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# The Effects of Prior Exposure to Amphetamine on Feeding Behavior in Rats

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A Thesis

in

The Department

of

Psychology

Presented in Partial Fulfilment of the Requirements
for the Degree of Master of Arts at
Concordia University
Montreal, Quebec, Canada

August 1998

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**Abstract**

The Effects of Prior Exposure to Amphetamine on Feeding Behavior in Rats

Isabella Moroz

The primary objective of this investigation was to explore the effects of prior exposure to amphetamine on subsequent stress-induced and free feeding of regular chow or palatable cereal. After treatment with 5 injections of either d-amphetamine (3 mg/kg IP) or saline, administered on alternate days, animals in Experiments 1 and 3 were permanently moved to computer-controlled test cages, whereas animals in Experiment 2 remained in the colony room and were transported to the test cages for testing only. After 14 drug-free days, animals were either briefly handled or restrained for 20 min, on 10 consecutive days, 6 hours into the light cycle. Consumption of regular chow or palatable cereal was monitored for 1 hour following the return of animals to the cages. Amphetamine preexposure enhanced stress-induced consumption of regular chow (Experiment 1) and, to some extent, of palatable cereal (Experiment 2), despite the stress-induced suppression of palatable food intake. Stress-induced feeding of regular chow increased progressively over initial stress sessions, indicating sensitization of the response to stress. Amphetamine preexposure also enhanced free consumption of palatable cereal, which similarly sensitized over repeated tests. The effects of amphetamine preexposure on stress-induced and free intake of palatable food were more pronounced in females than in males. When testing was conducted in home cage environment (Experiment 3), amphetamine preexposure had no effect on palatable food consumption,

however, it enhanced regular chow intake during the light cycle. Thus, prior exposure to sensitizing regimen of amphetamine exerts long-lasting effects on subsequent response of animals to motivationally significant stimuli.

**Acknowledgments**

First and foremost, I would like to express my appreciation to Dr. Jane Stewart, my supervisor, for her exceptional guidance, inspiring enthusiasm, and remarkable insight throughout this project.

I would also like to thank Steve Cabilio for his invaluable help and assistance with all problems encountered while operating the feeding apparatus.

My regards to Dr. Barbara Woodside and Dr. Jim Pfaus for helpful comments and suggestions during the final stage of writing and for serving as my oral examination committee.

Finally, I wish to express my deep gratitude to my husband Peter for his love, patience, and support at all times.

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## General Introduction

Stimulant drugs, such as the amphetamines, remain among the most widely used and abused of the many psychoactive compounds available, popular for their ability to decrease fatigue, elevate mood, and produce euphoria. Chronic excessive administration of these agents, however, can generate very different effects, eliciting a progressive augmentation in paranoid behaviors that can culminate in psychosis. Thus, there are hidden dangers associated with the use of amphetamines, not readily apparent to a naive user or anyone in the early stages of amphetamine use.

Typically, psychotic symptoms develop gradually, accompanying repeated stimulant abuse, eventually creating a profile indistinguishable from acute or chronic paranoid schizophrenia (Antelman & Chiodo, 1983). Although this amphetamine-induced psychosis usually dissipates with termination of drug use, former amphetamine users remain susceptible to reactivation of this psychosis even after years of abstinence, if administered small doses of amphetamine (Post & Weiss, 1988). These reports clearly suggest the long-lasting nature of amphetamine-induced changes in the nervous system.

Interestingly, there is evidence that 'physical or psychological stress' can precipitate a psychotic episode in 20-25% of former amphetamine abusers (Robinson & Becker, 1986; Sato, Numachi, & Hamamura, 1992). Stress has been thought to play a role in the onset of a number of neuropsychiatric disorders, and it has been suggested that the ability of amphetamine to induce a paranoid psychosis is due to the fact that it mimics the effects of nonpharmacological stressors (Antelman & Chiodo, 1983). In fact, according to

Antelman and Chiodo, the symptoms most characteristic of amphetamine psychosis, such as paranoia, fearfulness, stereotypy, social withdrawal, and hypervigiliance, are precisely the kind of hypersensitive responses one would find in an individual suffering from the effects of extreme stress. Taken together, the clinical observations described above suggest that repeated exposure to amphetamine produces long-lasting effects on brain function that result in an enhanced vulnerability to psychological stressors.

## Sensitization to Psychostimulants and Stressors

The clinical findings described above generated considerable interest in the study of the long-lasting effects of repeated amphetamine administration on brain and behavior, and in the development of an animal model of amphetamine-induced psychosis. In rodents, repeated intermittent administration of amphetamine has been shown to result in a progressive augmentation of locomotion and stereotyped behavior, a phenomenon termed behavioral sensitization (Robinson & Becker, 1986). Sensitization, however, is not unique to the psychopharmacology of stimulant drugs, and has been observed with repeated exposure to environmental stressors, such as food deprivation, self-stimulation, footshock, immobilization, and tail-pinch (see Antelman, 1988 for a review). Moreover, stress-induced behaviors are also enhanced in animals with a history of exposure to amphetamine (Antelman et al., 1980; Antelman & Chiodo, 1983; Hamamura & Fibiger, 1993), a phenomenon referred to as cross-sensitization.

**Cross-sensitization Between Amphetamine and Stress**

The extensive work of Antelman and colleagues on the similarities between the acute and long-term effects of amphetamine and stressors, prompted them to suggest that, in fact, amphetamine acts as a stressor (see Antelman & Chiodo, 1983 for review). This hypothesis received a great deal of support and stimulated numerous investigations of the effects of prior stress exposure on subsequent amphetamine challenges.

A variety of stressors, such as tail-pinch, food deprivation, footshock, nucleus accumbens and medial frontal cortex self-stimulation, and immobilization, whether administered acutely or repeatedly, have all been found to significantly enhance amphetamine-induced stereotypy (Antelman et al., 1980; Antelman & Eichler, 1979; Antelman & Chiodo, 1983), locomotion (Herman et al., 1984), rotation in animals with unilateral substantia nigra lesions (Robinson et al., 1985), and polydipsic or anorectic responses (Eichler & Antelman, 1979; Antelman & Chiodo, 1983), several weeks after stress. In fact, the behavioral responses to the subsequent injection of amphetamine appeared sensitized in a manner similar to that seen after repeated intermittent treatment.

In light of these findings, Antelman reasoned that amphetamine pretreatment should similarly be able to sensitize stress-induced behaviors. To address this issue, he and colleagues (1980) tested whether prior amphetamine administration would alter tail pinch-induced behavior. Since tail pinch-induced feeding occurs very rapidly and virtually in all animals subjected to this stressor, in order to observe any facilitation, the behavior had to be depressed first with haloperidol - dopamine antagonist. A single administration

of amphetamine was found to reduce the ability of haloperidol to suppress tail pinch-induced feeding 3-30 days later. The same researchers have also reported sensitization of self-stimulation in the nucleus accumbens and medial frontal cortex (Antelman & Chiodo, 1983) following repeated amphetamine administration.

More recently, Hamamura and Fibiger (1993) examined the effects of 14 daily amphetamine injections (2.0 mg/kg for the first 7 days, and 4.0 mg/kg for the next 7 days) on a number of behavioral and neurochemical responses to footshock stress, tested after a 7-day drug-free period. Footshock-induced running, flinching, jumping, and vocalizations were all significantly enhanced in amphetamine-sensitized animals, and were accompanied by elevated stress-induced increases in extracellular dopamine in the medial prefrontal cortex. Moreover, one hundred minutes after the last footshock, each animal received a challenge injection of amphetamine (2.0 mg/kg), after which stereotypy measures were taken. The group previously exposed to amphetamine displayed significantly augmented stereotyped behavior compared to the group preexposed to saline.

Effects of prior exposure to psychostimulants have also been tested in aversive conditioning or conditioned fear paradigms, where animals will respond with stress-like behavioral and biochemical changes when exposed to a neutral stimulus that had previously been paired with a stressful stimulus. Surprisingly, exposure to repeated cocaine prior to fear conditioning had an attenuating effect on fear potentiated startle tested 6 days after the last cocaine exposure (Borowski & Kokkinidis, 1994), and on behavioral (immobilization and defecation), as well as biochemical (dopamine metabolism in the mPFC, NAc, and

VTA) effects of aversive conditioning tested 21 days after the last cocaine exposure (Morrow, Taylor, & Roth, 1995). Interestingly, these effects were prevented when sensitization to cocaine was blocked by co-administration of an NMDA antagonist R-(+)-HA-966 (Morrow et al., 1995), suggesting that prior sensitization, and not just exposure to cocaine, was responsible for diminishing the stressful effect of conditioned fear.

On the other hand, in a different set of experiments, Kokkinidis and colleagues have found that when amphetamine or cocaine were administered repeatedly in the presence of fear conditioned stimulus (CS), the expression of fear potentiated startle was enhanced on tests conducted 24-72 hours after the last drug injection (Borowski & Kokkinidis, 1994; Willick & Kokkinidis, 1995; Borowski & Kokkinidis, submitted). Animals that received amphetamine or cocaine together with the fear-evoking CS (light), showed conditioned fear that was comparable to preextinction levels of fear potentiated startle, whereas saline-treated rats displayed extinction of fear responses. This enhancement of fear potentiated startle, apparent only when the drug was administered following fear acquisition and in the presence of the CS, indicates that the systemic effects of psychostimulants interact with the fear-eliciting properties of the CS to sustain significant levels of aversive emotional arousal during extinction.

The findings discussed thus far provide evidence that a variety of stressors can induce sensitization to a subsequent amphetamine challenge, and conversely, that amphetamine (or cocaine) pretreatment can similarly sensitize, or at least alter (see

Borowski & Kokkinidis, 1994; and Morrow et al., 1995), the response of an organism to subsequent stressors. Such 'cross-sensitization' between psychostimulant drugs and stressors suggests that intermittent exposure to amphetamine produces changes that are in some way related to those produced by exposure to environmental stressors, implying that common mechanisms or neural substrates could be involved in mediation of the enduring effects of these events on brain function and behavior.

Amphetamine-like psychostimulants act to block the reuptake and/or to promote the release of dopamine (Seiden, Sabol, & Ricaurte, 1993). It is this action of amphetamine within the mesolimbic dopamine system that is thought to mediate many of the behaviors, such as locomotion, stereotypy, and rotation, that show sensitization after repeated injections (see Robinson & Becker, 1986; and Kuczenski & Segal, 1988 for reviews). Similarly, exposure to experimental stressors, such as footshock, restraint, tail pinch, or conditioned fear, has also been reported to stimulate mesolimbic, as well as mesocortical, dopamine release (see Antelman, 1988; Cabib & Puglisi-Allegra, 1996; and Salamon, 1997 for reviews). Thus, given the evidence that both psychostimulant drugs and stressors promote dopamine transmission, and the postulated role of the dopamine hyperactivity in idiopathic and drug-induced psychosis, efforts to define underlying neuronal mechanisms responsible for sensitization to psychostimulants and stressors have focused on alterations in the dopamine systems. In the following section evidence for dopaminergic involvement in cross-sensitization between psychostimulants and stressors will be examined.

**Role of Dopamine in Cross-Sensitization Between Psychostimulants and Stress**

The mesocorticolimbic dopamine system is comprised of neurons originating in the VTA and substantia nigra that send their projections to various forebrain sites, such as the limbic cortex (medial prefrontal, cingulate and entorhinal areas) and other limbic structures (the regions of the septum, olfactory tubercle, nucleus accumbens septi, amygdaloid complex, and piriform cortex) (Cooper, Bloom, & Roth, 1996). As mentioned earlier, both amphetamine and stress are known to increase dopamine release and/or metabolism in the medial prefrontal cortex and nucleus accumbens, implicating the mesocorticolimbic dopaminergic system as a potential substrate mediating cross-sensitization. However, although most of the neurochemical changes associated with these phenomena have been searched for and observed in dopamine pathways, one must keep in mind that other (possibly all) neurotransmitter systems are likely to play a role (Antelman, 1988).

Robinson et al. (1987) reported that the neurochemical response to footshock stress was changed by prior amphetamine treatment. Seven days after rats received 10 amphetamine injections, spaced every 3 to 4 days, animals pretreated with amphetamine showed a greater initial reduction in tissue dopamine levels and greater elevation in DOPAC/DA ratios in medial frontal cortex, NAc, dorsolateral striatum, and hypothalamus, than nonhandled controls, providing evidence in support of the mesocorticolimbic dopamine system mediation of the cross-sensitization between amphetamine and stress.

As mentioned in the previous section, Hamamura and Fibiger (1993) reported an enhanced dopamine release in the medial prefrontal cortex in amphetamine-sensitized rats

after a 7-day abstinence period. Searching for other neural structures that might have contributed to this phenomenon, they examined the effects of repeated exposure to amphetamine on subsequent expression of c-*fos* induced by conditioned fear, given that changes in the expression of the immediate early gene c-*fos* have proven useful to mapping of activated cells by various pharmacological and physiological stimuli (Hamamura, Ichimaru, & Fibiger, 1996). Repeated exposure to amphetamine was found to significantly enhance the effects of conditioned fear on c-*fos* immunoreactivity in several brain regions (cingulate cortex, claustrum, piriform cortex, the shell region of NAc, medial striatum, and ventral lateral septum), nearly all of which were innervated by the mesocorticolimbic dopamine system. Similarly, cocaine has been reported to increase c-*fos* immunoreactivity in many of the same structures (Brown, Robertson, & Fibiger, 1992). These results indicate that treatment with psychostimulants can have long-lasting effects on the neural circuitries activated by conditioned stressors.

The most recent investigation of the neurobiological basis of interactions between drug exposure and stress examined the effects of chronic amphetamine, administered twice daily for 14 days, on stress-induced dopamine release in the ventral striatum after 12h and 7 days of abstinence (Weiss et al., 1997). In disagreement with the findings described so far, animals pretreated with amphetamine displayed a significant reduction of dopamine release in response to restraint stress and to release from restraint (both of which are associated with enhanced dopamine release in the NAc of control saline-pretreated rats; see Imperato et al., 1992). The reason for this discrepancy could be due to the continuous rather than intermittent mode of amphetamine administration, as well as to the short period

of withdrawal. After only 7 days of abstinence from this rather severe drug regimen, the dopamine neurons may be still adapting from the withdrawal of the drug; sudden withdrawal from psychostimulants has been noted to alter many factors involved in dopaminergic function, including dopamine overflow (Post & Contel, 1983). Accordingly, Rossetti, Hmaidan, and Gessa (1992) demonstrated that withdrawal from chronic amphetamine (1.5 mg/kg, twice a day, for 16 days) resulted in decreased extracellular dopamine concentrations in the ventral striatum on the first, third, and fifth, but not on the tenth day after termination of the treatment. In fact, the initial decrease observed on the first day progressively diminished until it was no longer evident after ten drug-free days.

A similar decrease in the extracellular dopamine levels in the NAc has been reported following chronic food deprivation and body weight reduction to 70-80% of normal (Pothos, Creese, and Hoebel, 1995). The authors proposed that the chronic stress associated with starvation may involve a state of "food withdrawal", characterized, just as after withdrawal from drugs of abuse, by dopamine depression. However, the underweight animals, although hypoactive in comparison to controls, were found to display a significant increase in the locomotor activity after systemic or local amphetamine infusion into the NAc, paralleled by significant increases in extracellular dopamine levels. The underweight rats were also found to consume a bigger size meal, which was accompanied by increased extracellular dopamine levels, although the dopamine response was weaker than in control rats. Thus, the authors suggested that the selective reduction of dopamine in the NAc may code a motivational "no-go" state, characterized by hypoactivity and lack

of goal oriented behavior, which may switch to a motivational "go" state, initiated by stimuli known to stimulate dopamine release, such as the prospect of a meal or familiar drug injections.

In conclusion, there is sufficient evidence to suggest that sensitization, which develops with repeated exposure to psychostimulants, produces enduring changes in dopamine functioning, changes characterized by hyperresponsivity to a variety of stressors that subsequently activate brain dopamine systems (but see Weiss et al., 1997).

