INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

**ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600**

UMI[®]

**Dissociating the Anorexic and Anhedonic Effects of Interleukin-1 β Administration:
Implications for the Study of Depression**

A thesis presented to the Faculty of Graduate Studies and Research, Carleton University, in
partial fulfillment of the requirements of the degree of Master of Science in Psychology

Carleton University
Ottawa, Ontario

**National Library
of Canada**

**Acquisitions and
Bibliographic Services**

**395 Wellington Street
Ottawa ON K1A 0N4
Canada**

**Bibliothèque nationale
du Canada**

**Acquisitions et
services bibliographiques**

**395, rue Wellington
Ottawa ON K1A 0N4
Canada**

Your file Votre référence

Our file Notre référence

The author has granted a non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of this thesis in microform, paper or electronic formats.

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de cette thèse sous la forme de microfiche/film, de reproduction sur papier ou sur format électronique.

The author retains ownership of the copyright in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

L'auteur conserve la propriété du droit d'auteur qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

0-612-66899-1

Canada

The undersigned recommend to the
Faculty of Graduate Studies and Research
Acceptance of the thesis:

" Dissociating the Anorexic and Anhedonic Effects of Interleukin-1 β Administration:
Implications For The Study of Depression "

submitted by

Kelly Brennan

in Partial fulfillment of the requirements for
the degree of Master of Science

Chair
Thesis Supervisor

Carleton University
September, 2001

Abstract

The infusion of cytokines is thought to evoke behaviors reflecting illness. It has been proposed, however, that these behaviors may arise from alterations of a centrally-mediated motivational state. The present set of experiments were conducted to determine whether the anorexic and anhedonic effects of cytokine administration could be distinguished using the concurrent measurement of free chow intake and performance on a Progressive Ratio (PR) schedule of reinforcement for sucrose reward. The first set of experiments assessed the validity of using PR to measure motivation for sucrose reward in High and Low sucrose consumers, as well as whether manipulation of the dopaminergic and/or opiodergic systems would alter the hedonic value of sucrose. The second set of experiments assessed the effects of Interleukin-1 β (IL-1 β) and gender with respect to free consumption of lab chow, and responding for sucrose reward on PR. In the first set of experiments High and Low consumer performance on PR was sensitive to the concentration of sucrose. Furthermore, the High sucrose consumers earned a greater number of reinforcements than the Low group. Treatment with the opioid antagonist, naloxone, attenuated PR performance for sucrose, whereas d-amphetamine (0.5 mg/kg) enhanced it. Furthermore, naloxone attenuated amphetamine-enhanced responding for sucrose reward, suggesting an interaction between the dopaminergic and opiodergic systems in the mediation of sucrose reward. In the second set of experiments, female rats consumed less chow, but when corrected for body weight, intake was greater in females. Relative to males, the breaking point on PR was lower in females. Systemic IL-1 β reduced chow consumption for 48 hr in both genders. Among males, breaking point on PR for sucrose reward was reduced for 24 hr, while in females the effect persisted for 72 hr. Evidently (a) the anhedonic and anorexic effects of IL-1 β are dissociable, and (b) in females the anhedonia was more persistent than the anorexia.

Table of Contents

Title.....	i
Recommendation.....	ii
Abstract.....	iii
Table of Contents.....	iv
List of Figures.....	v
Introduction.....	1
Experiments 1-6.....	8
Experiments 7-9.....	27
General Discussion.....	40
References.....	47

List of Figures

Figure 1: The impact of the concentration of sucrose reward on PR performance by the Low consumer group vs. the High consumer groups. Each point represents the mean $\pm$ S.E.M. of the reinforcements (0.1 ml of sucrose solution) earned on the PR schedule (n=8/group).

The High group earned significantly more reinforcements than the Low group. The between-group differences were greatest ($p < 0.01$) at the 20% sucrose concentration.

Figure 2: Dose-related effects of naloxone on PR performance for sucrose reward by Low vs. High sucrose consumers. Each point represents the mean $\pm$ S.E.M. of the reinforcements (0.1 ml of 20% sucrose solution) earned on the PR schedule (n=8/group). ** Significant difference between naloxone dose and saline for both groups using t-tests; $p < 0.01$ relative to baseline.

Figure 3: Effect of d-amphetamine on sucrose solution reinforcements earned by Low vs. High sucrose consumer groups. Each point represents the mean $\pm$ S.E.M. of the reinforcements (0.1 ml of 20% sucrose solution) earned on the PR schedule (n=8/group). ** Significant difference between dose of amphetamine and saline for the High sucrose consumers; $p < 0.01$ relative to baseline.

Figure 4: Effect of naloxone on d-amphetamine-elicited changes in PR performance for sucrose reward. Each point represents the mean $\pm$ S.E.M. of the reinforcements (0.1 ml of 20% sucrose solution) earned on the PR schedule (n=8/group). **Significant within group difference between d-amphetamine alone and d-amphetamine + naloxone condition, $p < 0.01$ relative to baseline.

Figure 5: Mean chow consumption (upper panel) and breaking point (lower panel) for responding for sucrose on a progressive ration schedule in male and female rats. Treatment with IL-1 β was administered only on Day 1 and testing continued for 4 successive days. * $p < 0.05$ and ** $p < 0.01$ relative to baseline.

Introduction

Cytokines and sickness behavior

It is well documented that the infusion of cytokines, such as Interleukin-1 β (IL-1 β) and Tumor necrosis factor- α (TNF α) will exert a number of behaviors thought to reflect sickness. Among other things, administration of these cytokines results in reduced activity and consumption of lab chow, diminished sexual activity, evokes soporific effects and at higher doses animals exhibit piloerection, ptosis, and adopt a curled body posture (reviewed in (Dantzer et al., 1996; Dantzer et al., 1999; Plata-Salaman, 2000)). The view has been proposed, however, that these behavioral changes are not necessarily a reflection of malaise evoked by cytokine exposure, but instead may arise due to alterations of a centrally-mediated motivational state (Dantzer et al., 1999). Indeed, the similarity between the behavioral and neurochemical effects associated with immune activation and the symptoms of depression has favored the position that cytokine activation may contribute to affective illness (Yirmiya et al., 1999; Maes et al., 1997).

Recent studies revealed that communication occurs between the immune, endocrine, autonomic and central nervous systems, and that activation of the immune system or its products greatly influence neuroendocrine and central neurotransmitter processes (Anisman et al., 1999; Besedovsky et al., 1991; Blalock, 1994; Dantzer et al., 2000; Dunn, 1995). In addition, cytokines, which have been shown to influence *in vivo* central monoamine (NE, DA and 5-HT) and neuropeptide activity (CRH and AVP), may act in a signalling capacity between the immune and central nervous systems, perhaps influencing behavioral outputs, such as affective illnesses. The data available, however, does not indicate whether any one cytokine in particular may be

focal in relation to depression, since these cytokines may have additive or synergistic actions (Brebner et al., 2000; Plata-Salaman, 1996).

Studies in animals have clearly raised the possibility that IL-1 may contribute to depression. However, owing to the complexity of the behavioral effects of this cytokine, it is difficult to disentangle potential depressogenic effects elicited by this cytokine from the numerous other actions of the compound. The present investigation was undertaken to assess whether the anorexic effects of IL-1 β administration could be distinguished from the anhedonic (a key symptom of depression) using the concurrent measurement of free chow intake and performance on a Progressive Ratio (PR) schedule of reinforcement for sucrose reward.

Behavioral effects of cytokine treatment

Of particular relevance to the present set of investigations is the finding that cytokines also disrupt operant responding for food reward (Bret-Dibat et al., 1995; Crestani et al., 1991). It is also possible that these cytokines promote disturbances of motivation (e.g., anhedonia; Anisman et al., 2000; Dantzer et al., 1999; Dantzer, 2001). Unfortunately, because anorexia and anhedonia are associated with both cytokine-induced malaise and depression, it is extremely difficult to determine the origin of these behaviors. This is compounded by the finding that illness is often accompanied by depressive symptoms (Yirmiya et al., 1999), and incidence of depression seems to be linked with increased cytokine presence (Maes et al., 1995; Maes et al., 1991; Maes et al., 1993). Moreover, while the individual behaviors elicited from cytokine exposure seem similar to those observed in depression, assessed together as a profile, no definite conclusions can be drawn. For example, the reduced activity and increased sleep associated with proinflammatory

cytokines is reminiscent of atypical depression, yet the reduced food intake is more closely associated with the typical form of depression. Therefore, it is not at all clear what, if anything, the behavioral effects of IL-1 β actually resemble.

A fundamental feature of depression is that of anhedonia (i.e., a diminution in the perceived reward or pleasure obtained from environmental stimuli). Hence it is of particular interest to assess the potential effects of cytokines on behaviors that might reflect anhedonia. However, few behavioral paradigms may be used to this end. One procedure available is that of measuring the consumption of a highly palatable substance to determine whether its rewarding value has been diminished following a particular treatment. Unfortunately, any observed effect may be confounded by cytokine-induced malaise. It has been demonstrated that IL-1 β and TNF- α individually induced a decrease in consumption of a highly palatable and preferred substance (chocolate milk), and also exerted additive effects when administered together (Brebner et al., 2000). With lipopolysaccharide (LPS) administration, however, it has been demonstrated that the reduced consumption of sucrose elicited by the endotoxin could be antagonized by repeated administration of an antidepressant (Yirmiya et al., 1999), suggesting that the effects of LPS were probably not simply due to sickness.

Even though paradigms measuring the consumption of a palatable substance or “snack” (Yirmiya, 1996) are valuable, these procedures cannot dissociate anorexic from anhedonic effects of a given treatment. An alternative method that has been applied in an attempt to assess the effects of cytokine treatment on anhedonia is to measure responding for electrical brain stimulation. Ordinarily, when electrodes are implanted in dopamine-rich brain regions (i.e. VTA, nucleus accumbens), animals will emit a large number of operant responses to obtain electrical stimulation, supporting the view that dopaminergic (DA) mechanisms (Wise et al., 1992) are

among those that mediate reward processes. If the rewarding value of the stimulation is reduced, the animal may increase responding, likely to compensate for the diminished value of the stimulation by increasing response frequency. In instances where responding is attenuated, it may be that the outcome was secondary to nonmotivational factors (i.e. motor disturbances). In an effort to assess the effects of IL-1 β on responding for rewarding stimulation from the lateral hypothalamus, Anisman et al. (1998) assessed performance at various times following IL-1 β treatment (1.0, 2.0 μ g, i.p.). It was found that doses that induced pronounced sickness behaviour (1 μ g) did not disrupt responding, while the higher dose did. This indicates that the anhedonia may not be brought on by sickness, raising the possibility that it is associated with an alteration of mood brought on by IL-1 β administration.

