THE SEROTONERGIC RELATIONSHIP BETWEEN FEEDING AND SEXUAL BEHAVIOUR IN THE MALE RAT

by

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ABSTRACT

Both feeding and sexual behaviour play a major role in the survival of a species. Research has revealed that there may be an antagonistic relationship between these two behaviours. For example, in times of limited food availability, priorities in energy partitioning favour individual survival (i.e., searching for food) over reproduction. Further, there appear to be significant interactions between the neuroendocrine control of reproduction and the regulation of energy balance. Research investigating the central sites that regulate sexual and feeding behaviour has shown the hypothalamus to be of prime importance for both these behaviours. Further, serotonin (5-HT) and norepinephrine (NE) have been shown to influence copulatory activity and food consumption in rats. There has been a considerable amount of controversy as to the relative involvement of different 5-HT receptor subtypes in both sexual and feeding behaviour. This thesis focusses on the importance of the 5-HT_2 receptor subtype in feeding and copulation in the male rat.

Experiments 1-3 were conducted in order to establish whether there is a natural relationship between sexual and feeding behaviour in the male rat. In these experiments, the involvement of 5-HT_2 receptors was investigated by recording the display of wet dog shakes, a behavioural indicator of 5-HT_2 activity. These experiments revealed that there may be a relationship between the natural level of sexual activity and feeding behaviour, and this may be due to an endogenous difference in 5-HT_2 receptors.

Experiments 4-9 were developed to investigate the specific receptor subtype important for the inhibition of sexual responding and feeding behaviour by 1-(2,5-dimethoxy-4-iodophenyl aminopropane) (DOI), a 5-$HT_{2/1C}$ receptor agonist. By employing selective 5-HT_2 antagonists, it was revealed that while 5-HT_2 receptors appear to be of prime importance in sexual activity in the male

rat, they are not as important in feeding behaviour. It may be that DOI's activity at 5-HT_{1C} receptors are more important for its influence on feeding behaviour than it's affect at 5-HT_2 receptors, while the reverse may be true of sexual responding. Further, 5-HT may be less important in feeding than copulation as a larger dose of DOI was required to inhibit feeding than that used in sexual behaviour experiments.

Taken together, these studies suggest that while 5-HT appears to be involved in both feeding and sexual responding, 5-HT may be more important in the regulation of sexual responding than in feeding behaviour and different receptor subtypes may mediate the influence of this neurotransmitter on feeding and copulatory activity.

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INTRODUCTION

The evolutionary success of a species relies on both the survival of the individual, and the ability of fit individuals to pass on their genetic information, or to produce offspring. Species maintenance and growth, therefore, require that individuals within a species survive, and individual survival relies on the availability of food and shelter. However, it is not enough for the success of a species that the individual merely survive, members of a species must also reproduce themselves, and, in many instances, successful reproduction requires copulation as well as adaptive paternal behaviours (Alcock, 1985). The importance of feeding and sexual behaviour for species survival is accentuated by research on the interaction between energy availability and reproductive behaviour. In *The Origin of Species* (1859), Charles Darwin noted that many domestic animals achieve reproductive success far greater that their wild counterparts, and speculated that this may be partially due to the fact that livestock receive an optimum food supply and expend minimal energy to acquire it. Research on the laboratory rat has revealed that under conditions of limited energy availability, priorities in energy partitioning are modified such that the processes necessary for individual survival are favoured over reproduction (Bronson, 1989). Conversely, in situations of food abundance, not only is more energy available to the individual, but less energy is needed to acquire food, leaving more energy for engaging in reproductive behaviours. From an adaptive standpoint, the interaction between food availability and copulatory behaviour is not surprising, for the individual must maintain itself in order to copulate, therefore the primary need of adequate energy for survival must be fulfilled before the need to pass on genes can be met (McFarland, 1989). Conclusions of this sort suggest that there must be some sort of neural/hormonal proximate mechanism involving regulation of an organism's behaviour depending on the availability of food energy. Indeed, there are significant interactions between the neuroendocrine control of reproduction and the regulation of energy balance (Wade &

Schneider, 1992). Furthermore, there appears to be a significant overlap in the neural circuitry controlling these functions (Wade & Schneider, 1992).

Research on the neuroendocrine control of feeding and sexual behaviour has found that gonadectomy in male and female rats leads to an increase in food intake and body weight, and this is reversed by administration of gonadal hormones (i.e., testosterone and estrogen, respectively) (e.g., female: Gentry & Wade, 1976a; Landau & Zucker, 1976; McElroy & Wade, 1987, male: Czaja, 1984; Gentry & Wade, 1976b;). These data are matched by sexual behaviour data showing that both castration and ovariectomy abolish sexual behavior while exogenous gonadal hormone replacement acts to reinstate sexual responding (e.g., male: Beach & Holz-Tucker, 1949; Bermant & Davidson, 1974; Everitt & Stacey, 1987; female: Boling & Blandau, 1939; Edwards, Whalen & Nadler, 1968; Whalen, 1974). In addition, supranormal levels of exogenous testosterone in male rats produce a further reduction in food intake beyond that induced by moderate levels of of testosterone (Gentry & Wade, 1976b).

Studies examining the central sites involved in hormonal reduction of food intake have revealed that estrogen implants into the ventromedial hypothalamus (VMH) and the paraventricular nucleus (PVN) of the hypothalamus act to decrease food intake in female rats (e.g., Beatty, O'Briant & Vilberg, 1974; Nunez, Gray & Wade, 1980). The hypothalamus has been pinpointed as a primary site for regulation of sexual behaviour in both the female and male rat (see Pfaff & Modianos, 1985). Further, feeding behaviour research has suggested that the primary neurotransmitters involved in controlling feeding behaviour may be norepinephrine (NE) and serotonin (5-HT) (Raible, in press). In general, NE acts to increase food intake, while 5-HT acts to inhibit feeding in rats (Raible, in press), and 5-HT administered to the PVN acts to inhibit feeding elicited by NE in this nucleus (Weiss, Papadakos, Knudson & Leibowitz, 1986). Due to this and other evidence, many researchers have proposed that these two monoamines work in an antagonistic fashion to control feeding behaviour (Leibowitz & Shor-Posner, 1986; Leibowitz, Weiss & Shor-Posner, 1988; Weiss, et al., 1986).