## Functions of Dopamine in the CNS

For years, considerable emphasis has been placed on the role of mesolimbic dopamine system in appetitive motivation and positive reinforcement. In fact, it has been proposed that dopamine release in the terminal regions (nucleus accumbens) is the critical substrate for all rewards, whether natural (i.e. food, sex) or artificial (i.e. intracranial self-stimulation, drugs of abuse) (Wise & Bozarth, 1984). Yet, it must be recognized that the mesolimbic dopamine system in general is activated by acute exposure to stressors (see Cabib and Puglisi-Allegra, 1996; and Salamone, 1997 for reviews). Thus, if the dopamine system is a common substrate mediating the enduring effects of psychostimulants and acute stressors, one needs to reconcile its paradoxical activation in response to both drug-induced (appetitive) and stress-induced (aversive) stimuli.

### Behavioral Significance of Stimuli Activating Dopamine Neurons

One view of the activation of the midbrain dopamine system proposes that this

system may serve to modulate or gate signals coming from the limbic brain structures involved in motivation, which are ultimately translated into motor acts via the output neuronal circuitry of the extrapyramidal motor system (Mogenson, 1980). The involvement of dopamine neurons in behavioral processes has been studied extensively by Schultz, using electrophysiological recordings of individual neurons in behaving animals. His studies revealed that dopamine neurons respond specifically to stimuli, which have alerting, arousing and attention-grabbing properties, such as: primary rewards and primary aversive stimuli, conditioned incentive appetitive and aversive stimuli, and unexpected and high intensity stimuli (Schultz, 1992; Schultz et al., 1993). More recently, Mirenowicz and Schultz (1996) reported that dopamine neurons are activated preferentially by appetitive rather than aversive stimuli. One may question, however, the true aversiveness of a mild air puff to the hand or hypertonic saline to the mouth.

Furthermore, Schultz has shown that although the mesolimbic dopamine neurons respond specifically to primary rewards, these responses gradually transfer to the conditioned stimuli that predict reward during the establishment of task performance. Thus, there is a transfer of stimulus salience from the primary reward to the conditioned stimulus predicting reward. Moreover, after overtraining, even the responses to the conditioned stimuli become strongly reduced, suggesting that dopamine neurons respond to the most significant stimuli for the behavior of the subject in a given situation.

Another observation made by Schultz is the homogeneity of response of dopamine neurons within their population, namely the fact that they are likely to respond to a

particular stimulus in the same manner or to not respond at all. This property enables the dopamine neurons to signal to their terminal domains the presence of an important stimulus in the subject's environment that needs to be processed with the highest priority. However, since the same neuron may respond to very different, and even opposing, stimuli, the message does not appear to specify any further details about the stimulus. Thus, the activity in the mesolimbic dopamine neurons may not be specifically related to either the incentive motivation or reward, but rather to an "alerting and attention-grabbing" mechanism. Such a mechanism might respond to both appetitive and aversive stimuli as long as they signal a stimulus of high significance, leading to motivational arousal and appropriate behavioral reactions.

Thus, the paradoxical activation of the dopamine system in response to both drug-induced (appetitive) and stress-induced (aversive) stimuli might be explained by evidence suggesting the involvement of dopamine neurons in mediating the salience of the stimuli that signal unpleasant consequences as well as those that signal pleasant ones. The salience of both positive and aversive stimuli is clearly mediated by the dopamine system, whereas the valence of that salience (attractive incentive vs frightening warning) may be determined by the coactivation of other neural systems (Robinson & Berridge, 1993).

Another view of the activation of the midbrain system is that if this is a "go" system, its activation by acute aversive stimuli may serve to suppress the aversion. Evidence for such a role comes from work on dopamine activation and pain suppression. In addition to their rewarding properties, many drugs of abuse also share analgesic potential

(see Franklin, 1998 for review). Similarly, a wide variety of stressors, such as footshock, tail- and body-pinch, food deprivation, cold and warm water swimming, environmental novelty, aggressive confrontation and defeat of a conspecific, have also been reported to induce analgesia (Amit & Galina, 1986; Terman et al., 1984). Furthermore, this drug- and stress-induced analgesia has been shown to depend upon the activation of dopamine transmission in the terminal regions. For example, amphetamine-induced analgesia in the formalin test for tonic pain has been blocked by dopamine D1 and D2 receptor antagonists (Morgan & Franklin, 1991) and by 6-OHDA lesions of the VTA and ventral striatum (Clarke & Franklin, 1992; Morgan & Franklin, 1990). Also, dopamine receptor antagonists administered into the NAc have been found to attenuate analgesia in the formalin test induced by intra-VTA infusions of Substance P analog or morphine and by intra-NAC infusions of amphetamine (Altier & Stewart, 1998). Similarly, bilateral infusions of an opioid receptor antagonist (naltrexone) into the VTA, known to inhibit the rate of firing of dopamine neurons, have been reported to block the analgesia induced by exposure to footshock stress (Altier & Stewart, 1996).

In summary, the functional significance of the overlap between the neural substrates of psychostimulant drugs, stress, and analgesia suggests a role for the mesolimbic dopamine system in survival. Typically, positive incentives facilitate approach and consummatory behaviors, whereas stress and pain elicit withdrawal and inhibit movement. The inhibitory effects of stress on pain may serve to promote survival by reducing competition between protective reflexes and the motor demands of fight and flight situations. However, the ability to overcome the impact of aversive and nociceptive

stimuli, through the activation of the same neural substrate in response to positive incentive stimuli, motivates the organism to engage in consummatory behaviors, such as feeding and copulation, which are no less essential to survival.

**Role of Dopamine in Sensitization**

If natural incentive stimuli are capable of generating an appetitive motivational arousal state, accompanied by enhanced dopamine activity, and if enhanced dopamine activity is associated with increased motivational arousal, then it follows that exposure to drugs that enhance activity within the mesolimbic dopamine system may serve to enhance motivational arousal, and in turn facilitate the effectiveness of incentive stimuli. It may be through this process that neutral stimuli paired with drugs that enhance dopamine transmission, come to be able to elicit, by themselves, the motivational states experienced while under the influence of the drug, and thus gate the expression of sensitization to both the behavioral and the biochemical effects of the drug (for a complete discussion of this topic see Stewart & Vezina, 1988; Anagnostaras & Robinson, 1996).

The evidence that the motivational states created through the interaction with an incentive stimulus may enhance the effectiveness of incentive stimuli in general, and facilitate behaviors other than those normally elicited by this particular incentive stimulus, poses a problem in identifying the degree to which motivational states are specific to a certain class of motivated behaviors. It has been shown, for instance, that tail-shock and tail-pinch increase both sexual behavior in male rats in the presence of receptive females (Sachs & Barfield, 1974; Leyton & Stewart, 1996) and feeding behavior when animals are

given access to food (Antelman & Szechtman, 1975; Levine & Morley, 1982). Those tail pinch-induced enhancements in motivational behavior are dependent on increases in the midbrain dopamine transmission (Antelman et al., 1975). One interpretation of the fact that tail-pinch is capable of eliciting seemingly unrelated behaviors, such as sexual activity or feeding in the presence of the appropriate stimuli, is that it serves to facilitate the motivational state of readiness with which the animal responds to salient incentive stimuli in its immediate environment.

Similarly, psychostimulant drugs, the behavioral activating effects of which depend on the activation of the mesocorticolimbic dopamine system, appear to facilitate forward locomotion and approach, but the particular behaviors engaged in while under the influence of a drug, are determined largely by the nature of the immediate environment surrounding the animal (Wise and Bozarth, 1987). Sensitization of activity in the mesolimbic dopamine system, accompanying repeated drug exposure, could serve to enhance the motivational arousal states created through the interaction with incentive stimuli having access to this system and, in turn, to facilitate behaviors appropriate to other incentive stimuli present in the animal's immediate environment.

In support of this idea, Mitchell and Stewart (1990) reported facilitation of male sexual behavior in the presence of stimuli previously paired with repeated morphine administration, a treatment known to sensitize the mesolimbic dopamine system. Male rats, some gonadally intact and some castrated, tested for sexual behavior in an environment previously associated with morphine, all displayed more frequent female-directed behavior

than males given saline in that environment. Thus, pairing of an environment with a drug known to have appetitive motivational effects, appeared to enhance the effectiveness of the sexually relevant stimuli, an effect possibly mediated by an increased activity within the mesolimbic dopamine system. It may be useful at this point to explain that opiates interact with the dopaminergic system at the level of VTA, where their receptors are located, leading to an increase in the rate of dopaminergic cell firing and in locomotor activity similar to that observed after systemic administration of dopamine agonists (Noel & Wise, 1993).

In another set of studies, repeated exposure to amphetamine, known to sensitize the mesolimbic dopamine system, has been reported to augment the prophagic effects of systemically administered morphine (Nencini, 1988; Nencini, Johanson, & Schuster, 1988), and morphine given into the VTA (Nencini & Stewart, 1990). Furthermore, rats treated with repeated amphetamine showed an enhancement of ingestive behaviors in response to intra-VTA infusions of DAMGO (mu opioid agonist), given 24 hours after the last amphetamine injection (Badiani et al., 1995). When tested in presence of gnawable objects and a drinking tube, in addition to food pellets, gnawing and drinking were also enhanced in amphetamine-pretreated animals, whereas the effects on feeding were negligible. Thus, amphetamine pretreatment amplified the effects of intra-VTA DAMGO, presumably by activation of the mesolimbic dopamine neurons, and facilitated not only ingestive behaviors, but also appropriate interactions with other incentive stimuli present in the immediate environment.

In the absence of a receptive female, or food stimuli, animals pretreated with amphetamine in a distinct environment, showed increased locomotor activity when they were administered morphine, either systemically or into the VTA, in that environment, starting 24 hours after the last preexposure (Stewart & Vezina, 1987). As mentioned earlier, the locomotor activating effects of intra-VTA morphine are also thought to be mediated by the increase in firing of the mesolimbic dopamine cells.

In conclusion, the activation of the mesolimbic dopamine system through pharmacological means, such as systemic or intra-VTA infusions of morphine and certain opioid agonists, as well as through exposure to environmental stimuli previously paired with the drug effects, were all found to facilitate behaviors appropriate to the testing situation. Male sexual behavior was enhanced in the presence of a sexually receptive female, feeding in the presence of food, and when neither was present, increased locomotion was found. Interestingly, the two latter effects were significantly enhanced in animals previously exposed to amphetamine in a manner known to sensitize functioning of the dopamine system.

These findings provide further support for the idea that the midbrain dopamine system is a behavioral facilitatory, or "go" system, the activity of which promotes approach and underlies the motivational properties of natural incentives, psychostimulant drugs, and non-pharmacological stressors. Sensitization of activity in this system, which accompanies repeated psychostimulant and stressor exposure, may arise from an exaggeration of processes that occur when an animal initially interacts with biologically significant stimuli,

processes that serve to augment the subsequent response of an animal to any stimuli having neural access to this system (Stewart & Badiani, 1993).

## Rationale of the Present Experiments

The present set of experiments was designed to explore further the idea that animals preexposed to stimulant drugs differ from drug-naive animals in their subsequent responses to motivationally significant stimuli, and, in particular, to stressors. Because there is ample evidence that mild stressors, such as tail pinch (Antelman et al., 1975; Rowland & Antelman, 1976) and restraint stress (Badiani et al., 1995) induce feeding and drinking behaviors in the rat, the main objective of the present investigation was to explore these stress-induced behaviors in response to restraint stress in animals that had been previously treated with intermittent injections of amphetamine. In Experiment 1, the effects of amphetamine preexposure on stress-induced feeding of regular lab chow was investigated. In Experiments 2 and 3, the role of palatability in stress-induced feeding in amphetamine- and saline-preexposed animals was examined by allowing them access to Honey Rice Crispies (Kellogg's) upon release from restraint. Furthermore, in Experiment 2 , possible sex differences in stress-induced consumption of palatable food in amphetamine- preexposed animals were examined by employing both male and female rats.

## General Method

### Subjects

A total of 88 male Wistar rats (Charles River, Canada), weighing 250-275g and 32 female rats weighing 200-225 g upon arrival, were used in the present experiments. Animals were housed individually, in wire-mesh cages, in a humidity- and temperature-controlled colony room, under a 12-h light/dark cycle. Water and food were continuously available. Animals were handled 4-5 times during their first week in the colony room. After completion of the drug treatment, the animals in Experiments 1 and 3 were moved permanently to a computer-controlled testing apparatus, whereas the animals in Experiment 2 remained in the colony room and were transported on testing days to the test boxes located on a different floor of the building for a period of approximately 1.5 hours.

### Apparatus

The testing apparatus consisted of 8 Plexiglass cages (26 X 34 X 34 cm) that allowed feeding and drinking to be monitored continuously every 0.1 second. The cages were equipped with a drinking bottle, a tunnel leading to a food cup, and 3 photo-electric beams. The food cup was a regular drinking glass glued to the bottom of a container that collected food spillage, and that rested on an electronic balance (precision: 0.1 g), equipped with an interface allowing communication with the computer. The computer recorded the weight reduction as a measure of food intake. Drinking was measured by a drinkometer

circuit that was closed when the animal licked a stainless steel drinking spout extended into the box, while standing on the grid floor. Two photo-electric beams, 17 cm apart on one side of the cage, allowed for the monitoring of general activity. Another photo-electric beam crossed the opening at the end of the food tunnel, just over the food cup, to monitor feeding-related activity. For a full description of this apparatus see Badiani, Mundl, and Cabilio (1995).

Drugs

D-amphetamine sulfate (Smith, Kline and French, I.A.C., Montreal, Quebec, Canada) was dissolved into 0.9% saline solution and administered IP at the dose of 3.0 mg/kg. Control treatment consisted of 1.0 ml/kg IP of saline.

Procedures

*Drug Treatment Procedures.* Animals were given 5 intraperitoneal (IP) injections of either 3mg/kg amphetamine dissolved in 0.9% saline solution, or saline (1.0 ml/kg IP). The injections were given once a day, every second day, and were terminated 14 days before the stress sessions. After being weighed, animals were placed in transportation buckets, two animals per bucket. They were then injected and placed back in the buckets, where they remained for about 30 minutes. Both animals in the bucket received the same injection (either amphetamine or saline). The buckets were used to increase the novelty of the environment where the drug was first experienced (see Badiani, Browman, & Robinson, 1995).

*Stress Tests.* Animals were stressed daily, for 10 days, 6 hours into the light cycle. At this time of day, the probability of spontaneous feeding and drinking is very low (Badiani et al., 1996). On each test day, animals were placed in plastic restrainers for a period of 20 minutes. Immediately after being released from the restrainers, they were placed in the test boxes, where feeding and drinking were monitored for 1 hour from the time of return for each animal. In Experiments 1 and 3, in which animals were being housed in the test boxes, feeding and drinking was monitored 23 hours per day. In the first hour of the light cycle the experimenter entered the room, refilled food and water containers, changed the bedding as needed, and then, reset the computer.

*Data Analysis and Statistics.* Total food and water intake in the first hour after restraint stress, on each of the 10 days, were analyzed using an ANOVA for Group (amphetamine or saline) by Day in Experiment 1, and with a 3-way ANOVA for Preexposure (amphetamine or saline) by Treatment (Stress or Brief Handling) by Day in Experiments 2 and 3. In order to determine the time course of food and water intake within the first hour after stress, the scores were analyzed in 15 min bins, averaged over the 10 test days. These scores were analyzed using an ANOVA for Group (amphetamine or saline) by Time Interval (0-15min, 15-30min, 30-45min, 45-60min) in Experiment 1, and with a 3-way ANOVA for Preexposure (amphetamine or saline) by Treatment (Stress or Brief Handling) by Time Interval (0-15min, 15-30min, 30-45min, 45-60min) in Experiments 2 and 3. In Experiments 1 and 3, in addition to monitoring changes in feeding and drinking in the first hour after stress, the total food intake during the light and dark cycles was calculated and analyzed using ANOVAs for Group by Day (Experiment 1) and

for Drug (amphetamine or saline) by Treatment (stress of brief pick-up) by day (Experiment 3).