A second approach to assess the anhedonic effects of a chosen treatment is that of measuring performance in an operant task in which animals must respond on a progressive ratio (PR) schedule of reinforcement. This schedule was first developed to measure the reinforcing efficacy of sweetened milk solutions in rodents (Hodos, 1961), and has since been adapted to measure the reinforcing properties of drugs of abuse such as psychomotor stimulants and opiates in rodents, as well as for food reward (Hodos, 1961; Fiefel et al., 1990; Rudski et al., 1994; Richardson et al., 1991). In a PR schedule, the “cost” of a reward is progressively increased over a number of trials to determine the maximal effort the animal will emit for the reward (Hodos, 1961). The final ratio completed within a designated period is defined as the “breaking point” (Hodos, 1961), which is a measure of the maximal effort a subject will put forth to “earn” the reward. The ratio in the PR series is developed based on the substance being studied (for the purposes of the

studies proposed, sucrose solution will be used), the type of subjects undergoing testing, and the length of each testing session (i.e. 24 hours).

The response requirements for a particular PR schedule of reinforcement can be modified over testing at one of two times. It can either be modified at the beginning of each testing session, or, as for the purposes of the following experiments, increased immediately following the delivery of a reward (sucrose solution). This allows for the swift establishment of a breaking point, allowing for a more efficient collection of data.

Concurrent measurement of “free” chow intake versus “earned” sucrose reward

By using the PR schedule of reinforcement, while providing test animals with free access to lab chow, it was believed that performance on PR would represent the effort the animal will put forth for a palatable solution, in other words, their motivation beyond that arising from hunger (i.e. energy requirements). It was anticipated that this concurrent measurement may reveal a dissociation of the anorexic and anhedonic effects of treatments administered, in particular that of IL-1 β .

Prior to undertaking these studies, however, it was necessary to evaluate the motivational value of a sucrose solution in non-deprived rats. Though the PR schedule has proven to be a reliable means of measuring motivation for drugs of abuse (Richardson et al., 1996), preliminary studies would determine (a) whether non-deprived animals will emit an increased number of bar presses to procure sucrose rewards, (b) at what rate the response requirements for each subsequent reward will increase, and (c) which concentration of the sucrose solution reward

elicits optimal performance from the test animals, avoiding difficulties with ceiling or floor effects in responding.

To this end, the first study evaluated PR performance alone. In addition, the study assessed whether PR performance of a group of high sucrose preferring rats differs from that of a group of low preferring rats. It is well established that a substantial interindividual variability in sucrose consumption exists in both humans and rodents. This difference, it has been suggested, is also indicative of an individual's propensity to self-administer drugs of abuse (Sills et al., 1996; DeSousa et al., 2000; Gosnell, 2000). It was of interest, therefore to examine the underlying mechanisms that contribute to these individual differences and whether pharmacological agents affecting the dopaminergic and/or opioidergic systems differentially alter the incentive value of sucrose reward.

Once it was established whether the animals would emit an increased number of bar presses on a PR schedule for sucrose solution, a PR ratio was established, and an optimal sucrose solution was ascertained, then the effects of IL-1 β administration on concurrent chow intake and PR performance could be examined. This study would assess whether the proposed anhedonic effects of IL-1 β on responding for a sucrose reward in a PR paradigm can be distinguished from the anorexia reflected by a decreased consumption of "free" chow. It has already been determined that IL-1 β may induce anorexia in rats (Gayle et al., 1997; Schwartz et al., 1997), as well as exert potential anhedonic effects (Anisman et al., 1998), yet it is difficult to dissociate the two. This study also assessed whether interactions between IL-1 β and gender would emerge, which may impact on the behavioral measures of interest. Females of most mammalian species exhibit a more pronounced immune response than males (Spitzer, 1999), therefore it is likely

that this is reflected in both their chow intake and propensity to seek out and consume sucrose reward.

Experiments 1-6

Individual Differences in Sucrose Consumption in the Rat: Motivational and Neurochemical Correlates of Hedonia

Substantial interindividual variability exists with respect to sucrose consumption, a phenomenon noted in rodents as well as humans. Thus when granulated sugar (sucrose) is freely available, the amount consumed varies widely across individual rats. Based on the amount consumed, these rats can be divided into High and Low sucrose consumers (Sills et al., 1996). It is of interest that these differences also appear to be correlated with other motivated behaviors, such as the propensity to self-administer drugs of abuse (Sills et al., 1996; DeSousa et al., 2000; Gosnell, 2000). The present study was undertaken to determine whether the interindividual variability in sucrose consumption was attributable to variations in the motivation to procure sucrose reward, and whether pharmacological agents affecting the dopaminergic and/or opioidergic systems differentially affected the incentive value of sucrose reward.

The progressive ratio (PR) schedule represents a well-established and effective means of measuring motivational factors. This schedule of reinforcement has been used to evaluate effects of various test conditions on motivation for drug and food reward (Fiefel et al., 1990; Maier et al., 1998; Rudski et al., 1994; Richardson et al., 1991). On a PR schedule, the “cost” of a reward is progressively increased over a number of trials to determine the maximal effort the animal will emit for the reward (Hodos, 1961). The final ratio completed within a designated period is defined as the “breaking point” (Hodos, 1961), which is a measure of the maximal effort a subject will put forth to “earn” the reward. It was hoped that the assessment of operant performance of the High and Low sucrose consumers for sucrose reward in the PR might help to

establish whether the individual variability in sucrose consumption is a reflection of their motivation to procure the sucrose reward (i.e. due to the “liking” and/or “wanting” of sucrose).

It has been hypothesized that the reward value of a stimulus (including sucrose) is influenced by reward learning, its incentive salience, and/or its hedonic impact (Dantzer et al., 1999). In the case of sucrose, the hedonic aspect(s) intrinsic to its sweet taste appears important in this context. Two neurochemical systems, namely, the endogenous opioidergic and dopaminergic systems, have been implicated in the hedonic aspects of sucrose consumption (Zurita et al., 1996; Rudski et al., 1997).

Ingestion of food has been reported to affect endogenous levels of opioid peptides in the brain, and conversely, administration of opioid agonists and antagonists have been found to increase or decrease food intake, respectively (Majeed et al., 1986; Jalowiec et al., 1981; Martin et al., 1963; Koch et al., 1995; Shabir et al., 1999). To determine whether the endogenous opioidergic system(s) has a differential role in the motivational aspects of sucrose consumption in the High vs. Low consumers, the effect of the opioid antagonist naloxone was assessed on PR responding for sucrose. It is of interest to note that whereas food intake for energy replenishment (in food deprived or hungry animals) is relatively insensitive to naloxone effects (Levine et al., 1995), the intake of a palatable, high-carbohydrate diet in non-deprived animals is highly sensitive to the suppressive effects of naloxone (Agmo et al., 1995; Philopena et al., 1996; Rudski et al., 1994).

Rewarding events, including feeding and self-administration of psychostimulants, are also accompanied by activation of the mesolimbic dopamine system (Cador et al., 1991; Wilson et al., 1995). In particular, dopamine release at the nucleus accumbens has been shown to be potentiated by rewarding stimuli (Hernandez et al., 1988; Phillips et al., 1993; Church et al.,

1987; Salamone et al., 1994). Conversely, lesions of the mesolimbic dopamine system or blockade of dopamine receptor(s) with dopamine antagonists can significantly influence feeding behavior (Yu et al., 2000; Pitts et al., 2000).

Sills and Crawley (1996) evaluated the hypothesis that High sugar consuming rats exhibit higher levels of amphetamine-stimulated accumbal dopamine release than Low consuming rats. Interestingly, these investigators observed that individual differences in sugar consumption predicted individual differences in amphetamine-stimulated dopamine release at the posterior-medial nucleus accumbens. It was proposed that administration of a low dose of amphetamine in High sugar consumers induced a state of dopamine hyperstimulation (resulting in anorexia), whereas in the Low consumers, the same dose of amphetamine produced dopaminergic activation sufficient to stimulate feeding. The mechanism(s) underlying the differential accumbal dopamine release in response to amphetamine in the High vs. Low consumers is not known, but it is speculated that such differences may be attributable to differences in dopamine synthesis and/or storage (Sills et al., 1996).

Inasmuch as opiates stimulate dopamine turnover and release (Kuschinsky et al., 1972; Matthews et al., 1984), the final objective of the current experiment was to assess whether the endogenous opioidergic and dopaminergic systems interacted at some level, to influence sucrose reward. It has been reported that both the nucleus accumbens and the ventral tegmental area are endowed with opioid peptides and their receptors, and are thought to be involved in ingestive behaviour (Pollard et al., 1977). Indeed, microinjections of morphine into these areas produce reinforcing effects, as reflected by induction of conditioned place preference, and appetitive effects as reflected by operant responding (Olds, 1982; Phillips et al., 1980). In support of the view that opioidergic and dopaminergic systems interact, it has been noted that pretreatment with

naloxone attenuates the neurochemical and locomotor effects of amphetamine (Jones et al., 1992; Schaad et al., 1995). As well, the effects of amphetamine on continuous avoidance behaviour in rats is attenuated (Holtzman, 1974).

The specific objectives of the present set of experiments were: 1) to differentiate rats into High and Low sucrose consumer groups, 2) to evaluate their motivation to procure sucrose solution reward using a PR schedule, and 3) to characterize the potential neurochemical differences between the two groups by using naloxone (an opioid antagonist) and/or d-amphetamine (an indirect dopamine agonist).