Similarly, central NE generally acts to increase sexual responding in the male rat (Melman, Fersel & Weinstein, 1984; Rodriguez, Castro, Hernandez & Mas, 1984; Segal, Shohami & Jacobwitz, 1984), while 5-HT generally acts to decrease sexual responding (Rodriguez, et al., 1984; Sheard, 1969; Shillito, 1969; Meyerson & Malmnas, 1971).

The above lines of evidence suggest that there may be a significant overlap between feeding and sexual behaviour in the rat in terms of mediating brain areas as well as neurotransmitter systems controlling these behaviours. The overall goal of this thesis is to investigate the existence of a serotonergic relationship between feeding and sexual behaviour in the male rat.

1 Serotonin and Sexual Behaviour in the Male Rat

Researchers looking for a neurochemical basis of male rat sexual behaviour in the 1970's concluded that a combined elevation in catecholamine levels (dopamine, norepinephrine and epinephrine) and the indoleamine, serotonin, lead to an inhibition of male rat sexual behaviour (e.g., Dewsbury, Davis, & Jansen, 1972; Malmnas & Meyerson, 1970). These conclusions were based on studies employing a variety of monoamine oxidase (MAO) inhibitors which inhibit the metabolism of both catecholamines and 5-HT, resulting in elevated levels of these neurotransmitters in the brain. It should be noted that this method of investigation did not allow researchers to determine which neurotransmitter(s) were specifically responsible for the inhibition of male rat sexual behaviour observed. Indeed, as mentioned above, recent research suggests that an increase in central NE acts to increase sexual responding in the male rat (Melman, et al., 1984; Rodriguez, et al., 1984; Segal, et al., 1984) suggesting that it was one of the other neurotransmitters or some combination of them acted to inhibit sexual responding in these early studies (e.g., Dewsbury, et al., 1972; Malmnas & Meyerson, 1970).

The importance of 5-HT in male rat sexual behaviour became apparent with the discovery of compounds that specifically altered 5-HT levels in the brain. The administration of 5-HTP (5-hydroxytryptophan), a metabolic precursor to serotonin, combined with an enzyme inhibitor (to prevent 5-HTP from being metabolized peripherally), leads to an inhibition of sexual behaviour in male rats (e.g., Ahlenius & Larsson, 1985; Ahlenius, Larsson, & Svensson, 1980; Tagliamonte, Fratta, Mercuro, Biggio, & Camba, 1972). These results were consistent with those of studies employing drugs that increase 5-HT release from nerve terminals in the brain (e.g., p-chloroamphetamine; Soderston,Berge, & Hole, 1978). Also, compounds that inhibit 5-HT reuptake at presynaptic membranes within the synapse (e.g., chlorimipramine, zimelidine, fluoxetine) allowing more stimulation of post-synaptic receptors, have been shown to produce a general inhibition of sexual behaviour in the male rat (Ahlenius, Heimann, & Larsson, 1979; Baum & Starr, 1980).

Before any strong conclusions could be made regarding serotonin's importance in copulation, it was also necessary to show that decreasing 5-HT activity would facilitate sexual behaviour. In order to investigate this, many researchers employed the compound para-chlorophenylalanine (PCPA) which inhibits 5-HT synthesis in the brain (Koe & Weissman, 1966). A few treatments with PCPA produces animals whose brains are nearly devoid of serotonin (Koe & Weissman, 1966). Experiments examining the effect of PCPA treatment on male rat sexual behaviour produced equivocal results. While many found an increase in sexual responding (e.g., Sheard, 1969, 1973; Shillito, 1969; Dahlof, 1980; Malmnas & Meyerson, 1971), others found no effect, especially when employing sexually experienced male rats (McIntosh & Barfield, 1984; Whalen & Luttge, 1970). However, other techniques that decrease 5-HT activity in the brain have produced results suggesting a general inhibitory effect of 5-HT on male sexual behaviour. For example, neurotoxic lesions of 5-HT neurons with 5,7-dihydroxytryptamine applied to the lateral ventricles of the brain produced an overall elevation of sexual responding in male rats (Sodersten, et al., 1978; McIntosh &

Barfield, 1984). In addition, when 5-HT is applied to the raphe nuclei of the brainstem (decreasing 5-HT release throughout the brain through feedback mechanisms) male rat sexual behaviour is facilitated (Hillegaart, Ahlenius, & Larsson, 1989).

Overall, the above data suggest that 5-HT has an inhibitory function in male rat sexual behaviour as an increase in brain 5-HT activity inhibits sexual responding while a decrease in 5-HT usually facilitates sexual behaviour.

2a. Serotonin and Feeding Behaviour in the Male Rat

The study of serotonin and feeding behaviour has also been a vigorous area of research that has lead to the development of effective pharmacological therapies for obese individuals. In general, agents that increase 5-HT activity in the brain decrease food intake in rats (Blundell, 1984) and humans (Silverstone & Goodall, 1986) . For example, fenfluramine (F) and its dextro isomer, *d*-fenfluramine (DF), which act to enhance the release of 5-HT from nerve terminals (e.g., Garattini & Samanin, 1976; Garattini, Mennini, Bendotti, Invernizzi & Samanin, 1986), and inhibit reuptake (Mennini, Borroni, Samanin & Garattini; Borroni, Ceci, Garattini & Mennini, 1983), have been shown to decrease food intake in rats (Invernizzi, Berettera, Garattini, & Samanin, 1986) and humans (Silverstone & Goodall, 1986). In addition, direct 5-HT postsynaptic receptor agonists (mimicking 5-HT), such as quipazine and *m*-chlorophenylpiperazine, have been reported to reduce food intake in animals (Samanin, Caccia, Bendotti, Borsini, Borroni, Invernizzi, Pataccini & Mennini, 1980; Samanin, Bendotti, Candelaresi & Garattini, 1977). Further, central application of 5-HT to the paraventricular nucleus (PVN) of the hypothalamus acts to decrease the size and duration of a meal with no effect on meal initiation. Findings such as these have lead many researchers to speculate that 5-HT acts as a satiety signal to terminate food consumption (Blundell, 1984, 1986; Leibowitz & Shor-Posner, 1986; Samanin & Garattini, 1989).

