediate environment to be a fixed factor constant across the various experiments that have been conducted. However, there are a number of studies which suggest that environmental conditions may contribute significantly to the overall determination of an animal's responses to drug exposure. For instance, studies were conducted examining the influence of different housing conditions on the effects in rodents of narcotic analgesics such as morphine, psychomotor stimulants such as amphetamine and cocaine, and sedative hypnotics such as ethanol and hexobarbital. Differences in response to drug exposure were found in aggregated animals as opposed to animals housed in isolation (e.g., Baumel, DeFeo, & Lal, 1970; Bonnet, Hiller, & Simon, 1976; Chance, 1946; Hadaway, Alexander, Coambs, & Beyerstein, 1979; Hill & Powell, 1976; Mohrland & Craigmill, 1980; Parker & Radow, 1974; Pilcher & Jones, 1981). These researchers suggest there to be an interaction of the environmental influences on the animal with the behavioral, physiological, and neurochemical effects of the various drugs indicated above.

Two relatively distinct aspects of exposure to different housing conditions appear in the literature. In the first, emphasis is placed on effects of chronic exposure to such conditions (e.g., Amir, Galina, & Amit, 1979; Bonnet et al., 1976; Hatch, Wiberg, Zawidzka, Cann, Airth, & Grice, 1965), while in the second, the emphasis is on the effects of acute exposure to different social living conditions (e.g., Blackshear, Wade, & Procter,

1979; Brister & Davis, 1974; Chance, 1946; Mohrland & Craigmile, 1980; Sklar & Amit, 1977).

Alterations in the distribution and activity of opiate receptors endogenous to the organism have been implicated in various behavioral and physiological changes attributed to chronic exposure of animals to different social environments (e.g., Amir et al., 1979; Bonnet et al., 1976; DeFeudis, DeFeudis, & Somoza, 1976; Hadaway et al., 1979). These changes are reported in such measures as body weight gain (e.g., Amir et al., 1979), opiate binding capacity (e.g., Bonnet et al., 1976; DeFeudis, Somoza, DeFeudis, Pugnaire, Munoz, Portal, Ibanez, & Bonnet, 1978; Schenk, Britt, & Atalay, submitted for publication), morphine-induced analgesia (e.g., Bonnet et al., 1976; DeFeudis, DeFeudis, & Somoza, 1976; Kostowski, Czlonkowski, Rewerski, & Piechocki, 1977), self-administration of morphine (e.g., Hadaway et al., 1979) and signs of morphine abstinence (Adler, Bendotti, Ghezzi, Samanin, & Valzelli, 1975).

Indirect evidence is also presented in the literature for mediation of such social behaviors as distress vocalizations induced by social separation, and maintenance of physical proximity, by endorphins (see Panksepp, Herman, Vilberg, Bishop, & De Eskinazi, 1980). Endorphins (opiate-like substances naturally occurring in the organism) are found to be active at the same receptor (the opiate receptor) as are opiate agonists such as morphine, and opiate antagonists such as naloxone (e.g., see Pert, 1981). Panksepp and his colleagues (1980) report finding reductions of separation-induced distress vocalizations with administration of opiates and endorphins, while opiate antagonists can increase these vocalizations. Morphine administration is reported to reduce the amount of time spent by aggregated rats in close physical proximity to one another (Panksepp, Najam, & Soares, 1979).

Thus, alterations involving opiate mechanisms are hypothesized to underlie differences in animals chronically exposed to different social environments and also are implicated in the mediation of social separation-induced distress and maintenance of social proximity. Social separation is intrinsic to chronic isolation housing conditions, while social proximity is intrinsic to chronic aggregation housing conditions. It therefore is apparent that opiate and endorphin-related mechanisms may be involved in some of the effects observed in animals chronically exposed to these conditions.

Alterations in adrenocortical activity (e.g., Hatch et al., 1965), and activity of central biogenic amines (e.g., Weinstock, Speiser, & Ashkenazi, 1978; Welch & Welch, 1969) are also reported in animals chronically exposed to different social environments. These alterations are, in turn, hypothesized to underlie some of the differences observed in animals exposed to different social environments in relation to the responses of these animals to administration of sedative hypnotics such as ethanol (e.g., Parker & Radow, 1974) and hexobarbital (Baumel, DeFeo, & Lal, 1970) and psychomotor stimulants, such as amphetamine (e.g., Segal, Knapp, Kuczenski, & Mandell, 1973). For instance, Hatch et al. (1965) report heightened activity of adrenocortical thyroid hormone (ACTH), as indicated by increased plasma corticosterone levels, in isolation-housed rats. Enlarged adrenal glands were found in isolation-housed rats that were also discovered to self-administer ethanol to a greater degree than did rats housed in groups (Parker & Radow, 1974). Segal et al. (1973) report finding altered activity of central biogenic amine enzymes tyrosine hydroxylase and tryptophan hydroxylase in isolation-housed rats that also exhibited enhanced locomotor activity in response to administration of amphetamine. These differences

in differently housed animals will be examined in the ensuing pages. As well, some consideration will be given to behavioral changes in locomotor activity (e.g., Bell, Miller, & Ordy, 1971) and aggression (e.g., Knutson & Krane, 1980) observed in animals living in conditions of social isolation or aggregation. These changes may be important in that, for example, Modigh (1974) observed differences in norepinephrine depletion in grouped animals exhibiting aggressive behavior as compared to those in which such aggression was not observed.

A second aspect of the study of effects of different social environments on responses of animals to drugs involves the examination of the effects of acute exposure to different social environments following drug administration. Acute exposure to aggregation conditions are reported to enhance the lethality of amphetamine (e.g., Chance, 1946; Lokiec, Rapin, Jacquot, & Cohen, 1978), and of morphine (e.g., Mohrland & Craigmill, 1980; Sklar & Amit, 1977). These phenomena will be considered below.

The findings described above, are of special interest in that both amphetamine and morphine have been found to be both positively reinforcing (as indicated by facilitated operant behavior) and aversive (as indicated by induction of a conditioned taste aversion) (Reicher & Holman, 1977; Wise, Yokel, & deWit, 1976; White, Sklar, & Amit, 1977; Switzman, Amit, White, & Fishman, 1978). For example, the same injection of morphine has been found to facilitate an operant behavior (running down an alleyway) and simultaneously, to induce a conditioned taste aversion (as indicated by reduced consumption of a novel-tasting substance) (Switzman et al., 1978). This seemingly "paradoxical" phenomenon has received considerable attention within the literature (e.g., see Goudie, 1979). The same neurochemical interventions which block self-administration of morphine and ethanol are

also reported to attenuate conditioned taste aversions (CTA) induced by these drugs (Sklar & Amit, 1977). It may therefore be possible that the positively reinforcing and aversive effects of these drugs are at least partially mediated by common neurochemical mechanisms. The final determination of the animal's response to the drug administration may then be dependent upon other factors beyond the initial pharmacological impact of the particular drug involved.

It may be that manipulation of environmental conditions, as outlined above, will make possible a clearer differentiation of the positively reinforcing and aversive effects of narcotic analgesics, such as morphine, psychomotor stimulants, such as amphetamine, and sedative hypnotics, such as ethanol. Changes in the responses of animals to these drugs as induced by exposure to different social environments may, in turn, result in alterations in these drugs' motivational properties. Understanding the nature of these alterations may provide important new insights into the capacity of these drugs to be aversive or positively reinforcing.

Effects of Chronic Exposure to Different Social Environments

Involvement of the Opiate Receptor

Endorphins, opiate agonists (such as morphine) and opiate antagonists (such as naloxone) are all known to act at the opiate receptor (e.g., see Pert, 1981). Administration of these substances is found to affect such social behaviors as separation-induced distress vocalizations (Hermann & Panksepp, 1978; Panksepp, Vilberg, Bean, Coy, & Kastin, 1978; Panksepp, Hermann, Vilberg, Bishop & DeEskinazi, 1980; Vilberg, Bean, Bishop, Porada, & Panksepp, 1977), maintenance of physical proximity (Panksepp et al., 1980; Panksepp, Najam, & Soares, 1979), play behavior (Panksepp et al., 1979) and maternal behavior such as pup retrieval (Panksepp et al., 1980). For

example, administration of low doses of morphine is found to attenuate distress vocalizations induced by social separation in puppies (Panksepp et al., 1978), guinea pigs (Hermann & Panksepp, 1978) and chicks (Vilberg et al., 1977). Endorphin administration resulted in similar attenuating effects in chicks (Panksepp et al., 1978). In addition, administration of the opiate antagonist, naloxone, was found to enhance these vocalizations in guinea pigs (Hermann & Panksepp, 1978), and chicks (Vilberg et al., 1977). A general decrease in behavioral activity cannot explain the morphine or endorphin-induced suppression of distress vocalizations. First, in guinea pigs, while no significant reduction in motor activity was found for two low doses of morphine, these significantly decreased vocalizations (Hermann & Panksepp, 1978). Second, non-opiate behavioral depressants such as sodium pentobarbital largely failed to influence distress vocalizations of infant puppies except at dosages which clearly incapacitated the animals. These two observations seem to militate against an explanation of general behavioral inactivity.

Also, Panksepp and his colleagues (Panksepp et al., 1979) demonstrated that administration of low doses of morphine reduces the amount of time paired rats spend in close physical proximity to one another.

Taken together, this evidence cited above concerning opiate modulation of separation-induced distress and maintenance of physical proximity can be seen to be congruent with a hypothesis of involvement of opiate mechanisms in the effects of isolated and aggregated housing conditions insofar as these involve social separation and physical proximity respectively.

Involvement of opiate systems in effects of different social environments is more directly implicated in a study by Amir, Galina, and Amit (1979). These authors found that chronic administration of naloxone reversed

the suppressive effects of body weight gain observed in rats housed in pairs as opposed to being kept in isolation. The suppressive effect of the aggregated housing was hypothesized to be due to an unspecified "crowding stress" inherent in this condition. An important role for endorphins has been widely implicated in relation to the mediation of the organism's response to stress (see Amir, Brown, & Amit, 1980). The term "stress" has been defined as "stimulation that requires behavioral and/or physiological adjustments" (Anisman & Bignami, 1978). Thus, chronic exposure to crowded housing conditions may be seen to constitute stimulation requiring repeated adaptive adjustments, given that social environments are dynamic. A number of studies report findings in support of this hypothesis. Recurrent exposure of young rats to heat stress during the early postnatal period was found to result in an increase in endorphin content and in opiate receptor binding capacity in the brain (Torda, 1977). As cited above, suppression of body weight gain induced by chronic crowding was determined to be reversed with chronic naloxone treatment (Amir et al., 1979). Enhancement of a naloxone-induced CTA by long-term social crowding is also reported (Pilcher & Jones, 1981). These authors compared aggregated animals chronically housed under crowded and non-crowded conditions. The crowded animals exhibited a stronger CTA, and this was not reversed by placing the rats in isolation three weeks prior to the experiment. The enhanced aversiveness of naloxone observed in the crowded animals was taken to be indicative of underlying changes in opiate-related systems in these animals as a result of exposure to crowding stress.

A prolonged increase in endorphin activity and alterations involving the opiate receptor, then, are hypothesized to be consequences of the stress associated with crowded living conditions. However, a body of evidence

exists which suggests that isolation-housing conditions may also result in altered opiate receptor binding capacity. Bonnet et al., (1976) reported alterations in opiate binding in mice chronically exposed to different housing conditions. Isolated mice of the C57BI/J7 strain showed a significant reduction in opiate binding and while exhibiting higher initial pain thresholds, the net analgesic potency of morphine was reduced in these animals. However, in Swiss albino mice, an increase in opiate binding was reported for the isolated animals, accompanied by a decreased initial pain threshold and more prolonged effects of morphine analgesia. Despite the strain difference, a positive correlation is evident between alterations in opiate receptor binding and analgesic potency of morphine in these differentially housed animals. DeFeudis et al., (1978) reported increased opiate receptor binding of morphine in aggregated Swiss albino mice in agreement with the earlier finding of Bonnet et al., (1976). Taken in concert with the finding of Torda (1977) cited above, of increased opiate receptor binding in young rats exposed to heat stress, it would appear that in at least one strain of mouse, isolation-housing may constitute a stress condition.

Several studies have reported differential responses to morphine analgesia due to different housing conditions of animals. Enhanced morphine analgesia is reported for mice reared in isolation, in agreement with the findings of Bonnet et al. (1976), (DeFeudis et al., 1976; Kostowski, Czlonkowski, Reyerski, & Piechocka, 1977). Interestingly, isolated rats were found by Kostowski et al., (1977) to exhibit decreased morphine analgesia, in concert with the binding results of Schenk et al., (submitted for publication). Panksepp et al., (1980) found that even brief 24 hour periods of isolation can result in decreased analgesic potency of morphine in young

rats. In contrast, however, Adler, Mauron, Samanin, and Valzelli (1975) reported no difference in morphine analgesia in differentially housed rats.

The effects of chronic exposure to different housing conditions is also reported in relation to self-administration of morphine (Alexander, Coambs, & Hadaway, 1978; Hadaway, Alexander, Coambs, & Beyerstein, 1979). It was found in these studies that isolated rats self-administered morphine to a significantly greater degree than did their group-housed counterparts. The authors suggest that this phenomenon could be explained either by the idea that grouped animals consumed less morphine because the drug effects would interfere with their species-specific social behavior, or alternatively, that for isolated rats morphine was less effective and so increased morphine consumption would result in order to achieve greater drug reinforcement. Either of these ideas could be accommodated within the body of opiate-related studies previously discussed. Hill and Powell, (1976) published a study in which no difference was found in self-administration of morphine between isolated and aggregated rats. The discrepancy with the Hadaway (1979) and Alexander (1978) studies could most parsimoniously be accounted for by a floor effect seen in the data, as neither of the two treatment groups in the Hill study consumed significant amounts of the morphine solution due perhaps to a difference in taste. However, an increased self-administration of cocaine was also observed in the aggregated animals studied by Hill and Powell (1976). An important procedural difference between these studies is that the aggregated animals in the Hadaway (1979) and Alexander (1978) studies were maintained in colony setting during drug presentation whereas, in the Hill and Powell (1976) study, the aggregated animals were individually housed when they reached 100 days of age, prior to presentation of the drug. This may help account for the discordant

findings. Differences in signs of morphine abstinence have also been reported in differentially housed rats (Adler, Bendotti, Ghezzi, Samanin, & Valzelli, 1975). In these animals, morphine dependence was produced by chronic pellet implantation and signs of naloxone-precipitated withdrawal were subsequently observed. Isolated rats demonstrated less jumping and less diarrhoea than did group-housed rats.

From the above studies it can be clearly seen that chronic exposure to different social environments can result in altered responses to opiate administration possibly reflecting underlying physiological adjustments induced by this exposure. In the next section, consideration will be given to some other alterations in drug responsiveness reported to be due to differential housing.