Methods

Animals

Sprague-Dawley rats ($n = 60$), weighing approximately 250-275 g, were individually housed in a temperature- and light-controlled environment, on a 12L:12D schedule with lights on at 0700 h and off at 1900 h. Rats had *ad libitum* access to water and lab chow throughout the experiment, except where noted. All experimental protocols followed the guidelines of the Canadian Council on Animal Care and were approved by the Research Ethics Committee at the University of Ottawa.

Procedure

Experiment 1: Procedure for identifying High vs. Low sucrose consuming rats. For 7 consecutive days, granulated sugar was provided in each animal's home-cage for 30 min, and the

amount consumed was recorded daily. The rats had *ad libitum* access to regular lab chow and water for the remaining 23.5 h of each day. Initially, the sugar intake increased progressively over days, to eventually reach a plateau (around day 5). Rats were divided into the High and Low sugar consumer groups ($n = 12/\text{group}$), based on their individual intake (mean over the final 2 days) being either two standard deviations ($SD=.99$) above (for High group) or below (for the Low group) the mean intake of the entire population (1.67 grams; $n=60$). The remaining animals were excluded from the study.

Experiment 2: Operant performance for water reward under deprivation conditions.

Testing cages

Testing was conducted in Plexiglas rat chambers (35 x 25 x 34 cm) with a lever located in the center of one of the walls, 6.5 cm from the floor. A drinking spout was located 3.5 cm directly to the right of the lever. Initially, each response on the lever (30 g force required for activation) automatically activated a syringe driver to deliver 0.1 ml of liquid reward into the spout protruding into the cage. A white stimulus light located 6 cm above the spout was activated concurrently with delivery of reward.

Training

Animals were trained to lever press for reward, on a fixed ratio (FR-1) schedule. Training was continued until stable bar-pressing behavior was established.

PR performance for water in water-deprived rats

Once shaping sessions were completed, animals (n=12/group) were deprived of water for 11 h and then placed in the operant chambers to bar press for water reward, to verify that they were able and willing to work for water reward on a PR schedule. The reinforcement schedule was changed from an FR1 to a PR response schedule, in which the initial lever press was reinforced, but the number of presses required for subsequent rewards increased sequentially according to the following progression: 1, 3, 6, 10, 16, 23, 32, 44, 58, 75, 96, 121, 151, 187, 229, 281, 350, 431. If rats failed to emit any responses for 10 consecutive minutes, the session was terminated. The dependent variable constituted of the “breaking point” values, defined as the number of reinforcements received prior to the cessation of responding. Animals that did not respond for water were eliminated from the study.

To determine whether naloxone administration interfered with PR performance for water reward in water deprived rats, the remaining animals (n = 8/group) were again deprived of water for 11 h, for two consecutive testing sessions, one session in which all animals were administered saline (0.1 ml/kg; i.p.), then another in which all water deprived animals were administered naloxone (3.0 mg/kg; i.p.) 15 min before testing.

Experiment 3: Performance dependent on sucrose concentrations

The incentive value of various concentrations of sucrose reward (0% (water), 10%, 20% and 30%) was evaluated using the PR schedule described earlier (n = 8/group). Each rat underwent two sessions of testing with each of the concentrations of sucrose. The volume of the reward delivered remained constant (0.1 ml). The various sucrose concentrations were presented in a randomized order (one concentration/test day) on two separate occasions spaced 24 hr apart.

Rats had access to lab chow and water, *ad libitum*, except during testing. Following the sucrose concentration PR performance study, it was of interest to determine whether similar group differences also existed when the sucrose solution was available freely. Thus, on two separate occasions (two weeks and one month following study) a 20% sucrose solution was presented *ad libitum* (for 30 min) in the home-cage, through a standard water-spout (connected to a 50 ml bottle). The mean of the two days was used for statistical analysis.

Experiment 4: Effects of naloxone on PR performance

Using the PR schedule as before, several doses of naloxone (0, 0.03, 0.3, 1, 3 mg/kg; i.p.) were administered 15 min before the test sessions, with a day off between each injection (n = 8/group). As before, rats were given the opportunity to obtain the sucrose reward (0.1 ml of 20% sucrose solution) and the session continued until 10 min elapsed with no responding, up to a maximum of 60 min.

Experiment 5: Effects of d-amphetamine on PR performance for sucrose reward

Several doses of d-amphetamine (0 (control), 0.1, 0.25, 0.5, 1.0 mg/kg; i.p.) were administered immediately before each PR test session, with a day off between each dose (n = 8/group).

Experiment 6: Naloxone and d-amphetamine interactions on PR performance

Naloxone (0 (control), 0.1, 0.3, 3.0 mg/kg; i.p.) was administered 15 min prior to d-amphetamine (0.5 mg/kg; i.p.). Animals were tested in the operant chambers immediately following d-amphetamine injection (n = 8/group).

Statistical Analyses

Consumption of freely available sucrose solution (over 30 min) was analyzed through a one-way analysis of variance (ANOVA). In the remaining experiments breaking point values were defined as the number of reinforcements received prior to the cessation of responding, and mixed measures ANOVAs were performed on these values. As animals were tested repeatedly, this factor was treated as a within group variable. Where significant interactions were observed, the simple effects were examined using paired sample t-tests (with Bonferroni corrections).

Results

Experiment 1: Procedure for differentiating High vs. Low sucrose consuming rats

Rats were allowed free access to granulated sugar (sucrose) daily for 7 consecutive days, and the amounts consumed during the final 2 days were analyzed. On average, the High group consumed 3.07 ± 0.12 g of granulated sucrose, whereas the Low group consumed 0.53 ± 0.11 g over the 30 min test period. When given the opportunity to freely consume (*ad libitum*) the 20% sucrose solution (Experiment 3), group differences were also evident with respect to the volume

consumed, $F(1,13)=6.1$, $p<0.05$, where the High group ($5.79 \pm .57$ ml) consumed a significantly greater volume of sucrose solution than rats designated as Low consumers ($4.08 \pm .39$ ml).

Experiment 2: Operant performance for water in water deprived rats

In order to ascertain that there were no confounding differences between the two groups in their ability to emit motor responses, water deprived rats were allowed to procure water reward on the PR schedule of reinforcement. Subsequently, the impact of naloxone on PR performance for water on the two groups was assessed. A mixed measures ANOVA, with High and Low sucrose consumers as the between-group variable and naloxone vs. saline treatment as the within-groups variable was conducted to evaluate the effects on the number of rewards earned on the PR schedule. The analysis revealed a significant main effect of the Naloxone treatment, $F(1,14)=19.5$, $\eta^2=.58$, $p<0.001$, indicating that naloxone (3 mg/kg) significantly attenuated bar pressing for water for both the High and Low consumers.

Experiment 3: PR performance dependent on sucrose concentrations

The incentive value of various concentrations of sucrose reward (0% (water), 10%, 20% and 30%) was evaluated on the PR schedule. The ANOVA values revealed a main effect of group, $F(1,14)=4.86$, $\eta^2=.26$, $p<0.05$, wherein the High sucrose consumers earned significantly more reinforcements than the Low consumers. The analysis also revealed a significant main effect of sucrose concentration, $F(3, 42)=10.83$, $\eta^2=.44$, $p<0.0001$, attributable to increased breaking points in a concentration-dependent manner for both groups. The breaking point was

highest at the 20% concentration, in both groups. The between-group difference was also the greatest ($p < 0.01$) at this concentration of sucrose (see Figure 1).

Experiment 4: Effects of naloxone on PR performance

The ANOVA revealed a significant Group effect, $F(1, 14) = 5.97$, $\eta^2 = .30$, $p < 0.05$, whereby the High sucrose consumers emitted more bar presses than the matched Lows, regardless of naloxone dose administered. Analysis also revealed a significant main effect of Naloxone dose, $F(4, 49) = 14.92$, $\eta^2 = .52$, $p < 0.001$, which was attributable to a naloxone-induced decrease in the breaking point. Follow-up pair-wise comparisons revealed that at doses of 0.03, 1 and 3 mg/kg naloxone attenuated bar pressing in both groups ($p < 0.01$) relative to the baseline (see Figure 2).

Experiment 5: Effects of d-amphetamine on PR performance

The ANOVA of the breaking points failed to reveal any significant overall effects. Due to *a priori* decisions, however, follow-up comparisons were conducted between each dose and the control (saline) condition, revealing that there was a significant effect of amphetamine dose with the High consumers ($F(3, 21) = 5.54$, $p = 0.01$). Paired sample t-tests indicated that bar pressing by High consumers was significantly increased with amphetamine (0.5 mg/kg; $8.63 \pm .46$) from baseline ($7.25 \pm .53$). The behavioral output of the Low consumers was not affected by amphetamine (see Figure 3).

Experiment 6: Naloxone and d-amphetamine (0.5 mg/kg) interactions in performance

The effects of naloxone on d-amphetamine-elicited changes in PR performance varied as a function of the Treatment condition, $F(3,32)=11.9$, $\eta^2=.46$, $p<0.0001$. Pairwise comparisons revealed that 0.3 mg/kg and 3.0 mg/kg of naloxone significantly attenuated the effects of the 0.5 mg/kg d-amphetamine-stimulated bar pressing (see Figure 4).

Figure 1: The impact of the concentration of sucrose reward on PR performance by the Low consumer group vs. the High consumer groups. Each point represents the mean $\pm$ S.E.M. of the reinforcements (0.1 ml of sucrose solution) earned on the PR schedule ($n=8/\text{group}$).

The High group earned significantly more reinforcements than the Low group. The between-group differences were greatest ($p<0.01$) at the 20% sucrose concentration.

Figure 2: Dose-related effects of naloxone on PR performance for sucrose reward by Low vs. High sucrose consumers. Each point represents the mean $\pm$ S.E.M. of the reinforcements (0.1 ml of 20% sucrose solution) earned on the PR schedule ($n=8/\text{group}$). ** Significant difference between naloxone dose and saline for both groups using t-tests; $p<0.01$ relative to baseline.

Figure 3: Effect of d-amphetamine on sucrose solution reinforcements earned by Low vs. High sucrose consumer groups. Each point represents the mean $\pm$ S.E.M. of the reinforcements (0.1 ml of 20% sucrose solution) earned on the PR schedule (n=8/group). ** Significant difference between dose of amphetamine and saline for the High sucrose consumers; $p < 0.01$ relative to baseline.