In order to establish the mechanism through which serotonergic drugs (particularly F and DF) exert their effect on feeding behaviour, studies employing 5-HT

antagonists (compounds that block 5-HT receptors so that agonists cannot bind to them) and lesions of 5-HT neurons in conjunction with F and DF administration have been conducted. Initially, electrolytic lesions of the medial raphe nucleus in the brainstem (where some of the 5-HT neurons innervating the forebrain originate), were shown to block F induced anorexia (Samanin, Ghezzi, Valzelli & Garattini, 1972). Further, the hypothesis that DF reduces food intake via central mechanisms was confirmed through studies showing that the anorectic activity of DF can be blocked by metergoline, a potent central 5-HT antagonist, but not by xylamidine, a 5-HT antagonist that does not cross the blood-brain barrier (Borsini, Bendotti, Aleotti, Samanin & Garattini, 1982; Carruba, Mantegazza, Memo, Missale, Pizzi & Spano, 1986; Fletcher & Burton, 1986).

Further support for an inhibitory role of 5-HT in feeding comes from studies employing techniques that decrease brain 5-HT activity. For example, a low dose of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), causing a decrease in brain 5-HT through feedback mechanisms in the brainstem (Samanin & Garatinni, 1990), increases feeding (Dourish, Hutson & Curzon, 1985). Simply stated, the above data appear to confirm the role of 5-HT as an appetite suppressant.

2b. Nutrient Components and Brain Serotonin

Another compelling line of research has found that while 5-HT may act as a satiety signal, it may only do so for particular nutrients. In 1989, Leibowitz, Weiss, Walsh & Viswanath published a series of experiments in which they examined the effect of 5-HT applied to the PVN on the choice between 3 pure macronutrient diets (protein, carbohydrate and fat). In addition, animals were monitored for any differences in macronutrient preferences throughout the nocturnal cycle. These researchers found that animals consumed the majority of their carbohydrate (CHO) intake during the first hour after dark onset, and the amount of CHO consumed within the first hour accounted for the largest percentage of the rats' total macronutrient intake within that

hour (44% CHO; 37% fat; 19% protein). Further, 5-HT in the PVN inhibited CHO intake in a dose-dependent fashion only during the first hour of the nocturnal period, while having no effect on protein or fat intake during any portion of the dark phase of the light cycle. Leibowitz and colleagues also found that 5-HT in the PVN had no effect on the total calories consumed at any time during the dark cycle. Therefore, it appears that 5-HT in the PVN selectively acts to decrease rats' appetite for CHO's and this effect is only evident during the early part of the dark cycle when CHO appetite is normally quite high.

The mechanism through which CHO acts to increase brain 5-HT has been determined (Spring, 1984). In general, CHO intake induces a variety of changes in the system that act to potentiate the uptake of tryptophan (the amino acid precursor of 5-HT) into the brain. Initially, CHO causes insulin levels in the blood to rise (as does the ingestion of any food). Insulin causes the uptake of large neutral amino acids (LNAA's), such as leucine, tyrosine, and tryptophan, and non-esterified fatty acids (NEFA's) largely into skeletal muscle. Prior to the ingestion of CHO, the NEFA's are bound to albumin, a protein carrier molecule. In order to be taken up by the skeletal muscle cells, NEFA's are stripped from the albumin. Tryptophan, which has a fairly high affinity for albumin, binds to NEFA-free albumin molecules. Further, the tryptophan stored in skeletal muscle is taken up by the the free albumin, thereby increasing plasma tryptophan. Albumin bound tryptophan is not available for uptake into the cells, leaving it free to circulate in the system. In addition, the brain has a higher affinity for tryptophan than albumin does, therefore, once albumin-bound tryptophan reaches the blood-brain barrier, tryptophan dissociates from albumin and enters the brain. The enzyme that converts tryptophan to 5-hydroxytryptophan (the precursor to 5-HT) in the brain has a low affinity for tryptophan and because of this is not saturated. It is the amount of tryptophan in the brain that limits the conversion of tryptophan to 5-HT, therefore, an increase in brain tryptophan leads to an increase 5-HT synthesis.

Conversely, in a balanced diet, where all three macronutrients are present (CHO, protein, and fat), plasma tryptophan increases relative to the amount of tryptophan ingested, however brain tryptophan does not increase. This is largely due to the fact that the other LNAA's are much more abundant in protein than is tryptophan and these LNAA's compete with tryptophan for entrance into the brain via the blood-brain barrier. In addition, if fats are ingested, less albumin is available to bind tryptophan such that more free tryptophan is available for uptake into the muscle cells. Therefore, recent research suggests that it is the percent composition of CHO to protein, and to a lesser extent, fat, that is important in a diet's ability to increase brain 5-HT.

3. Serotonin Receptor Subtypes

More current research has revealed that there are multiple 5-HT receptor subtypes that, when activated, appear to have differing effects on food intake as well as sexual responding. Within the last decade or so, technology has allowed specific binding profiles of various drugs to be established. In a binding profile analysis, compounds are characterized by their affinity for receptor subtypes and their selectivity across different types of receptors. Researchers may also investigate the intrinsic activity of a compound when bound to a particular receptor in order to determine whether the drug activates the receptor (an agonist) or blocks the receptor (an antagonist). Agonists for a particular receptor subtype act as the neurotransmitter (e.g., 5-HT) would at that receptor and are considered to have high intrinsic activity. Antagonists, on the other hand, simply block the receptor from being bound by any other compound or neurotransmitter while having little or no effect on their own (i.e., low intrinsic activity) (Kalant, Roschlau & Sellers, 1985).