Involvement of Adrenocortical and Biogenic Amine Systems

Pronounced behavioral differences between chronically aggregated and isolated animals have been observed in measures of aggression (e.g., Brain & Nowell, 1969; Knutson & Krane, 1980; Sigg, Day, & Columbo, 1966; Welch & Welch, 1969), and locomotor activity (e.g., Bell, Miller, & Ordy, 1971; Del Pozo, DeFeudis, & Jimenez, 1978; Weinstock, Speider, & Ashkenazi, 1978). Physiological alterations in adrenocortical function (e.g., Bell et al., 1971; Brain & Nowell, 1969; Hatch, Wiberg, Zaividzka, Cann, Airth, & Grice, 1965), and in biogenic amines (e.g., Modigh, 1973; 1974; Segal, Knapp, Kuczenski, & Mandell, 1973; Welch & Welch, 1969; Weinstock et al., 1978) are hypothesized to underlie these behavioral changes. These physiological changes are also hypothesized to underlie changes in self-administration of ethanol (Ellison, 1981; Parker & Radow, 1974) and metabolism of hexobarbitol (Baumel et al., 1970) and amphetamine (Segal et al., 1973).

Heightened aggression is reported to be induced by chronic isolation

in studies of rats (Hatch et al., 1965; Knutson & Krane, 1980; Sigg et al., 1966) and of mice (e.g., Baumel et al., 1970; Brain & Nowell, 1969; Sigg et al., 1966; Welch and Welch, 1969). Several authors explained this phenomenon in relation to isolation-induced changes in adrenocortical activity which, in turn, are suspected to possibly mediate differences in drug effects due to differential housing (e.g., Baumel et al., 1970; Ellison, 1981; Parker & Radow, 1974). Baumel et al (1970) reported finding reductions of hexobarbital narcosis in isolated mice, indicative of enhanced hepatic detoxification. Also, recovery of these isolation-housed animals from the drug-induced narcosis was found to take place at higher brain concentrations of the hexobarbital. These changes in drug sensitivity were found to be unrelated to the development of increased aggression. While altered drug sensitivity was observed for both males and females, the heightened aggression was only seen in the male mice. Also, while the decline in hexobarbital narcosis was greatest after the first week of isolation, male aggressive behavior increased progressively over the five week period of isolation. Thus it appears that explanation of this altered hexobarbital sensitivity must be sought elsewhere.

In the study by Hatch et al (1965) isolated rats, in addition to having decreased body weights, also exhibited greater adrenocortical response to exogenously administered adrenocortical thyroid hormone (ACTH) as measured by plasma corticosterone levels. In addition, isolated females had greater mean weights of the adrenal, thyroid, and pituitary glands as compared to aggregated animals. Enlarged adrenal glands were also reported in isolated rats by Parker and Radow (1974). These authors reported finding increased consumption of ethanol in the isolated rats and it was suggested that increased adrenocortical activity may be responsible for the phenomenon of

increased ethanol consumption observed in these rats. More recently, this finding has been replicated and extended by Ellison (1981) who reported that while isolated rats voluntarily consumed more ethanol than did rats housed in a colony setting, individual colony rats exhibited extremes of ethanol preference not seen in the isolated animals. The important influence of social environment on ethanol self-administration, then, is clearly demonstrated in these studies. While isolation-induced changes in adrenocortical activity may account for differences in ethanol consumption, these physiological changes do not seem, however, to underlie the changes seen by Baumel et al (1970) in regard to isolation-induced alterations in hexobarbital metabolism. An explanation of this phenomenon may be related to isolation-induced changes in brain biogenic amine activity to be discussed below.

In a study by Bell et al (1971) the mean brain and pituitary weights of aggregated mice were found to increase with increased group size in a non-crowded condition (in which cage size was increased in conjunction with increased group size). In a crowded condition (in which living space alloted per animal decreased with larger group size), these weights decreased with increased group size of up to sixteen animals per cage. With crowded mice housed thirty-two per cage, however, mean pituitary weights were found to be heavier than those of crowded mice in groups of sixteen. Thus it appears that the degree of crowding inherent in aggregated housing (as determined by restriction of living space) may be a critical factor in regard to consideration of its effects on endocrinological function. In this study by Bell et al (1971), the effects of crowding or aggregation were examined in relation to possible effects on locomotor activity.

It was found that exploratory behavior in a maze decreased with larger

group size in the home cage regardless of whether the mice were crowded or not. Whereas a similar decrease in locomotor activity as measured on an activity wheel was observed with increasing group size in both crowded and non-crowded treatment groups, the largest non-crowded group of thirty-two animals unexpectedly exhibited a relative increase in locomotor activity. This corresponds to the relative increase previously mentioned for this group in regard to pituitary weight. Thus a curvilinear function of group size with locomotor activity and pituitary weight is indicated by this study, a phenomenon which merits further investigation. The overall finding of Bell et al (1971) indicating an inverse relationship between group size and locomotor activity is supported in a complimentary fashion by the findings of Del Pozo et al (1978), and Weinstock et al (1978). These authors reported increased activity in isolated mice as compared to those housed in groups when each animal was individually placed in activity test chambers. Del Pozo et al (1978) also reported an enhanced sensitivity in the isolated mice to amphetamine's stimulatory effect on locomotor activity. This phenomenon has also been established in rats isolated for as little as five days (Segal et al., 1973). In this latter study, differences between isolated and aggregated animals were also found in brain levels of tyrosine hydroxylase and tryptophan hydroxylase, enzymes involved in the metabolism of catecholamines and serotonin respectively. Isolated rats exhibited elevated activity of midbrain and striatal tyrosine hydroxylase and decreased septal tryptophan activity. The decrease in tryptophan activity in conjunction with increased motor activity found by Segal et al (1973) is in agreement with the findings of Appel, Lovell, and Freedman (1970) who reported an inverse relationship between brain levels of serotonin and spontaneous motor activity. The isolation-induced elevation in tyrosine hydroxylase activity

complements the findings of Weinstock et al (1978) who reported changes in catecholamine turnover induced by isolation. Hyperactivity in isolated rats was observed by these authors to be accompanied by increases in forebrain dopamine and hindbrain norepinephrine levels as compared to those of group-housed rats. After inhibition of catecholamine synthesis by alpha-methyl-para-tyrosine (AMPT), norepinephrine concentrations declined to a lesser extent in the isolated than in the aggregated rats when the animals were allowed to remain in their home cages, but these levels were diminished to a greater extent in the isolated animals when the rats were transferred to a novel open field environment. It can be seen from these results that familiarity with the test environment and prior housing conditions may be critical factors in the determination of the effects of drugs such as amphetamine which act on brain biogenic amines. Increased levels of brain norepinephrine in isolated rats are also reported by Tanaka and Noda (1974) and Stolk, Conner, and Barchas (1974). Stolk et al (1974) also reported increased norepinephrine turnover in isolated rats. In contrast to these data, Garrattini, Giacolini, and Valzelli (1969) and Welch and Welch (1968) reported finding increased turnover of norepinephrine and serotonin in isolated mice. It is possible that enzymatic differences may account for this discrepancy between species, but such speculation must await further study.

Neurochemical changes in biogenic amine activity induced by exposure to additional stress have also been investigated in differentially housed animals (Modigh, 1973, 1974; Welch & Welch, 1966, 1968a, 1968b, 1969). Modigh (1973) isolated aggregated male mice for six to eight weeks after which they were exposed to brief periods of aggregation in groups of twenty-five to thirty animals. Intensive fighting occurred during this time. It

was found that the rate of synthesis of brain catecholamines was lower in isolated than in previously grouped mice and that intensive fighting rapidly accelerates the synthesis of both the catecholamines and serotonin in the brain. In a subsequent study, Modigh (1974) observed that depletion of norepinephrine by AMPT was least in isolated mice; slightly greater in grouped-non-fighting animals, and greatest in grouped-fighting mice. In contrast, pretreatment with a tryptophan hydroxylase inhibitor had no differential effect on serotonin levels between isolated, grouped or fighting mice, suggesting that this amine may not be involved in the differences observed between treatment groups. This last finding is in conflict with those of Garrattini et al (1969) and of Welch and Welch (1968b), although differences in intensity of the treatment stress may partially account for this discrepancy. Welch and Welch (1968a, b) reported that restraint stress can cause a greater elevation of brain catecholamines and serotonin in isolated than in grouped mice, reflective of the hyperexcitability of the former animals. After inhibition of catecholamine synthesis by pretreatment with AMPT, exposure to restraint stress retarded the consequent depletion of norepinephrine and dopamine in the group-housed mice, but facilitated this depletion in the isolated mice. Welch and Welch (1970) have suggested that the isolated mice, due to their enhanced reactivity to the additional stress, may not benefit from a postulated compensatory mechanism activated by mild stress involving partial inhibition of monoamine oxidase, which would then result in temporarily increased levels of brain amines. Thus, additional stimulation requiring physiological adjustments may differentially affect isolated and aggregated animals. Differences in this regard may ultimately be reflected in altered responses to centrally acting drugs.

Finally, a recent study has reported increased dopamine receptor bind-

ing in the rat striatum following long-term isolation (Guisado, Fernandez-Tome, Garzon, & Del Rio, 1980). This finding, taken in concert with the other changes in catecholamine and serotonin activity discussed above, clearly emphasizes the importance of considering the social environment in any determination of the effects of centrally-acting drugs administered to these animals. Adrenocortical changes due to differential housing conditions may also be important here as evidenced by the studies on ethanol consumption by Parker and Radow (1974) and Ellison (1980). Further investigation is required in order to more fully integrate the data concerning isolation-induced changes in adrenocortical and biogenic amine activity and to clarify their respective contributions to the differences observed in drug responses of animals housed in different social environments.

Acute Aggregation and Enhanced Drug Toxicity

In the previous section various behavioral, physiological, and neurochemical differences induced by prolonged isolation or aggregated living conditions were discussed. Generally, it was found that comparison of isolated versus group-housed rodents exhibited important differences including altered responsivity to morphine across several different behaviors including analgesia and self-administration, increased behavioral reactivity as measured by locomotor activity and aggressive behavior, and altered neurochemical and adrenocortical responses to stress. These findings demonstrate the importance, within the animal literature on drug effects, of taking into consideration the nature of prior housing conditions. In this present section, the influence on drug effects of acutely aggregating or isolating animals subsequent to drug administration is examined.

Drug lethality is found to be markedly enhanced in aggregated animals as compared to those left in isolation after drug exposure (e.g., Chance,

1946; Davis & Brister, 1971; Mohrland & Craigmill, 1980; Sklar & Amit, 1977). The two drugs most extensively studied in this context are amphetamine and morphine. Separate consideration of the various factors postulated to be involved in the enhanced lethality of each of these drugs will be presented. These factors include changes in behavioral reactivity (e.g., Chance, 1946), and locomotor activity (Greenblatt & Osterberg, 1961; Swinyard, Clark, Niyahana & Wolf, 1961), increases in ambient temperature and hyperthermia (e.g., Craig & Kupferberg, 1972; Menon & Dandiya, 1967; Mohrland & Craigmill, 1980), alterations in brain amine levels (e.g., Davis & Brister, 1971; Mennear & Rudzik, 1966; Stolk & Rech, 1968) as well as changes in a number of other physiological indices such as blood glucose levels (Clark, Blackman, & Preston, 1967; Stolk et al., 1970) and brain polyribosomes (Blackshear, Wade, & Procter, 1979). Given that these various alterations in the organism's responses to these drugs are at least to some degree overlapping, it becomes quite clear that the animal's social environment, at the time when the animal is under the drug's influence, can be of considerable relevance in determining these drugs' lethal effects. It would seem important to establish how this acute aggregation phenomenon might influence drug effects using other measures aside from that of lethality, with an eye to understanding the mechanism underlying a drug's motivational effectiveness. The term "toxicity" is used here as being synonymous with "lethality", that is to say meaning "causing death".

It is unfortunate that within the literature on acute aggregation lethality, few studies report on chronic housing conditions prior to the acute differential housing manipulation. That such a consideration is important is indicated from the findings discussed in the previous section.

Several studies, however, have addressed this issue. Consolo,

Garrattini, and Valzelli, (1965a, b) found the toxicity of dexamphetamine was greater in aggregated mice made aggressive by prior chronic isolation than in aggregated mice which were normal whether or not they had been previously isolated or group-housed. Enhanced lethality was also reported for fencamfamin, an amphetamine-like drug without apparent peripheral sympathomimetic activity. Pentobarbitone sleep-time was also increased in the aggressive animals. A direct comparison of chronic and acute differential housing effects on amphetamine toxicity was conducted by Welch and Welch (1966). They found that while acutely aggregated mice died more quickly than acutely isolated mice, pre-isolated mice placed in isolation after drug administration died more quickly than did pregrouped mice isolated after drug administration. Similarly, it was found that pre-isolated mice grouped subsequent to drug injection died faster than did the pregrouped mice grouped after the drug. Also, group size in the chronically aggregated mice was inversely related to the reductions in subsequent drug toxicity seen in the pregrouped condition. Chronic isolation for a brief period of one week was sufficient to produce an increase in response to the amphetamine. These studies clearly demonstrate the complex relationship between chronic and acute housing manipulations on the effect of psychoactive drugs.

Amphetamine Toxicity

An early study on the excitatory effects of benzedrine and several related compounds reported that mice placed together in groups were considerably more susceptible to the excitatory effects of the drugs than were mice left isolated after the drug administration (Gunn & Gurd, 1940). In 1946, Chance observed that the toxicity of amphetamine was increased ten-fold by aggregation. It was also noted that as the area per mouse

in the aggregated condition was decreased, the number of mortalities was progressively increased. In addition to this crowding effect, an increased room temperature was also found to markedly increase the amphetamine toxicity in the aggregated animals. Since this original study first appeared, a considerable number of studies have been published addressing the "amphetamine aggregation effect". The mediation of toxicity has been attributed to several factors including increased behavioral excitation, alterations in brain amine levels, hypoglycemia, and hyperthermia. It seems apparent that these factors are not necessarily mutually exclusive and that each may contribute significantly to the overall toxicity phenomenon. In the following pages, the relative contribution of each of these factors to aggregation-induced enhancement of amphetamine lethality will be discussed.