Figure 4: Effect of naloxone on d-amphetamine-elicited changes in PR performance for sucrose reward. Each point represents the mean $\pm$ S.E.M. of the reinforcements (0.1 ml of 20% sucrose solution) earned on the PR schedule ($n=8$ /group). **Significant within group difference between d-amphetamine alone and d-amphetamine + naloxone condition, $p<0.01$ relative to baseline.

Discussion

Sucrose consumption varies substantially across individuals, a phenomenon common to both humans and rodents. What is less clear, however, is (are) the underpinning(s) for the differences. In this context, we sought to determine whether this variability was due purely to differences in their consummatory response to a readily available “snack” or whether it was due to differences in their motivation or drive to procure the sucrose reward (its incentive value). In order to gain a better insight into the neurochemical substrates of these differences, we also explored whether pharmacological agents affecting the opioidergic and dopaminergic systems differentially affected the incentive value of sucrose in the High and Low sucrose consumers.

Rats could be distinguished on the basis of the amount of granulated sugar consumed over a 30 min test session, a finding concordant with those of earlier reports (Sills et al., 1993; Sills et al., 1994). When these groups were given *ad libitum* access to a sucrose solution (20%) instead of the granulated sugar, the differences in intake were still clearly evident. In addition to increased consumption of “free” sucrose (in granulated or solution form), increased consumption was apparent on the PR schedule, where the “cost” of reward increased progressively until the “breaking point” was reached (Hodos, 1961; Maier et al., 1998).

It has been hypothesized that the reward value of a stimulus (including sucrose) is influenced by reward learning, its incentive salience, and/or its hedonic impact (Dantzer et al., 1999). In terms of potential neurochemical underpinnings, the opioidergic system(s) has been implicated in the hedonic aspects of palatable food or reward procurement. The next set of experiments assessed whether the reward value of sucrose in the two groups of rats was differentially affected by naloxone (an opioid antagonist). The results demonstrated that

naloxone suppressed PR performance for sucrose as well as water reward, at equivalent doses. This is concordant with previous findings reporting that naloxone suppressed both food and water intake (Rudski et al., 1994; Philopena et al., 1996; Rudski et al., 1997). Since the animals in the present study were not food deprived (controlling for hunger), it could be surmised that naloxone reduced the incentive value of sucrose reward, thus diminishing PR performance. This observation is consistent with the argument that the opioid system controls the hedonic aspect of food reward (palatability). Although naloxone has been shown to decrease free feeding of palatable food (Baile et al., 1981; Jalowiec et al., 1981; Shabir et al., 1999), feeding driven by energy deficit appears resistant to naloxone blockade (Levine et al., 1995). In the present study, both high and low doses of naloxone attenuated bar pressing for sucrose. At the higher dose (3 mg/kg), however, naloxone was also capable of attenuating bar pressing for water in water-deprived animals. This finding is in keeping with various reports demonstrating that naloxone (and related antagonists) suppress water intake, both in water deprived and non-deprived animals, depending on the doses utilized (Ukai et al., 1987; Sanger et al., 1980; Holtzman, 1974; Maickel et al., 1977).

In order to test the hypothesis that the dopaminergic system may also influence the motivation for sucrose procurement related to the reinforcing (hedonic) aspects of sucrose, we assessed the effects of d-amphetamine (an indirect dopamine agonist) on sucrose intake in the High and Low sucrose consumers. Whereas amphetamine failed to affect the PR performance of the Low consumers, it markedly elevated the number of reinforcements earned by the High consumers. Conversely, individual differences in oral sucrose consumption have been shown to be predictive of the reinforcing properties of amphetamine or cocaine, as revealed by the propensity to self-administer these drugs (DeSousa et al., 2000; Gosnell, 2000). In the current

study, the effect on sucrose self-administration was specific to the medium dose of amphetamine (0.5 mg/kg), as doses lower or higher failed to affect PR performance for sucrose (an inverted-U dose-response relationship). This effect would appear paradoxical as amphetamine is known to have anorectic properties (Colle et al., 1988; Thurlby et al., 1983). However, such effects of amphetamine have been noted in the past as well; low doses suppress feeding according to some, but stimulate feeding according to others (Sills et al., 1996; Sills et al., 1993). In the context of sucrose intake, low doses (<0.25 mg/kg) of amphetamine have been reported to stimulate sugar consumption in Low sucrose consumers while having little or no effect in High consumers (Sills et al., 1996). In self-administration procedures, effects of amphetamine have been shown to be dependent upon the operant basal rates. Low doses increase low-rate responding, whereas high doses suppress response rates. Although the current results are not entirely concordant with those of Sills and Vaccarino (1996), these differences may well be related to the differences in experimental protocols (free feeding versus operant PR performance) used.

The final set of experiments addressed the contention that the opioidergic system may influence hedonic effects through the dopaminergic system. Thus rats pretreated with naloxone were exposed to d-amphetamine. Globally speaking, the data revealed that naloxone significantly attenuated amphetamine-stimulated bar pressing, in a dose-dependent manner. This suggests that, in addition to attenuating the locomotor effects of amphetamine (Holtzman, 1974; Schaad et al., 1995), naloxone attenuates amphetamine-stimulated bar-pressing for sucrose reward, particularly in High sucrose consumers. However, these findings should be considered preliminary as the rats had been exposed to d-amphetamine repeatedly, and may have become sensitized to the effects of this drug. In order to assess whether sensitization had occurred, responsiveness to d-amphetamine was reassessed after the amphetamine/naloxone studies were

completed. It was observed that with the repeated dose, performance on PR was not significantly altered from that observed after the initial administration of 0.5 mg/kg amphetamine.

Experiments 7-8

Effects of Gender, Estrous Cycle and Interleukin-1 β on “Free” Chow and “Earned” Sucrose Intake

Like bacterial endotoxins, proinflammatory cytokines, such as interleukin-1 β (IL-1 β), elicit a constellation of behavioral changes including soporific effects, ptosis, piloerection, and profoundly reduce locomotor activity, sexual activity, food intake, operant responding for food reward, exploration and social interaction (Dantzer, 2001; Plata-Salman, 2000). It has been surmised that these “sickness behaviors” are of adaptive significance, in that they help maintain body temperature and reduce energy expenditure that may be needed to deal with immunological insults (Dantzer, 2001; Hrupka et al., 1997). The behavioral changes exerted by IL-1 β are not simply a reflection of malaise, but may arise owing to alterations of a centrally-mediated motivational state (i.e. anhedonia: diminution in the perceived reward or pleasure obtained from environmental stimuli; Anisman et al., 1999; Dantzer, 2001). Indeed, it has been maintained that immune activation may contribute to psychological illness, such as depression, where anhedonia is a fundamental characteristic (Anisman et al., 1999; Maes, 1999). Unfortunately, it is exceedingly difficult to dissociate the sickness behaviors elicited by cytokines from those related to anhedonia. In fact, the depressive-like effects induced by such treatments may be secondary to the malaise elicited by cytokines.

In assessing the wide range of effects induced by IL-1 β , it may be important to consider that interactions may exist between IL-1 β and gender effects (including gonadal hormones), which may impact on behavioral outcomes. Females of most mammalian species exhibit a more pronounced immune response than males (Spitzer, 1999), and such responses may vary over the

estrous cycle (Mouihate et al., 1998). Likewise, IL-1 β challenge elicited a more pronounced increase of plasma adrenocorticotrophic hormone (ACTH) or corticosterone in females than in males (Rivier, 1994), and the febrile response provoked by IL-1 β was more pronounced in females during proestrus than diestrus (Mouihate et al., 1998). Behaviorally, IL-1 β may have particularly marked inhibitory effects on various aspects of sexual behavior of female rats (Avitsur et al., 1999), and the disturbed sexual behavior was modifiable by progesterone alterations (Avitsur et al., 1995). Whether these effects reflect outcomes specific to sexual behaviors, general motivation states, or sickness, remains to be determined.

The present investigation assessed the potential anhedonic and anorexic effects of IL-1 β on responding for a rewarding substance, namely that of sucrose. As rate of emitting an operant response may be confounded by factors unrelated to motivation, performance was assessed using a progressive ratio (PR) schedule. In this paradigm the number of responses required to obtain a reward becomes progressively greater, making it possible to determine the amount of work animals are willing or prepared to emit in order to obtain a fixed reward. The PR schedule has been used to evaluate the effects of various environmental and pharmacological manipulations on an animal's motivation for drug and food reward (Hodos, 1961; Richardson et al., 1991). It was of interest in the present investigation to assess whether the effects of IL-1 β on responding for an earned sucrose reward in a PR paradigm could be distinguished from the anorexia reflected by consumption of "free" chow. Given the influence of gonadal hormones on consummatory behaviors, coupled with the differential effects of IL-1 β on gonadal hormones and male-female behaviors, the effects of IL-1 β were determined in both sexes.

Ordinarily, food intake in males exceeds that of females (Wade et al., 1979), but this difference is virtually eliminated in response to a palatable food (Zylan et al., 1996). The difference of food intake between males and females may be related to the actions of gonadal steroids, as intake in females varies over the estrous cycle (Wingkar, 1992), and is modified by estrogen and progesterone (Hrupka et al., 1997). In view of the potential influence of gender and gonadal functions, a first study assessed chow consumption and PR performance for sucrose reward in males and females (and at different phases of the estrous cycle in female rats). The second experiment assessed the effects of IL-1 β on chow consumption and PR performance in the two genders.

Methods

Animals

Naïve male and female Long Evans rats ($n = 7$ /group in each of the experiments), 250-275 g, were individually housed in their testing cages on a 12L:12D schedule (lights on at 0100 hr) and had *ad libitum* access to water and lab chow, except where noted. All experimental procedures followed the guidelines of the Canadian Council on Animal Care and were approved by the University Research Ethics Committee.