## Experiment 1. Effects of amphetamine pretreatment on stress-induced consumption of regular rat lab chow in the home cage environment.

Sustained mild tail-pinch has been reported to reliably induce a variety of oral behaviors in the rat, such as gnawing, eating, and licking, in almost every animal tested (Antelman et al., 1975; Antelman & Szechtman, 1975). Levine and Morley (1982) have shown that a mild pinch stimulus applied to other body parts, such as ears, scruff of the neck, hind paws, induces similar behaviors. A brief period of restraint stress has been also reported to facilitate feeding and drinking behaviors in the rat (Badiani et al., 1996). Furthermore, in the case of tail-pinch, the latency to eat decreases, and in the case of restraint stress, the amount of food eaten increases progressively, with repeated exposure to these stressors, suggesting development of sensitization of these stress-induced behaviors.

Based on the evidence reviewed in the General Introduction, showing that exposure to psychostimulants produces long-lasting hypersensitivity to a variety of stress-induced behaviors, it was hypothesized that the effects of restraint-stress on feeding might be enhanced in animals previously exposed to intermittent treatment with amphetamine. This possibility was addressed in the present experiment, in which animals previously given repeated exposure to amphetamine or saline were restrained daily, starting 14 days after the last drug exposure.

## Method

### Subjects

Twenty-four male Wistar rats (Charles River, Canada) were used in the present experiment, maintained as described in General Methods. The animals were divided into two groups based on the preexposure drug: Amph (n=12) and Sal (n=12), and both groups were subjected to daily restraint stress.

### Procedure

Seven to eight days after completion of drug treatment, animals were moved permanently to the test boxes, where they were allowed 5-6 days to habituate to the new conditions. Fourteen days after the last injection of amphetamine or saline, the stress regimen was initiated. All animals were stressed daily, for 10 days, at a time of day when the probability of spontaneous feeding and drinking was low. Each day, animals were removed from the cages and taken to a separate nearby room to be restrained in plastic restrainers. After 20 minutes, they were released from the restrainers and returned to the test boxes. The exact time each animal was returned to its cage was recorded, so that feeding and drinking could be monitored from the time of return of each animal individually. Baseline intake of food and water was calculated as the mean intake in one hour, from the time when animals were released from 20 minutes of restraint stress, administered 6 hours into the light cycle, on the last three days of the habituation period.

## Results

Fig. 1A shows the total food intake during the first hour after restraint stress, plotted over the 10 test days. Close inspection of the figure revealed two things: first, animals previously treated with amphetamine ate more in response to restraint stress after the initial exposure to the stress than animals previously treated with saline, and second, the group differences were apparent only during the first four of the ten days of testing. Thus all statistical analyses were conducted on the data from the first four days only. The ANOVA for Group by Day revealed a significant effect of Group ($F(1, 22) = 5.67$, $p = 0.03$), indicating that overall, amphetamine-preexposed animals consumed more laboratory chow than saline-preexposed animals in the first hour following the 20-min restraint period. The analysis also revealed a significant effect of Day ($F(3, 66) = 3.14$, $p = 0.03$), but no significant interaction, indicating that for both groups, stress-induced feeding increased progressively over the initial 4 test sessions.

Fig. 2A shows the time course of food intake in the first hour after restraint stress. The data shown are mean food intake in each 15 min interval during the first hour after restraint stress, averaged over the first four test days. The ANOVA for Group by Time Interval, in addition to the significant Group effect, revealed a significant effect of Time Interval ($F(3, 66) = 4.50$, $p = 0.006$). There was, however, no significant Group by Time Interval interaction, indicating that the time course of feeding for both groups followed the same pattern, peaking in the second 15 min interval and gradually decreasing to baseline levels by the end of the hour.

*Figure 1.* Mean (±SEM) **A.** food intake (grams), and **B.** water intake (number of licks), during the first hour after restraint stress, over the 10 test days for groups Amph and Sal. Also shown are the pre-stress baseline hourly intake levels, taken on the last three days of habituation period, at the time at which animals were released from 20 minutes of restraint stress, administered 6 hours into the light cycle (Experiment1). * different from saline-preexposed group, $p<.05$.

Daily consumption of regular lab chow and water in response to restraint stress

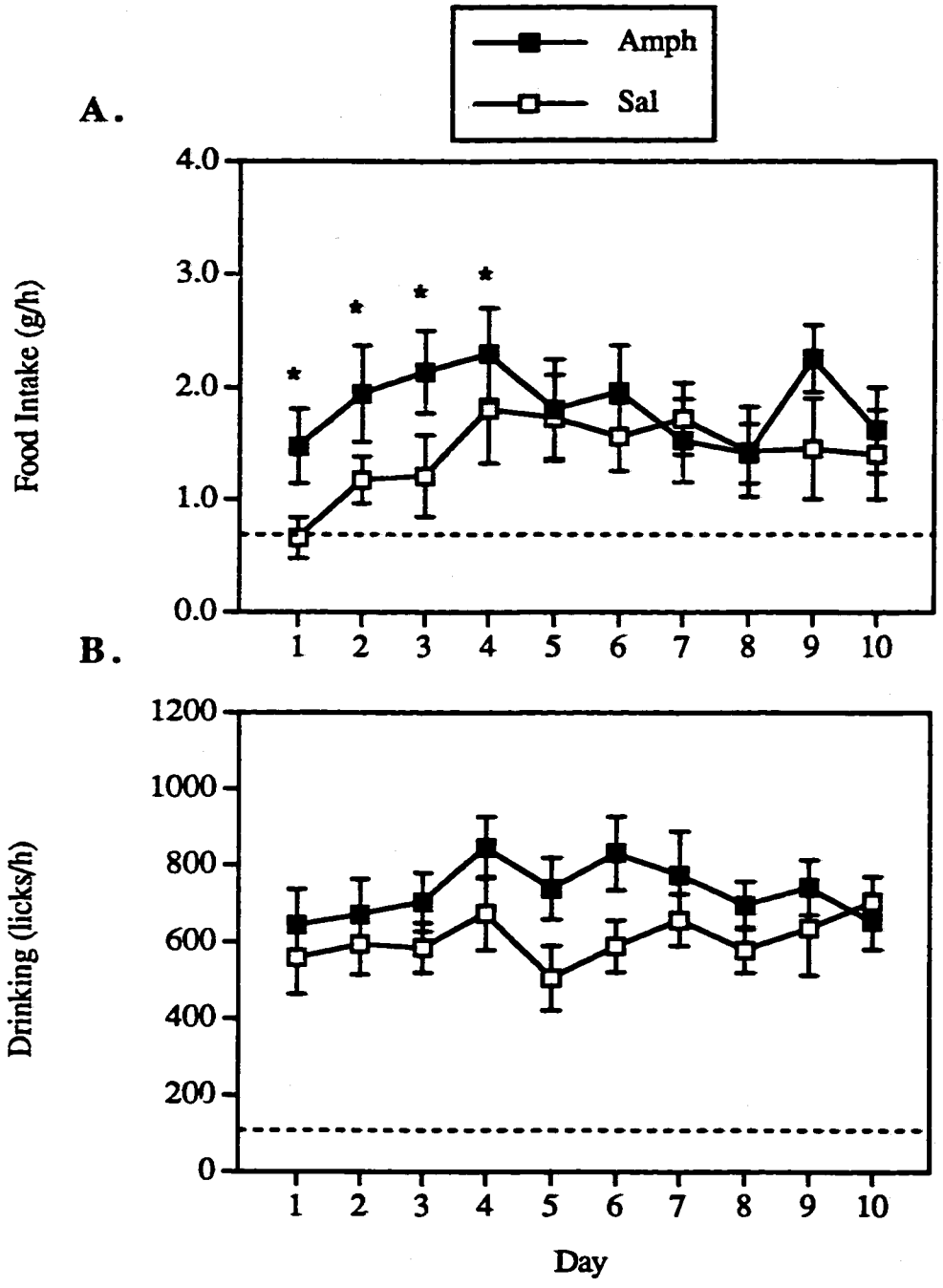


*Figure 2.* Mean (±SEM) **A.** food intake (grams), and **B.** water intake (number of licks), in each 15 min interval, during the first hour after restraint stress, averaged over the 10 test days for groups Amph and Sal (Experiment 1).

Time course of feeding and drinking in response to restraint stress

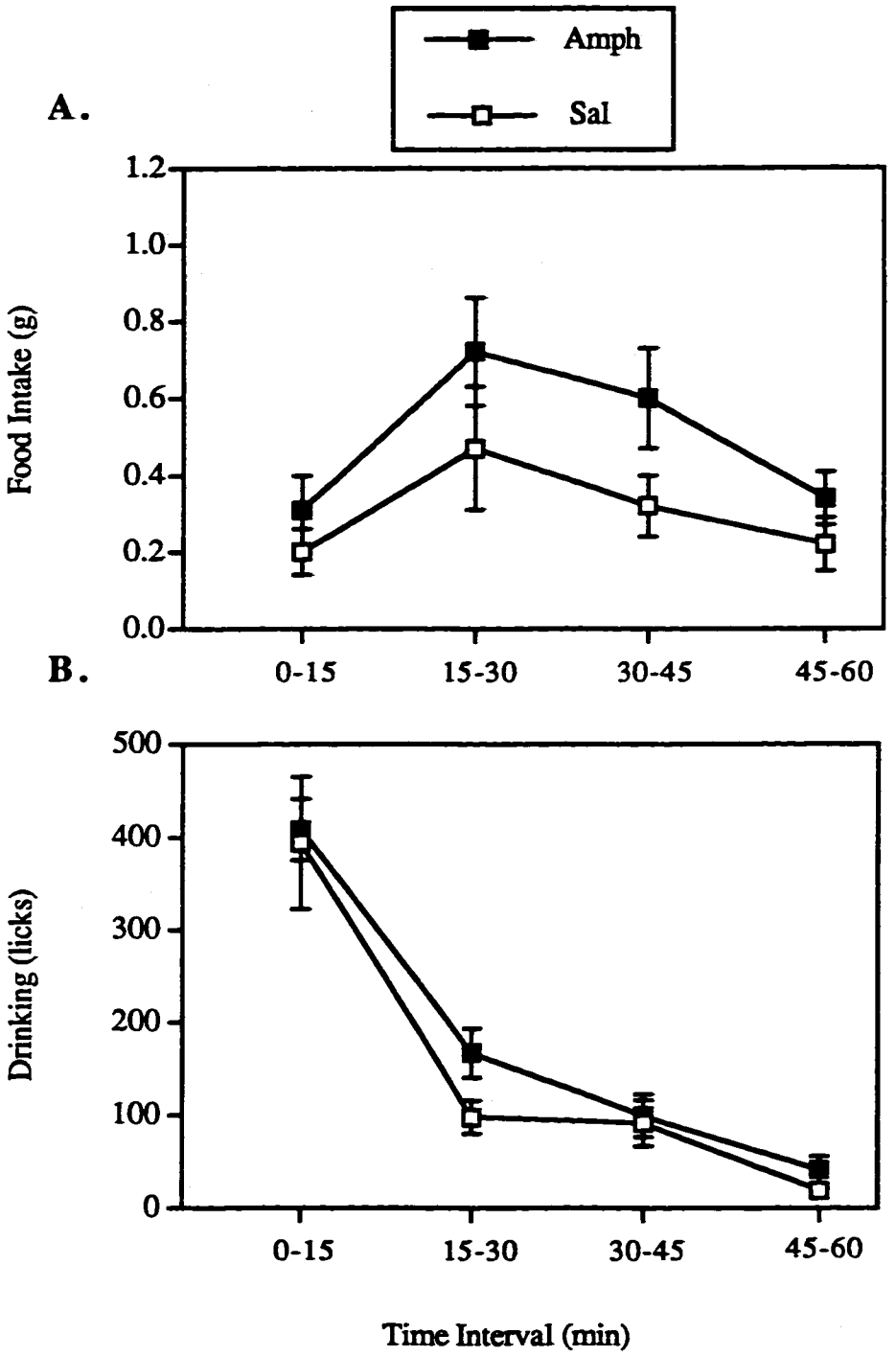


Fig. 1B shows the total number of licks in the first hour after restraint stress, plotted over the 10 test days. Since feeding analyses were conducted only for the first four test days, it seemed appropriate to analyze drinking in the same way, although it made little difference to the outcome. The ANOVA for Group by Day revealed no effect of Group, indicating that amphetamine-preexposed animals did not drink more than the saline-preexposed animals. It can be seen, however, that both groups drank above the baseline in response to restraint. The analysis also revealed a significant effect of Day ($F(3, 66) = 3.27$, $p = 0.03$), but no significant interaction, indicating that for both groups, stress-induced drinking increased progressively over the initial 4 test sessions.

Fig. 2B shows the time course of drinking in the first hour after restraint stress. The data shown are mean number of licks in each 15 min interval during the first hour after restraint stress, averaged over the first four test days. The ANOVA for Group by Time Interval revealed a significant effect of Time Interval ($F(3, 66) = 52.02$, $p = 0.0001$), but again, no significant interaction, indicating that the time course of drinking was similar groups. Interestingly, unlike feeding, drinking peaked in the first 15 min interval following restraint stress and gradually decreased to baseline levels by the end of the hour.

## Discussion

The major finding of the present experiment was that previous exposure to amphetamine enhanced stress-induced intake of regular lab chow, at the time of day when the probability of spontaneous feeding was low, providing further support for the hypothesis that treatment with amphetamine produces long-lasting hypersensitivity to a

variety of stress-induced behaviors. The difference between amphetamine- and saline-preexposed animals was evident starting on day 1 and was maintained until day 4, after which the stress-induced food intake by both groups dropped somewhat and remained at about the same level for the remainder of the experiment.

Interestingly, throughout those initial four days, both amphetamine- and saline-preexposed animals showed a progressive increase (sensitization) in their stress-induced feeding, replicating previous findings of Badiani et al. (1996). Unlike in Badiani's experiment, however, in which stress-induced feeding reached its maximum value on day 10 of testing, in the present experiment, the maximum intake was reached by both amphetamine- and saline-preexposed animals on day 4. It is tempting to speculate that, since saline injections have been shown to be stressful to the animals (Sakellaris & Vernikos-Danellis, 1975), and since prior exposure to stressors can sensitize subsequent stress-induced behaviors (see Antelman, 1988 for review), in the present experiment, both amphetamine- and saline-preexposed animals may have developed some degree of sensitization induced either by repeated administration of amphetamine or by repeated exposure to the stress of the repeated saline injections, both of which are capable of leaving the animals hypersensitive to a subsequent stressor.

Support for this idea comes from Robinson et al. (1987), who reported that footshock stress produced a greater initial reduction in tissue concentrations of dopamine and a greater elevation in DOPAC/DA ratios in a number of brain regions in amphetamine-pretreated, as well as in saline-pretreated animals, as compared to nonhandled control rats.

Thus, if both amphetamine- and saline-preexposed rats had already developed sensitization to the stressor, it might explain why sensitization of stress-induced feeding proceeded so rapidly in the present experiment, whereas in the Badiani et al. (1996) experiment, conducted with injection-naive rats, it took much longer.

In the case of stress-induced drinking, animals preexposed to amphetamine did not differ from those preexposed to saline, although animals in both groups drank almost immediately after being placed back in the cages upon release from restraint. The fact that drinking peaked before feeding for both groups suggests that drinking was not feeding-induced. The drug preexposure manipulation in the present experiment clearly affected only the feeding, supporting the idea that feeding and drinking behaviors can be activated independently (Badiani & Stewart, 1993; Badiani et al., 1996).

It may be of importance that eating of regular laboratory rat chow was employed in the present experiment. Based on anecdotal reports, one may speculate that exposure to stressors might preferentially increase the intake of highly palatable foods, and that such effect, if true, may be further amplified in by previous exposure to intermittent amphetamine. This possibility was addressed in Experiment 2.

## Experiment 2. Effects of amphetamine pretreatment on stress-induced consumption of palatable food in male and female rats in the novel environment.