In accord with the description of Chance of a rapid alteration of "defensive encounters" and "escape reactions" in aggregated mice, Swinyard and his colleagues (Swinyard, Clark, Niyahana, & Wolf, 1961) reported that thresholds for pentylenetetrazol seizures were reduced in aggregated mice. The authors also noted that adaptation to aggregation just prior to amphetamine administration attenuated the enhanced toxicity effect, and that, while mortalities for the isolated animals given amphetamine all occurred within two hours, mortalities in the aggregated animals occurred as long as twelve hours after drug administration. These data were taken to support the idea that heightened excitability due to introduction into a novel, crowded environment involving unavoidable high levels of stimulus contact, mediated the enhanced toxicity. Further support for this idea is found in a study in which it was found that the incidence of toxicity for mice aggregated with either untreated or sedated mice was intermediate to that of isolated or amphetamine-injected aggregated mice (Wang, Hasagawa, Peters,

& Rimm, 1969). Using a wide range of excitatory drugs including methylphenidate and caffein, Greenblatt and Osterberg (1961) found a significant correlation between increased lethality and actions on motor activity and body temperature in grouped but not in isolated mice. In contrast, Abdallan, Lumsford, and Burnell (1981), in examining aggregation lethality across many different drugs, concluded that the phenomenon cannot be explained by central nervous stimulation alone in that enhanced lethality was also seen in such clearly non-excitatory drugs as aspirin. However, these authors also report no aggregation enhancement of morphine lethality, an effect reported by several other experimenters (e.g., Davis & Brister, 1971; Mohrland & Craigmill, 1978, Sklar & Amit, 1977). Lasagna and McCann (1957) reported that pretreatment with phenobarbital, chlorpromazine or reserpine successfully protected aggregated mice from enhanced amphetamine toxicity. This effect was attributed to the tranquilizing effects of these drugs.

Moore (1964) extended the findings of Lasagna et al (1957) and postulated that the aggregation-enhanced amphetamine toxicity may be mediated by enhanced depletion of endogenous peripheral norepinephrine observed in this condition. As previously shown, pretreatment of aggregated mice with chlorpromazine and to some degree, with phenobarbital, reduced the initial behavioral excitation seen in these animals and also served to protect them from increases in mortality as compared to isolated mice. Additionally however, pretreatment with phenoxybenzamine, alpha-methyl-m-tyrosine and reserpine tended to exaggerate the excitement phase yet effectively protected the aggregated animals from enhanced amphetamine toxicity. It was postulated that reductions in norepinephrine release induced by these drugs protected the aggregated mice from increased amphetamine toxicity. Also, as reserpine reduced peripheral (heart) but not brain norepinephrine levels,

it was suggested that enhanced depletion in peripheral amines may play an important role in the increased toxicity seen in aggregated animals. Protective effects of pretreatment with reserpine and alpha-methyl-tyrosine have been reported elsewhere (e.g., Menon & Dandiya, 1967; Mennear & Rudzik, 1966). Chronic reserpine pretreatment has been shown to enhance amphetamine toxicity in aggregated animals (Mennear et al., 1966; Stolk & Rech, 1968) possibly due to peripheral adrenergic receptors developing supersensitivity to sympathomimetic agents during the chronic pretreatment. Stolk and Rech (1968), also reported that acute reserpine pretreatment may only delay amphetamine lethality in mice, in that while acute reserpine pretreatment protected against amphetamine toxicity over a four hour period post-injection, the number of fatalities over a twenty-four hour period were not different for saline pretreated as versus reserpine pretreated mice. A triphasic dose-mortality curve was obtained in aggregated and in isolated mice given amphetamine, suggesting that multiple actions of amphetamine may be involved. This effect has been reported by others (George & Wolf, 1966; Gardocki, Schuler, & Goldstein, 1966). Amphetamine lethality was found to gradually increase with dosage, reaching a peak at a lower dosage, (less than 30 mg/kg), and then as the dosage was increased, lethality was observed to decrease followed by a second peak at a higher dose range (greater than 100 mg/kg).

Differences in the characteristics of amphetamine toxicity have been reported between rats and mice (Stolk & Rech, 1968; Stolk, Burnett & Rech, 1970). In these studies, no difference in amphetamine-induced mortalities were found between aggregated as versus isolated rats, nor did reserpine pretreatment protect rats from dying. Also, the trimodal dose-mortality curve observed in mice given amphetamine was not seen in the rats. It is

not clear at present what factors may mediate these differences.

In addition to differences in behavioral excitation and in levels of biogenic amines, a third factor which has been reported to influence amphetamine toxicity has been the depletion in glycogen observed in peripheral tissues following acute amphetamine injections. Differential response patterns in mice have been reported in regard to tissue glycogen levels following higher (64 mg/kg) or lower (32 mg/kg) dosages of amphetamine (Stolk et al., 1970). While reductions in liver, heart, and skeletal muscle, glycogen stores were observed at the lower dosage, at the higher dosage reduction of liver reduction was less severe and heart and skeletal muscle stores actually increased. It was noted by Stolk and his associates that this differential response pattern corresponds favourably with the trimodal dose-lethality curve exhibited by mice given amphetamine. Although hypoglycemia has been associated with amphetamine toxicity by a number of different experimenters (e.g., Blackshear, Wade, & Proctor, 1979; Clark, Blackman, & Preston, 1967; Moore, 1964) it appears that this factor alone cannot account for the phenomenon. Clark et al (1967), demonstrated that pretreatment with glucose reduced but did not fully prevent amphetamine-induced mortalities. These authors reported that fatalities occurred only in mice with body temperatures above 104 degrees Fahrenheit. It seems that other factors such as hyperthermia may work in concert with glycogen depletion in determining amphetamine toxicity.

Hyperthermia has been postulated to be a contributing factor in relation to amphetamine toxicity in several of the studies mentioned above in regard to other factors such as enhanced motor activity (Greenblatt & Osterberg, 1961) and the protective actions of alpha-methyl tyrosine (AMPT) or reserpine pretreatment (Menon & Dandiya, 1967). In this latter study, elevating

body temperature by exposure to heat stress was shown to markedly reduce the protective effect of AMPT on amphetamine toxicity and as well, by maintaining reserpine-treated mice at a higher environmental temperature, the protective effect of reserpine was reduced. In addition, in the original study on the amphetamine aggregation phenomenon, Chance (1946) noted that environmental temperature was an important factor in determining the degree of amphetamine toxicity. It would seem apparent that the degree of crowding within an enclosed space could also contribute to changes in the immediate environmental temperature of the animal. Askew (1962) reported that a rise in body temperature of mice given amphetamine above a critical maximum value was a reliable predictor of ensuing death. The hyperactivity observed in aggregated mice was also suggested to contribute to increased body temperature. Craig and Kupferberg (1972) investigated strain differences in response of aggregated mice at three distinct ambient temperatures, and found that while at the lowest temperature, one strain exhibited aggregate toxicity and the other did not, at increasing temperatures aggregate toxicity appeared for the second strain also. It was suggested that a difference in thermoregulation rather than in pharmacokinetics may account for this strain difference. More recently, Blackshear, Wade, and Procter (1979) observed that amphetamine-induced disaggregation of brain polyribosomes was enhanced by crowding. This effect could be mediated by lowered blood glucose levels and has been shown to be facilitated by hyperthermia (Moskowitz, Rubin, Liebschutz, Munro, Nowak, & Wurtman, 1977).

In contrast to the study by Stolk et al (1968), cited above in which it was reported that no amphetamine aggregation was found with rats, Lokiec and his colleagues have more recently reported an enhancement by aggregation of dextro- but not of levo- amphetamine lethality (Lokiec, Jacquot, Rapin,

& Cohen, 1977; Lokiec, Rapin, Jacquot, & Cohen, 1978). The greater sensitivity of aggregated rats to dextro-amphetamine was ascribed to change in serotonin metabolism seen in the aggregated but not the isolated rats. Also, it was found that in the aggregated animals the disappearance curves for labelled amphetamine were biexponential whereas in isolated animals they were monoexponential. This corresponds to the trimodal dose-mortality and hypoglycemic response pattern mentioned above (Gardocki et al., 1966; George and Wolf, 1966; Stolk and Rech, 1968.

Considered together, the data cited above relating to aggregate amphetamine toxicity emphasize that consideration of the multiple actions of both drug and of its interaction with environmental conditions is necessary in order to achieve a comprehensive appraisal of amphetamine's effects. Moreover, those interactions such as result in altered responsiveness of peripheral amine systems (Mennear & Rudzik, 1966; Moore, 1964; Stolk & Rech, 1968), altered glycogen levels in response to drug administration (Blackshear et al., 1979; Clark et al., 1967; Moore, 1964; Stolk et al., 1970), altered body temperature (Askew, 1962; Craig & Kupferberg, 1972; Greenblatt & Osterberg, 1961; Menon & Dandiya, 1967; Moskowitz et al., 1977) and central serotonin levels (Lokiec et al., 1977, 1978) all may actually alter the behavioral effectiveness of psychoactive drugs.

Morphine Toxicity

In contrast to the rather extensive literature on amphetamine aggregation toxicity, only a relatively small number of studies have specifically considered the effect of acute aggregation on opiate lethality. Evidence for a distinct demarcation between morphine and amphetamine toxicity has accumulated and will be discussed below. As well, two major areas of emphasis have emerged in regard to acute aggregation and morphine lethality.

The first addresses possible mechanisms which may mediate the lethal effects of morphine in aggregated mice or rats. Respiratory depression is usually considered to be the cause of morphine mortality, but there is also some evidence for other contributory factors. The second area of interest is focused on the quite striking dependence upon ambient temperature of the morphine aggregation toxicity phenomenon. As in the previous discussion of acute aggregation and amphetamine action, accent will be placed upon the importance of including consideration of environmental factors such as level of exogenous stimulation, or of ambient temperature, in determination of overall drug effects.

Aggregation enhancement of morphine toxicity in mice was first demonstrated by Spoerlein (1968), and while Verdernikov (1970) consequently failed to demonstrate this effect, the latter study is compromised by the fact that the "nonaggregated" condition was comprised of grouped rats placed in a cage larger than that used in the aggregation condition. Thus the results of this study are more properly considered to represent a failure to find any effect of differential crowding on morphine lethality rather than of an aggregation effect per se. Enhanced morphine lethality has also been demonstrated in aggregated rats (Sklar & Amit, 1977). This study also found that aggregation can enhance morphine lethality at relatively low dosages which are clearly non-lethal when administered to rats kept in isolation. This latter finding emphasizes the potential impact that aggregation can have on the actions of psychoactive drugs such as morphine.

In accord with previous studies investigating the role of brain catecholamines in the amphetamine toxicity phenomenon (e.g., Mennear et al., 1966; Menon et al., 1967), Davis and Brister (1971) examined the ability of pretreatment with AMPT to protect aggregated mice against amphetamine or

morphine-induced toxicity. AMPT has been shown to block the stimulatory effects of morphine on locomotor activity in mice (Menon, Dandiya, & Bapna, 1967) and in rats (Ayaahan & Randrup, 1973) in a manner similar to that seen with AMPT pretreatment reduction of amphetamine-induced motor excitation. Enhanced lethality of morphine or of one of several morphine-like analgesics was found for aggregated as versus isolated mice although the potency of this effect was significantly less than was found with amphetamine. Pretreatment with AMPT effectively protected aggregated mice against enhanced amphetamine toxicity but did not reduce the lethal toxicity of the narcotic analgesics. This finding is clearly indicative of a basic difference between the amphetamine and morphine toxicity in regard to the role of catecholamines in the respective phenomenon. In a subsequent study, Brister and Davis (1974), found that pretreatment with pilocarpine, a cholinomimetic agent, successfully protected aggregated mice from enhanced lethal effects of morphine but not of amphetamine. Moreover, in isolated mice, an increase in toxicity was found with pilocarpine pretreatment, and acute morphine administration. Simultaneous administration of a peripheral anticholinergic agent, methylatropine bromide, assured that the effects of the pilocarpine pretreatment were centrally and not peripherally mediated. The synergistic effect in isolated but not in aggregated animals may in part be due to the higher dosages required in the nonaggregated condition. However, it is clear that pilocarpine appears to have both central depressant and excitatory effects, and that within the aggregated condition, the central cholinomometic action of this drug serves to reduce morphine-induced lethality while not affecting aggregation-enhanced lethality of amphetamine. This, then, is a clear example of how the aggregation phenomenon may serve to elucidate differential effects of centrally acting drugs.

The lethal effects of morphine have most commonly been associated with the drug's depressive effect on respiratory activity (Jaffé, 1970; Tatum, Seevers & Collins, 1929). Morphine-induced respiratory depression has been clearly associated with changes in brain levels of serotonin and noradrenaline (Meldrum & Isom, 1981). Mohrland and Craigmill (1980a) however, were unable to find any difference between aggregated and isolated mice, given acute injections of morphine, in brain levels of serotonin, noradrenaline, dopamine, or morphine. These data suggest that aggregation enhancement of morphine lethality may involve other mechanisms of morphine lethality aside from those involved in morphine's depressive actions upon respiratory activity as mediated by alterations in brain amine levels. Mohrland and Craigmill (1978), reported that an aggregation effect on morphine lethality was found at an ambient temperature of 29° C but not at 19° C. At 29° C room temperature, the time course of the hyperthermia exhibited by aggregated mice closely followed that of enhanced locomotor activity observed in these mice. Also, whereas morphine produced significant hypothermia in both isolated and aggregated mice at 19° C and in isolated mice at 29° C, aggregated mice at 29° C were hyperthermic after morphine administration. In addition, no apparent threshold body temperature was found for these hyperthermic aggregated mice. Thus, it appears that the enhanced morphine lethality was not due to heat exhaustion alone. In a subsequent study, Mohrland and Craigmill (1980b) reported that the incidence of convulsions as well as the degree of morphine lethality was significantly greater in aggregated than in isolated mice at a room temperature of 29° C. When the room temperature was maintained at 19° C, no aggregation enhancement of lethality was found and no significant difference was found in the incidence of convulsions observed in the two differentially housed groups. In a

second study with ambient temperature at 29° C, isolated mice given morphine were stressed by means of continuous gentle prodding for five seconds every minute over a two hour period post-injection. It was found that the incidence of convulsions was significantly greater in stressed than in non-stressed mice. Therefore, it seems that the enhanced lethality of morphine in aggregated mice may be due to a higher incidence of convulsions induced by the increased tactile stimulation present in this condition.

From the studies discussed above it can clearly be seen that the environmental factors introduced by aggregation can dramatically alter the characteristics of pharmacological and behavioral response of such psychoactive drugs as amphetamine and morphine. It seems apparent that greater understanding of this phenomenon will provide valuable insight into important physiological and pharmacological mechanisms underlying these drugs' effects. Also, the role of environmental factors in the determination of drug responses constitutes an essential component of a better overall understanding of drug-related behavior. It would appear important to extend the investigation of the acute aggregation phenomenon beyond studies of lethal toxicity to address motivational aspects of drug administration as well. One such measure is discussed in the following section.

Drug-Induced Conditioned Taste Aversion

Conditioned taste aversion (CTA) induced by drug administration involves initially allowing the subject to ingest a novel tasting fluid of food immediately following which a drug treatment is administered. After full recovery from the drug's pharmacological effects, the subject is again exposed to the taste and a reduction in intake is assumed to indicate an association between the taste and some aversive consequence of the drug administration.