Testing cages

Rats were both housed and tested in Plexiglas chambers (33.5 x 25.5 x 33 cm), with a removable stainless steel grid floor. Food was available through a 5.75 x 11.5 cm rectangular

opening in one side of the cage (8 cm above the floor) fitted with a tunnel extending 12.5 cm out from the cage wall. The bottom of the tunnel comprised a grid through which the animal could reach powdered chow contained in a stainless steel weigh-boat, which rested on computerized scale. A retractable lever extended into the center of the front of each cage, 8 cm above the grid floor. Polyethylene tubing (PE 10) inserted into a modified drinking spout was located to the right of the lever, 6 cm above the floor. Initially, each response on the lever (30 g force) activated a syringe driver to deliver 0.1 ml of 20% sucrose solution. The drinking spout for water was located on the side opposite the reward spout.

Experiment 7: Male and female chow intake and PR performance for 20% sucrose

Training

Animals were trained on a fixed-ratio schedule where each lever press produced a reward (FR1) comprising 0.1 ml of sucrose solution. Rats were maintained on this schedule for 7 days, which permitted stable responding to be established. The reinforcement schedule was then changed to a PR response schedule, wherein the initial lever press was reinforced, but the number of bar presses required for subsequent rewards increased according to the following progression: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219. Motivation for reward was measured using breaking points, defined as the last ratio of bar presses achieved before responding ceased for at least 10 minutes.

The daily 24 hr sessions were divided into 6, four-hour portions. The procedures were only conducted during 5 of these 6 periods (3 night and 2 day), the sixth being used to conduct housekeeping. During the initial 3 hr of each portion, the lever was available to the animal and the number of bar presses emitted and rewards obtained was recorded. After the third hour, the

levers retracted and remained unavailable for 1 hr. Upon reintroduction of the lever the PR schedule was reset (i.e., initial lever press was reinforced) permitting the determination of the breaking point once again.

Swabbing

In order to monitor the female rats' estrous cycles, samples of vaginal cells were collected once each day prior to testing. A small cotton swab dipped in 0.9% saline solution was gently inserted into the vagina, then rolled on a slide and sprayed with cytoprep to preserve the cells. The slides were stained with a Wright–Giemsa solution and examined under a microscope.

Experiment 8: Influence of IL-1 β administration on chow intake and PR performance

Training: Rats underwent the same training as in Experiment 1, except that the 24 hr testing session was divided into 8-3 hr portions. During the initial 2 hrs of each portion, the lever was available to the animal, after which the lever automatically retracted and remained unavailable for 1 hour. Once more, the PR schedule was reset at the start of each portion.

IL-1 β administration: After 7 days of PR training, IL-1 β was administered to all animals (2.0 μ g/kg; i.p.) 3 hr prior to the start of the testing session (i.e. 3 hr before lights off). This time was selected on the basis of earlier studies indicating that despite the short half-life of IL-1 β , the central neurochemical changes elicited by the treatment were still marked at this time (Song et al., 1999). The cytokine was administered on only a single occasion, but animals were tested on each of 4 consecutive days following IL-1 β treatment. This procedure was used in order to

determine whether protracted effects of the cytokine would be evident, as previously observed in response to IL-2 (Anisman et al., 1996).

Recombinant human IL-1 β , produced by E.I. Dupont De Nemours, was provided by Dr. Craig Reynolds (Biological Response Modifiers Program, National Cancer Institute, USA). The specific activity of the cytokine was 1.81×10^4 units/ μ g with negligible endotoxin. The IL-1 β was suspended in pyrogen-free, phosphate buffered sterile saline (0.9%).

Statistical analyses

Chow intake was recorded throughout the 24 hr periods; however, as consumption occurred primarily during the 12 hr of the dark cycle, only these data were reported. Chow consumption was analyzed as a mixed measures analysis of variance with Gender as a between group variable and both Days and Portion of the dark cycle as within group variables. As consumption in males and females may have been related to the relative weight of the animals, chow consumption was also analyzed as a proportion of body weight. For the analysis of PR performance, breaking point values were defined as the number of reinforcements received prior to the cessation of responding. The breaking points were analyzed using mixed measures ANOVAs as described for chow consumption. In Experiment 2, Gender was again included as a between groups measure and Days (relative to IL-1 β treatment) and Portion of the Dark cycle as within group variables. The performance on the Day prior to cytokine treatment was considered as the baseline. Where significant main effects or interactions were observed, follow-up comparisons with baseline performance were conducted using Bonferonni corrected t-tests.

Results

Experiment 7: Male and female chow intake and concurrent PR performance

Chow intake: Chow consumption (in grams) was greater in males ($5.15 \pm .29$) than in females ($4.46 \pm .23$), $F(1,12)=6.11$, $p<.05$, $\eta^2=.34$ and varied over the portion of the dark cycle $F(2,36)=8.25$, $p<.01$, $\eta^2=.41$. Follow-up tests revealed more chow was consumed during the early portion (5.44 ± 0.29) than the late portion of the dark cycle (3.9 ± 0.29). When chow consumption was converted to percentage of body weight, it was observed that the females consumed more chow per gram of body weight than did the males ($1.36 \pm .07$ vs. $0.82 \pm .05$ % of body weight), $F(1,12)=181.20$, $p<0.001$, $\eta^2=.94$.

Progressive Ratio: Breaking points varied as a function of the interaction between Gender and Portion of the dark cycle, $F(2,19)=3.08$, $\eta^2=.24$, $p<0.05$. Follow-up t-tests of the simple effects revealed that during the early portion of training the males showed a higher breaking point than did the females. The females' PR performance increased between the early and mid-portion of the dark cycle, and remained high through the late portion. Thus, the magnitude of the male-female difference was diminished.

Effects of Estrus on Chow intake and PR performance: Female rats consumed more chow during diestrus (3.20 ± 0.10 gm) than during estrus (2.70 ± 0.13 gm), $F(1,7)=27.7$, $p<0.001$, $\eta^2=.80$. As well, consumption varied over the course of the test session, $F(3,16)=33.48$, $p<0.0001$, $\eta^2=.83$, with chow intake being higher during the three dark test portions (4.98 ± 0.34 gm) than during the light portions (0.89 ± 0.16 gm).

The breaking point was found to vary over the portion of testing, $F(4,21)=13.08$, $p<.0001$, $\eta^2=.65$ in that the high breaking point (14.50 ± 0.71) evident during the dark portion of testing declined precipitously during the light portion (6.88 ± 2.36). However, estrus phase did not influence PR performance ($F < 1$).

Experiment 8: Chow intake and PR performance for sucrose reward following IL-1 β

Chow intake (g): Chow consumption was again found to be greater in males than in females, $F(1, 12)=5.64$, $\eta^2=.32$, $p<0.05$, and varied as a function of the interaction between Days (relative to IL-1 β treatment) and Portion of the dark cycle, $F(6,68)=5.74$, $\eta^2=.32$. Pairwise comparisons indicated that relative to baseline, chow intake in both genders declined on the day of IL-1 β treatment and remained depressed the following day. These reductions were most pronounced during the initial two portions of the dark cycle. Chow intake normalized on the ensuing days, being comparable to that seen at baseline (Figure 5, upper panel). When consumption was converted on the basis of bodyweight, the interaction between Day (relative to IL-1 β treatment) and Portion of the dark cycle was maintained, $F(3,27)=12.11$, $\eta^2=.50$, $p<0.01$. In addition, females consumed more food as a percent of body weight (0.64 ± 0.04) than did the males (0.44 ± 0.04), $F(1, 12)=11.85$, $\eta^2=.50$, $p<0.01$.

PR performance: Breaking point varied as a function of the Day (relative to IL-1 β treatment) x Gender interaction, $F(4,48) = 2.64$, $p < 0.05$, $\eta^2=.18$. Follow-up tests indicated that relative to baseline, IL-1 β induced a decline of responding on the day of treatment so that both male and female rats received fewer rewards. Thereafter, responding in males returned to baseline values,

whereas in females responding remained depressed for 2 further days (Figure 5, lower panel).

The analysis revealed a main effect of Portion of the Dark cycle, $F(3,36)=3.25$, $\eta^2=.21$, $p<0.05$, indicating that PR performance was reduced during the early portions of the dark cycle.

Figure 5: Mean chow consumption (upper panel) and breaking point (lower panel) for responding for sucrose on a progressive ration schedule in male and female rats. Treatment with IL-1 β was administered only on Day 1 and testing continued for 4 successive days. * $p < 0.05$ and ** $p < 0.01$ relative to baseline.

Discussion

Performance on a progressive ratio schedule, like chow consumption, varied over the diurnal cycle, with breaking points being higher during the dark than during the light cycle.

Furthermore, males consumed more chow than did females and exhibited higher breaking points. However, when adjusted for body weight, chow was greater in females than males. These data are consistent with the suggestion that female rats are more motivated to consume carbohydrates (Sclafani et al., 1987). This phenomenon, however, may simply be related to differences in taste preference between male and female animals, as females have also been reported to display a greater preference for saccharin and glucose solutions (Di Lorenzo et al., 1989).

It has been reported that food consumption was reduced in females to a greater extent during estrus than diestrus, supporting an association between estrogen and diminished food intake (Eckel, 1999). It has been suggested that estrogens reduce food intake by intensifying processes responsible for the short-term satiation of hunger (Drewett, 1974), possibly by increasing availability of triglycerides as a metabolic fuel (Wade et al., 1979). In the present investigation, differences of chow intake between diestrus and estrus were modest, and were restricted to the first two portions of the dark cycle. As well, PR performance was unaffected by the estrous cycle, suggesting that the propensity to work for sucrose reward was unrelated to hormone levels. These findings are inconsistent with evidence indicating that shifts in carbohydrate intake occur at different stages of the estrous cycle (Geiselman et al., 1981). As the estrus phase only lasts approximately 12 hours in rats, it is possible that some of the females exited estrus during the testing session, thus influencing consumption. It should also be noted that only a single concentration of sucrose was employed in the PR test, and it is possible that experimental

manipulation of the concentration or magnitude of the sucrose reward might have permitted detection of effects related to the estrous cycle.