Binding studies employing a variety of 5-HT receptor antagonists and agonists have allowed for the definition of as many as 11 distinct 5-HT receptor subtypes, and evidence of new subtypes continues to accumulate (Bradley, Handley, Cooper, Key,

Barnes & Coote, 1992; Glennon & Dukat, 1991; Zifa & Fillion, 1992). However, many of these sites have only been characterized pharmacologically, in isolated tissue preparations, and their functional significance remains unknown. *In vivo* studies of 5-HT receptor activation in the intact organism have been limited by the lack of compounds that are truly specific for any one type of 5-HT receptor subtype. However, drugs that exhibit moderate selectivity do exist and have been used for studies of the functions of the six best-established 5-HT receptor subtypes: the 5-$HT_{1A, 1B, 1C,}$ and $_{1D}$ receptors; 5-HT_2 receptors; and 5-HT_3 receptors (Bradley, et al., 1992; Glennon & Dukat, 1991; Zifa & Fillion, 1992). Research has revealed that these receptors are often localized in different areas of the brain, and may be either presynaptic (autoreceptors) or postsynaptic in nature (e.g., Palacios, Waeber, Hoyer & Mengod, 1990). For example, the 5-HT_{1A} receptor has been found to be primarily located in the dorsal raphe nucleus (DRN) of the brainstem, located on the presynaptic membrane of neurons (Palacios, et al., 1990), however there are postsynaptic 5-HT_{1A} receptors in other areas of the brain (Palacios, et al., 1990) but these possess a lower affinity for 5-HT_{1A} selective compounds. Activation of 5-HT_{1A} receptors in the brainstem leads to a decrease in 5-HT release from presynaptic membranes in the DRN. These 5-HT_{1A} receptors are autoreceptors which act to regulate the amount of neurotransmitter activity in the synapse. A high level of autoreceptor binding informs the neuron that there is an excessive amount of neurotransmitter available, causing the neuron to inhibit further release of the neurotransmitter into the synapse. When there is a low level of 5-HT in the DRN, little 5-HT is available for binding to postsynaptic receptors that activate neurons whose cell bodies reside here. Since the serotonergic neurons whose cell bodies are located in the dorsal raphe project to many forebrain areas, the effect of a 5-HT_{1A} agonist in the brainstem is to decrease serotonergic activity throughout the brain. Therefore, 5-HT_{1A} agonists at relatively low doses (so as not to activate any postsynaptic receptors) act as overall 5-HT antagonists in the brain.

This thesis is concerned with the functional activity of 5-HT_2 receptors in the male rat brain. The 5-HT_2 receptor is postsynaptic in nature, and is located largely in the forebrain (e.g., hypothalamus, neocortex) (Palacios, et al., 1990), however some 5-HT_2 receptors are found in certain midbrain (e.g., substantia nigra) and hindbrain areas (e.g., pons) (Palacios, et al., 1990; Palacios, Mengod, Hoyer, Waeber, Pompeiano, Niclou & Bruinvels, 1992). Pharmacological data suggest that there may be multiple 5-HT_2 receptor subtypes, in particular, it appears that the 5-HT_{1C} receptor belongs to the same class as the 5-HT_2 receptor (Glennon & Dukat, 1991; Hoyer, 1988, 1992). Indeed, cloning studies have revealed an 80% homology between the 5-HT_2 and 5-HT_{1C} receptor subtypes (Hartig, 1989; Glennon & Dukat, 1991). Given the great physical similarity between these two receptor subtypes, it is not surprising that compounds that show an affinity for 5-HT_2 receptors, tend to show similar affinity for 5-HT_{1C} receptors (Hoyer, 1988, 1992; Glennon & Dukat, 1991). For example, 1-(2,5-dimethoxy-4-iodophenyl aminopropane) (DOI) is classified as a 5-HT_2 agonist, however, it shows similar affinity for the 5-HT_{1C} receptor (Pranzatelli, 1990), making it impossible to determine whether the behavioural effects of this compound are due to its activity at 5-HT_2 or 5-HT_{1C} receptors. Recently, a variety of new 5-HT_2 antagonists have been synthesized whose binding profiles have been characterized as showing a high affinity for 5-HT_2 receptors, and no or little affinity for 5-HT_{1C} receptors (Doble, Girdlestone, Piot, Allam, Betschart, Boireau, Dupuy, et al., 1992; Haskins, Muth & Andree, 1987; Rinaldi-Carmona, Congy, Santucci, Simiand, Gautret, Neliat, Labeeuw, et al., 1992). In order to determine whether DOI's inhibitory effect on feeding and sexual behaviour is due to its effect at 5-HT_2 or 5-HT_{1C} receptors, the combined effect of DOI with these newly synthesized 5-HT_2 antagonists will be investigated in this thesis. If DOI's inhibitory effect is not observed when a 5-HT_2 antagonist is co-administered, then one could conclude that the effect of DOI on feeding and sexual behaviour is due to its effect at 5-HT_2 receptors. However, if DOI-induced inhibition of

feeding and mating is still observed after administration of 5-HT_2 antagonists, then 5-HT_{1C} mechanisms must be considered.

A more natural method of determining the importance of 5-HT_2 receptors in male copulatory and ingestive behaviour is through the presence or absence of a behaviour resembling a wet dog shake (WDS). The WDS is considered part of the "Serotonin Behavioural Syndrome" which occurs when rats receive treatments that increase serotonergic activity in the brain (Corne, Pickering & Warner, 1963; Grahame-Smith, 1971). The WDS is characterized by a paroxysmal, rapid rotational shudder of the head and shoulders, reminiscent of a dog shaking water from its coat. In the rat, a single WDS may last for 500 to 1000 msec. It appears that the WDS is primarily mediated by the activation of 5-HT_2 receptors (Green, 1989; Green & Heal, 1985; Pranzatelli, 1990). WDS may be activated in isolation from other symptoms of the serotonin behavioural syndrome by 5-HT_2 agonists (Darmani, Martin, Pandey & Glennon, 1990; Pranzatelli, 1990) and inhibited by 5-HT_2 antagonists (Colpaert & Janssen, 1983; Meert, Niemegeers, Gelders & Janssen, 1989; Pranzetelli, 1990). In addition, the WDS has been shown to be a part of the animal's natural repetoire of behaviours (Watson & Gorzalka, 1990). Research in our laboratory has shown that the level of WDS is inversely related to sexual activity in the male rat (Watson & Gorzalka, 1990). Therefore, one of the objectives of the present thesis is to investigate whether or not animals show elevated levels of WDS during a copulatory bout or a feeding bout. This finding would suggest the central activation of 5-HT_2 receptors in the brain. If animals that displayed different levels of sexual responding and feeding behaviour also showed predictable differences in the level of WDS, one could speculate that a 5-HT_2 mechanism is important in the natural display of feeding and sexual behaviour.