Early studies of CTA usually involved administration of treatments such as X-ray-irradiation, which possess well-known toxic effects (e.g., Garcia, Kimeldorf, & Koelling, 1955). Interest here was focused on certain facets of CTA in relation to learning theory. CTAs have been demonstrated with long delays between presentation of the conditioned (taste) stimulus and the unconditioned (drug) stimulus, (Garcia, Ervin, & Koelling, 1966) and are reliably induced after only one trial (Nachman & Ashe, 1973). It is not the aim of this section to address these issues associated with CTA but rather, emphasis will be placed on studies within the CTA literature which have provided further understanding of the motivational properties of drugs and of the pharmacological mechanisms which underlie these properties. Major consideration will be given, therefore, to CTA studies involving psychoactive drugs. In particular, the following discussion will address studies which involve the induction of CTA by drugs such as amphetamine and morphine which have also been found to be self-administered by animals (e.g., Yokel & Pickens, 1974; Weeks & Collins, 1964). These two drugs are also those most extensively investigated within the acute aggregation paradigm as was outlined in the previous section. Several factors which may potentially modulate CTAs induced by these drugs will also be considered.

CTA may be induced by a wide range of drugs (see Riley & Clarke, 1977) including psychoactive drugs such as amphetamine (Cappell & LeBlanc, 1971; Stolerman & D'Mello, 1978) opiates (Cappell, LeBlanc, & Endrenyi, 1973; Jacquet, 1973), ethanol (Cappell, LeBlanc, & Endrenyi, 1973; Eckardt, 1975), barbiturates (Vogel & Nathan, 1975) and benzodiazepines (Cappell, LeBlanc, & Endrenyi, 1973; Vogel & Nathan, 1973), amongst others.

Several authors have attempted to explain drug-induced CTA in terms of "toxicity", a hypothesis originally postulated by Garcia and his associates

(Garcia, Hankins, & Rusinak, 1974). No clear interpretation of this term has been reached however, and several lines of evidence militate against such an explanation. Berger (1972) observed that there is only a poor correlation between overt behavioral signs of drug-induced toxicity (e.g., sedation, diarrhoea) and potency to induce a CTA. Ionescu and Buresova (1977) demonstrated that severe poisoning by several drugs, such as cyanide, fail to induce CTA. Perhaps the most convincing evidence to indicate the inadequacy of a toxicity notion to explain the CTA phenomenon is to be found in the studies demonstrating induction of a CTA by administration of drugs which have also been shown to be self-administered at comparable dosages. Morphine is self-administered (Weeks & Collins, 1964) and can also induce a CTA within a similar dose range (Cappell et al., 1973). Amphetamine is self-administered (Yokel & Pickens, 1974) and a similar dose has been shown to induce a CTA (D'Mello, Stolerman, Booth, & Pilcher, 1977). Even more convincing evidence is to be found in studies in which the same drug administration mediates both positive reinforcement and aversion (Reicher & Holman, 1977; Wise, Yokel, & DeWit, 1976; White, Sklar, & Amit, 1977; Switzman, Amit, White, & Fishman, 1978). In the study of Switzman et al (1978) for instance, food-deprived rats were allowed to run down an alleyway for novel-tasting food, consumption of which was followed by an injection of morphine. It was shown that in subsequent trials, rats would run faster down the alley and eat less food as compared to control group rats. Furthermore, a direct relationship was found between the morphine-produced positive reinforcement (as indicated by faster running speeds) and aversion (as indicated by reductions in food intake). Rats increasing their running speed over trials also evidenced a CTA whereas those rats not demonstrating an increase in running speed also did not exhibit a CTA-mediated reduction in

food intake.

In addition to this behavioral evidence for the presence of both positively reinforcing and aversive characteristics of self-administered drugs, morphine and amphetamine, a number of studies have indicated a common pharmacological mechanism for these effects. The catecholamine system has been widely implicated in the mediation of positive reinforcement (see Fibiger, 1978; Wise, 1980). Studies have demonstrated that functional disruptions of catecholamine systems which can attenuate or block CTA-formation are identical to those which disrupt positive reinforcement in studies of self-administration. For instance, pretreatment with AMPT (a tyrosine hydroxylase blocker) has been found to block formation of a CTA induced by morphine, ethanol (Sklar & Amit, 1977), and amphetamine (Goudie, Thornton, & Wheatley, 1975). Intraventricular administration of 6-hydroxydopamine (a catecholamine neurotoxin) has been reported to attenuate an amphetamine-induced CTA (Roberts & Fibiger, 1975). In a subsequent study (Roberts & Fibiger, 1977) these authors found that injections of 6-hydroxydopamine into the dorsal tegmental noradrenergic pathway attenuated a morphine CTA while not affecting an amphetamine CTA, thereby implicating a role for norepinephrine in the CTA-induced properties of morphine. Pimozide, (a dopaminergic receptor blocker) has been reported to block a CTA induced by morphine, ethanol (Sklar & Amit, 1977) and amphetamine (Grupp, 1977). Sklar and Amit have also demonstrated that FLA57 (a dopamine-beta-hydroxylase inhibitor) attenuated morphine and ethanol CTAs. This accumulation of evidence suggests that similar mechanisms may underlie CTA and positive reinforcement in self-administered drugs.

Mechanisms involved in pituitary-adrenal responses to stress have also been implicated in the mediation of CTA (Riley, Jacobs & Lolordo, 1976;

Riley, Zellner, & Duncan, 1980). A large number of drugs used to induce CTA, result in the secretion of ACTH (Riley et al., 1976). Activation of the pituitary-adrenal axis has been found to occur when animals are forced to consume substances paired earlier with toxic substances (Ader, 1976; Smotherman, Hennessy, & Levine, 1976a, b). Avoidance of the paired taste, on the other hand, is not accompanied by this activation, as indicated by increased levels of plasma corticosterone (Smotherman et al., 1976b). Early handling, a treatment demonstrated to reduce the elevation of plasma corticosterone in response to novel stimuli observed in adult rats (Ader, 1970; Levine, Haltmeyer, Karas, & Denenberg, 1967) has also been shown to attenuate a lithium chloride CTA (Weinberg, Smotherman, & Levine, 1978). Administration of ACTH or one of its fragments devoid of steroidogenic activity ($ACTH_{4-10}$) has been found to delay the extinction of a CTA with lithium chloride (Smotherman & Levine, 1978). Pretreatment with $ACTH_{4-10}$ has also been shown to potentiate a lithium chloride CTA at a dosage with which otherwise no CTA was observed (Dray & Taylor, 1979). More recently, Sinyor, Switzman, and Amit (1980) found that simultaneous injection of ACTH along with morphine administration resulted in a potentiation of a sub-threshold dose of this drug to elicit a CTA. It is interesting to note, in the context of the previous discussion of CTA with self-administered drugs, that ACTH has also been implicated to play a role in mediation of morphine self-administration. For example, it was found by Amit, Ziskind, Gelfand, and Hébert, (1977) that administration of ACTH reinstated oral consumption in hypophysectomized rats. One possible explanation of ACTH effects in morphine-related behaviors has been that these may reflect altered attention to particular components of the narcotic cue (Colpaert, Niemegeer, Janssen, Van Rie, & DeWied, 1978). Clear interpretation of this hypothesis in rela-

tion to the "paradox" of CTAs induced by self-administered drugs as outlined before has not yet been articulated.

Environmental factors have also been found to modulate CTAs. Extinction of a lithium chloride CTA was shown to be specific to the environmental context in which the taste was initially paired with the drug administration, (Archer, Sjoden, Nilsson, & Carter, 1979). Krane (1980) has reported finding that environmental cues previously paired with drug-induced toxicosis subsequently served to block acquisition of a CTA taking place in the presence of these cues. In contrast, Stewart and Eikelboom (1978) examined the role of situational cues in drug pre-exposure effects on morphine CTA and analgesia. They found that while attenuation of morphine analgesia by pre-exposure to the drug was specific to the pairing environment, this situation-specificity was not observed with morphine-induced CTA. Moreover, while pre-exposure to morphine successfully attenuated formulation of a CTA with this drug regardless of the environmental context, when the pre-exposure drug administration was paired with a distinctive taste stimulus, this prevented the attenuation of a CTA to a second different taste stimulus. Procedural differences between these studies by Archer, Krane, and Stewart, and Eikelboom, make consolidation difficult. Most importantly, perhaps, whereas in the first two studies cited, lithium chloride is used, in the Stewart paper, morphine is used as the conditioning drug. The need for further research into the modulation of drug-induced CTAs by environmental factors is, however, clearly underlined by these studies.

Chronic aggregation conditions have recently been shown to affect a CTA with naloxone, an opiate antagonist. Naloxone was demonstrated to induce a CTA (LeBlanc & Cappell, 1975; Van der Kooy & Phillips, 1977). Pilcher and Jones (1981) differentially housed rats from time of weaning in either crow-

ded or non-crowded conditions. When these rats reached adulthood, a two-flavour discrimination procedure for conditioning taste aversion was administered. Results indicated an enhancement of the naloxone-induced CTA in those animals that had been reared in crowded conditions. In a second experiment, it was found that individual housing initiated three weeks prior to the conditioning procedure did not alter the enhancement by crowded housing exposure of the naloxone CTA. In view of the considerable evidence for altered responsiveness to psychoactive drugs in animals housed individually or in groups discussed in previous sections of this introduction, it would seem appropriate to further evaluate possible influences of differential housing in the CTA paradigm.

Overall Considerations

In summary, there is considerable evidence to suggest that exposure of animals to different social environments can alter their responses to drug administration. One critical component of this alteration in drug sensitivity appears to involve opiate-related mechanisms. Enhanced morphine analgesia is reported for mice reared in isolation (Bonnet et al., 1976; DeFeudis, et al., 1976; Kostowski et al., 1977), while isolated rats exhibit a decreased morphine analgesia (Kostowski et al., 1977). A positive correlation is found here with alterations in central opiate binding capacity in isolation-housed mice and rats (Bonnet et al., 1976; DeFeudis et al., 1978; Schenk et al., submitted for publication), although there is inconsistency in this effect across strains of mouse. Decreases in two signs of the morphine abstinence syndrome were reported in rats housed in isolation (Adler et al., 1975). Chronically isolated rats are reported to self-administer morphine to a greater degree than do chronically aggregated rats (Alexander et al., 1978; Haddway et al., 1979).

Another component of the pattern of alterations in drug sensitivity observed in animals chronically exposed to different social environments involves altered adrenocortical activity in these animals. For instance, Hatch et al., (1965) reports greater adrenocortical response to exogenously administered adrenocortical thyroid hormone (ACTH), as measured by plasma corticosterone levels, in chronically isolated rats. Isolated rats were also found by Parker and Radow (1974) to exhibit enlarged adrenal glands and were also found to voluntarily consume ethanol to a greater degree than did aggregated rats. Ellison (1981) recently replicated this finding. Baumel et al. (1970) reported reduced sensitivity to the sedative hypnotic, hexobarbital, in isolated mice as indicated by reduced drug-induced sleep-time in these animals.

Alterations in activity of central biogenic amines are also reported in animals exposed to different social environments (e.g., Modigh, 1973, 1974; Stolk et al., 1974; Welch & Welch, 1969; Weinstock et al., 1978). Enhanced sensitivity to amphetamine's stimulating effects on locomotor activity is reported in isolated mice (Del Pozo et al., 1978) and rats (Segal et al., 1973). In this latter mentioned study, elevated activity of the catecholamine enzyme, tyrozine hydroxylase and decreased activity of the serotonin enzyme tryptophan hydroxylase was reported in the isolated rats. Increased levels of brain norepinephrine were found in isolated rats (Stolk et al., 1974; Tanaka & Noda, 1974; Weinstock et al., 1978). Isolated mice are reported to exhibit decreased turnover rates of norepinephrine and serotonin (Garrattini et al., 1969; Welch & Welch, 1968).

Exposure to acute aggregation conditions is reported to enhance the toxicity of amphetamine in mice (e.g., Chance, 1946; Blackshear et al., 1979; Moore, 1964; Stolk & Rech, 1978) and in rats (Lokiec et al., 1978).

Factors hypothesized to be involved in this phenomenon of altered responses to amphetamine in aggregated animals include changes in: responsiveness of peripheral amine systems (e.g., Mennear et al., 1966; Moore, 1964), glycogen levels (e.g., Blackshear et al., 1979; Clark et al., 1967), thermoregulation (e.g., Askew, 1962; Menon & Dandiya, 1967) and central serotonin levels (Lokiec et al., 1978).

Exposure to acute aggregation is also reported to enhance morphine lethality in mice (e.g., Brister & Davis, 1974; Davis & Brister, 1971; Spoerlein, 1968) and in rats (Sklar & Amit, 1977). Brister and Davis (1974) reported that while pretreatment with an anticholinergic agent, pilocarpine, successfully protected aggregated mice from enhanced morphine toxicity, no such protection was realized for the enhanced lethality of amphetamine. Mohrland and Craigmill (1980) clearly demonstrated the importance of ambient temperature in the determination of this morphine aggregation toxicity phenomenon. While enhanced morphine toxicity was observed in aggregated mice at a room temperature of 29° C, no such enhancement was observed at a room temperature of 19° C. Also, it was found that by exposing isolated mice to continuous tactile stress following drug administration, an enhancement in lethality similar to that observed in the aggregated mice could be seen.

In a recent study by Pilcher and Jones (1981), a CTA induced by the opiate antagonist naloxone was found to be enhanced by crowded housing conditions maintained prior to conditioning. Within the CTA literature, an apparent "paradox" presently exists concerning the capacity of some drugs such as morphine and amphetamine to mediate positive reinforcement and also aversion (e.g., Reicher & Holman, 1977; Wise et al., 1976; Switzman et al., 1978). It is proposed in the present thesis that by providing acute exposure to different social environments to animals administered one of these

drugs, important additional information concerning the complex motivational properties of these drugs may come to light. It is presently unclear as to how possible alterations in the responsiveness of animals to drug exposure induced by acute exposure to different social environments may affect the capacity of such drugs as morphine to induce a CTA. This question is investigated in the first experiment of this thesis.

EXPERIMENT I

An enhancement of the excitatory effects of amphetamine in aggregated mice as compared to mice kept in isolation was first reported by Gunn and Gurd (1940). Chance (1946) subsequently reported finding a ten-fold increase in the lethal effects of amphetamine in aggregated mice. Since these initial studies first appeared, numerous reports have been published demonstrating that aggregation of mice potentiated the lethal effects of amphetamine (e.g., Lasagna & McCann, 1957; Moore, 1964; Mennear & Rudzik, 1961; Swinyard, Clark, Miyahana, & Wolf, 1961; Wang, Hasagawa, Peters, & Rimm, 1969). More recently, this phenomenon was reported in rats (Lokiec, Rapin, Jacquot, & Cohen, 1978), although an earlier study reported failure to find such an effect (Stolk & Rech, 1968).

Enhanced group toxicity of morphine is also reported in mice (Davis & Brister, 1971; Brister & Davis, 1974; Mohrland & Craigmill, 1980a, b; Spoerlein, 1968), and in rats (Sklar & Amit, 1977). There is one report of a failure to find enhanced morphine lethality in aggregated mice (Vedernikov, 1970), but comparisons of this study with other studies of acute aggregation effects is made problematic by the lack of an isolation-housed control group in this study.