In the study of stress-induced feeding, one important question to be asked is whether the quality of food plays a role in the phenomenon. It is typically assumed that

individuals who eat under stress will prefer certain nutrients over others. There is a suggestion that the consumption of sweet (Grunberg & Straub, 1992) or high fat (Michaud et al. 1990) foods increases preferentially under stress, at least in women. Support for such anecdotal evidence comes from animal studies, reporting increased intake of sucrose in rats submitted to an inescapable shock (Strongman et al., 1970), increased intake of sweetened water and sweetened milk (Antelman, Rowland, & Fisher, 1976), or chocolate cookies (Morley et al., 1982) in response to tail-pinch, and finally, increased intake of sweet Fruit Loops in response to restraint stress (Ely et al., 1997).

In Experiment 1, stress-induced consumption of regular lab chow was enhanced by preexposure to amphetamine 14 days prior to stress sessions. This effect, however, was evident only during the first 4 days of stress. Since activation of the dopamine system that occurs in response to incentive stimuli has often been attributed to their appetitive or rewarding nature, and since food reward depends in part on the hedonic component of its sensory properties, namely palatability, it was speculated that amphetamine pretreatment may preferentially enhance stress-induced intake of more palatable food. The present experiment addressed this possibility using Honey Rice Crispies cereal as palatable food.

In addition, Experiment 2 addressed the possibility of sex differences in stress-induced feeding in animals previously exposed to amphetamine. Interestingly, both psychostimulants and stressors have been reported to produce more robust sensitization in female rats (see Robinson, 1988 for review), and there is some evidence that women are more vulnerable to stress-induced eating than men (Grunberg & Straub, 1992; Michaud et

al., 1990). In addition, there is some evidence that female rats are more sensitive to the palatability of food (Zylan & Brown, 1996).

In light of such findings, it was hypothesized that the enhancement of stress-induced feeding seen in amphetamine-preexposed animals may be even greater in female rats, especially when they are given access to more palatable food.

## Method

### Subjects

Thirty-two male Wistar rats and thirty-two female Wistar rats were used in the present experiment (Charles River, Canada). The animals were maintained as described in General Methods.

### Procedure

After completion of drug treatment, animals were habituated to the test boxes for 1 hour on two occasions, where they were exposed to the new taste of highly palatable food - Honey Rice Crispies (Kellogg's). They were subsequently assigned to one of the two treatment groups (Restrain Stress or Brief Handling), matched on the basis of Honey Rice Crispies consumption averaged over the two habituation days. In total, there were 4 groups created: Amph-Restraint Stress (n=8), Amph-Brief Handling (n=8), Sal-Restraint Stress (n=8), and Sal-Brief Handling (n=8).

Two weeks after the last amphetamine or saline injection, the animals were

transferred daily from their home cages to the test boxes, right after being subjected to either 20 minutes of restraint stress or to brief handling. The intake of Honey Rice Crispies and water was monitored for one hour after animals were placed in the test boxes, for 10 consecutive test days.

*Estrus cycle verification.* Vaginal smears were collected daily from female rats to determine the day of the cycle (either proestrus, estrus, metestrus or diestrus), on which amphetamine and stress treatments were given. Both treatments were distributed throughout the cycle, and thus were not associated with any particular phase. Since the intake of Honey Rice Crispies displayed consistent trends both within- and between-groups, it is reasonable to conclude that there was no systematic relationship between the observed effects of both drug and stress treatments on palatable food intake and the stage of the cycle at which the behavior was tested.

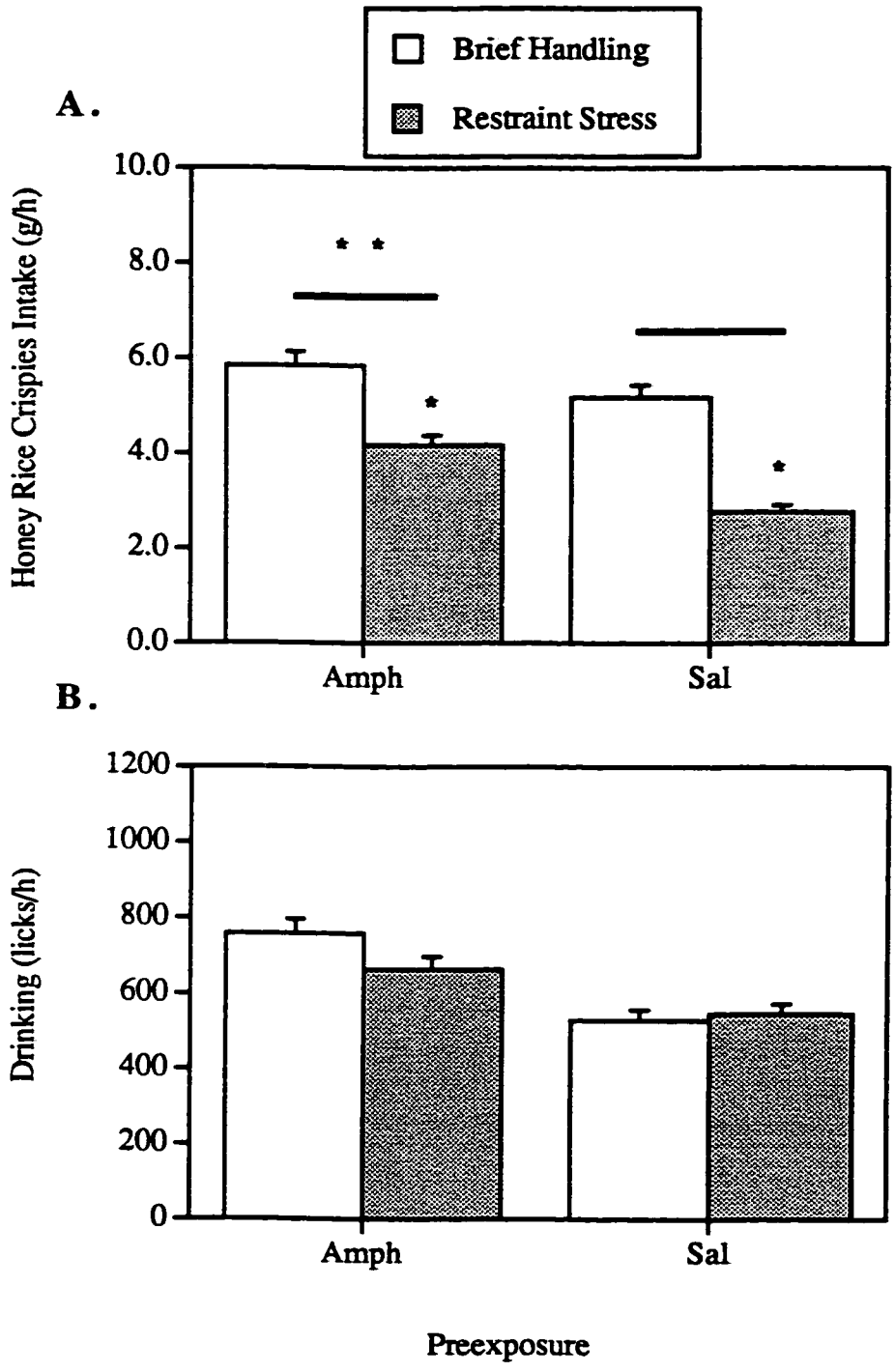
## Results

Males. Fig. 3A shows the mean daily consumption of Honey Rice Crispies in response to restraint stress or brief handling, in amphetamine- and saline-preexposed animals. The 3-way ANOVA for Preexposure by Treatment by Day, revealed a significant effect of Preexposure ($F(1, 28) = 4.12$, $p = 0.05$), indicating that regardless of the stress condition, amphetamine-preexposed males consumed significantly more Honey Rice Crispies than saline-preexposed males, during the test hour (Fig. 4A). A close inspection of Fig. 3A revealed that amphetamine-preexposed males ate more in response to restraint stress than saline-preexposed males, however, this difference did not reach

*Figure 3.* Mean (±SEM) **A.** Honey Rice Crispies intake (grams), and **B.** water intake (number of licks), during the first hour after restraint stress or brief handling in male rats previously exposed to amphetamine or saline (Experiment 2).

** different from saline-preexposed group, $p \leq .05$; * different from briefly handled group.

Mean daily consumption of Honey Rice Crispies and water in male rats



statistical significance when ANOVA for Preexposure by Day was conducted on the data ($F(1, 14) = 3.64$, $p = 0.08$).

The 3-way ANOVA also revealed a significant effect of Stress ($F(1, 28) = 16.22$, $p = 0.0004$), indicating that both amphetamine and saline-preexposed animals consumed significantly less Honey Rice Crispies in response to restraint stress than animals that were briefly handled. There was also a significant effect of Days ($F(9, 252) = 14.91$, $p = 0.0001$), reflecting the fact that all animals consumed progressively more Honey Rice Crispies across test sessions. Furthermore, a significant Treatment by Day interaction ($F(9, 252) = 3.63$, $p = 0.0003$), reflected the fact that the main effect of Days was due primarily to the increase in intake of Honey Rice Crispies over days in the briefly handled animals, whereas intake in stressed animals remained more or less at the same level over the 10 days.

The analysis of the time course of food intake in the first hour after restraint stress, in 15 min intervals averaged over the 10 test days, revealed, in addition to significant Preexposure and Treatment effects, a significant effect of Time ($F(3, 84) = 127.59$, $p = 0.0001$), reflecting the fact that feeding peaked in the first 15 min interval, after which it decreased to about the same level for all groups by the end of the hour (Fig. 5A). There was also a significant Treatment by Time interaction ($F(3, 84) = 13.80$, $p = 0.0001$), reflecting a difference in the time course of eating for stressed and briefly handled animals. It can be seen that in the briefly handled animals, feeding peaked in the first 15 min interval and remained elevated during the second 15 min interval, gradually decreasing throughout

*Figure 4.* Mean (±SEM) **A.** Honey Rice Crispies intake (grams), and **B.** water intake (number of licks), during the first hour after restraint stress or brief handling, over the 10 test days, in male rats previously exposed to amphetamine or saline (Experiment 2).

Consumption of Honey Rice Crispies and water in amphetamine- and saline-preexposed male rats

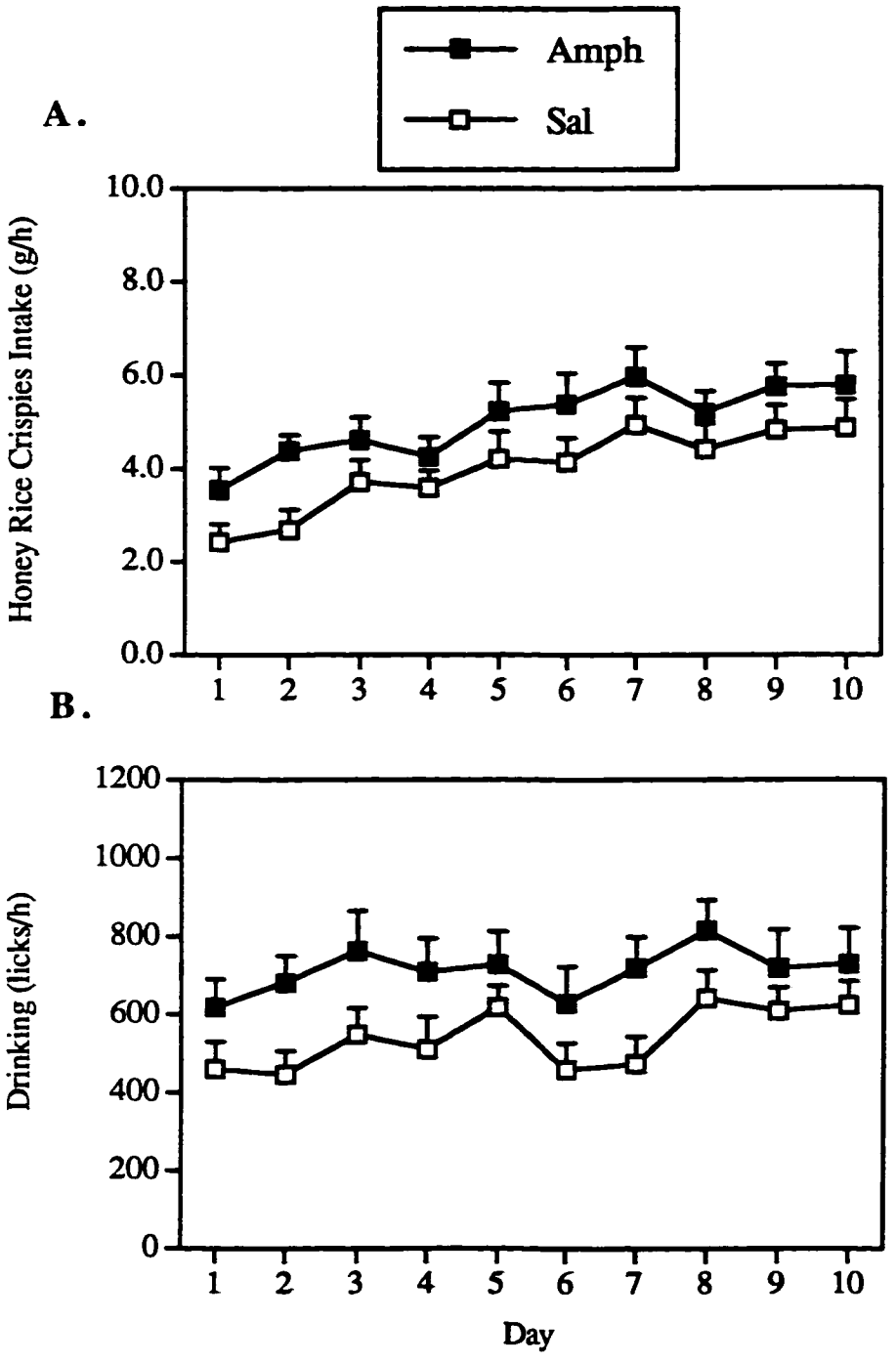
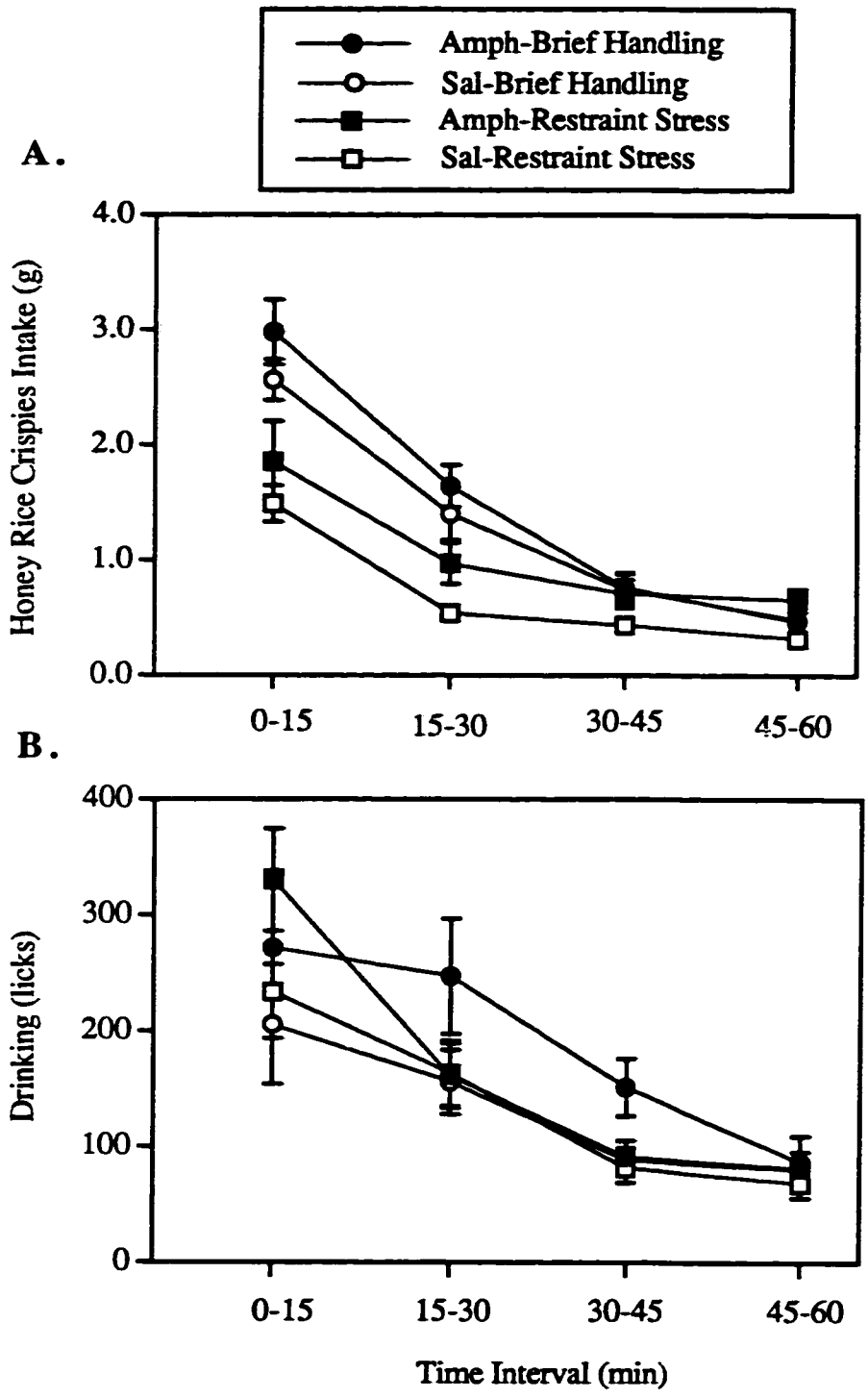


*Figure 5.* Mean (±SEM) **A.** Honey Rice Crispies intake (grams), and **B.** water intake (number of licks), in each 15 min interval, during the first hour after restraint stress or brief handling, averaged over the 10 test days, for male rats previously exposed to amphetamine or saline (Experiment 2).