4. Serotonin Receptor Subtypes and Male Rat Sexual Behaviour

While early work employing serotonergic compounds suggested a general inhibitory effect of 5-HT on sexual behaviour in the male rat, more recent research has

suggested that 5-HT may inhibit or facilitate male copulation depending on the receptor subtypes activated (reviewed in Gorzalka, Mendelson & Watson, 1990; Zifa & Fillion, 1992).

A large amount of the research in this area has focussed on the roles of 5-HT_1 receptors in male sexual responding. For example, the highly selective 5-HT_{1A} agonist 8-OH-DPAT [8-hydroxy-2-(di-n-propylamino)tetralin] has been found to produce an net facilitation of sexual behaviour (e.g., Ahlenius & Larsson, 1984a, 1984b, 1985; Ahlenius, Larsson, Svensson, Hjorth, Carlsson, Lindberg, et al., 1981; Dahlof, Ahlenius & Larsson, 1988; Mendelson & Gorzalka, 1986). Conversely, compounds that are fairly selective for the 5-HT_{1B} receptor [1-(3-trifluoromethylphenyl)piperazine and m-chlorophenylpiperazine] have been reported to inhibit sexual responding in the male rat (Fernandez-Guasti, Escalante & Agmo, 1989; Fernandez-Guasti & Rodriguez-Manzo, 1992; Mendelson & Gorzalka, 1990).

Recent evidence suggests that central 5-HT_3 receptors play no role in male rat sexual responding (Tanco, Watson & Gorzalka, 1993, 1994; Watson, Tanco & Gorzalka, 1991).

In contrast to the vast amount of research conducted on the behavioural effects of 5-HT_1 receptors, relatively little is known about the role of 5-HT_2 receptors in sexual responding, and data that have been collected thus far are often contradictory. For example, the 5-$HT_{2/1C}$ receptor antagonists ketanserin and pirenperone have been reported to inhibit male rat sexual behaviour (Mendelson & Gorzalka, 1985), suggesting that 5-$HT_{2/1C}$ receptors mediate a facilitatory role in male sexual responding. However, the 5-$HT_{2/1C}$ antagonists cyproheptadine and LY 53857 have been reported to facilitate male rat copulation, suggesting 5-$HT_{2/1C}$ activation is inhibitory (Abraham, Viesca, Plaza & Marin, 1988; Foreman, Hall & Love, 1989). This latter conclusion is supported by research employing the 5-$HT_{2/1C}$ agonist DOI, which has been shown to inhibit copulatory behaviour in the male rat (Foreman, et al., 1989). One of the objectives of the present thesis is to determine the relative contributions of 5-HT_2 and

5-HT_{1C} receptors in male rat copulation by employing highly selective 5-HT_2 antagonists in combination with DOI, and observing the cumulative effect on sexual behaviour. If 5-HT_2 receptors mediate DOI's effect on sexual responding, then antagonists that have a high affinity for 5-HT_2 receptors and low affinity for 5-HT_{1C} recpeptors should block the effect of DOI. If, however, 5-HT_{1C} receptors mediate the DOI-induced inhibition of sexual behaviour, then selective 5-HT_2 antagonists will have no effect.

5. Serotonin Receptor Subtypes and Feeding Behaviour in the Male Rat

The role of serotonin as a general anorectic came into question with the discovery of multiple 5-HT receptor subtypes. As with copulation, studies of feeding in rats revealed that the effect of 5-HT depended on the receptor subtypes activated (Samanin & Garattini, 1990). Most of the research to date has been devoted to investigating the roles of 5-HT_{1A} and 5-$HT_{2/1C}$ receptors on feeding in the male rat.

Many experiments have been conducted employing the 5-HT_{1A} agonist 8-OH-DPAT (e.g., Dourish, Hutson & Curzon, 1985; Dourish, Clark & Iversen, 1988; Fletcher, Zack & Coscina, 1991; Montgomery, Willner & Muscat, 1988). In general, low doses of peripherally administered 8-OH-DPAT tend to increase feeding, while high doses act to decrease feeding as well as induce motor disturbances (indicative of high levels of 5-HT in the brain, i.e., the "Serotonin Behavioural Syndrome") (Dourish, et al., 1985). In addition, when 8-OH-DPAT is applied to the dorsal raphe, feeding behaviour is enhanced (Bendotti & Samanin, 1986). These and similar data, have encouraged some researchers to conclude that the increase in feeding by 8-OH-DPAT is likely due to the activation of autoreceptors in the brainstem producing a decrease in 5-HT release throughout the brain (Fletcher & Davies, 1990). Since 5-HT_{1A} receptor stimulation generally acts to deplete the brain of 5-HT, these data do little to inform us of the postsynaptic receptors involved in serotonin's role in satiety.

Many researchers have also investigated the role of 5-$HT_{2/1C}$ receptors in feeding behaviour. In 1988, Hewson, Leighton, Hill & Hughes found that ketanserin (a 5-$HT_{2/1C}$ antagonist) blocks the anorectic effect of fenfluramine, while having no effect on its own. In addition, the inhibitory effect of DOI on feeding was blocked by the administration of ketanserin (Hewson, Leighton, Hill & Hughes, 1989). It has been theorized that this result was due to these drugs binding at peripheral 5-HT_2 sites (Dourish, 1992; Massi & Marini, 1987), however, Schechter & Simansky (1988) provided evidence that challenged this theory. These researchers administered DOI in combination with a variety of central and one peripheral 5-$HT_{2/1C}$ antagonists. They found that while the antagonists (ketanserin and LY 53857) that were able to bind to central 5-HT_2 neurons blocked the inhibitory effect of DOI on feeding, the antagonist that bound only to peripheral 5-$HT_{2/1C}$ receptors (xylamidine) had no effect. Further, the 5-$HT_{2/1C}$ agonist, DOI, has been found to preferentially inhibit CHO intake in rats when compared to agonists which act at other 5-HT receptor subtypes (e.g., mCPP=5-$HT_{1B/1C}$, RU24969=5-$HT_{1A/1B}$) (Lawton & Blundell, 1993a), suggesting that the role of 5-HT to inhibit food intake may be due to its effect at 5-HT_2 and/or 5-HT_{1C} receptors.