A number of effects of chronic aggregation or isolation are also reported. These include changes in morphine-induced analgesia (e.g., DeFeudis, DeFeudis, & Somoza, 1976; Kostowski, Czlenkowski, Reverski & Piechocki, 1977), signs of morphine abstinence (Adler, Bendotti, Ghezzi, Samanin, & Valzelli, 1975), and self-administration of morphine (Hadaway, Alexander, Coambs, & Beyerstein, 1979), of cocaine (Hill & Powell, 1976), and of ethanol (Parker & Radow, 1974). Baumel, DeFeo, and Lal, (1970)

reported alterations in metabolism of hexobarbital in differentially housed animals. Differences between chronically aggregated and isolated animals are also reported in a naloxone-induced conditioned taste aversion (CTA) (Pilcher & Jones, 1981). In addition, an alteration in the density of brain opiate receptors due to chronic differential housing has been observed in mice (Bonnet, Hiller, & Simon, 1976) and in rats (Schenk, Britt, & Atalay, 1981).

Acute aggregation was demonstrated to enhance the lethality of morphine in rats at dosages which are clearly non-lethal in isolated rats (Sklar & Amit, 1977a). Morphine is also known to reliably induce a CTA (Cappell, LeBlanc, & Endrenyi, 1973; Farber, Gorman, & Reid, 1976; Sklar & Amit, 1977b). Moreover, the same morphine injection was also shown to induce a CTA as well as positively reinforce an operant behavior (White, Sklar, Amit, White, & Fishman, 1978). This phenomenon is also reported in regard to amphetamine (Reicher & Holman, 1977; Wise, Yokel, & deWit, 1976).

In the light of these findings, it would be interesting to examine whether exposure to acute aggregation might influence the ability of morphine to induce a CTA. No study to date has investigated the possible effects of acute exposure to different social environments on the ability of psychoactive drugs such as morphine to induce a CTA. Such an investigation may serve to elucidate the apparently complex motivational properties of these drugs. The present experiment investigates possible effects of acute aggregation following administration of morphine within a standard CTA paradigm.

Method

Subjects

Subjects were 130 male Wistar rats weighing 250-300 g at the start of

the experiment. The animals were individually housed in stainless steel cages with free access to Purina laboratory chow and water.

Drugs and Apparatus

Morphine hydrochloride (May and Baker Can. Ltd.) was dissolved in a vehicle of injectable Ringer's solution (Abbott Laboratories Ltd.).

Animals exposed to acute aggregation after drug administration were placed in large, opaque plastic baskets (26.5 cm x 26.5 cm x 22 cm).

Procedure

Following arrival from the breeding farm, a period of 7 days was observed to allow for adaptation of the animals to laboratory housing conditions. During this period, the animals were handled and weighed on three separate occasions in order to familiarize them with this procedure. On the next day, following this adaptation period, water was removed from the home cages and for the remainder of the experiment, the animals were maintained on a 23 hr and 30 min water deprivation schedule. For the following 7 consecutive days, tap water was available to the rats for a 30 min period between 1000 and 1200 each day in the home cage. The water was presented in stoppered plastic test tubes fitted with stainless steel ball-bearing spouts inserted through the front of the cage. The rats were weighed on two separate days during this period.

On day 8 (first conditioning day) the animals were presented with a 0.1% saccharin solution as their drinking fluid for a 30 min period. Fluid consumption was measured to the nearest ml. Following within a minute of the termination of the drinking period, animals were intraperitoneally (ip) injected with either Ringer's solution (n = 40) or one of three dosages of morphine. The morphine dosages administered were 8 mg/kg (n = 30), 15 mg/kg (n = 30), or 21 mg/kg (n = 30). Immediately after injection, half of the

animals in each treatment group were returned to their respective home cages, and the remaining animals in each group were placed in plastic baskets in groups of five. These aggregated animals remained in the baskets for the following 2 hr and 15 min after which time they were returned to their home cages. The injection volume for all treatment groups was 1 ml/kg body weight.

For 3 days following the first conditioning day, tap water continued to be available for 30 min daily drinking periods. On day 12, the fourth day after initial conditioning, the saccharin solution was again presented to the animals, and the identical procedure was subsequently conducted as on the first conditioning day (day 8). On the ensuing 3 days, tap water was again presented. On day 16, the saccharin solution was once more presented to the animals.

Results

Saccharin intake for each test day, expressed as a percentage of change from baseline levels, is presented in Figure 1. Baseline levels were determined for each rat by their individual levels of fluid consumption on the first day of saccharin presentation (day 8) and percentage change from these values were then calculated for individual levels of fluid intake on the subsequent saccharin test days (days 12 and 16). Two animals in the isolated-saline group and one in the isolated-morphine (15 mg/kg) group fell ill during the course of the initial water deprivation period. A fourth animal in the aggregated-morphine (21 mg/kg) group died at the time of the first aggregation. Data for these animals were dropped from the statistical analysis.

A three-way ANOVA indicated a significant interaction between dosage and days ($F(3,118) = 5.69$, $p < .0011$) and significant main effects for dosage ($F(3,118) = 11.06$, $p < .0000$) and days ($F(1,118) = 8.97$, $p < .0034$). No significant difference was found between aggregated and isolated groups ($F(1,118) = .17$, $p < .6774$).

Individual two-way ANOVAs were also conducted for each dosage. In the saline group, no significant change was found in saccharin intake over test days ($F(1,36) = 2.48$, $p < .1239$) nor was there any significant effect due to aggregation ($F(1,36) = .0097$, $p < .9223$). In the groups administered morphine, significant decreases in saccharin intake were found at the dosages of 8 mg/kg ($F(1,28) = 21.63$, $p < .0001$) and 15 mg/kg ($F(1,27) = 5.10$, $p < .0323$). However, no change in saccharin intake was indicated at the 21 mg/kg dose of morphine ($F(1,27) = .65$, $p < .4269$).

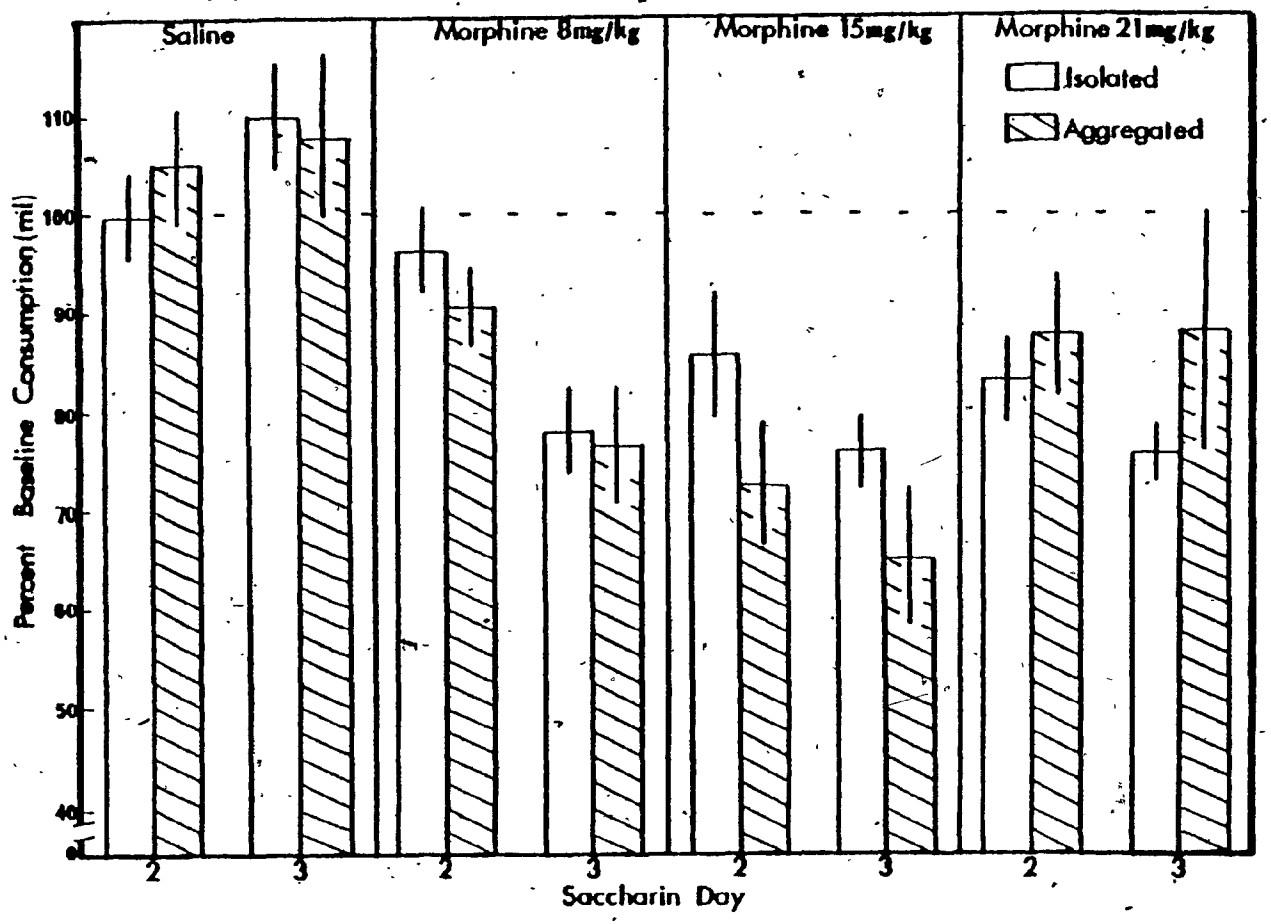

Figure 1. Percentage of baseline saccharin intake for aggregated and isolated rats receiving injections of saline or morphine on saccharin days 1 and 2.

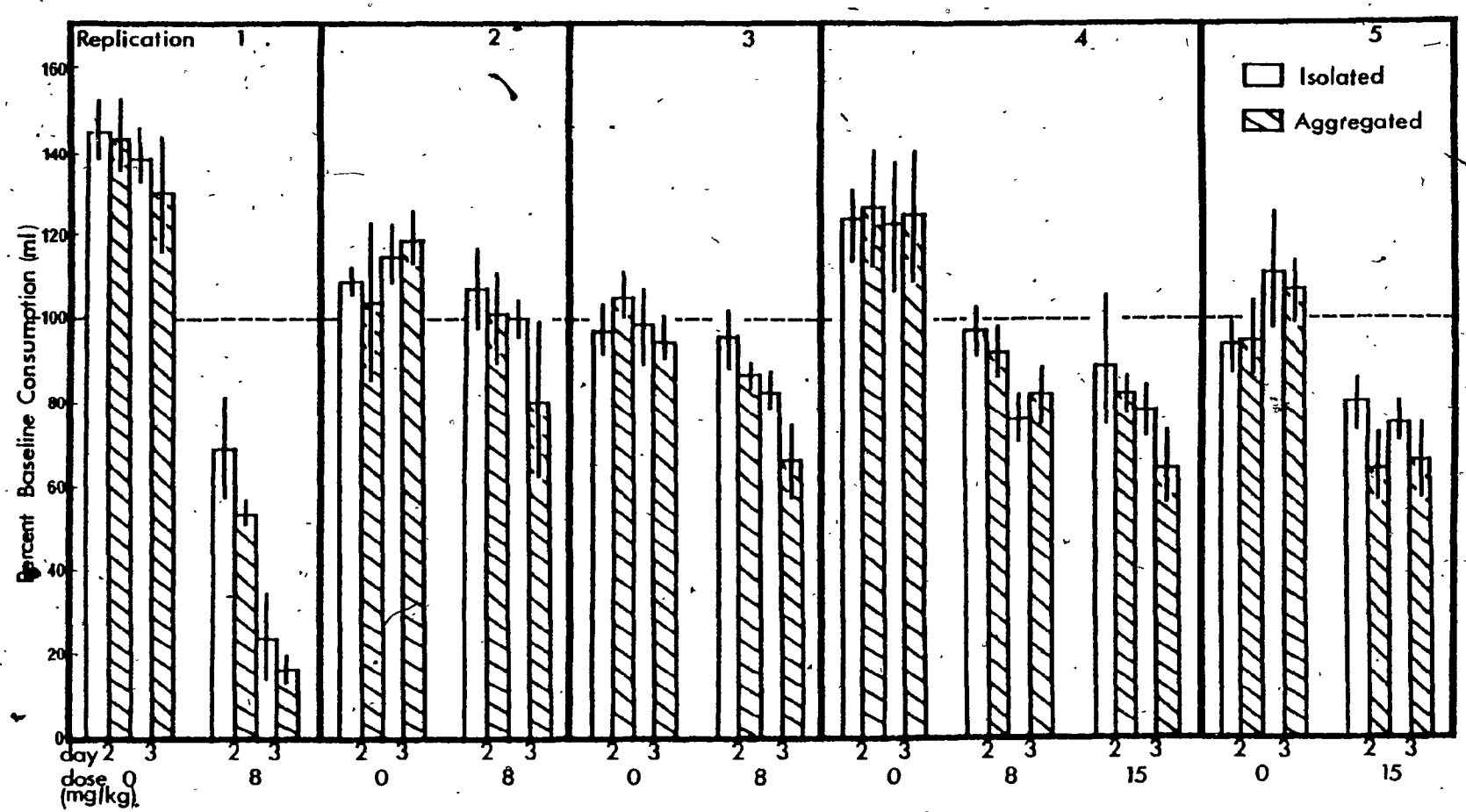

Figure 2. Percentage of baseline saccharin intake for aggregated and isolated rats receiving injections of saline or morphine on saccharin days 1 and 2 considered across five independent replications.

No significant effect was found for aggregation either at the 8 mg/kg ($F(1,28) = .44$, $p < .5148$), 15 mg/kg ($F(1,27) = 2.74$, $p < .1095$) or 21 mg/kg ($F(1,27) = .86$, $p < .3608$). The raw data for this experiment appear in Appendix A.

Although a statistically significant effect of aggregation was not found in the present experiment, the same pattern of aggregation-induced enhancement of a morphine CTA has been observed across five separate replications (see Figure 2). Data obtained from Replications 3, 4, and 5 were later combined to make up the data base of Experiment II. Exactly the same procedure was followed on each of these three separate occasions, with animals of the same "new colony" Wistar strain (Canadian Breeding Farm and Laboratories Ltd.) being exposed to identical experimental conditions. In Replication 3, data for Ringer's and morphine (8 mg/kg) groups ($n = 5$) for both aggregated and isolated conditions are presented. In Replication 4, data for Ringer's groups for aggregated ($n = 5$) and isolated ($n = 4$) conditions are presented as well as for morphine (8 mg/kg) aggregated ($n = 10$) and isolated ($n = 10$) conditions and for morphine (15 mg/kg) aggregated ($n = 5$) and isolated ($n = 4$) animals. In Replication 5, data are presented for Ringer's groups for both aggregated ($n = 10$) and isolated ($n = 9$) conditions as well as for morphine (15 mg/kg) groups ($n = 10$). In Replications 1 and 2, an identical experimental procedure was followed as was presented for Experiment II of this thesis with the exception that five days (instead of three days) intervened between the first and second saccharin exposures and only two days (instead of three days) elapsed between the second saccharin presentations and the final saccharin exposures. Additionally, in Replication 1, it is not clear whether the Wistar rats used in the study were from the "new colony" or "old colony" breeding condi-

tions recently instigated at Canadian Breeding Farms. "New colony" rats are raised in a more pathogen-free environment and are handled less frequently than "old colony" animals (personal communication, Canadian Breeding Farms). In Replication 1 Ringer's and morphine (8 mg/kg) groups were run for both aggregated (n = 10) and isolated (Ringer's: n = 10, morphine: n = 7) conditions. In Replication 2, Ringer's and morphine (8 mg/kg) groups were run for aggregated (n = 5) and isolated (Ringer's n = 4; morphine n = 7) conditions using the "new colony" Wistar animals.