As previously reported (Sonti et al., 1996), acute IL-1 β administration markedly reduced *ad libitum* chow intake in both the males and females. Given the soporific effects of IL-1 β (Slisli et al., 1999) such an outcome was expected. However, it ought to be underscored that despite the short-half life of IL-1 β , the diminished chow consumption persisted on the day following cytokine treatment. Thus, these data suggest that the cytokine either had protracted effects on motivational processes, possibly relating to sustained central neurochemical changes (Song et al., 1999) or that consumption following IL-1 β treatment was perceived as being aversive, hence leading to the protracted effects. While there have been reports that IL-1 β may induce a conditioned taste aversion (Goehler et al., 1995), in the present investigation the ingested substance (sucrose) was not novel, which is typically a prerequisite for the establishment of a conditioned taste aversion. Moreover, unlike conditioned taste aversions, the diminished sucrose consumption was a relatively transient effect. Together, these facts would argue against the present findings reflecting the establishment of a conditioned taste aversion. However, this does not belie the possibility that sick animals did not find the sucrose rewarding and hence ceased or limited their responding, and this extinction-like effect persisted for several days.

The impact of IL-1 β on PR performance in male and female rats did not parallel that of chow intake. In particular, while IL-1 β treatment initially provoked a precipitous decline of the breaking point in both genders, among male rats PR responding returned to basal levels on the ensuing day, whereas among female rats the breaking point was reduced for two additional days. In effect, the diminished chow consumption was unrelated to the animal's propensity to respond

for sucrose reward. Thus, it appears that the anorexic effects of IL-1 β can be dissociated from its anhedonic effects. Further, these data are consistent with the suggestion (Dantzer, 2001) that this cytokine may have effects on motivational processes beyond those related to anorexia or sickness.

Conclusion

The present findings indicate that systemic administration of IL-1 β may provoke illness characterized by reduced food consumption. Despite the brief half-life of IL-1 β , the anorexia may persist over a 48 hr period. Although chow intake was markedly reduced by the cytokine, male rats were sufficiently motivated to respond for the sucrose reward. In females, however, the diminished responding for sucrose reward, likely reflecting the anhedonic effects induced by the cytokine, lasted longer than the diminished food intake. The finding that the IL- β effects were longer lasting in females is consistent with reports showing that the cytokine induces more pronounced effects in females than in males (Avitsur et al., 1999; Mouihate et al., 1998; Rivier, 1994). Moreover, given that anhedonia is a key feature of depressive illness, and females exhibit depression at rates twice that of males, the present findings concerning IL-1 β effects on anhedonic processes is consistent with the supposition that immune activation contributes to depressive illness (Maes, 1999).

General Discussion

Under a number of test conditions, the PR schedule was validated as an effective tool to assess the incentive value of sucrose reward in High and Low sucrose preferring rats, just as it was previously shown to be effective in assessing motivational value of food reward (Hodos, 1961; Fiefel et al., 1990) and drug self-administration (Richardson and Roberts, 1991). Specifically, employing this paradigm it was found that the difference in sucrose consumption between these two groups might be related to a difference in their motivation to procure the reward. Furthermore, variations in performance on PR following administration of an opioid antagonist and an indirect dopamine agonist indicate that changes within these systems may contribute to motivational processes, both individually and in conjunction with each other.

Despite the weaker incentive salience of sucrose compared with that of substances commonly evaluated on the PR response schedule (i.e. psychostimulants, opiates), it was still sufficiently rewarding to elicit responding from the High and Low consumer rats. Furthermore, an optimum concentration of sucrose reward was ascertained, and using this concentration, we were able to distinguish the incentive salience of sucrose in High and Low consumer rats, and to modify their response to it by dopaminergic and opioidergic manipulations.

As observed in the third of the preliminary experiments, when animals were in a water-deprived state, their PR performance for procurement of water reward increased markedly. Clearly, the PR performance is sensitive to the motivational (or the “wanting”) component of reward. When given the opportunity to “earn” sucrose on the PR schedule, rats responded in a concentration-dependent manner (i.e. the breaking point increased as sucrose concentrations increased from 0 to 20%). Furthermore, rats previously categorized as High sucrose consumers

earned a significantly greater number of rewards (i.e. increased breaking point) than did the Low sucrose consumers. Evidently, sucrose has a higher incentive value for animals that normally consume more granulated sugar when freely available. In effect, the High sucrose consumers were more motivated to consume sucrose than their Low sucrose-consuming counterparts. The difference between the two groups was unrelated to differences in the ability to emit motor responses, since comparable responding was observed for water reward in animals that had been water-deprived. Parenthetically, the number of water reinforcements earned under the water-deprived state was about equivalent to that of the 20% sucrose solution in non-deprived High sucrose consumers. In the case of the Low sucrose consumers, the breaking point for water under a water-deprived state was higher than for sucrose under the non-deprived condition.

Using Naloxone to manipulate the opioidergic system, it was found that the hedonic value of sucrose was decreased similarly for both High and Low sucrose consumers. These findings are consistent with evidence indicating that the opioid system influences the hedonic impact of palatable food intake. Our results, however, demonstrate that while lower doses of naloxone seem to specifically affect motivation for sucrose, at higher doses the distinction between motivation for water (thirst-driven) and motivation for sucrose was no longer apparent. These effects do not seem attributable to non-specific motor effects, as previous studies indicated that naloxone does not decrease food or water intake as a consequence of a delay in the initiation of ingestion or by affecting motor performance (Brown et al., 1981; Kirkham et al., 1984).

As noted earlier, although amphetamine failed to affect the breaking point of the Low sugar consumers, it clearly increased the breaking point in the High consumers (at the 0.5 mg/kg dose). As the effect was limited to the 0.5 mg/kg dose, there is some question about the reliability of the results, though in other paradigms, this dose has been found to be effective in

stimulating response rates. Lucki and DeLong (1983) found that although the 0.25 and 1.0 mg/kg doses of amphetamine did not significantly alter response rates, the 0.5 mg/kg dose increased the breaking point. It is possible that the 0.5 mg/kg dose represents an optimal dose in the inverted-U relationship between dopamine activity and feeding behavior of the High group. In contrast, the Low consumers may have lower sensitivity to amphetamine, and thus the 0.5 mg/kg dose may fall at a different point on the dose-response curve (Heffner et al., 1974). The decrease in response rate with the higher dose (1.0 mg/kg) may reflect the emergence of competing motor behaviors (such as stereotypy), although no gross motor effects were apparent during testing. These results suggest that (1) the pharmacological response to d-amphetamine is dependent upon whether the animal normally consumes high or low amounts sucrose, and (2) that the dopaminergic system may be relevant to the individual differences in sucrose consumption noted across animals.

The interindividual variability in sucrose consumption observed in the present study is not unique to rats. This phenomenon has also been observed in humans (Looy et al., 1992; Thompson et al., 1977). Adult humans can also be grouped into High and Low sucrose preferers (Looy et al., 1992; Thompson et al., 1977). Of particular interest is the observation that opiate addicts tend to crave sweet flavor and substitute foods rich in fat and proteins with those rich in sucrose (Morabia et al., 1989). This raises the possibility that there exists a link between the neurochemical mechanisms that influence opiate addiction and sucrose consumption.

As indicated earlier, the reward value of sucrose (or drugs) is influenced by several components, including the hedonic aspect. Thus consideration must also be given to differences in taste *perception* in High and Low sucrose consumers. Though taste perception and taste preference are distinct concepts (Crystal et al., 1995; Flynn et al., 1993), taste perception may

influence the incentive value of the reward and influence sucrose consumption, and may impact the relationship with mechanisms mediating addictive behaviors and obesity. Interestingly, active heroin addicts estimate sweet taste as being more pleasant than detoxified former addicts and matched controls (Perl et al., 1997). Similarly, cocaine-exposed infants show a significantly greater preference for sweet taste (Maone et al., 1992). The preference for a higher sucrose concentration (sweet liking) is also characteristic of alcoholics as well as alcohol-preferring rats (Kampov-Polevoy et al., 1997; Kampov-Polevoy et al., 1998; Stewart et al., 1994). More recently, humans have been categorized into three distinct groups according to their taste sensitivity: Non-tasters (weak taste perception), medium tasters, and super tasters (heightened perception of taste (Bartoshuk et al., 1996), a phenomenon attributable to the genetic differences in the expression of the “sweet” receptors on the tongue. Thus differences in preference for sweet carbohydrates may have a genetic basis (Bachmanov et al., 1997).

Cytokines, anhedonia and depression

When male and female groups were administered IL-1 β , differences in the anorexic and anhedonic effects of cytokine administration emerged between the sexes. Though the effects on free chow intake were similar for the two groups, the decrease in bar pressing for sucrose reward remained low for the females longer than for the males. These findings are in line with evidence that females exhibit a greater immune response than males (Spitzer, 1999), and also suggests that an animal's consumption of free chow may be unrelated to the motivation to procure sucrose reward.

Since both major depression and exposure to cytokines share several common behavioral expressions, it was of interest to determine whether two behaviors closely associated with both states are dissociable. Frequently, the presence of anhedonia and anorexia following exposure to cytokines is believed to reflect malaise. However, a growing amount of evidence indicates that activation of the immune system and alterations in mood states (i.e. depression), are closely linked, to the degree that it has been proposed that the release of cytokines may actually contribute to the onset and exacerbation of depressive illness (Yirmiya, 1996); (Maes et al., 1997).

There are strong arguments that cytokines are linked with depression, since there are many similarities in the neurochemical variations associated with each. Activation of the IRS influences activity of the Hypothalamic-Pituitary-Adrenal axis, enhancing its activity through stimulation of corticotrophin releasing hormone (CRH) and hence stimulating release of ACTH . The IRS also influences 5-HT and catecholaminergic turnover, as administration of proinflammatory cytokines increase extracellular concentrations of 5-HT in several brain regions (Clement et al., 1997). Major depression is also accompanied by HPA axis hyperactivity, as well as serotonergic disturbances (for review see Maes, 1993).

Incidence of depression seems to be linked to increased cytokine presence (Maes, 1995); (Maes et al., 1991). In addition, a number of studies have found indications of an acute phase response in association with depression, including increased serum concentrations of positive acute phase proteins (i.e. haptoglobin, alpha-1 antichymotrypsin), as well as a decrease in negative acute phase proteins (i.e. albumin, transferrin; Swartz, 1990; Maes et al., 1996). There is also evidence of increased leukocytes, monocytes, neutrophils, and activated T-lymphocytes in patients suffering from major depression (Maes et al., 1995).