Time course of feeding and drinking in response to restraint stress or brief handling in male rats



the hour, whereas in stressed animals, feeding peaked in the first 15 min interval, after which it dropped and remained low for the remainder of the hour.

Fig. 3B shows the total number of licks in the first hour after restraint stress, across the 10 test days. There was some suggestion that amphetamine-preexposed animals, regardless of the stress condition, consumed more water during the test than saline-preexposed animals (Fig. 4B), however, the effect of preexposure was not statistically significant ($F(1, 28) = 3.50$, $p = 0.07$). There was a significant effect of Days ($F(9, 252) = 3.95$, $p = 0.0001$), reflecting the fact that all groups progressively increased their water intake across the 10 test sessions, and a significant Treatment by Day interaction ($F(9, 252) = 3.63$, $p = 0.0003$), reflecting, just as with feeding, that the main effect of Days was due primarily to the change in intake in the briefly handled animals.

An ANOVA carried out on the mean number of licks in each 15 min interval of the first hour after restraint stress revealed a significant effect of Time Interval ($F(3, 84) = 35.92$, $p = 0.0001$), reflecting that for all animals drinking peaked in the first 15 min of the test hour, after which it gradually decreased to about the same level in all groups by the end of the hour (Fig. 5B).

Females. Fig. 6 shows the mean daily consumption of Honey Rice Crispies in response to restraint stress and brief handling in amphetamine and saline-preexposed females. The 3-way ANOVA for Preexposure by Treatment by Day, revealed a significant effect of Preexposure ($F(1, 28) = 6.31$, $p = 0.018$), indicating that regardless of the stress condition, amphetamine-preexposed females consumed significantly more

*Figure 6.* Mean (±SEM) Honey Rice Crispies intake (grams) during the first hour after restraint stress or brief handling in female rats previously exposed to amphetamine or saline (Experiment 2).

* different from saline-preexposed groups, $p<.05$.

Mean daily consumption of Honey Rice Crispies in
in female rats

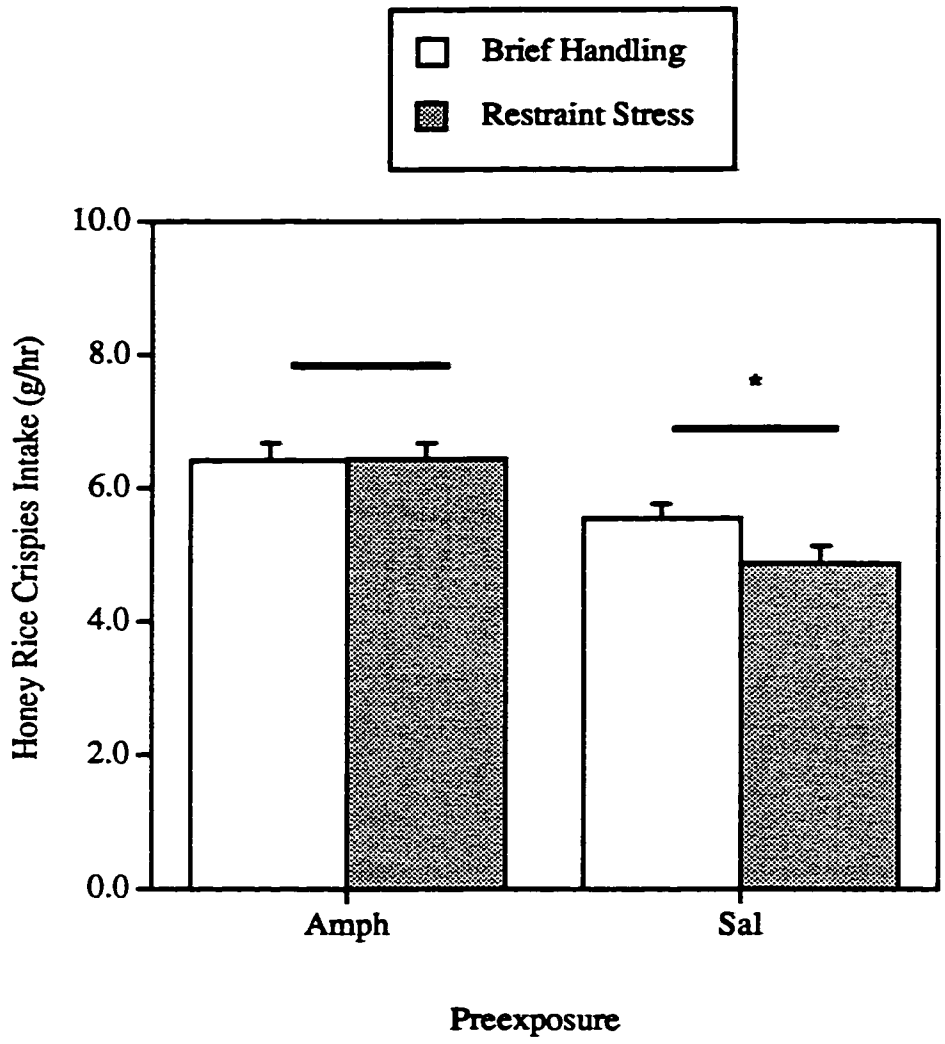
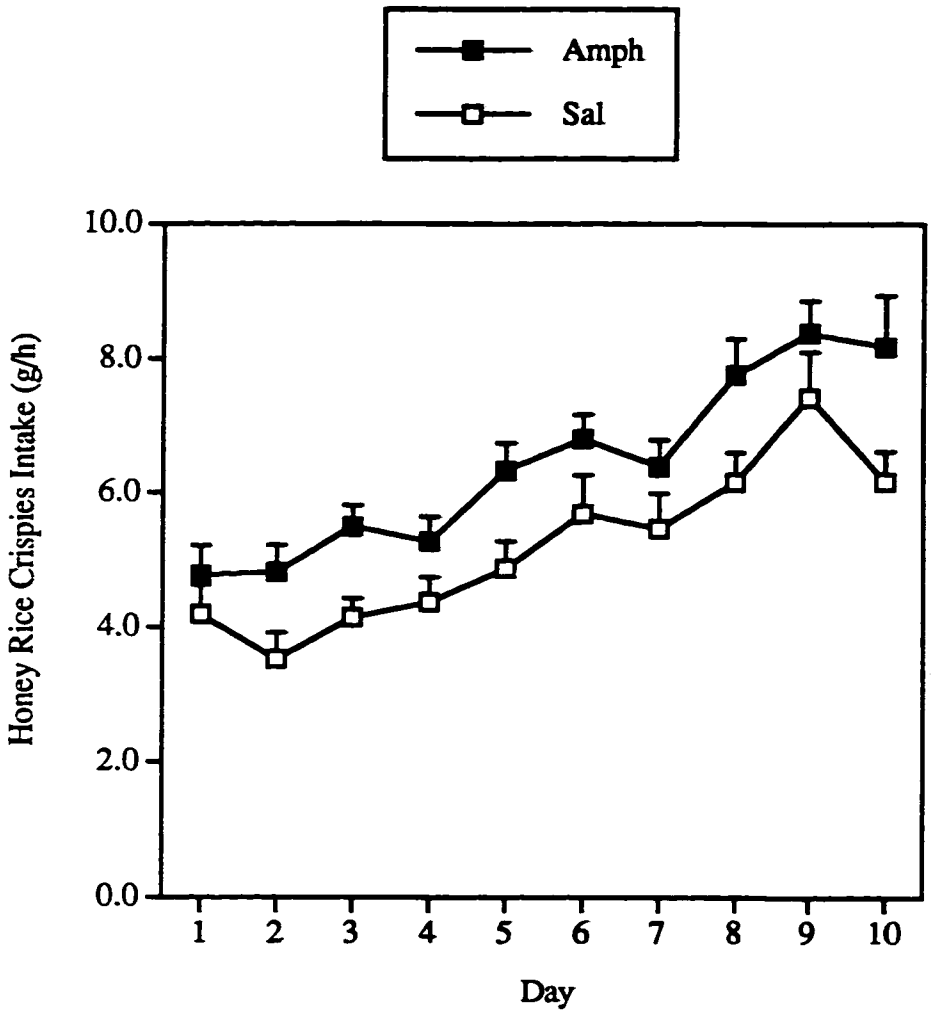


*Figure 7.* Mean (±SEM) Honey Rice Crispies intake (grams) during the first hour after restraint stress or brief handling, over the 10 test days, in female rats previously exposed to amphetamine or saline (Experiment 2).

Consumption of Honey Rice Crispies in amphetamine- and saline-pretreated female rats



Honey Rice Crispies than saline-preexposed females during the test hour (Fig. 7).

A close inspection of Fig. 6 revealed that this difference occurred entirely in stressed animals. In fact, when a separate analysis for Preexposure by Day was conducted on data from stressed animals only, a significant effect of preexposure was found ($F(1, 14) = 5.88$, $p = 0.03$), indicating that in response to restraint stress, amphetamine-preexposed females consumed more Honey Rice Crispies than saline-preexposed females.

There was also a significant effect of Day ($F(9, 252) = 27.87$, $p = 0.0001$), reflecting that all groups progressively increased their consumption of Honey Rice Crispies throughout the 10 test days. Surprisingly, there was no effect of restraint stress on intake of Honey Rice Crispies in these female rats.

The time course analysis of food intake in the first hour after restraint stress (not shown), revealed a significant Time Interval effect ($F(3, 84) = 148.75$, $p = 0.0001$), indicating that the intake of Honey Rice Crispies peaked in the first 15 min interval for all animals, after which it gradually decreased to about the same level in all groups by the end of the hour. The ANOVA carried out on water intake showed that there was no significant effect of either Preexposure or Treatment (data not shown).

## Discussion

The major finding of the present experiment was that previous exposure to amphetamine, in both males and females, regardless of the stress condition, enhanced the intake of palatable food, at the time of day when the probability of spontaneous feeding

(but perhaps not the eating of sweets) was low. It is worth noting that the animals ate large quantities of Honey Rice Crispies, consuming an average of 4.5 g and 5.8 g (32 grains per 1 gram) in the case of males and females, respectively. Furthermore, the amount of Honey Rice Crispies consumed during the test hour increased progressively over the 10 day test period in both male and female rats, and this change over days appeared to be greater in females than in males. Moreover, the difference in consumption of palatable food between the amphetamine- and saline-preexposed groups was evident throughout the ten days of testing, indicating that the effect of previous amphetamine treatment on a subsequent intake of palatable food was long-lasting and persistent. By the end of the experiment, the amphetamine-preexposed males and females were consuming 5.3 g and 7.2 g of Honey Rice Crispies respectively, as compared with 3.0 g and 5.3 g at the very beginning of the experiment.

Little effect of either amphetamine preexposure or restraint stress was found on drinking behavior under the conditions of this experiment.

A somewhat surprising finding was that stressed male rats consumed less of the palatable food than male rats subjected to brief handling. This finding was unexpected in light of evidence suggesting that exposure to brief mild stressors typically induces feeding, especially when highly palatable food is available to the animals. On the other hand, a chronic, more severe stress regimen, proposed to serve as an animal model of depression, has been reported to produce anhedonia, illustrated by a marked decrease in consumption and preference for a sucrose solution (Willner, Muscat, & Papp, 1992). It should be

noted, however, that in the present experiment, even among the stressed males, those that were preexposed to amphetamine displayed a tendency to consume more of the palatable food than those that were saline-preexposed, pointing once again to a long-lasting effect of amphetamine preexposure (see Fig. 3A).

Interestingly, this suppression of consumption of palatable food after stress was not seen in female rats. There was, again, a tendency (greater than seen in males), for the amphetamine-preexposed females to consume more of the Honey Rice Crispies than for those that were saline-preexposed (see Fig 6). Nonetheless, there was no difference between stressed and briefly handled groups in their intake of Honey Rice Crispies. A similar finding was reported by Zylan and Brown (1996), who found no difference between stressed and nonstressed females, when they were given access to a variety of palatable foods after release from 20 min restraint stress. Another interesting finding from the present study, as well as from the Zylan and Brown study was that, even though males generally eat more than females, when offered more palatable food this effect was abolished. In fact, in the present experiment the females were consuming more of the palatable food than males throughout all ten test days. Zylan and Brown suggested that this finding may indicate that females are more sensitive to the palatability of food. Thus, it is possible that in the present experiment the palatability of the food overcame any suppressive or enhancing effect that stress may have had in female rats.

Even it this were the case for females, it does not explain the decreased consumption of the palatable food following stress in males. One possible source of the

difference seen between this experiment and Experiment 1 is that the animals did not live in the test cages. It may be that due to the difference in the housing conditions during testing, the daily transportation to the test boxes, in addition to the daily restraint, provided a source of stress severe enough to lead to a reduction of a high probability behavior such as consumption of the palatable Honey Rice Crispies. Thus, in Experiment 3, the effect of place of housing on the consumption of Honey Rice Crispies following restraint stress was studied in male rats preexposed to amphetamine or saline.

## Experiment 3. Effects of amphetamine pretreatment on stress-induced consumption of palatable food in the home cage environment.

Under the conditions of Experiment 1, a facilitation of regular lab chow intake was seen in response to restraint stress, and this effect was stronger in amphetamine-preexposed male rats. In contrast, under the conditions of Experiment 2, a reduction in intake of palatable food was seen in response to restraint stress but, at the same time, there was a tendency for amphetamine-preexposed animals to consume more than saline-preexposed males.

There were two major differences between Experiments 1 and 2. One was the food employed - regular lab chow in Experiment 1 versus Honey Rice Crispies in Experiment 2; the other was the familiarity of the testing environment - in Experiment 1, animals lived in the test cages for the duration of the stress regimen, whereas in Experiment 2, they were transported to the test boxes daily, where they remained for about 1.5 h. Because stress-induced facilitation of intake of palatable food has been frequently found in

the past, it seemed unlikely that the reduction observed in Experiment 2 was due to the nature of the food. Rather, given that the stress-induced feeding was enhanced under the conditions of Experiment 1, where the testing environment served as home cage for the duration of the stress regimen, it seemed reasonable to speculate that, in Experiment 2, daily transportation of the animals to the test boxes could have been a stressor on its own, and together with the daily restraint was adding up to a more severe experience, thus leading to the reduction in intake of palatable foods reported under conditions of more severe stress (Willner et al., 1992). It was hypothesized, therefore, that if the rats were to be housed in the testing environment for the duration of the stress regimen, without daily trips to the test boxes, one would see a facilitation of stress-induced feeding of palatable food, typically reported after exposure to brief, mild stressors. This possibility was addressed in Experiment 3, which was conducted in an identical manner to Experiment 1, except that the rats were given access to Honey Rice Crispies in the hour after stress.