As no difference in the strength of the aggregation-induced alteration of a morphine CTA was evident for 8 mg/kg and 15 mg/kg doses, these data were considered together. A chi square analysis (Winer, 1971) of the percent baseline data for each saccharin test day over the five independent replications was then performed. This was done under the hypothesis that the observed probabilities associated with the levels of saccharin consumption of aggregated as compared to isolated animals are a random sample from a population of probabilities having a mean of .50. The observed probabilities of obtaining a greater reduction in saccharin intake in aggregated as versus isolated animals on each of the five replications on each of the two test days were calculated using individual t-tests. These data were then tested against a one-tailed alternative hypothesis. This analysis revealed a significant effect of aggregation in the morphine-treated animals on saccharin day 2 (X^2 = 19.26, df = 10, $p < .05$). An effect of aggregation approached but did not reach statistical significance on saccharin day 3 for the drug-exposed group (X^2 = 16.54, df = 10, $p < .10$). No differences were found for the saline-treated control animals for saccharin day 2 (X^2 = 10.10, df = 10, $p < .49$) or saccharin day 3 (X^2 = 9.72, df = 10; $p < .49$).

Discussion

It appears from the data collected in this experiment that acute aggregation has no effect on a morphine-induced CTA. A significant reduction in saccharin intake from baseline was recorded for the 8 mg/kg and 15 mg/kg morphine dosages, indicative of effective CTAs. In the most effective dosage of 15 mg/kg, exposure to acute aggregation appeared to enhance the CTA, although this did not reach statistical significance. This pattern of CTA enhancement by exposure to acute aggregation also was apparent to a lesser degree at the 8 mg/kg dosage, although again this was not statistically significant. This same pattern of acute aggregation-induced enhancement of morphine CTA has been replicated at this dosage level on five previous occasions in pilot studies and appears therefore to be a reliable effect, albeit one in which differences between scores of isolated and aggregated animals are not established statistically on any one occasion.

Several factors may help to explain the failure to establish a strong effect of aggregation within the present paradigm. First, Welch and Welch (1966) reported the chronic housing conditions prior to an acute aggregation or isolation manipulation served to influence the effect of this manipulation on the lethality of delta-amphetamine in mice. While enhanced lethality was observed in acutely aggregated mice as compared to those placed in isolation after drug administration, those aggregated animals which had been housed in isolation for a period of 5 weeks prior to the experiment died up to 8.5 times faster than did those that had lived in groups for this same period. It may be that, in the present experiment the period of isolation prior to the onset of the experiment was not sufficiently prolonged to enable observation of maximal effects of the exposure of acute aggregation. Second, there have been several reports emphasizing differ-

ences in strain and species in differential housing effects. For instance, Bonnet et al. (1976) reported reduction in opiate binding in chronically isolated mode of the C57B1/J7 strain, while in Swiss albino mice, an increase in opiate binding was reported. Chance (1947) reported differences between strains of mouse in the degree of enhanced toxicity seen in aggregated animals. Stolk and Rech (1968) reported species differences in amphetamine toxicity seen in acutely aggregated rats and mice. Thus it may be important in the context of the present study to investigate the acute aggregation phenomenon in other strains of rat. Third, it can be seen from the results of the present experiment that a high degree of variability in the saccharin intake scores over the test was present, making it problematic to clearly establish any statistically significant differentiation between the treatment groups. This phenomenon of relatively high degrees of variability in a morphine-induced CTA as compared to CTAs induced by non self-administered drugs such as lithium chloride has been noted elsewhere in the literature (e.g., Gorman, De Obaldia, Scott, & Reid, 1978). Also, the absence of a graded effect in which higher morphine doses would induce stronger CTAs than at lower doses, as evidenced by the lack of a reliable reduction in saccharin intake at the 21 mg/kg dose, is a phenomenon which has been previously reported (Riley, Jacobs, & LoLordo, 1978). This aspect is most evident in the case of the 21 mg/kg morphine dosage which clearly appears to demonstrate a reduction in saccharin intake (see Figure 1) although this reduction did not reach levels of statistical significance.

Another interesting aspect of the pattern of saccharin intake at the 21 mg/kg dosage is the apparent reversal of the trend of possible CTA enhancement by aggregation discussed above. This is of particular interest in that one of the rats administered this dosage died during initial aggre-

gation. The facts appear to argue for a disassociation of the effects of acute aggregation on morphine-induced lethality and possible effects of acute aggregation on morphine-induced CTA. However, a low baseline saccharin intake for one animal in the aggregated group resulted in inflated scores on the ensueing test days so that while the aggregated group scores are not lower than isolated group scores, it can not be irrevocably stated that a reversal from the pattern of the lower dosages scores was indicated even at a statistically non-significant level.

While not reaching statistical significance, in the present study, a mild yet reliable effect of acute aggregation is clearly indicated by the data presented from five independent replications. It seems clear that the possible effects of acute aggregation on CTAs induced by psychoactive drugs merit further investigation. In the following experiment, an investigation is conducted into the possible influence of acute aggregation on a CTA induced by lithium chloride, a non self-administered psychoactive drug.

EXPERIMENT II

In the previous experiment, enhancement of a morphine-induced CTA was indicated across a series of five independent replications in those animals exposed to acute aggregation (as opposed to being kept in isolation) follow-drug administration. The high variability typical of this kind of data may have contributed to the failure to achieve statistical significance in any single experiment.

The present study investigates the effects of acute aggregation upon a CTA induced by lithium chloride (LiCl). LiCl is an emetic drug, with central and peripheral effects, which is commonly used within the CTA literature (Riley & Clarke, 1977). In contrast to morphine, there are no reports of LiCl being self-administered. It has been shown to possess unpleasant side-effects as indicated, for example, by sedation and diarrhoea observed in animals exposed to the drug (e.g., Garcia & Koelling, 1967; Nachman & Ashe, 1973). Specifically, LiCl is reported to exhibit several properties which make it particularly advantageous for an investigation of effects of acute aggregation on a CTA induced by this drug. First, LiCl induces a CTA which is greater in magnitude and is less variable than a CTA induced by morphine (Gorman, Ricardo, De Obaldia, Scott, & Reid, 1978; Riley, Jacobs, & LoLordo, 1978). Possible modulation of a LiCl-induced CTA by acute aggregation may therefore by easier to detect. Second, Sklar and Amit (1977) reported that pharmacological disruptions of catecholaminergic systems which block a morphine CTA failed to block a CTA induced by LiCl. These same pharmacological disruptions are also reported to attenuate morphine's positively reinforcing properties (e.g., Davis & Smith, 1972,

1974; Glick, Zimmerberg, & Charap, 1973; Meade & Amit, 1974). For instance, pretreatment of rats with alpha-methyl-para-tyrosine (AMPT, a tyrosine hydroxylase inhibitor) blocked a morphine CTA but failed to block a LiCl CTA. Pretreatment with AMPT is also known to attenuate self-administration of morphine in rats (e.g., Davis & Smith, 1972; Glick et al., 1973). The findings cited above, then, suggest a significant difference between CTAs induced by morphine and LiCl. Investigation of the effects of acute aggregation on CTAs induced by morphine and LiCl may serve to further delineate this difference. Furthermore, this study may result in a clearer understanding of the possible effects of acute aggregation upon the behavioural properties of psychoactive drugs.

Method

Subjects

Subjects were 60 male Wistar rats weighing 250-300 g at the start of the experiment. The animals were individually housed in stainless steel cages with free access to Purina laboratory chow and water.

Drugs and Apparatus

Lithium chloride (Abbott Laboratories Ltd.) was dissolved in a vehicle of distilled water. Animals exposed to acute aggregation after drug administration were placed in large (26.5 cm x 26.5 cm x 22 cm) plastic baskets as in Experiment I.

Procedure

The procedure used in this experiment was the same as that used in Experiment I. Fifteen animals were randomly assigned to each of the four treatment groups. A 15 ml/kg dose of lithium chloride (0.15 molar solution) was administered intraperitoneally. Control animals received an injection of distilled water in the same 15 ml/kg volume. Half of the animals in both

the LiCl and control groups were immediately returned to their respective home cages following injection. The remaining animals were placed in plastic baskets in groups of five for a period of 2 hrs and 15 minutes following injection.

Results

Levels of fluid intake for each day of saccharin presentation are presented in Figure 1. Baseline values and percentage change from these values are calculated as in Experiment 1.

Due to illness, six animals were eliminated from the study prior to the first conditioning day. Due to a procedural error, a seventh animal exposed to both isolated and aggregated conditions was also eliminated from the statistical analysis.

A three-way ANOVA performed on the data for the remaining 55 rats revealed significant main effects of drug ($F(1,51) = 70.36$, $p < .0000$) and of days ($F(1,51) = 20.33$, $p < .0000$). No significant difference between aggregated and isolated animals was observed ($F(1,51) = .41$, $p < .6250$). The interaction between aggregation and drug factors also failed to reach significance ($F(1,51) = 1.44$, $p < .2350$). Raw data are presented in Appendix B.

Discussion

A clear reduction in saccharin intake over the three days of saccharin presentation in animals receiving LiCl indicates the formation of a strong CTA. Comparison with the data in Experiment 1 demonstrates that the CTA induced by LiCl is greater in magnitude than that induced by morphine. These findings support earlier reports of CTAs induced by morphine and LiCl (e.g., Gorman et al., 1978; Riley et al., 1978).

In contrast to the findings reported in Experiment 1 with regard to morphine, no trend of aggregation-induced CTA enhancement was observed in animals given LiCl in the present experiment. Indeed, a slight tendency toward an aggregation-induced attenuation of the LiCl CTA is suggested by the data for saccharin day 3, although this was not significant statistically.

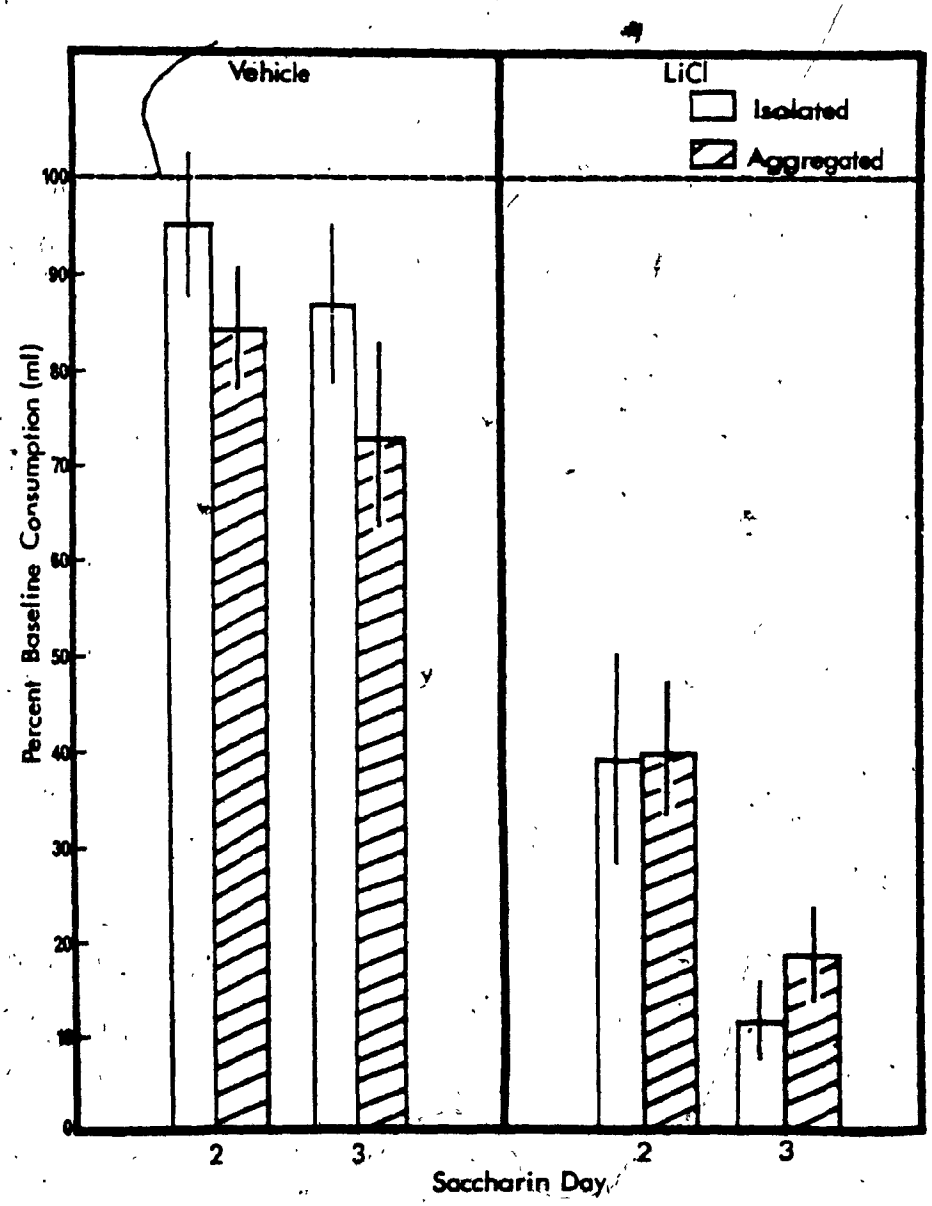

Figure 2. Percentage of baseline saccharin intake for aggregated and isolated rats receiving injections of vehicle or LiCl on saccharin days 1 and 2.