Increased production of proinflammatory cytokines may contribute to the symptomatology of depression (Yirmiya, 1996; Maes et al., 1997). In patients undergoing cytokine immunotherapy, severe depressed mood has been reported (McDonald et al., 1987; Valentine et al., 1998), and depressive symptoms have resulted from increased dose and duration of treatment (Valentine et al., 1998; Pavol et al., 1995) and disappeared completely within 2-3 weeks following the end of treatment. This would suggest a causal role for cytokines in depression.

One of the primary symptoms of depressive illness is that of anhedonia. Yet, in studies assessing the relationship between cytokine treatments and depression there have been few efforts to assess the effects on anhedonia. Instead, most studies have evaluated “sickness behaviors” in which the lethargy seen in animals was attributed to anhedonia, as well as other sickness characteristics (Dantzer et al., 1999). Using concurrent measurement of free chow intake and performance on a PR schedule of reinforcement, the present set of experiments demonstrated that the anorexic and anhedonic effects of peripherally administered IL-1 β can be dissociated from one another. The second set of experiments revealed that despite its short half-life (~30 min) following peripheral administration of IL-1 β , the anorexic effects of the cytokine persisted into the day following administration. In addition, the effects exerted by IL-1 β on PR performance did not parallel that of free chow intake in males and females. In both males and females chow consumption was reduced for 48 hr following cytokine administration; however, sucrose consumption was attenuated for only 24 hr in males, but was relatively persistent (72 hr) in females. Thus, it appears that the anhedonic effects of cytokine exposure are not necessarily an expression of malaise (possibly reflected as anorexia), but may be the product of a depression of mood.

In conclusion, the present experiments demonstrate that not only is performance on PR an effective tool to measure an animal's motivation to procure sucrose reward, but when paired with free access to regular chow, it may dissociate the anorexic and anhedonic effects of acute cytokine treatment. The disparate effects in the males and females indicate that motivation to consume free chow and to procure sucrose reward may be mediated through different mechanisms. This suggests that malaise is not necessarily solely responsible for the anorexic and anhedonic effects of IL-1 β administration. These findings support the emerging evidence linking activation of the immune system and the onset of affective disorders. Further study to discern the precise relationship between cytokines and depression will hopefully lead to more effective treatment of major depression.

References

- Agmo, A., Galvan, A., & Talamantes, B. (1995). Reward and reinforcement produced by drinking sucrose: two processes that may depend on different neurotransmitters. Pharmacology, Biochemistry & Behavior, 52, 403-414.
- Anisman, H., Kokkinidis, L., Borowski, T., & Merali, Z. (1998). Differential effects of interleukin (IL)-1beta, IL-2 and IL-6 on responding for rewarding lateral hypothalamic stimulation. Brain Research, 779(1-2), 177-187.
- Anisman, H., Kokkinidis, L., & Merali, Z. (1996). Interleukin-2 decreases accumbal dopamine efflux and responding for rewarding lateral hypothalamic stimulation. Brain Res, 731(1-2), 1-11.
- Anisman, H., & Merali, Z. (1999). Anhedonic and anxiogenic effects of cytokine exposure. Adv Exp Med Biol, 461, 199-233.
- Anisman, H., & Merali, Z. (2000). Induction of a "learned helplessness effect" in mice. In J. N. Crawley (Ed.), Current Protocols in Neuroscience. (pp. in press New York: Wiley.
- Avitsur, R., Donchin, O., Barak, O., Cohen, E., & Yirmiya, R. (1995). Behavioral effects of interleukin-1B: modulation by gender, estrous cycle, and progesterone. Brain Behavior and Immunity, 9, 234-241.
- Avitsur, R., & Yirmiya, R. (1999). The immunobiology of sexual behavior: gender differences in the suppression of sexual activity during illness. Pharmacol Biochem Behav, 64(4), 787-796.
- Baile, C.A., Keim, D.A., Della-Fera, M.A., & McLaughlin, C.L. (1981). Opiate antagonists and agonists and feeding in sheep. Physiology & Behavior, 26, 1019-1023.
- Besedovsky, H.O., & del Rey, A. (1991). Feed-back interactions between immunological cells and the hypothalamus. Netherlands Journal of Medicine, 39(3-4), 274-280.
- Blalock, J.E. (1994). Shared ligands and receptors as a molecular mechanism for communication with neuroendocrine systems. Annals of the New York Academy of Sciences, 741, 292-298.
- Brebner, K., Hayley, S., Zacharko, R., Merali, Z., & Anisman, H. (2000). Synergistic effects of interleukin-1 beta, interleukin-6, and tumour necrosis factor alpha on monoamine, corticosterone, and behavioral variations. Neuropsychopharmacology, 22(6), 566-580.
- Bret-Dibat, J.L., Bluthe, R.M., Kent, S., Kelley, K.W., & Dantzer, R. (1995). Lipopolysaccharide and interleukin-1 depress food-motivated behavior in m mechanism. Brain Behavior and Immunity, 9(3), 242-246.

- Cador, T., & Robbins, T.W. (1991). Potentiation of the effects of reward-related stimuli and the dopaminergic-dependent mechanisms of the nucleus accumbens. Psychopharmacology, 104, 377-385.
- Church, W.H., Justice, J.B., & Neill, D.B. (1987). Detecting behaviorally relevant changes in extracellular dopamine with microdialysis. Brain Research, 412, 397-399.
- Colle, L., & Wise, R. (1988). Concurrent facilitory and inhibitory effects of amphetamine on stimulation-induced eating. Brain Research, 459, 356-360.
- Crestani, F., Seguy, F., & Dantzer, R. (1991). Behavioral effects of peripherally injected interleukin-1: role of prostaglandins. Brain Research, 542(2), 330-335.
- Dantzer, R. (2001). Cytokine-induced sickness behavior: where do we stand? Brain Behav Immun, 15(1), 7-24.
- Dantzer, R., Aubert, A., Bluthé, R.M., Gheusi, G., Cremona, S., Laye, S., Kinsman, J.P., Goodall, G., Bret-Dibat, J.L., Kent, S., Goujam, E., Parnet, P., & Kelley, K.W. (1996). Cytokine actions on behavior I. In N. J. Rothwell (Ed.), Cytokines and the Nervous System. (pp. 117-144). London: Landes.
- Dantzer, R., Aubert, A., Bluthé, R.M., Gheusi, G., Cremona, S., Laye, S., Kinsman, J.P., Parnet, P., & Kelley, K.W. (1999). Mechanisms of the behavioral effects of cytokines. Advances in Experimental Medicine & Biology, 461, 83-105.
- Dantzer, R., Kinsman, J.P., Bluthé, R.M., & Kelley, K.W. (2000). Neural and humoral pathways of communication from the immune system convergent? Autonomic Neuroscience, 85(1-3), 60-65.
- Dantzer, R., Wollman, E., Vitkovic, L., & Yirmiya, R. (1999). Cytokines and depression: fortuitous or causative association? [Review] [38 refs]. Molecular Psychiatry, 4(4), 328-332.
- DeSousa, N.J., Bush, D.E., & Vaccarino, F.J. (2000). Self-administration of intravenous amphetamine is predicted by individual differences in sucrose feeding in rats. Psychopharmacology (Berl), 148(1), 52-58.
- Di Lorenzo, P.M., & Monroe, S. (1989). Taste responses in the parabrachial pons of male, female and pregnant rats. Brain Research Bulletin, 23(3), 219-227.
- Drewett, R.F. (1974). The meal patterns of the oestrous cycle and their motivational significance. Quarterly Journal of Experimental Psychology, 26, 489-494.
- Dunn, A.J. (1995). Interactions between the nervous system and the immune system. In F. E. Bloom & D. J. Kupfer (Eds.), Psychopharmacology: The fourth generation of progress. (pp. 719-731). New York: Raven Press.

- Eckel, L.A., & Geary, N. (1999). Endogenous cholecystokinin's satiating action increases during estrus in female rats. Peptides, 20(4), 451-456.
- Fiefel, D., & Vaccarino, F.J. (1990). Central injections of growth hormone-releasing factor increase operant responding for food reward. Progress in Neuro-Psychopharmacology and Biological Psychiatry, 14, 813-820.
- Gayle, D., Ilyin, S.E., & Plata-Salaman, C.R. (1997). Central nervous system IL-1 beta system and neuropeptide Y mRNAs during IL-1 beta-induced anorexia in rats. Brain Research Bulletin, 44(3), 311-317.
- Geiselman, P.J., Martin, J.R., Vanderweele, D.A., & Novin, D. (1981). Dietary self-selection in cycling and neonatally ovariectomized rats. Appetite, 2(2), 87-101.
- Goehler, L.E., Busch, C.R., Tartaglia, N., Relton, J., Sisk, D., Maier, S.F., & Watkins, L.R. (1995). Blockade of cytokine induced conditioned taste aversion by subdiaphragmatic vagotomy: further evidence for vagal mediation of immune-brain communication. Neuroscience Letters, 185, 163-166.
- Gosnell, B.A. (2000). Sucrose intake predicts rate of acquisition of cocaine self-administration. Psychopharmacology (Berl), 149(3), 286-292.
- Hernandez, L., & Hoebel, B. (1988). Feeding and hypothalamic stimulation increase dopamine turnover in the accumbens. Physiology & Behavior, 44, 599-606.
- Hodos, W. (1961). Progressive ratio as a measure of reward strength. Science, 134, 943-944.
- Holtzman, S.G. (1974). Behavioral effects of separate and combined administration of naloxone and d-amphetamine. Journal of Pharmacology and Experimental Therapeutics, 189(1), 51-60.
- Hrupka, B.J., Smith, G.P., & Geary, N. (1997). Ovariectomy and estradiol affect postingestive controls of sucrose licking. Physiology & Behavior, 61(2), 243-247.
- Jalowiec, J.E., Panksepp, J., Zolovick, A.J., Najam, N., & Herman, B.H. (1981). Opioid modulation of ingestive behavior. Pharmacology, Biochemistry & Behavior, 15, 477-484.
- Jones, D.N.C., & Holtzman, S.G. (1992). Interaction between opioid antagonists and amphetamine: evidence for mediation by central delta opioid receptors. Journal of Pharmacology and Experimental Therapeutics, 262, 638-645.
- Koch, J.E., Glass, M.J., Cooper, M.L., & Bodnar, R.J. (1995). Alterations in deprivation, glucoprivic and sucrose intake following general, mu and kappa opioid antagonists in the hypothalamic paraventricular nucleus of rats. Neuroscience, 66(4), 951-957.