## Method

### Subjects

Thirty-two male Wistar rats were used in the present experiment (Charles River, Canada), maintained as described in General Methods. The animals were randomly assigned to four groups, identical to those in Experiment 1, Amph-Stress (n=8), Amph-Brief Handling (n=8), Sal-Stress (n=8), and Sal-Brief Handling (n=8).

Procedure

After 4-5 days of handling, but before drug treatment, the animals were introduced to the new food by being given a small bowl filled with Honey Rice Crispies in their home cages, once a day, for 4 days. There was no time limit imposed on the consumption of Honey Rice Crispies during those habituation days.

The testing procedure was identical to the one described in Experiment 1, except, after being released from restraint or after brief handling, the animals were placed back in the testing boxes, where they had access to Honey Rice Crispies for one hour, after which the experimenter entered the room and switched the food to the regular powdered laboratory chow.

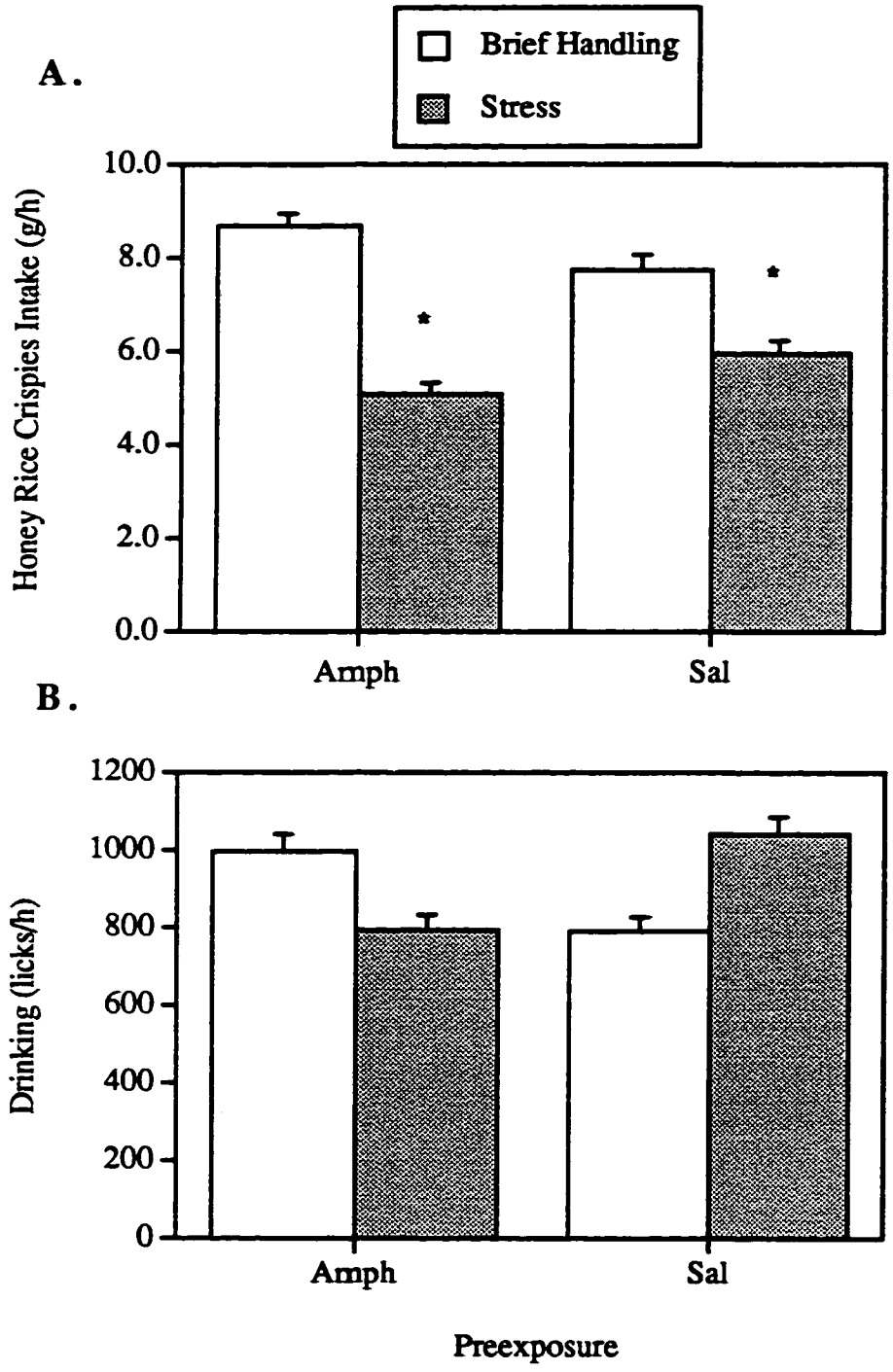
## Results

Fig. 8A shows the mean daily consumption of Honey Rice Crispies in response to restraint stress and brief handling in amphetamine and saline-preexposed animals. The 3-way ANOVA for Preexposure by Treatment by Day, revealed no effect of Preexposure, but a significant effect of Treatment ($F(1, 28) = 14.31$, $p = 0.0008$), reflecting the observation that regardless of the drug preexposure condition, stressed animals consumed significantly less Honey Rice Crispies than animals that were briefly handled. There was also a significant effect of Day ($F(9, 252) = 2.95$, $p = 0.002$), reflecting an increase in consumption of Honey Rice Crispies seen in all groups over the ten daily test sessions. The time course analysis of food intake during the test hour (not shown), revealed a

*Figure 8.* Mean (±SEM) **A.** Honey Rice Crispies intake (grams), and **B.** water intake (number of licks), during the first hour after restraint stress or brief handling in male rats previously exposed to amphetamine or saline, housed in the test environment for the duration of the stress treatments (Experiment 3).

* different from briefly handled groups, $p<.05$.

Mean daily consumption of Honey Rice Crispies
and water in male rats tested in home cage environment



significant effect of Time Interval ($F(3, 84) = 39.59$, $p = 0.0001$), reflecting a decrease in consumption of Honey Rice Crispies over the course of the test hour.

Fig. 8B shows the mean number of licks in the first hour after restraint stress, across the 10 test sessions. The ANOVA for Preexposure by Treatment by Day revealed a significant Preexposure by Treatment interaction ($F(1, 28) = 4.81$, $p = .04$). Among stressed animals, those that were amphetamine-preexposed drank less than those that were saline-preexposed, whereas this effect was reversed within the briefly handled animals. There was also a significant effect of Days ($F(9, 252) = 8.80$, $p = 0.0001$), reflecting an increase in drinking over days in all groups.

The time course analysis carried out on the mean number of licks for each animal taken at 15 min intervals during the testing hour, averaged over the ten test sessions (not shown), revealed a significant Treatment by Time Interval interaction ($F(3, 84) = 10.82$, $p = 0.0001$), reflecting a different time course of drinking between the stressed and briefly handled animals. Namely, drinking peaked in the first 15 min interval in stressed animals, whereas in briefly handled animals, it peaked in the second 15 min interval.

Finally, in this experiment the total food intake during the light cycle was analyzed using the ANOVA for Preexposure by Treatment by Day. Significant effect of Preexposure was found ($F(1, 28) = 4.32$, $p = 0.05$), showing that regardless of the stress condition, amphetamine-preexposed rats consumed more of the regular powdered chow during the light cycle than saline-preexposed animals. There was no difference in food consumption during the dark part of the cycle.

## Discussion

The main finding of Experiment 3 was that, in contrast to Experiment 2, amphetamine preexposure did not enhance the intake of highly palatable food. It is important to note, however, that when animals were housed in the test cages, as they were in Experiment 3, all animals consumed much larger quantities of Honey Rice Crispies than they did under the conditions of Experiment 2, suggesting a possible ceiling effect, making it impossible to see an effect of amphetamine preexposure.

There was, however, in this experiment just as in Experiment 2, a significant reduction in the intake of more palatable food in response to restraint stress, even though the animals were housed in the test boxes. This would suggest that regardless of the housing conditions, restraint stress produced a decrease in the intake of palatable food. This effect seen in both experiments may well reflect a disruption, by stress, of a high rate and high probability response, rather than a direct effect on food intake. Perhaps only when the probability of feeding is low, as in the case of the eating of regular lab chow in the middle of the light cycle, will food consumption be increased after exposure to mild stress.

Although, to my knowledge, the effects of stress on behavioral response rates have not been directly addressed in past research, it has been frequently suggested that the effects of amphetamines on schedule-controlled behavior are dependent upon the rate of the response under investigation. Typically, in low to moderate doses, amphetamine has been shown to increase low rates of responding, but to decrease or not affect high rates of

responding (Sanger & Blackman, 1975; Slawecki & Samson, 1996; van Haaren et al., 1986). Interestingly, when total intake of regular lab chow by animals during the light cycle was analyzed, amphetamine-preexposed animals were found to have consumed significantly more than saline-preexposed animals, revealing again a long-lasting effect of amphetamine preexposure. There was no such effect during the dark cycle, indicating that the enduring effects of amphetamine were seen during the time of day when the probability of spontaneous feeding was low to begin with.

## General Discussion

### Effects of amphetamine preexposure on *stress-induced* food intake

When allowed access to regular laboratory chow after a brief period of restraint stress (Experiment 1), animals that had been given repeated intermittent injections of amphetamine and then withdrawn from the drug for 14 days showed enhanced stress-induced feeding compared to a saline-treated control group. This enhancement was evident on day 1 and persisted for 4 days. During this time, animals in both groups showed a progressive increase (sensitization) in the magnitude of stress-induced feeding, a finding that replicates that of Badiani et al. (1996). The fact that amphetamine-preexposed animals exhibited enhanced stress-induced feeding, which showed sensitization across initial test sessions, without affecting total daily food intake, provides further support for the hypothesis that treatment with amphetamine exerts long-lasting effects on the subsequent response of an organism to stress, and may be of significance for understanding brain substrates of cross-sensitization between psychostimulants and stressors.

In Experiment 2, the effects of amphetamine on feeding were studied in both stressed and non-stressed animals. Testing was carried out in a setting different from the home cage environment and using highly palatable food. Amphetamine-preexposure was found to enhance the consumption of palatable food, regardless of the stress condition, however, there was slight tendency for amphetamine-preexposed animals, and particularly amphetamine-preexposed females, to consume more of the palatable food than saline-pretreated animals, despite the general stress-induced suppression of palatable food intake. This tendency was evident throughout all 10 days of testing, suggesting once again a long-lasting and persistent effect of amphetamine preexposure. In contrast, when rats were tested in their home cage environment, no effect of amphetamine preexposure on consumption of palatable food in response to restraint stress was evident (Experiment 3). The fact that all animals consumed much larger quantities of the palatable food under these living conditions points to a possible ceiling effect, beyond which animals were unable to eat more.

The argument made in the General Introduction was that stressors, as well as psychostimulant drugs, are capable of eliciting a motivational state of arousal and readiness, which in turn facilitates an animal's response to salient incentive stimuli in its immediate environment. Consistent with this idea, it appears that in the present experiments restraint stress elicited a motivational state sufficient to initiate, or facilitate, the previously low probability response of eating regular chow. Furthermore, it was proposed that the hypersensitivity of the mesolimbic dopamine system, that develops following repeated exposure to psychostimulants, may serve to augment the subsequent response of an

organism to stimuli known to be capable of activating this system. And, although the present experiments did not assess changes in the response of the dopamine system directly, it was found that preexposure to sensitizing injections of amphetamine served to augment the subsequent response of an animal to restraint stress, facilitating stress-induced consumption of regular chow and, to a lesser degree, stress-induced consumption of highly palatable food.

It may be useful at this point to examine what is known about changes in dopamine release in response to restraint stress. It has been reported that the beginning of restraint is characterized by an increase in dopamine release in the NAc, and then followed by a return to pre-stress baseline levels after about 60 min of continued restraint. Upon release from restraint, there is again a marked increase in dopamine release in the NAc (Puglisi-Allegra et al., 1991). In another experiment, it was found that after repeated restraint stress there was a gradual reduction in the initial dopamine rise, which was abolished from the fourth day onward, whereas the post-release rise in dopamine remained unchanged (Imperato et al.,1992). Interestingly, in these microdialysis experiments, it was found that dopamine was elevated in the period 0-30 min post-stress, at a time when in the present experiments stress-induced feeding of both types of food peaked.

In summary, the findings of the present experiments provide evidence that preexposure to a drug known to have long-lasting effects on the mesolimbic dopamine system may indeed serve to enhance the motivational arousal state produced in response to subsequent restraint stress, during a phase characterized by increased dopamine release in

the NAc. The behavioral manifestation of this state was increased consumption of food, probably the most salient incentive stimulus in the immediate environment of the animals.

**Effects of amphetamine preexposure on *free feeding* of highly palatable food**

In addition to the effect of amphetamine preexposure on stress-induced feeding discussed above, amphetamine preexposure significantly increased the consumption of Honey Rice Crispies cereal in both males and females, regardless of the stress condition, when the animals were tested in a setting different from home cage environment (Experiment 2). The difference in consumption of palatable food between the amphetamine- and saline-preexposed groups was evident throughout all ten days of testing, during which the amount of Honey Rice Crispies consumed increased progressively in all animals, but especially in females. These results, again, reveal the long-lasting and persistent nature of previous exposure to amphetamine on the subsequent response of an organism to incentive stimuli.

It will be recalled, however, that when testing was carried out in the home-cage environment (Experiment 3), there was no effect of amphetamine preexposure on the subsequent intake of the highly palatable food, probably due to the fact that all animals were consuming very large quantities of the food. It was found, nonetheless, that amphetamine-preexposed animals consumed significantly more regular chow during the remaining part of the light cycle (a low probability response compared to the consumption of Honey Rice Crispies) than did the saline-preexposed animals, revealing again a long-lasting effect of amphetamine preexposure.

These experiments appear to be the first on the long-lasting changes of amphetamine preexposure on the subsequent intake of palatable food. There have been several investigations of the acute effects of amphetamine on ingestive behaviors, which showed that systemic administration of amphetamine selectively stimulated sugar intake, while having no significant effect on standard rat chow intake (Evans & Vaccarino, 1987; 1990). Similarly, Kanarek and Marks-Kaufman (1988) reported that ingestion of amphetamine in the drinking water, produced significant increases in the intake of granulated sucrose, while at the same time, leading to significant decreases in the intake of regular chow. These findings indicate that the direct action of amphetamine is not to facilitate food intake in general, but rather, to enhance selectively the consumption of foods associated with preferred sensory or postingestional hedonic properties.

The purpose of the present experiments, however, was to investigate the long-lasting, as opposed to acute, effects produced by amphetamine treatment on the subsequent responses of animals to motivationally significant stimuli in their environments. It was suggested that the enhanced consumption of palatable food observed in amphetamine-preexposed animals might be explained by the long-lasting consequences of amphetamine treatment on the mesolimbic dopamine system. Experiments on dopamine release associated with food intake have focused mainly on the examination of changes in dopamine release in animals having access to regular chow. Moreover, in most experiments, the animals were either food deprived or maintained at 70-80% of their basal body weight, making it hard to determine whether the enhanced dopamine release in the NAc was due to food intake per se or to the rewarding aspect of feeding for hungry

animals. The issue of palatability was addressed by Martel and Fantino (1996), who examined the effects of the ingestion of regular chow (low palatable food) and short cakes (highly palatable food) on the activity of the mesolimbic dopamine system using microdialysis in non-deprived rats. Their findings revealed that during feeding, the dopamine rise in the NAc was considerably greater for the highly palatable than for the low palatable food and that increases in the dopamine metabolites, DOPAC and HVA, reached significance only with the highly palatable food. These results suggest that the mesolimbic dopamine system is activated upon ingestion of food and that the activity in this system is associated with its rewarding properties, related to palatability, as well as hunger.