Two possible explanations of this apparent contrast in aggregation effects for morphine and LiCl-induced CTAs may be established by considering differences in neurochemical mechanisms underlying the particular effects of these two drugs. One hypothesis would suggest that the different degree of catecholaminergic involvement in mediating the aversive properties of LiCl and morphine (Sklar & Amit, 1977b) may help explain the distinct difference in aggregation effects observed in the present studies. Sklar and Amit (1977b) reported that pharmacological disruption of catecholaminergic systems by pretreatment with such drugs as AMPT blocked a CTA induced by morphine but did not affect a LiCl-induced CTA. This same pharmacological disruption was found to attenuate morphine's positively reinforcing properties (e.g., Davis & Smith, 1972; Glick et al., 1973). There are also studies reporting a direct relationship between morphine's positively reinforcing properties and its ability to induce a CTA (Switzman et al., 1978; White et al., 1977). In these studies, the same morphine injection was found to mediate both positive reinforcement (indicated by running down an alleyway) and aversion (indicated by induction of a CTA). Before further elaboration of the hypothesis of catecholaminergic involvement in the reversal of acute aggregation effects in morphine and LiCl-induced CTAs would be justifiable, it would appear to be necessary to examine effects of acute aggregation on LiCl CTA at several doses lower than that used in the present experiment. It is possible that, at these lower doses, an enhancement rather than a possible attenuation, of the LiCl-induced CTA may be observed in aggregated animals. This might then lead to a second possible explanation of the divergent findings in Experiments I and II. A model for such an explanation may be found in studies of adrenocortical activity induced by morphine and LiCl in relation to their respective capacities to induce a CTA (e.g., Hennessy,

Smotherman, & Levine, 1976, 1980; Rigter & Popping, 1976; Riley, Zellner, & Duncan, 1980). It has been postulated that the release of adrenocorticotrophic hormone (ACTH) may play a critical role in the formation of drug-induced CTAs (Braveman, 1977; Riley et al., 1976, 1978; Riley et al., 1980). For instance, it is reported that preexposing a rat to a particular drug attenuates a subsequent CTA induced by that drug (e.g., Braveman, 1975; Cappell, Leblanc, & Herling, 1975). Riley et al. (1976) argue that this CTA attenuation may be mediated by decreased levels of drug-induced ACTH release observed in animals previously exposed to the drug. This argument is, however, based on correlational data alone. Riley et al. (1978) also reported that the relative weakness of a morphine CTA as compared to a LiCl CTA may be attributable to relatively lower levels of plasma corticosterone (indicative of ACTH release) associated with morphine administration. Support for this argument is found in reports that ACTH administration prior to extinction trials delays extinction of a LiCl-induced CTA (e.g., Hennessy et al., 1976, 1980; Smotherman & Levine, 1978; Rigter & Popping, 1976). Additionally, however, while administration of dexamethasone (which suppresses ACTH release) just prior to conditioning was found to attenuate a LiCl CTA, no effect was found with ACTH treatment at time of conditioning (Hennessy et al., 1976, 1980).

In contrast, Sinyor, Switzman, and Amit (1980) report potentiation of a morphine CTA with ACTH administration at time of conditioning. Also Sinyor (1979) found no effect of dexamethasone pretreatment on a morphine CTA. Thus from the studies just cited above, it can be seen that alterations in ACTH levels can serve to modulate CTAs induced by LiCl and morphine. But it appears that these effects are distinctly different for each drug. There are also reports of biphasic effects of ACTH. Amir, Galina, Blair, Brown,

and Amit (1980) found that low doses of ACTH stimulated locomotor activity while high ACTH doses suppressed this activity. Sands and Wright (1979) studied rats' performance in a Y-maze task of active avoidance and discovered that a low ACTH dose enhanced, while a high ACTH dose disrupted, retention of a previously learnt behaviour. In congruence with these findings of ACTH effects, it may be that the possible reversal of acute aggregation effects observed in this thesis in regard to morphine and LiCl CTAs may be mediated, at least in part, by a difference in ACTH activity associated with these two drugs. The aggregation-induced enhancement of morphine-induced CTAs presented in Experiment I may be mediated by an aggregation-induced elevation in ACTH levels, in accordance with the findings of Sinyor et al. (1980). Due to the relatively greater drug-induced ACTH release reported with LiCl administration (Riley et al., 1978), the LiCl-treated aggregated animals may be subject to an elevation in ACTH levels which might be expected to surpass some hypothetical critical threshold. This, then, might tentatively be expected to result in an attention -- rather than an enhancement -- of the LiCl CTA in accord with the inverted U-shape patterns of ACTH response reported by Amir et al. (1980) and Sands and Wright (1979). This hypothesis must be considered, however, to be purely speculative at this time. For example, Hennessy et al. (1978, 1980) failed to find any attenuation of a LiCl CTA with ACTH administered at the time of initial conditioning.

Two additional factors should be taken into consideration in the interpretation of the data presented in Experiment 2. The first such factor is constituted in the reduction in saccharin intake of the control animals given injections of the LiCl vehicle. This unexpected trend may reflect the effects of illness unrelated to the drug treatment which was observed in a number of the rats used in this study. Unfortunately, no systematic record

was kept for individual animals of the signs of illness such as diarrhoea and minor respiratory difficulties observed in some of the rats. Such systematic observation should be made in future studies to control for this possible confound. A second consideration which may be important in achieving a comprehensive appraisal of the data of Experiment 2 is to be found in the extremely low levels of saccharin intake observed in the drug-treated animals on saccharin day 3. This may constitute a situation in which any further reduction in saccharin intake to be expected in either aggregated or isolated animals would not be realizable. For example, after two conditioning days using a similar LiCl dose, several experimenters failed to report reductions of a novel tasting fluid to levels of under two milliliters (e.g., Gorman et al., 1978; Nachman & Ashe, 1973; Riley et al., 1976).

Although no definitive explanation of the divergent findings in Experiments 1 and 2 can be attempted at present, it nevertheless is apparent that acute environmental manipulations may differentially influence the motivational properties of psychoactive drugs such as lithium chloride and morphine. Such a finding has important implications. As discussed above, further understanding of this phenomenon may well lead to greater appreciation of the critical pharmacological mechanisms underlying the important differences between behavioral effects of drugs such as morphine and lithium chloride.

General Discussion

In Experiment I of this thesis, data was presented from five independent replications which indicated that the magnitude of a morphine-induced CTA in rats was found to be enhanced by acute aggregation of the animals immediately following the drug administration. This phenomenon was observed at morphine doses of 8 mg/kg and 15 mg/kg. At the 21 mg/kg morphine dose, a slight attenuation of the morphine-induced reduction in saccharin fluid consumption was observed in aggregated animals. However, a morphine-induced CTA was not evident at this dose. In addition, one aggregated animal died following injection of the 21 mg/kg morphine dose. In Experiment II, the magnitude of a LiCl-induced CTA was found to be unchanged or even slightly attenuated in rats aggregated following the LiCl administration. The greater overall magnitude of the CTA resulting from this second conditioning trial may have served to mask any attenuating effect of the aggregation exposure.

It therefore appears from these studies that exposure to acute aggregation enhances a morphine-induced CTA while serving to attenuate a CTA induced by LiCl. At present it is not clear to what factors this divergence of acute aggregation effects can be properly attributed. However, it is known that pharmacological disruptions of central catecholaminergic systems serve to block formation of a morphine-induced CTA while these do not affect formation of a CTA induced by LiCl in rats (Sklar & Amit, 1977b). So, for example, formation of a morphine CTA, but not a LiCl CTA, was found to be blocked by pretreatment with AMPT. Thus, it is conceivable that acute aggregation serves to enhance a morphine CTA by acting on the central catecholaminergic system, while serving to attenuate a LiCl CTA by acting on

some other, perhaps peripheral, system. This hypothesis must remain purely speculative at this time however. A second, more likely, explanation for the reversal of aggregation effects across the CTAs induced by LiCl and morphine involves consideration of the adrenocortical activity resulting from exposure to each of these two drugs. Riley et al. (1978) reported that an acute administration of LiCl results in a relatively high elevation of plasma corticosterone levels (indicative of ACTH release). In contrast, these authors found that acute morphine administration results in only a comparatively low elevation in levels of plasma corticosterone. Sinyor et al. (1980) reported a potentiation of a CTA induced by a sub-threshold dose of morphine when ACTH was injected at the time of the morphine administration. This then suggests that the aggregation-induced enhancement of a morphine CTA reported in Experiment I may be mediated by increases in ACTH release. The basis for an explanation of the possible reversal of the aggregation effect on a LiCl CTA, as presented in Experiment II, may be found in two recent studies. An inverted U-shaped dose-response curve for ACTH administration was found on measures of locomotor activity (Amir et al., 1980) and active avoidance (Sands & Wright, 1979). Low ACTH doses were found to stimulate locomotor activity, while high ACTH doses suppressed this activity (Amit et al., 1980). Similarly, low doses of ACTH were found to enhance retention of a previously learnt avoidance response, while high doses disrupted this retention (Sands & Wright, 1979). Thus, it is possible to argue that a similar inverted U-shaped dose-response function for ACTH may underlie the reversal of aggregation effects from enhancement to attenuation across the CTAs induced by morphine and LiCl respectively. Such an argument is supported by the findings of Riley et al. (1978), cited above, in which low ACTH release was associated with morphine exposure while considerably higher

ACTH release was associated with exposure to LiCl. However, before this model can be firmly established in order to explain the results of Experiments I and II, several issues must be addressed. First, the assumption is made that acute aggregation enhances release of ACTH in previously isolated rats. This has not yet been established. A biochemical assay is presently being conducted in order to find out whether this assumption is valid or not. Second, it is reported that ACTH administration at time of conditioning has no effect on a LiCl-induced CTA (Kendler et al., 1976; Hennessy et al., 1980). However, in these experiments ACTH was administered prior to presentation of the taste exposure. It would appear to be important to investigate effects of ACTH administration given following the taste exposure, that is, at the time of LiCl administration. This would then follow the procedure used by Sinyor et al. (1980) in their investigation of ACTH's effect on a morphine CTA. Finally, it would be important that the effect of acute aggregation on lower doses of LiCl also be investigated in order to find out how consistent the attenuation effect may be within a full range of LiCl doses. Such information would help to clarify what factors may be critically involved in the reversal of aggregation effects on morphine and LiCl CTAs.

From the above considerations, it can be seen that the reversal of aggregation effects observed in Experiments I and II carries important theoretical implications in regard to the mechanisms possibly underlying CTA induction by morphine and LiCl. The findings of Experiment I also represent an important extension of previous reports on aggregation-induced alterations of opiate-related effects. Acute aggregation is reported to enhance the lethality of morphine in mice (e.g., Brister & Davis, Davis & Brister, 1971; Mohrland & Craigmill, 1978, 1980), and rats (Sklar & Amit, 1977a). Effects of chronic aggregation are also reported in relation to

morphine-induced analgesia (e.g., DeFeudis et al., 1976; Kostowski et al., 1977), signs of morphine abstinence (Adler et al., 1975) and self-administration of morphine (Alexander et al., 1978; Hadaway et al., 1979). Recently, Pilcher and Jones (1980) reported an enhancement of a CTA induced by the opiate antagonist, naloxone, in rats previously housed in crowded conditions. The results of Experiment I, then, represent an important extension of the scope of these studies. The enhancement of a morphine CTA in rats by acute aggregation provides additional evidence that alterations in social environment can influence the motivational properties of morphine, as was previously demonstrated in the studies by Alexander et al., (1978) and Hadaway et al. (1979). The results of Experiment I also add to the knowledge concerning effects of acute aggregation on the responsiveness of animals to morphine (e.g., Brister & Davis, 1974; Davis & Brister, 1971; Mohrland & Craigmill, 1978, 1980; Sklar & Amit, 1977a).

Another aspect of the findings presented in Experiment I, which may be of potential theoretical significance, involves the nature of the effects at the highest morphine dose. Although a reduction of saccharin fluid consumption was observed for both isolated and aggregated groups, these reductions did not reach statistical levels of significance. Thus in contrast to the two lower morphine doses, the high morphine dose used in this study induced, at best, only a weak CTA. This finding is congruent with the study by Riley et al. (1978) who reported an absence of a graded effect of morphine dose for induction of CTA. In contrast, increasing doses of LiCl are reported to exhibit increasing CTA induction (Nachman & Ashe, 1973). Additionally, in the findings of Experiment I, a potential aggregation-induced enhancement of morphine lethality was observed at the highest morphine dosage. This would be in agreement with the aggregation-induced

enhancement of morphine lethality reported by Sklar and Amit (1977a). Taken together, these two dose-related patterns of morphine-induced CTA and lethality would appear to argue in favour of a disassociation of morphine's aversive (as indicated by induction of CTA) and lethal effects. Such a disassociation would be in accord with reports of earlier studies within the CTA literature. Berger (1972) demonstrated that only a poor correlation could be established for overt behavioral signs of drug-induced toxicity (e.g., sedation, diarrhoea) and capacity of the drug to induce a CTA. Ionescu and Buresova (1977) found that severe poisoning by drugs such as cyanide, failed to induce a CTA. Also, psychoactive drugs such as amphetamine and morphine, which are self-administered by animals (e.g., Yokel & Pickens, 1974; Weeks & Collins, 1964) are also known to induce a CTA (e.g., Cappell et al., 1973; D'Mello et al., 1977). Moreover, the same single injection of each of these drugs is reported to mediate both positive reinforcement and aversion within the same paradigm (e.g., Switzman et al., 1978; Wise et al., 1976). It appears, then, from the data presented in Experiment I, that effects of acute aggregation on a CTA induced by morphine cannot be simply a function of acute aggregation enhancement of morphine's lethal properties. Additional evidence in support of this contention is to be found within the literature on acute aggregation-induced enhancement of morphine lethality. Davis and Brister (1971) reported that AMPT pretreatment failed to block aggregation-induced enhancement of morphine toxicity in mice. However, as previously mentioned above, Sklar and Amit (1977b) reported that AMPT pretreatment successfully blocked a morphine CTA in rats. Thus it appears likely that clear differences in mechanism exist for aggregation-induced enhancement of morphine lethality and of morphine CTA.

A tendency was observed for the aggregated animals receiving the high

morphine dose to exhibit a slightly attenuated reduction in saccharin intake in comparison to isolated animals receiving this dose. This pattern of aggregation-induced attenuation of CTA, then, is similar to that found in Experiment II in which the aggregated animals receiving LiCl also exhibited an attenuated CTA. It would be tempting, therefore, to suggest that the physiological mechanisms mediating the reduction in saccharin intake observed in those animals receiving the high morphine dose may be comparable to those mechanisms mediating a LiCl-induced CTA. It may be, then, that for instance, peripheral mechanisms implicated in LiCl-induction of CTA (e.g., Gorman et al., 1978) are also involved in mediating the reduction of saccharin intake observed in animals receiving the high morphine dose. However, such speculation must await more statistically reliable validation.

Finally, it would seem important to examine the phenomenon of acute aggregation effects on CTAs induced by morphine and LiCl in another strain of rat in addition to the one studied in the present experiments. While strain differences have not been emphasized within the acute aggregation literature, such genetic differences are reported in studies of chronic aggregation or isolation. For instance, Bonnet et al, (1976) reported important differences between strains of mouse in the effects of chronic isolation on morphine CTA. While Swiss albino mice demonstrated increased morphine analgesia, this effect was not established in mice of the C57B1/65 strain. Also, in Experiment II, a number of rats were eliminated from the statistical analysis due to sickness. It would appear to be important to replicate this study using a different strain of rat which would be, amongst other things, hopefully, less vulnerable to disease.