- Kuschinsky, K., & Hornykiewicz, O. (1972). Morphine catalepsy in the rat: relation to striatal dopamine metabolism. European Journal of Pharmacology, 19, 119-122.
- Levine, A.S., Weldon, D.T., Grace, J.P., Cleary, J., & Billington, C.J. (1995). Naloxone blocks that portion of feeding driven by sweet taste in food-restricted rats. American Journal of Physiology, 268, R248-R252
- Maes, M. (1999). Major depression and activation of the inflammatory response system. Adv Exp Med Biol, 461, 25-46.
- Maickel, R.P., Braude, M.C., & Zabik, J.E. (1977). The effects of various narcotic agonists and antagonists on deprivation-induced fluid consumption. Neuropharmacology, 16, 863-866.
- Maier, S.F., & Watkins, L.R. (1998). Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. Psychol Rev, 105(1), 83-107.
- Majeed, N.H., Lason, W., Przewlocka, B., & Przewlocki, R. (1986). Brain and peripheral opioid peptides after changes in ingestive behavior. Neuroendocrinology, 42, 267-272.
- Martin, W.R., Wikler, A., Eades, C.G., & Prescor, F.T. (1963). Tolerance to and physical dependence on morphine in rats. Psychopharmacology, 4, 247-260.
- Matthews, R.T., & German, D.C. (1984). Electrophysiological evidence for excitation of rat ventral tegmental area dopamine neurons by morphine. Neuroscience, 11(3), 617-625.
- Mouihate, A., Chen, X., & Pittman, Q.J. (1998). Interleukin-1B fever in rats: gender difference and estrous cycle influence. American Journal of Physiology, 275(44), R1450-R1454
- Olds, M.E. (1982). Reinforcing effects of morphine in the nucleus accumbens. Brain Research, 237, 429-440.
- Phillips, A.G., Atkinson, L.J., Blackburn, J.R., & Blaha, C.D. (1993). Increased extracellular dopamine in the nucleus accumbens of the rat elicited by a conditional stimulus for food: an electrochemical study. Canadian Journal of Physiology and Pharmacology, 71, 387-393.
- Phillips, A.G., LePiane, A., & Fredrik, G. (1980). Reinforcing effects of morphine microinjection into the ventral tegmental area. Pharmacology, Biochemistry & Behavior, 12, 965-968.
- Philopena, J., Greenberg, D., & Smith, G.P. (1996). Naloxone decreases intake of 10% sucrose in preweanling rats. Pharmacology, Biochemistry & Behavior, 54(2), 333-337.

- Pitts, S.M., & Horvitz, J.C. (2000). Similar effects of D(1)/D(2) receptor blockade on feeding and locomotor behavior. Pharmacol Biochem Behav, 65(3), 433-438.
- Plata-Salaman, C.R. (2000). Central nervous system mechanisms contributing to the cachexia-anorexia syndrome. Nutrition, 16(10), 1009-1012.
- Plata-Salaman, C.R., Sonti, G., Borkoski, J.P., Wilson, C.D., French-Mullen, J.M., & brahms. (1996). Anorexia induced by chronic central administration of cytokines at estimated pathophysiological concentrations. Physiology & Behavior, 60(3), 867-875.
- Pollard, H., Llorens-Cortes, C., & Schwartz, J.C. (1977). Enkephalin receptors on dopaminergic neurons in rat striatum. Nature, 268, 745-747.
- Richardson, N.R., & Roberts, D.C. (1991). Fluoxetine pretreatment reduces breaking points on a progressive ratio schedule reinforced by intravenous cocaine self-administration in the rat. Life Sciences, 49(11), 833-840.
- Richardson, N.R., & Roberts, D.C. (1996). Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J Neurosci Methods, 66(1), 1-11.
- Rivier, C. (1994). Stimulatory Effect of Interleukin-1B on the Hypothalamic-Pituitary-Adrenal Axis of the Rat: Influence of Age, Gender and Circulating Sex Steroids. Journal of Endocrinology, 140, 365-372.
- Rudski, J.M., Billington, C.J., & Levine, A.S. (1994). Naloxone's effects on operant responding depend upon level of deprivation. Pharmacology, Biochemistry & Behavior, 49(2), 377-383.
- Rudski, J.M., Billington, C.J., & Levine, A.S. (1997). A sucrose-based maintenance diet increases sensitivity to appetite suppressant effects of naloxone. Pharmacology, Biochemistry & Behavior, 58(3), 679-682.
- Salamone, J.D., Cousins, M.S., McCullough, L.D., Carriero, D.L., & Berkowitz, R.J. (1994). Nucleus accumbens dopamine release increases during instrumental lever pressing for food but not free food consumption. Pharmacology, Biochemistry & Behavior, 49(1), 25-31.
- Sanger, D.J., & McCarthy, P.S. (1980). Differential effects of morphine on food and water intake in food deprived and freely-feeding rats. Psychopharmacology, 72, 103-106.
- Schaad, C.A., Justice, J.B., & Holtzman, S.G. (1995). Naloxone reduces the neurochemical and behavioral effects of amphetamine but not those of cocaine. European Journal of Pharmacology, 275, 9-16.

- Schwartz, G.J., Plata-Salaman, C.R., & Langhans, W. (1997). Subdiaphragmatic vagal deafferentation fails to block feeding-suppressive effects of LPS and IL-1 beta in rats. American Journal of Physiology, 273(3 Pt 2), R1193-R1198
- Sclafani, A., Hertwig, H., Vigorito, M., & Feigin, M.B. (1987). Sex differences in polysaccharide and sugar preferences in rats. Neurosci Biobehav Rev, 11(2), 241-251.
- Shabir, S., & Kirkham, T.C. (1999). Diet-induced enhancement of naloxone sensitivity is independent of changes in body weight. Pharmacology, Biochemistry & Behavior, 62(4), 601-605.
- Sills, T., Baird, J.P., & Vaccarino, F.J. (1993). Individual differences in the feeding effects of amphetamine: role of nucleus accumbens dopamine and circadian factors. Psychopharmacology, 112, 211-218.
- Sills, T., & Crawley, J.N. (1996). Individual differences in sugar consumption predict amphetamine-induced dopamine overflow in the nucleus accumbens. European Journal of Pharmacology, 303, 177-181.
- Sills, T., & Vaccarino, F.J. (1994). Individual differences in sugar intake predict the locomotor response to acute and repeated amphetamine administration. Psychopharmacology, 116, 1-8.
- Sills, T., & Vaccarino, F.J. (1996). Individual differences in sugar consumption following systemic or intraaccumbens administration of low doses of amphetamine in nondeprived rats. Pharmacology, Biochemistry & Behavior, 54(4), 665-670.
- Slisli, Y., & de Beaurepaire, R. (1999). Interleukin-1 B and calcitonin, but not corticotropin-releasing factor, alter sleep cycles when injected into the rat hypothalamic lateral paraventricular area. Neuroscience Letters, 265, 29-32.
- Song, C., Merali, Z., & Anisman, H. (1999). Variations of nucleus accumbens dopamine and serotonin following systemic interleukin-1, interleukin-2 or interleukin-6 treatment. Neuroscience, 88(3), 823-836.
- Sonti, G., Ilyin, S.E., & Plata-Salaman, C.R. (1996). Anorexia induced by cytokine interactions at pathophysiological concentrations. American Journal of Physiology, 270(6 Pt 2), R1394-R1402
- Spitzer, J.A. (1999). Gender differences in some host defense mechanisms. Lupus, 8, 380-383.
- Thurlby, P.L., Grimm, V.E., & Samanin, R. (1983). Feeding and satiation observed in the runway: the effects of d-amphetamine and d-fenfluramine compared. Pharmacology, Biochemistry & Behavior, 18, 841-846.

- Ukai, M., & Holtzman, S.G. (1987). Suppression of deprivation-induced water intake in the rat by opioid antagonists: central sites of action. Psychopharmacology, 91, 279-284.
- Wade, G.N., & Gray, J.M. (1979). Gonadal effects on food intake and adiposity: a metabolic hypothesis. Physiol Behav, 22(3), 583-593.
- Wilson, C., Nomikos, G.C., Collu, M., & Fibiger, H.C. (1995). Dopaminergic correlates of motivated behavior: importance of drive. The Journal of Neuroscience, 15(7), 5169-5178.
- Wingkar, K.C. (1992). Alterations in feeding and sexual behaviour during reproductive cycle in female rats. Indian Journal of Physiology & Pharmacology, 36(3), 174-176.
- Wise, R.A., Bauco, P., Carlezon, W.A., & Trojnar, W. (1992). Self-stimulation and drug reward mechanisms. Annals of the New York Academy of Sciences, 28(654), 192-198.
- Yirmiya, R., Weidenfeld, J., Barak, O., Avitsur, R., Pollak, Y., Gallily, R., Wohlman, A., & Ovadia, H. (1999). The role of brain cytokines in mediating the behavioral and neuroendocrine mycoplasma fermentans. Brain Research, 829(1-2), 28-38.
- Yirmiya, R., Weidenfeld, J., Pollak, Y., Morag, M., Morag, A., Avitsur, R., Barak, Reichenberg, A., Cohen, E., Shavit, Y., & Ovadia, H. (1999). Cytokines, "depression due to a general medical condition," and antidepressant drugs. [Review] [235 refs]. Advances in Experimental Medicine & Biology, 461, 283-316.
- Yu, W.Z., Silva, R.M., Sclafani, A., Delamater, A.R., & Bodnar, R.J. (2000). Pharmacology of flavor preference conditioning in sham-feeding rats: effects of dopamine receptor antagonists. Pharmacol Biochem Behav, 65(4), 635-647.
- Zurita, A., Murua, S., & Molina, V. (1996). An endogenous opiate mechanism seems to be involved in stress-induced anhedonia. European Journal of Pharmacology, 299(1-3), 1-7.
- Zylan, K.D., & Brown, S.D. (1996). Effect of stress and food variety on food intake in male and female rats. Physiolo