In summary, previous treatment with amphetamine, in contrast to previous saline treatment, was found to enhance feeding in animals stimulated to feed by availability of highly palatable food. The argument presented is that prior exposure to repeated intermittent amphetamine injections, known to sensitize functioning within the mesolimbic dopamine system, served to enhance the effectiveness or salience of incentive stimuli. The fact that these interactions appeared progressively enhanced (or sensitized) over repeated encounters with the incentive stimuli may be of significance for understanding the phenomenon of sensitization and the nature of changes in the dopamine system, activated in common by psychostimulant drugs, stressors, and natural incentives.

### *Sex differences* in effects of amphetamine preexposure on intake of palatable food

Finally, when offered more palatable food in a setting different from the home-cage environment, both male and female rats preexposed to amphetamine consumed more of

the palatable food than those preexposed to saline. This enhanced intake, however, increased progressively over repeated test sessions to a greater extent in females than in males. There was also a greater tendency in females than in males for amphetamine-preexposed animals to consume more Honey Rice Crispies following restraint stress than for saline-preexposed animals.

These findings are in agreement with reports that repeated administration of amphetamine, as well as repeated stress, produces more robust behavioral sensitization in female than in male rats (Robinson, 1988). Furthermore, past research has revealed not only that female animals show greater behavioral sensitization than males, but that as a population, they are relatively more homogeneous in this regard. It might follow then that the female population offers certain advantages with respect to studying and identifying the neurochemical and behavioral correlates of sensitization.

**Concluding Remarks**

The results of the present experiments revealed long-lasting effects of previous amphetamine treatment on subsequent responses of animals to stress-induced feeding and to free consumption of palatable foods. Amphetamine-preexposed animals were found to consume more regular chow in response to restraint stress than saline-preexposed animals. Furthermore, this effect increased progressively across initial stress sessions, suggesting the development of sensitization of the response to stress. In addition, amphetamine-preexposure increased the consumption of highly palatable food, an effect that also showed sensitization over repeated test sessions. The effects of amphetamine preexposure on

consumption of palatable food were more pronounced in female than in male rats. The argument presented is that previous exposure to a sensitizing regimen of amphetamine exerts long-term effects on the neural systems activated in common by psychostimulant drugs, stressors and natural incentive stimuli, increasing the subsequent behavioral impact of such stimuli on an organism.

## References


Altier, N., & Stewart, J. (1996) Opioid receptors in the ventral tegmental area contribute to stress-induced analgesia in the formalin test for tonic pain. *Brain Research, 718*, 203-206.

Altier, N., & Stewart, J. (1998) Dopamine receptor antagonists in the nucleus accumbens attenuate analgesia induced by ventral tegmental area Substance P or morphine and by nucleus accumbens amphetamine. *Journal of Pharmacology and Experimental Therapeutics, 285*, 208-215.

Amit, Z., & Galina, Z.H. (1986) Stress-induced analgesia: Adaptive pain suppression. *Physiological Reviews, 66*, 1091-1120.

Anagnostaras, S.G., & Robinson, T.E. (1996) Sensitization to the psychomotor stimulant effects of amphetamine: Modulation by associative learning. *Behavioral Neuroscience, 110,* 1397-1414.

Antelman, S.M. (1988) Stressor-induced sensitization to subsequent stress: Implications for the development and treatment of clinical disorders. In: *Sensitization in the Nervous System,* P.W. Kalivas & C.D. Barnes, eds., Telford Press, Caldwell, NJ, pp. 227-254.

Antelman, S.M. & Chiodo, L.A. (1983) Amphetamine as a stressor. In:

*Stimulants: Neurochemical, Behavioral, and Clinical Perspectives,* I. Creese, ed, Raven Press, New York, pp.269-299.

Antelman, S.M. & Eichler, A.J. (1979) Persistent effects of stress on dopamine-related behaviors: Clinical implications. In: *Catecholamines: Basic and Clinical Frontiers*, E. Usdin, I.J. Koppin, & J. Barchas, eds., Pergamon Press, New York, pp. 1759-1761.

Antelman, S.M., Eichler, A.J., Black, C.A., & Kocan, D. (1980) Interchangeability of stress and amphetamine in sensitization. *Science, 207,* 329-331.

Antelman, S.M., Rowland, N.E., & Fisher, A.E. (1976) Stimulation bound ingestive behavior: A view from the tail. *Physiology and Behavior, 17,* 743-748.

Antelman, S.M., & Szechtman, H. (1975) Tail pinch induces eating in sated rats which appears to depend on nigostriatal dopamine. *Science, 189,* 731-733.

Antelman, S.M., Szechtman, H., Chin, P., & Fisher, A. (1975) Tail pinch-induced eating, gnawing and licking behavior in rats dependence on the nigrostriatal dopamine system. *Brain Research, 99,* 319-337.

Badiani, A., Browman, K.E., & Robinson, T.E. (1995) Influence of novel versus home environments on sensitization to the psychomotor stimulant effects of

cocaine and amphetamine. *Brain Research, 674,* 291-298.

Badiani, A., Jacob, A., Rodaros, D., & Stewart, J. (1996) Sensitization of stress-induced feeding in rats repeatedly exposed to brief restraint: The role of corticosterone. *Brain Research, 710,* 35-44.

Badiani, A., Leone, P., Noel, M.B., & Stewart, J. (1995) Ventral tegmental area opioid mechanisms and modulation of ingestive behavior. *Brain Research, 670,* 264-276.

Badiani, A., Mundl., W., & Cabilio, S. (1995) A computerized system for the continuous recording and analysis of feeding, drinking, diuresis, and locomotor activity. *Physiology and Behavior, 57,* 973-981.

Badiani, A., & Stewart, J. (1993) Enhancement of the prophagic but not the antidipsogenic effect of U-50,488H after chronic amphetamine pretreatment. *Pharmacology, Biochemistry, and Behavior, 44,* 77-86.

Borowski, T.B., & Kokkinidis, L. (1994) Cocaine preexposure sensitizes conditioned fear in a potentiated acoustic startle paradigm. *Pharmacology, Biochemistry, and Behavior, 49,* 935-942.

Borowski, T.B., & Kokkinidis, L. The effects of cocaine, amphetamine, and the dopamine D1 receptor agonist SKF 38393 on fear extinction in a potentiated

startle paradigm: Implications for psychomotor stimulant psychosis, submitted.

Brown, E.E., Robertson, G.S., & Fibiger, H.C. (1992) Evidence for conditioned neuronal activation following exposure to a cocaine-paired environment: Role of forebrain limbic structures. *Journal of Neuroscience, 12*, 4112-4121.

Cabib, S., & Puglisi-Allegra, S. (1996) Stress, depression and the mesolimbic dopamine system. *Psychopharmacology, 128*, 331-342.

Caul, W.F., Jones, J.R., & Barrett, R.J. (1988) Amphetamine's effects on food consumption and body weight: The role of adaptive processes. *Behavioral Neuroscience, 102*, 441-450.

Clarke, P.B.S., & Franklin, K.B.J. (1992) Infusions of 6-hydroxydopamine into the nucleus accumbens abolish the analgesic effect of amphetamine but not of morphine in the formalin test. *Brain Research, 580*, 106-110.

Cooper, J.R., Bloom, F.E., & Roth, R.H. (1996) *The Biochemical Basis of Neuropharmacology*. Oxford University Press, New York.

Eichler, A.J., & Antelman, S.M. (1979) Sensitization to amphetamine and stress may involve nucleus accumbens and medial frontal cortex. *Brain Research, 176*, 412-416.

Ely, D.R., Dapper, V., Marasca, J., Correa, J.B., Gamaro, G.D., Xavier, M.H., Michalowski, M.B., Catelli, D., Rosat, R., Ferreira, M.B.C., & Dalmaz, C. (1997) Effect of restraint stress on feeding behavior of rats. *Physiology and Behavior, 61*, 395-398.

Evans, K.R., & Vaccarino, F.J. (1987) Effects of d- and l-amphetamine on food intake: Evidence for a dopaminergic substrate. *Pharmacology, Biochemistry, and Behavior, 27*, 649-652.

Evans, K.R., & Vaccarino, F.J. (1990) Amphetamine- and morphine-induced feeding: Evidence for involvement of reward mechanisms. *Neuroscience and Biobehavioral Reviews, 14*, 9-22.

Franklin, K.B.J. (1998) Analgesia and abuse potential: An accidental association or a common substrate? *Pharmacology, Biochemistry, and Behavior, 59*, 993-1002.

Grunberg, N.E., & Straub, R.O. (1992) The role of gender and taste class in the effects of stress on eating. *Health Psychology, 11*, 97-100.

Hamamura, T., & Fibiger, H.C. (1993) Enhanced stress-induced dopamine release in the prefrontal cortex of amphetamine-sensitized rats. *European Journal of Pharmacology, 237*, 65-71.

Hamamura, T., Ichimaru, Y., & Fibiger, H.C. (1997) Amphetamine sensitization enhances regional c-*fos* expression produced by conditioned fear. *Neuroscience, 76*, 1097-1103.

Herman, J.P., Stinus, L., & Le Moal, M. (1984) Repeated stress increases locomotor response to amphetamine. *Psychopharmacology, 84*, 431-435.

Imperato, A., Angelucci, L., Casoni, P., Zocchi, A., &Puglisi-Allegra, S. (1992) Repeated stressful experiences differently affect limbic dopamine release during and following stress. *Brain Research, 577*, 194-199.

Kanarek, R.B., & Marks-Kaufman, R. (1988) Dietary modulation of oral amphetamine intake in rats. *Physiology and Behavior, 44*, 501-505.

Kuczenski, R., & Segal, D.S. (1988) Psychomotor stimulant-induced sensitization: Behavioral and neurochemical correlates. In: *Sensitization in the Nervous System*, P.W. Kalivas & C.D. Barnes, eds., Telford Press, Caldwell, NJ, pp. 175-205.

Levine, A.S., & Morley, J.E. (1982) Tail pinch-induced eating: Is it the tail or the pinch? *Physiology and Behavior, 28*, 565-567.

Leyton, M., & Stewart, J. (1996) Acute and repeated activation of male sexual behavior by tail pinch: Opioid and dopaminergic mechanisms. *Physiology*

*and Behavior, 60*, 77-85.

Martel, P., & Fantino, M. (1996) Mesolimbic dopaminergic system activity as a function of food reward: A microdialysis study. *Pharmacology, Biochemistry, and Behavior, 53*, 221-226.

Michaud, C., Kahn, J.P., Musse, N., Burlet, C., Nicolas, J.P., & Mejean, L. (1990) Relationships between a critical life event and eating behaviour in high-school students. *Stress Medicine, 6*, 57-64.

Mirenowicz, J., & Schultz, W. (1996) Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. *Nature, 379*, 449-451.

Mitchell, J.B. & Stewart, J. (1990) Facilitation of sexual behaviors in the male rat in the presence of stimuli previously paired with systemic injections of morphine. *Pharmacology, Biochemistry, and Behavior, 35*, 367-372.

Mogenson, G.J., Jones, D.L., & Yim, C.Y. (1980) From motivation to action: Functional interface between the limbic system and the motor system. *Progress in Neurobiology, 14*, 69-97.

Morgan, M.J., & Franklin, K.J.B. (1990) 6-hydroxydopamine lesions of the ventral tegmentum abolish d-amphetamine and morphine analgesia in the formalin test but not in the tail-flick test. *Brain Research, 519*, 144-149.

Morgan, M.J., & Franklin, K.J.B. (1991) Dopamine receptor subtypes and formalin test analgesia. *Pharmacology, Biochemistry, and Behavior, 40*, 317-322.

Morley, J.E.A., Levine, A.S., Murray, S.S., Kneip, J., & Grace, M. (1982) Peptidergic regulation of stress-induced eating. *American Journal of Physiology, 243*, R159-R163.

Morrow, B.A., Taylor, J.R., & Roth, R.H. (1995) Prior exposure to cocaine diminishes behavioral and biochemical responses to aversive conditioning: Reversal by glycine/N-methyl-D-aspartate antagonist co-treatment. *Neuroscience, 69*, 233-240.

Nencini, P. (1988) The role of opiate mechanisms in the development of tolerance to the anorectic effects of amphetamines. *Pharmacology, Biochemistry, and Behavior, 30*, 755-764.

Nencini, P., Johanson, C.E., & Schuster, C.R. (1988) Sensitization to kappa opioid mechanisms associated with tolerance to the anorectic effect of cathinone. *Journal of Pharmacology and Experimental Therapeutics, 245*, 147-154.

Nencini, P., & Stewart J. (1990) Chronic systemic administration of amphetamine increases food-intake to morphine, but not to U-50,488H, microinjected into the ventral tegmental area in rats. *Brain Research, 527*, 254-258.

Noel, M.B., & Wise, R.A. (1993) Ventral tegmental injections of morphine but not U-50,488H enhance feeding in food-deprived rats. *Brain Research, 632*: 68-73.

Post, R.M. & Weiss, S.R.B. (1988) Sensitization and kindling: Implications for the evolution of psychiatric symptomatology. In: *Sensitization in the Nervous System,* P.W. Kalivas and C.D. Barnes, eds., Telford Press, Caldwell, NJ, pp. 257-292.

Post, R.M., & Contel, N.R. (1983) Human and animal studies of cocaine: Implications for development of behavioral pathology. In: *Stimulants: Neurochemical, Behavioral, and Clinical Perspectives,* I. Creese, ed, Raven Press, New York, pp. 169-203.

Pothos, E.N., Creese, I., & Hoebel, B.G. (1995) Restricted eating with weight loss selectively decreases extracellular dopamine in the nucleus accumbens and alters dopamine response to amphetamine, morphine, and food intake. *Journal of Neuroscience, 15*, 6640-6650.

Puglisi-Allegra, S., Imperato, A., Angelucci, L., & Cabib, S. (1991) Acute stress induces time-dependent responses in dopamine mesolimbic system. *Brain Research, 554,* 217-222.

Robinson, T.E. (1988) Stimulant drugs and stress: Factors influencing individual differences in the susceptibility to sensitization. In: *Sensitization in the Nervous System*, P.W. Kalivas and C.D. Barnes, eds., Telford Press, Caldwell, NJ, pp. 145-173.

Robinson, T.E., Angus, A.L., & Becker, J.B. (1985) Sensitization to stress: The enduring effects of prior stress on amphetamine-induced rotational behavior. *Life Sciences, 37*, 1039-1042.

Robinson, T.E. & Becker, J.B. (1986) Enduring changes in brrain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. *Brain Research Reviews, 11*, 157-198.

Robinson, T.E., Becker, J.B., Young, E.A., Akil, H., & Castaneda, E. (1987) The effects of footshock stress on regional brain dopamine metabolism and pituitary B-endorphin release in rats previously sensitized to amphetamine. *Neuropharmacology, 26*, 679-691.

Robinson, T.E., & Berridge, K.C. (1993) The neural basis of drug craving: An incentive sensitization theory of addiction. *Brain Research Reviews, 18*, 247-291.

Rossetti, Z.L., Hmaidan, Y., & Gessa, G.L. (1992) Marked inhibition of mesolimbic dopamine release: A common feature of ethanol, morphine, cocaine, and amphetamine abstinence in rats. *European Journal of Pharmacology, 221,* 227-234.

Rowland, N.E., & Antelman, S.M. (1976) Stress-induced hyperphasia and obesity in rats: A possible model for understanding human obesity. *Science, 191,* 310-312.

Sachs, B.D., & Barfield, R.J. (1974). Copulatory behavior of male rats given intermittent electric shocks: Theoretical implications. *Journal of Comparative Physiological Psychology, 86,* 607-615.

Sakellaris, P.C., & Venikos-Danellis, J. (1975) Increased rate of response of pituitary-adrenal system in rats adapted to chronic stress. *Endocrinology, 97,* 597-602.

Salomone, J.D. (1994) The invovement of nucleus accumbens dopamine in appetitive and aversive motivation. *Behavioural Brain Research, 61,* 117-133.