It can be seen from the findings of Experiments I and II that acute response of rats to conditions of social aggregation can differentially

affect the responsiveness of these animals to formation of CTAs induced by morphine and LiCl. A marked difference in the aggregation-induced alteration of CTA was observed in rats receiving morphine as versus those receiving LiCl. It appears, therefore, that an important difference exists in the physiological and/or neurochemical mechanisms underlying the induction of CTAs by these two psychoactive drugs. Further investigation is required, however, in order to firmly establish what may constitute the nature of this difference. Nevertheless, it is clear that consideration of the effect of altered social environments on the motivational properties of psychoactive drugs such as morphine and LiCl promoses to lead to meaningful insights into the processes underlying these critical drug properties.

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Appendix A

Raw Scores for Experiment I

Table 1

Levels of Fluid Intake (ml) from the Day Prior to the First Saccharin Injection Pairing Onward

Group	Day 7	8[a]	9	10	11	12[a]	13	14	15	16[a]
Saline (Isolated)	13	16	12	13	11	12	14	19	14	12
	21	24	21	24	22	24	23	20	18	29
	19	19	18	20	18	22	17	22	19	25
	19	22	19	18	21	21	21	20	16	22
	17	20	21	20	20	20	20	20	19	30
	18	20	16	19	20	20	18	17	15	20
	16	19	14	14	19	21	17	18	15	25
	19	21	17	16	15	15	12	14	5	15
	16	15	14	11	14	13	15	20	15	18
	21	22	24	18	19	20	18	18	20	23
	22	30	20	18	19	25	18	17	20	26
	22	21	18	18	18	24	17	18	20	20
	22	23	20	17	20	23	16	15	20	21
	18	19	16	17	18	20	17	15	17	22
	19	18	15	15	17	20	12	16	14	20
	12	12	9	9	15	17	14	16	18	20
	15	17	17	17	20	21	23	14	15	17
	16	17	17	15	18	20	15	17	15	19

Table 1

(Continued)

Group	Day									
	7	8[a]	9	10	11	12[a]	13	14	15	16[a]
Morphine 8 mg/kg (Isolated)	22	28	17	16	23	22	16	18	18	20
	14	25	18	21	18	24	15	20	21	23
	22	22	17	16	16	20	17	17	19	17
	23	21	20	20	20	23	17	14	20	20
	20	21	19	18	17	22	17	17	18	16
	21	22	17	19	24	23	20	20	20	24
	19	20	15	12	15	18	15	13	15	14
	18	21	11	13	15	20	12	17	14	14
	17	19	20	12	19	22	15	16	17	18
	18	23	14	18	16	19	12	15	16	13
	15	17	15	15	15	13	12	18	15	14
	17	16	19	12	18	20	12	15	13	8
	15	18	13	17	18	21	13	19	15	18
	20	21	15	15	18	17	15	16	16	12
	21	22	12	14	20	18	14	11	20	16

Table 1

(Continued)

Group	Day									
	7	8[a]	9	10	11	12[a]	13	14	15	16[a]
Morphine 15 mg/kg (Isolated)	19	21	20	16	21	22	17	17	15	15
	21	22	17	20	21	18	18	19	17	20
	16	24	20	20	19	20	17	20	18	21
	20	23	19	20	20	25	17	19	17	25
	20	23	18	18	17	18	13	9	10	15
	23	23	18	18	19	8	13	9	7	17
	19	17	12	15	17	14	14	13	9	11
	21	24	19	14	21	21	16	18	15	16
	18	17	18	13	14	11	10	7	10	12
	17	22	15	17	16	17	14	10	13	12
	14	16	15	13	16	19	13	16	14	15
	19	20	19	9	13	18	16	13	15	14
	11	15	16	14	21	19	17	16	15	13
	18	16	15	12	16	10	16	16	15	13
	18	16	15	12	16	10	16	14	14	10

Table 1

(Continued)

Group	Day									
	7	8[a]	9	10	11	12[a]	13	14	15	16[a]
Morphine 21 mg/kg (Isolated)	20	24	15	15	17	21	14	15	20	16
	24	24	15	19	20	20	12	17	18	18
	25	26	17	20	22	24	17	18	20	20
	16	18	15	17	19	16	15	12	17	13
	18	22	15	14	20	19	16	12	19	15
	22	26	16	16	16	24	15	15	17	22
	16	24	17	16	16	23	13	13	17	20
	22	26	17	18	20	26	14	16	21	21
	20	22	13	15	17	23	13	18	16	20
	19	22	16	16	17	9	15	5	13	12
	19	17	10	11	10	13	13	12	11	13
	18	21	9	10	13	15	15	12	16	17
	18	20	18	12	17	14	14	15	17	11
	21	22	12	9	19	16	18	16	19	22

Table 1

(Continued)

Group	Day									
	7	8[a]	9	10	11	12[a]	13	14	15	16[a]
Saline (Aggregated)	18	20	19	21	18	22	18	2	14	24
	13	19	13	17	18	18	14	12	18	20
	19	21	16	15	17	20	18	18	15	22
	18	17	13	19	18	17	7	9	10	9
	21	21	21	19	19	20	16	21	14	25
	12	18	13	7	12	9	12	12	18	7
	17	15	16	19	20	23	21	18	15	29
	16	17	10	15	13	13	13	16	15	15
	17	16	12	15	13	11	11	11	12	14
	17	14	12	11	15	15	16	21	16	22
	22	22	15	18	15	23	16	15	17	17
	23	21	21	22	16	24	16	15	19	19
	24	24	18	20	23	21	20	19	20	24
	19	21	19	16	17	21	15	19	14	23
	19	21	15	18	18	25	17	16	18	20
	18	20	18	15	18	19	16	17	16	17
	14	15	11	14	19	17	16	17	14	19
	15	11	3	9	15	20	17	15	18	20
	18	17	12	15	14	21	15	14	18	21
	15	18	18	18	19	21	17	17	14	19

Table 1

(Continued)

Group	Day 7	8[a]	9	10	11	12[a]	13	14	15	16[a]
Morphine 8 mg/kg (Aggregated)	18	21	19	15	20	17	16	16	16	7
	22	22	16	16	18	17	16	16	18	17
	23	24	18	17	17	22	15	16	18	19
	19	20	15	16	18	19	15	17	15	15
	21	22	15	15	18	20	13	16	16	14
	13	14	14	13	14	12	14	16	17	13
	15	17	15	12	17	16	16	16	17	13
	16	18	15	13	14	12	10	14	15	10
	16	21	15	12	15	15	15	19	15	11
	22	18	8	15	17	21	17	16	18	17
	15	17	14	16	13	15	12	15	15	12
	16	13	6	10	12	15	14	15	17	18
	19	18	12	14	16	20	16	15	16	14
	18	20	20	17	18	13	15	15	17	17
	16	21	12	15	15	23	12	17	17	17

Table 1

(Continued)

Group	Day									
	7	8[a]	9	10	11	12[a]	13	14	15	16[a]
Morphine 15 mg/kg (Aggregated)	22	18	12	15	16	18	16	17	14	19
	21	20	12	14	17	17	14	16	13	14
	23	22	18	19	20	19	13	17	19	20
	22	20	21	14	20	19	16	18	16	16
	22	23	13	20	16	13	10	22	2	5
	17	24	15	16	14	8	8	9	7	3
	22	18	10	17	20	9	21	20	18	14
	16	19	15	15	13	13	13	16	14	15
	19	21	14	17	18	7	18	20	17	12
	18	18	14	15	13	13	11	15	10	12
	17	20	17	14	20	13	12	12	7	8
	16	20	15	12	16	15	14	16	13	12
	14	18	17	22	21	16	19	17	15	17
	15	20	17	16	18	18	15	15	15	12
	17	21	13	16	18	19	18	15	17	14

Appendix B

Raw Scores for Experiment II

Table 1

(Continued)

Group	Day 7	8[a]	9	10	11	12[a]	13	14	15	16[a]
	21	22	15	13	20	18	20	17	15	17
	23	27	17	17	16	19	17	11	16	16
	24	24	23	19	20	27	20	18	19	23
	19	23	18	11	16	21	18	20	16	22
	20	21	17	16	19	19	16	16	16	14
	20	25	10	14	18	20	12	15	18	14
	18	22	15	16	17	20	4	18	14	6
Morphine	22	21	17	18	20	22	19	21	18	21
21 mg/kg	21	23	----------			died	----------			
(Aggregated)	22	25	15	20	19	13	17	17	20	18
	21	22	13	14	19	12	15	14	15	17
	20	24	15	15	19	18	11	15	17	17
	14	19	16	15	15	17	17	17	16	18
	11	7	15	11	16	10	19	19	17	16
	18	14	13	13	17	13	17	12	16	15

[a]Saccharin presented on these days

Table 1

Levels of Fluid Intake (ml) from the Day Prior to the First Saccharin-Injection Pairing Onward

Group	Day									
	7	8[a]	9	10	11	12[a]	13	14	15	16[a]
Vehicle (Isolated)	15	18	15	18	14	20	15	15	12	15
	15	21	15	21	13	19	13	19	18	22
	17	18	14	14	5	10	13	10	10	9
	15	19	15	18	15	14	12	12	10	13
	17	20	12	19	13	20	15	17	15	17
	15	22	17	17	20	22	15	17	13	8
	13	20	12	14	11	11	5	9	13	10
	18	20	16	16	18	18	15	21	15	22
	10	15	17	12	15	18	15	16	12	18
	11	19	15	22	20	21	12	22	13	18
	5	18	18	17	18	17	20	19	14	19
	14	18	17	14	17	22	15	20	10	19
	12	18	17	20	17	15	15	18	13	15

Table 1

(Continued)

Group	Day 7	8[a]	9	10	11	12[a]	13	14	15	16[a]
LiCl (Isolated)	16	18	10	17	12	3	15	5	5	1
	16	18	10	10	15	3	0	15	18	4
	16	17	16	10	9	1	10	11	8	4
	17	26	12	15	18	5	20	17	16	1
	14	19	10	17	15	1	18	18	17	1
	16	18	13	20	12	7	11	10	14	0
	12	16	10	11	15	1	9	7	7	0
	13	21	8	16	9	11	16	18	12	1
	14	15	16	14	15	2	20	20	12	1
	13	15	14	17	19	9	17	21	14	0
	15	14	16	14	18	4	18	22	17	1
	13	12	18	18	18	2	23	23	15	1
	11	14	18	19	20	15	20	18	15	9

Table 1

(Continued)

Group	7	8	9	10	11	12	13	14	15	16
Vehicle (Aggregated)	15	20	20	19	20	20	17	16	18	19
	15	18	13	16	18	18	13	22	15	13
	9	16	20	15	18	18	17	23	17	20
	12	18	15	17	16	16	15	21	14	14
	12	13	18	15	19	18	20	23	18	23
	14	20	17	15	13	17	15	15	19	18
	17	18	11	15	10	16	10	11	9	11
	16	20	15	18	15	16	15	15	13	10
	15	16	13	16	8	6	7	9	11	11
	19	19	15	17	16	16	11	10	9	10
	16	17	11	14	13	12	10	9	13	15
	16	22	12	20	20	11	14	10	8	8
	13	22	14	19	15	19	6	10	9	8
	15	18	13	13	0	12	2	2	1	5
	20	16	17	16	11	12	15	7	2	10

Table 1

(Continued)

Group	Day 7	8[a]	9	10	11	12[a]	13	14	15	16[a]
LiCl (Aggregated)	15	17	15	17	21	12	15	24	19	9
	14	19	21	16	19	12	17	19	18	2
	11	15	14	17	20	12	17	21	15	10
	16	15	18	15	18	8	17	21	14	2
	12	15	15	16	17	12	16	19	15	1
	16	20	12	18	16	8	17	13	10	2
	20	20	17	18	19	6	20	21	19	3
	20	24	10	15	12	9	21	17	15	1
	13	15	11	15	11	9	15	15	12	2
	13	21	9	15	15	2	18	16	18	2
	16	21	8	10	8	1	6	1	4	2
	12	12	10	11	8	1	8	3	6	1
	16	21	8	15	17	3	15	19	12	0
	15	20	10	12	10	1	2	10	15	8

[a]Saccharin presented on these days

Three-Way ANOVA Summary Table

Source	df	SS	MS	F Ratio	Sign. Level
Aggregation (A)	1	171.322	171.322	.1738	.6774
Drug (B)	3	32701.0	10900.30	11.063	.0000
A x B	3	3368.54	1122.85	1.139	.3361
Subjects (S)	118		116268.	985.32	
Days (D)	1	2038.91	2038.91	8.967	.0034
A x D	1	84.0554	84.0554	.3696	.5444
B x D	3	3883.91	1294.64	5.6935	.0011
A x B x D	3	364.11	121.37	.5337	.6600
D x S	118	26831.7	227.387		

Appendix C

ANOVA Summary Tables for Experiment I

Two-Way ANOVA Summary Tables

Saline

Source	df	SS	MS	F Ratio	Sign. Level
Aggregation (A)	1	13.5645	13.5645	.0097	.9223
Subjects (S)	36	50555.3	1404.31		
Days (D)	1	538.491	538.491	2.4816	.1239
A x D	1	135.524	135.524	.6246	.4345
D x S	36	7811.68	216.991		

Morphine (8 mg/kg)

Source	df	SS	MS	F Ratio	Sign. Level
Aggregation (A)	1	217.361	217.361	.4353	.5148
Subjects (S)	28	13981.6	499.341		
Days (D)	1	4018.02	4018.02	21.6343	.0001
A x D	1	72.60	72.60	.3909	.5369
D x S	28	5200.27	185.724		

Morphine (15 mg/kg)

Source	df	SS	MS	F Ratio	Sign. Level
Aggregation (A)	1	2081.38	2081.38	2.7387	.1095
Subjects (S)	27	20519.3	759.97		
Days (D)	1	1013.06	1013.06	5.0956	.0323
A x D	1	20.8552	20.8552	.10489	.7485
D x S	27	5367.92	198.812		

Morphine (21 mg/kg)

Source	df	SS	MS	F Ratio	Sign. Level
Aggregation (A)	1	999.206	999.206	.8644	.3608
Subjects (S)	27	31211.6	1155.98		
Days (D)	1	203.719	203.719	.6508	.4269
A x D	1	223.36	223.36	.7135	.4057
D x S	27	8451.80	313.03		

Appendix D

ANOVA Summary Table for Experiment II

ANOVA Summary Table for Experiment II

Source	df	SS	MS	F Ratio	Sign. Level
Aggregation (A)	1	525.213	525.213	.4097	.6250
Drug (B)	1	90829.3	90829.3	70.3596	.0000
A x B	1	1851.44	1851.44	1.4444	.2350
Subjects (S)	51	65372.3	1281.32		
Days (D)	1	3132.85	3132.85	20.3327	.0000
A x D	1	20.4904	20.4904	.0512	.8218
B x D	1	1364.92	1364.92	3.4124	.0705
A x B x D	1	136.849	136.849	.3421	.5612
D x S	51	20399.3	399.936		