6. Objectives

As discussed earlier, 5-HT plays a role in the regulation of feeding and sexual behaviour in the male rat. In addition, there appears to be a natural inverse relationship between food availability (and consumption) and copulatory activity. In order for this relationship to exist, there must be some endogenous mechanism that allows the animal to determine when to modify its behaviour in relation to the availability of food. Since 5-HT participates in the regulation of both behaviours, it is possible that the relationship between energy availability and copulatory activity is mediated through the serotonergic system. The overall goal of this thesis is to determine whether a relationship exists between feeding behaviour and sexual behaviour in the male rat

through a serotonergic mechanism, and more specifically, through activity at the 5-HT_2 receptor. This goal lead to four general objectives:

1) Initially, we set out to determine whether there was a natural relationship between feeding behaviour and sexual behaviour in the male rat. The literature would suggest that an animal with naturally elevated levels of 5-HT would eat less and be less sexually active than a conspecific with lower 5-HT levels. Further, it may be possible to determine whether 5-HT_2 receptors mediate both sexual behaviour and feeding behaviour through the observation of naturally occurring WDS. For example, if a male naturally shows low levels of both feeding and sexual behaviour, does he also show elevated WDS levels in comparison to his more active counterparts? These hypotheses will be tested in Experiment 1.

2) Since CHO consumption increases brain 5-HT levels, while protein/CHO consumption does not, one might expect that a rat which eats only CHO prior to a copulatory session would show less sexual activity than a male that consumes a protein/CHO meal. This hypothesis will be tested in Experiments 2 and 3 by exposing males to two test diets (CHO or protein/CHO), and measuring their sexual behaviour during the period when brain 5-HT levels have been shown to be influenced by these diets.

3) Since 5-HT_2 activation has been shown to inhibit both sexual behaviour and feeding behaviour in the male rat, it appears necessary to rule out the possibility that a certain level of 5-HT_2 receptor activity simply acts to inhibit any motivated behaviour. This possibility will be indirectly tested in Experiments 4-10 by comparing the effect of the same dose of DOI (5-$HT_{2/1C}$ agonist) on sexual behaviour and feeding behaviour. If this dose acts to inhibit both feeding and sexual behaviour, it is possible that increased 5-HT_2 activity generally inhibits motivated behaviour. However, if feeding and sexual behaviour are differentially influenced by a single dose of DOI, then this hypothesis is not tenable.

4) As stated in the introduction, 5-HT_2 agonists and antagonists used to date have had comparable affinity for the type 2 and type 1C receptor. Recently, antagonists that show high affinity for 5-HT_2 receptors and little, if any, affinity for 5-HT_{1C} receptors have been synthesized. The final objective of this thesis is to determine whether the influence of the 5-$HT_{2/1C}$ agonist DOI on sexual and feeding behaviour in the male rat is through its effect on 5-HT_2 or 5-HT_{1C} receptor activation by blocking 5-HT_2 receptors with these new antagonists in Experiments 4-10.

GENERAL METHODS: Sexual Behaviour

Surgery

Female rats were bilaterally ovariectomized while under sodium pentabarbital (43 mg/kg) and ketamine (4 mg/kg) anesthesia. These animals were allowed at least one week to recover before administration of exogenous hormones and exposure to male rats.

Materials

Estradiol benzoate (10 µg/0.1 cc; 44-48 hours prior to testing) and progesterone (500 µg/0.1 cc; 3-4 hours prior to testing) dissolved in peanut oil were injected subcutaneously to induce behavioural estrus in female rats. Both of these hormones were obtained from Sigma Chemical Company, St. Louis, Missouri. Sexual behaviour was scored with animals in Plexiglas testing chambers (30 x 30 x 60 cm), or clear glass testing chambers (30 cm dia., 60 cm ht.) lined with a layer of San-i-cel bedding.

Behavioural Testing

Initially, test males were placed in plastic carrying cages with wire lids and stimulus females were placed on top of the carrying cages for 5 minutes. Males were then placed in the testing chambers, one male per chamber, and allowed 5 minutes to habituate to their surroundings prior to the introduction of a stimulus female. Stimulus

females were rotated between cages every 10 minutes. In addition, if a female was not receptive to the male's copulatory attempts, she was removed from the test session and replaced with another stimulus female. Sexual behaviour was recorded using an in-house computer program. Each male was scored on the following behaviours: mount latency (ML), the latency in seconds from the introduction of the stimulus female into the testing chamber to the time of the first mount by the male; the intromission latency (IL), the latency in seconds from the introduction of the female into the testing chamber to the time of the first intromission by the male; the ejaculation latency (EL), the latency in seconds from the time of the first intromission to the time of the first ejaculation; the post-ejaculatory interval (PEI), the interval in seconds between an ejaculation and the first subsequent intromission; the mount frequency (MF), the number of mounts displayed during a single test session; and the intromission frequency (IF), the number of intromissions displayed during a single test session. Length of testing varied depending on the individual experiment.

GENERAL METHODS: Feeding Behaviour
(EXPERIMENTS 7-9)

Housing

At least two weeks prior to diet exposure, experimental male rats were housed in a colony maintained on a reverse 12:12 hour dark/light cycle, with lights off at 11 a.m. and a temperature of 21±1°C. One week prior to diet exposure, animals were rehoused into single wire mesh cages from their original group housing environment.

Materials

Animals were exposed to a milk diet composed of one part "Western Families Sweetened Condensed Milk" to one part water, and a small amount of powdered multivitamins (Vitamin Mixture 76; ICN). Milk diet was prepared just prior to diet exposure. The milk diet was provided in plastic drinking bottles fitted with rubber

stoppers and curved stainless steel spouts containing a few ball bearings to decrease dripping.