Sanger, D.J., & Blackman, D.E. (1975) Rate-dependent effects of drugs on the variable-interval behavior of rats. *Journal of Pharmacology and*

*Experimental Therapeutics, 194*, 343-350.

Sato, M., Numachi, Y., & Hamamura, T. (1992) Relapse of paranoid psychotic state in methamphetamine model of schizophrenia. *Schizophrenia Bulletin, 18*, 115-122.

Schultz, W. (1992) Activity of dopamine neurons in the behaving primate. *Seminars in the Neurosciences, 4*, 129-138.

Schultz, W., Apicella, P., & Ljungberg, T. (1993) Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *Journal of Neuroscience, 13*, 900-913.

Seiden, L.S., Sabol, K.E., & Ricaurte, G.A. (1993) Amphetamine: Efects on catecholamine systems and behavior. *Annual Reviews in Pharmacology and Toxicology, 32*, 639-677.

Slawecki, C.J., & Samson, H.H. (1996) Effect of amphetamine on behavior maintained by sucrose: Interaction of reinforcement schedule and food restriction. *Pharmacology, Biochemistry, and Behavior, 54*, 595-600.

Stewart, J., & Badiani, A. (1993) Tolerance and sensitization to the behavioral effects of drugs. *Behavioural Pharmacology, 4*, 289-312.

Stewart, J., and Vezina, P. (1987) Environment-specific enhancement of the hyperactivity induced by systemic or intra-VTA morphine injections in rats preexposed to amphetamine. *Psychobiology, 15*, 144-153.

Stewart, J., & Vezina, P. (1988) Conditioning and behavioral sensitization. In: *Sensitization in the Nervous System,* P.W. Kalivas and C.D. Barnes, eds., Telford Press. Caldwell, NJ, pp. 207-225.

Strongman, K.T., Coles, M.G.H., Remington, R.E., & Wookey, P.E. (1970) The effect of shock duration and intensity on the ingestion of food of varying palatability. *Quarterly Journal of Experimental Psychology, 22,* 521-525.

Terman, G.W., Shavit, Y., Lewis, J.W., Cannon, J.T., & Liebeskind, J.C. (1984) Intrinsic mechanisms of pain inhibition: Activation by stress. *Science, 226,* 1270-1277.

Weiss, F., Imperato, A., Casu, M.A., Mascia, M.S., & Gessa, G.L. (1997) Opposite effects of stress on dopamine release in the limbic system of drug-naive and chronically amphetamine-treated rats. *European Journal of Pharmacology, 337,* 219-222.

Wise, R.A, and Bozarth, M.A. (1984) Brain reward circuitry: Four circuit elements "wired" in apparent series. *Brain Research Bulletin, 12,* 203-208.

Wise, R.A., and Bozarth, M.A. (1987) A psychomotor stimulant theory of addiction. *Psychological Review, 94*, 469-492.

Willick, M.L., & Kokkinidis, L. (1995) Cocaine enhances the expression of fear-potentiated startle: Evaluation of state-dependent extinction and the shock-sensitization of acoustic startle. *Behavioral Neuroscience, 109*, 929-938.

Willner, P., Muscat, R., & Papp, M. (1992) Chronic mild stress-induced anhedonia: A realistic animal model of depression. *Neuroscience and Biobehavioral Reviews, 16*, 525-534.

Van Haaren, F., Heinsbroek, R.P.W., Louwerse, A., & van de Poll, N.E. (1986) D-Amphetamine differentially affects low, but not high response rates of male and female Wistar rats. *Psychopharmacology, 89*, 73-76.

Zylan, K.D., & Brown, S.D. (1996) Effect of stress and food variety on food intake in male and female rats. *Physiology and Behavior, 59*, 165-169.

# Appendices

Appendix A

**Analysis of Variance for stress-induced consumption of regular chow (Experiment 1)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Group (G) | 1 | 13.5 | 13.50 | 5.67 | .03 |
| between-group error | 22 | 52.40 | 2.38 | | |
| Day (D) | 3 | 11.74 | 3.91 | 3.14 | .03 |
| G x D | 3 | .61 | .20 | .16 | .92 |
| within-group error | 66 | 82.23 | 1.25 | | |

**Analysis of Variance for time course of stress-induced consumption of regular chow (Experiment 1)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Group (G) | 1 | .84 | .84 | 5.67 | .03 |
| between-group error | 22 | 3.27 | .15 | | |
| Time Interval (TI) | 3 | 1.82 | .61 | 4.50 | .00 |
| G x TI | 3 | .14 | .05 | .34 | .79 |
| within-group error | 66 | 8.90 | .13 | | |

**Analysis of Variance for stress-induced water consumption (Experiment 1)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Group (G) | 1 | 306908.17 | 306908.17 | 1.29 | .27 |
| between-group error | 22 | 5243601.67 | 238345.53 | | |
| Day (D) | 3 | 349044.42 | 116348.14 | 3.27 | .03 |
| G x D | 3 | 33285.25 | 11095.08 | .31 | .82 |
| within-group error | 66 | 2352241.83 | 35640.03 | | |

**Analysis of Variance for time course of stress-induced water consumption (Experiment 1)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Group (G) | 1 | 19181.76 | 19181.76 | 1.29 | .27 |
| between-group error | 22 | 327725.10 | 14896.6 | | |
| Time Interval (TI) | 3 | 1915812.65 | 638604.22 | 52.02 | .00 |
| G x TI | 3 | 13831.16 | 4610.39 | .38 | .77 |
| within-group error | 66 | 810157.16 | 12275.11 | | |

## Appendix B

**Analysis of Variance for Honey Rice Crispies consumption in male rats (Experiment 2)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Preexposure (P) | 1 | 84.77 | 84.77 | 4.12 | .05 |
| Treatment (T) | 1 | 333.54 | 333.54 | 16.22 | .00 |
| P x T | 1 | 10.99 | 10.99 | .53 | .47 |
| between-group error | 28 | 575.97 | 20.57 | | |
| Day (D) | 9 | 194.15 | 21.57 | 14.91 | .00 |
| D x P | 9 | 5.73 | .64 | .44 | .91 |
| D x T | 9 | 47.33 | 5.26 | 3.63 | .00 |
| D x P x T | 9 | 4.75 | .53 | .37 | .95 |
| within-group error | 252 | 364.73 | 1.45 | | |

**Analysis of Variance for stress-induced consumption of Honey Rice Crispies in amphetamine- or saline-pretreated males (Experiment 2)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Preexposure (P) | 1 | 78.40 | 78.40 | 3.64 | .08 |
| between-group error | 14 | 301.56 | 21.54 | | |
| Day (D) | 9 | 29.71 | 3.30 | 4.07 | .00 |
| P x D | 9 | 4.42 | .49 | .61 | .79 |
| within-group error | 126 | 102.22 | .81 | | |

**Analysis of Variance for the time course of Honey Rice Crispies consumption in male rats (Experiment 2)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Preexposure (P) | 1 | 2.12 | 2.12 | 4.12 | .05 |
| Treatment (T) | 1 | 8.34 | 8.34 | 16.22 | .00 |
| P x T | 1 | .28 | .28 | .53 | .47 |
| between-group error | 28 | 14.40 | .51 | | |
| Time Interval (TI) | 3 | 59.08 | 19.69 | 127.59 | .00 |
| TI x P | 3 | .37 | .12 | .80 | .50 |
| TI x T | 3 | 6.39 | 2.13 | 13.80 | .00 |
| TI x P x T | 3 | .17 | .06 | .37 | .77 |
| within-group error | 84 | 12.97 | .15 | | |

**Analysis of Variance for water consumption in male rats (Experiment 2)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Preexposure (P) | 1 | 2378085.61 | 2378085.61 | 3.50 | .07 |
| Treatment (T) | 1 | 118118.45 | 118118.45 | .17 | .68 |
| P x T | 1 | 242770.61 | 242770.6 | .36 | .56 |
| between-group error | 28 | 19045250.5 | 680187.5 | | |
| Day (D) | 9 | 1185318.74 | 131702.08 | 3.95 | .00 |
| D x P | 9 | 192830.45 | 21425.61 | .64 | .76 |
| D x T | 9 | 756389.24 | 84043.25 | 2.52 | .01 |
| D x P x T | 9 | 131610.95 | 14623.44 | .44 | .91 |
| within-group error | 252 | 8398218.23 | 33326.26 | | |

**Analysis of Variance for the time course of water consumption in male rats (Experiment 2)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Preexposure (P) | 1 | 59452.14 | 59452.14 | 3.50 | .07 |
| Treatment (T) | 1 | 2952.96 | 2952.96 | .17 | .68 |
| P x T | 1 | 6069.27 | 6069.27 | .36 | .56 |
| between-group error | 28 | 476131.26 | 17004.69 | | |
| Time Interval (TI) | 3 | 652932.57 | 217644.19 | 35.92 | .00 |
| TI x P | 3 | 20764.36 | 6921.45 | 1.14 | .34 |
| TI x T | 3 | 34058.77 | 11352.93 | 1.87 | .14 |
| TI x P x T | 3 | 18877.71 | 6292.57 | 1.04 | .37 |
| within-group error | 84 | 508937.42 | 6058.78 | | |

**Analysis of Variance for Honey Rice Crispies consumption in female rats (Experiment 2)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Preexposure (P) | 1 | 118.83 | 118.83 | 6.31 | .02 |
| Treatment (T) | 1 | 8.32 | 8.32 | .44 | .51 |
| P x T | 1 | 9.66 | 9.66 | .51 | .48 |
| between-group error | 28 | 527.45 | 18.84 | | |
| Day (D) | 9 | 454.23 | 50.47 | 27.87 | .00 |
| D x P | 9 | 12.02 | 1.34 | .74 | .67 |
| D x T | 9 | 25.27 | 2.8 | 1.55 | .13 |
| D x P x T | 9 | 24.36 | 2.71 | 1.50 | .15 |
| within-group error | 252 | 456.29 | 1.81 | | |

**Analysis of Variance for stress-induced consumption of Honey Rice Crispies in amphetamine- or saline-pretreated females (Experiment 2)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Preexposure (P) | 1 | 98.13 | 98.13 | 5.88 | .03 |
| between-group error | 14 | 233.59 | 16.69 | | |
| Day (D) | 9 | 247.17 | 27.46 | 13.15 | .00 |
| P x D | 9 | 15.26 | 1.70 | .81 | .61 |
| within-group error | 126 | 263.13 | 2.09 | | |

**Analysis of Variance for the time course of Honey Rice Crispies consumption in female rats (Experiment 2)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Preexposure (P) | 1 | 2.97 | 2.97 | 6.31 | .02 |
| Treatment (T) | 1 | .21 | .21 | .44 | .51 |
| P x T | 1 | .24 | .24 | .51 | .47 |
| between-group error | 28 | 13.19 | .47 | | |
| Time Interval (TI) | 3 | 106.10 | 35.37 | 148.75 | .00 |
| TI x P | 3 | .88 | .29 | 1.23 | .30 |
| TI x T | 3 | .50 | .17 | .70 | .56 |
| TI x P x T | 3 | .58 | .19 | .81 | .49 |
| within-group error | 84 | 19.97 | .24 | | |

**Analysis of Variance for water consumption in female rats (Experiment 2)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Preexposure (P) | 1 | 696764.45 | 696764.45 | .71 | .41 |
| Treatment (T) | 1 | 980580.61 | 980580.61 | 1.00 | .32 |
| P x T | 1 | 1247751.01 | 1247751.01 | 1.27 | .27 |
| between-group error | 28 | 27502577.08 | 982234.90 | | |
| Day (D) | 9 | 1260979.86 | 140108.87 | 3.82 | .00 |
| D x P | 9 | 294107.24 | 32678.58 | .89 | .53 |
| D x T | 9 | 505609.95 | 56178.88 | 1.53 | .14 |
| D x P x T | 9 | 511604.18 | 56844.91 | 1.55 | .13 |
| within-group error | 252 | 9249847.18 | 36705.74 | | |

**Analysis of Variance for the time course of water consumption in female rats (Experiment 2)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Preexposure (P) | 1 | 17419.11 | 17419.11 | .71 | .41 |
| Treatment (T) | 1 | 24514.52 | 24514.52 | 1.00 | .32 |
| P x T | 1 | 31193.76 | 31193.76 | 1.27 | .27 |
| between-group error | 28 | 687564.43 | 24555.87 | | |
| Time Interval (TI) | 3 | 1379084.30 | 459694.77 | 68.87 | .00 |
| TI x P | 3 | 3656.99 | 1219.00 | .18 | .91 |
| TI x T | 3 | 438.05 | 146.02 | .02 | .99 |
| TI x P x T | 3 | 29859.43 | 9953.14 | 1.50 | .22 |
| within-group error | 84 | 560675.70 | 6674.71 | | |

Appendix C

**Analysis of Variance for Honey Rice Crispies consumption in male rats (Experiment 3)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Preexposure (P) | 1 | .12 | .12 | .00 | .96 |
| Treatment (T) | 1 | 580.77 | 580.77 | 14.30 | .00 |
| P x T | 1 | 65.61 | 65.61 | .1.62 | .21 |
| between-group error | 28 | 1136.61 | 40.59 | | |
| Day (D) | 9 | 81.26 | 9.03 | 2.95 | .00 |
| D x P | 9 | 12.18 | 1.35 | .44 | .91 |
| D x T | 9 | 19.07 | 2.12 | .69 | .72 |
| D x P x T | 9 | 17.41 | 1.93 | .63 | .77 |
| within-group error | 252 | 771.27 | 3.06 | | |

**Analysis of Variance for the time course of Honey Rice Crispies consumption in male rats (Experiment 3)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Preexposure (P) | 1 | .003 | .003 | .003 | .96 |
| Treatment (T) | 1 | 14.52 | 14.52 | 14.31 | .00 |
| P x T | 1 | 1.64 | 1.64 | 1.62 | .21 |
| between-group error | 28 | 28.42 | 1.02 | | |
| Time Interval (TI) | 3 | 72.36 | 24.12 | 39.59 | .00 |
| TI x P | 3 | 1.09 | .36 | .60 | .62 |
| TI x T | 3 | 3.60 | 1.20 | 1.97 | .12 |
| TI x P x T | 3 | .20 | .07 | .11 | .95 |
| within-group error | 84 | 51.18 | .61 | | |

**Analysis of Variance for water consumption in male rats (Experiment 3)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Preexposure (P) | 1 | 36018.83 | 36018.83 | .04 | .84 |
| Treatment (T) | 1 | 44911.50 | 44911.50 | .05 | .82 |
| P x T | 1 | 4123001.03 | 4123001.03 | 4.81 | .04 |
| between-group error | 28 | 24024623.81 | 858022.28 | | |
| Day (D) | 9 | 4499322.84 | 499924.76 | 8.80 | .00 |
| D x P | 9 | 469867.77 | 52207.53 | .92 | .51 |
| D x T | 9 | 696749.84 | 77416.65 | 1.36 | .21 |
| D x P x T | 9 | 405293.19 | 45032.58 | .79 | .62 |
| within-group error | 252 | 14308875.06 | 56781.25 | | |

**Analysis of Variance for the time course of water consumption in male rats (Experiment 3)**

| Source of Variation | Degrees of Freedom | Sum of Squares | Mean Square | F-Value | p-Value |
|---|---|---|---|---|---|
| Preexposure (P) | 1 | 900.47 | 900.47 | .04 | .83 |
| Treatment (T) | 1 | 1122.79 | 1122.79 | .05 | .82 |
| P x T | 1 | 103075.03 | 103075.03 | 4.81 | .04 |
| between-group error | 28 | 600615.60 | 21450.56 | | |
| Time Interval (TI) | 3 | 1003707.99 | 334569.33 | 29.82 | .00 |
| TI x P | 3 | 176.15 | 58.72 | .01 | 1.00 |
| TI x T | 3 | 364328.79 | 121442.93 | 10.82 | .00 |
| TI x P x T | 3 | 33758.95 | 11252.98 | 1.00 | .40 |
| within-group error | 84 | 942465.13 | 11219.82 | | |