Procedure

Animals were exposed to the milk diet for 6 hours/day (11 a.m. to 5 p.m.) for nine days prior to testing and maintained on this diet between test sessions. Animals were deprived of their usual food pellets, but not water, during the diet exposure and testing periods. Each animal was given approximately 80 ml of milk diet each exposure day (this amount was employed to ensure that no animal would run out of diet during the 6 hour exposure period). On test days, animals were given approximately 50 ml of the milk diet in plastic graduated drinking tubes. The amount of milk consumed was measured 30 minutes after presentation of the milk diet. A between-within design of drug treatments was employed such that animals were placed in either the DOI or saline treatment group, and all animals received the antagonist or vehicle during one of the two tests. Testing was conducted on a weekly basis, on day 10 of the diet exposure and again on day 17. After experiments were complete, animals were returned to a group housing environment and given free access to Purina Rat Chow and water.

NATURAL FEEDING AND SEXUAL BEHAVIOUR RELATIONSHIP (EXPERIMENTS 1-3)

EXPERIMENT 1

Early findings in feeding behaviour research suggested that elevated 5-HT levels decreased feeding. This experiment was conducted to determine whether there is a natural difference between sexually active males (studs) and sexually inactive males (duds) in their eating behaviour. Specifically, do duds eat less than studs as serotonergic data would suggest? In addition, is there a relationship between feeding

behaviour and WDS, as there is between sexual behaviour and WDS, suggesting that 5-HT_2 receptors participate in 5-HT's effect on feeding behaviour?

Animals

Forty male Sprague-Dawley rats obtained from the Animal Care Unit, U.B.C., were 60 days old at the beginning of the experiment. Long-Evans female rats that had been bilaterally ovariectomized, and treated with exogenous hormones were used to elicit sexual behaviour from the test males. Animals were group housed in standard wire mesh cages. Males were housed 3-4 animals/cage, while females were housed 5-6 animals/cage. Colonies were kept on a 12 hour reverse light cycle with lights off at 9 a.m., and maintained at a temperature of 21±1^{o}C.

Procedure

a) Sexual Behaviour:

Males were exposed to stimulus females for one hour/week for three weeks prior to the beginning of the experiment. Each test of sexual behaviour was 60 minutes in duration. Tests occurred during the dark phase of the light cycle, using the scoring procedure described in the General Methods section. In addition, males were scored, by hand, on the number of WDS displayed during the 60 minute test session.

b) Feeding Behaviour:

Feeding behaviour testing commenced two days after the sexual behaviour testing and continued for three consecutive days. Food, but not water, was removed from the males' home cages a minimum of 18 hours prior to behavioural testing. Since rats are photophobic (Zuckerman, 1984) the testing room was illuminated with red lights to decrease the rats' anxiety and reduce interference with feeding behaviour. Experimental males were placed on top of wire grids 1 cm above the floor of clear Plexiglas testing chambers, and allowed 5 minutes to habituate. Newspaper was used to line the bottom of the testing chambers so any food spillage could be collected and

weighed for more accurate food consumption measurement. After habituation, pre-weighed food pellets were placed in the cages. Animals were scored on the amount of food consumed (grams) in 60 minutes; the number of feeding bouts; the total amount of time spent eating, in seconds, during the 60 minute session; and the number of WDS displayed. Animals were weighed in order to determine possible correlations between feeding behaviour and weight. At the end of the 60 minute test, males were returned to their home cages and given free access to food and water.

Data were analyzed using the student's t-test statistic and Bartlett's test for homogeneity of variance.

Results

Males were categorized as either studs, if they ejaculated during the sexual behaviour testing session, or duds, if they failed to ejaculate. Table 1 shows that there are significant differences between the studs and the duds in terms of their sexual responding, specifically, duds show longer ML and IL's and fewer intromissions, which is consistent with an elevated level of endogenous brain 5-HT. T-tests revealed a significant difference between studs and duds on the amount of food consumed, with the studs consuming more food than the duds (see Table 1). In addition, duds exhibited a higher number of WDS during sexual behaviour scoring than the studs (Table 1). None of the other feeding behaviours showed any significant differences between duds and studs (see Table 1). Bartlett's tests for homogeneity of variance revealed that the variance between groups for WDS was significantly different, however none of the other significantly different measures showed heterogeneous variances. The t-test and degrees of freedom calculation were modified to deal with the heterogeneous variances in the WDS measure. No correlations were found between feeding behaviour and the number of WDS displayed during sexual behaviour testing. In addition, animals' weights were not correlated with the amount of food consumed.

Table 1. A comparison of "Duds" and "Studs" on the level of sexual responding, feeding behaviour and WDS displayed during sexual behaviour testing. Data are presented as mean scores ± standard deviations. Student's t-test values and significance levels are cited in the final column of the table (NS=nonsignificant).

Comparison between Studs and Duds on Sexual Responding, Feeding Behaviour, and WDS.

Behavioural Measure	DUDS	STUDS	T-TEST
Sexual Behaviour:			
ML	1917.3 ± 1471.5 s	341.9 ± 516.7 s	t(38)=4.52, p<.01
MF	22.8 ± 25.0	32.5 ± 26.5	t(38)=1.19, NS
IL	2594.5 ± 1040.6 s	517.7 ± 519.4 s	t(38)=7.99, p<.01
IF	4.2 ± 4.8	12.9 ± 4.6	t(38)=5.84, p<.01
#WDS	6.7 ± 6.4	1.6 ± 1.1	t'(39)=3.50, p<.01
Feeding Behaviour:			
Duration	611.9 ± 229.9 s	759.8 ± 320.3 s	t(38)=1.68, NS
Amount Consumed	4.10 ± 1.39 g	5.45 ± 2.01 g	t(38)=2.47, p<.05
# Feeding Bouts	6.95 ± 3.15	7.60 ± 2.80	t(38)=0.11, NS
n	20	20	

Discussion

This study revealed a relationship between naturally displayed copulatory activity and feeding behaviour in the male rat. In addition, the data are consistent with a serotonergic mechanism mediating the relationship. For example, males displaying high levels of sexual activity (studs) consumed significantly more food than males displaying low levels of sexual activity (duds), suggesting that studs may have lower levels of central 5-HT than duds. Chronically lower levels of endogenous 5-HT could act to increase sexual responding since pharmacologically elevated 5-HT has been shown to decrease sexual behaviour (e.g., Ahlenius & Larsson, 1985; Baum & Starr, 1980; Sodersten, et al., 1978) while decreased brain 5-HT acts to elevate sexual responding (Hillegaart, et al., 1989; Malmnas & Meyerson, 1971; Sheard, 1969; Sodersten, et al., 1978). These data are also consistent with the role of 5-HT serving as a satiety signal in feeding, as rats with low levels of brain 5-HT may need to ingest more food to elevate brain 5-HT sufficiently to induce satiety. Indeed, feeding has been shown to increase brain 5-HT levels (Fernstrom & Wurtman, 1971), and elevated brain 5-HT acts to inhibit feeding (e.g., Blundell, 1984; Invernizzi, et al., 1986).

EXPERIMENT 2

Food consisting of pure CHO increases brain 5-HT synthesis and a peak in brain 5-HT is reached 2-3 hours after the commencement of feeding (Fernstrom & Wurtman, 1971). Since sexual behaviour in the male rat has been shown to be inhibited by elevation of brain 5-HT levels, the following experiment was conducted in order to determine whether consumption of a pure CHO meal would elevate brain 5-HT levels sufficiently to inhibit sexual responding in male rats.

Animals

Male Sprague-Dawley rats obtained from the Animal Care Unit, U.B.C., were 60 days old at the beginning of the experiment. Long-Evans female rats that had been bilaterally ovariectomized, and treated with exogenous hormones were used to elicit sexual behaviour from the test males. Males were housed 3-4 animals/cage, females were housed 5-6 animals/cage. Colonies were kept on a 12 hour reverse light cycle with lights off at 9 a.m., and maintained at a temperature of $21 \pm 1^{o}C$.

Materials

Sugar cubes were used for the CHO diet, and Purina Rat Chow pellets were used as the combination CHO/protein diet.

Procedure

Males were exposed to stimulus females for three weeks prior to the commencement of the experiment in order to establish a consistent level of sexual responding. One week prior to the beginning of the experiment, test males were fed sugar cubes to familiarize them with the test food. Animals were food deprived a minimum of 18 hours prior to testing. Feeding behaviour was scored as described in Experiment 1, however, only half the animals received the sugar diet, while the other

half received pellets. Feeding behaviour was observed for 30 minutes, then the males were returned to their home cages, still devoid of food.

The animals remained in their home cages for 100 minutes, after which they were placed in Plexiglas testing chambers lined with a layer of San-i-cel and allowed 5 minutes to habituate. The 100 minute rest period was necessary to allow brain 5-HT levels to reach their maximum after CHO consumption (Fernstrom & Wurtman, 1971). After the habituation period, a stimulus female was placed with each male in the testing chambers. Sexual behaviour and WDS were scored for 30 minutes as outlined in the General Methods section. After the 30 minute test session, males were returned to their home cages and allowed free access to food and water.

Data were analyzed using the student's t-test statistic and bartlett's test for homogeneity of variance.

Results

T-tests revealed that the animals in the CHO group consumed significantly less food than animals in the pellet group; spent significantly less time consuming food; and exhibited fewer feeding bouts (see Table 2). Bartlett's tests for homogeneity of variance were not significant. No significant differences in sexual responding and WDS were found between the CHO and the pellet groups (Table 2). The mean scores and standard deviations for these results are presented in Table 2. It is interesting to note that when the copulatory behaviours of the CHO group were analyzed, the amount of CHO consumed was positively correlated with the IL ($r=0.415$, $p<.05$), while animals that were fed regular food pellets showed no such correlation.

Table 2. A comparison of the effect of a pure carbohydrate (sugar) or a carbohydrate/protein meal (pellets) on the level of sexual responding, feeding behaviour and WDS (displayed during sexual behaviour testing). Data are presented as mean scores ± standard deviations. Student's t-test values and significance levels are cited in the final column of the table (NS=nonsignificant).

Comparison between a CHO and CHO/protein meal on Sexual Responding, Feeding Behaviour, and WDS.

Behavioural Measure	CHO	CHO/protein	T-TEST
Sexual Behaviour:			
ML	809.9 ± 678.8 s	661.9 ± 596.5 s	t(38)=0.73, NS
MF	11.3 ± 13.3	13.5 ± 12.4	t(38)=0.54, NS
IL	1149.1 ± 665.6 s	1037.3 ± 696.3 s	t(38)=0.52, NS
IF	4.1 ± 4.7	6.0 ± 6.3	t(38)=1.08, NS
#WDS	2.0 ± 1.6	1.4 ± 1.3	t(38)=1.30, NS
Feeding Behaviour:			
Duration	295.2 ± 176.4 s	680.9 ± 261.1 s	t(38)=5.47, $p<.01$
Amount Consumed	1.74 ± 1.25 g	4.52 ± 2.08 g	t(38)=5.12, $p<.01$
# Feeding Bouts	3.80 ± 2.04	4.90 ± 1.33	t(38)=2.02, $p<.05$
n	20	20	

Discussion

While the CHO group did not differ from the pellet group in sexual responding or the display of WDS, the animals in the CHO group displayed less feeding behaviour and food consumption. It is possible that the animals in the CHO group consumed significantly less than the animals in the pellet group due to a lack of familiarity with the sugar cube (CHO) diet. In addition, there are several qualitative differences between the two diets (e.g., palitability, texture) that may have affected feeding behaviour. However, it is also possible to interpret the decreased consumption of the CHO group in terms of serotonergic mechanisms. Recall that CHO consumption leads to an increase in brain 5-HT which acts to inhibit the animal's appetite for CHO, an effect that does not occur when animals are exposed to a meal composed of protein and CHO (Spring, 1984). It is possible that such a mechanism was activated in this instance, inducing satiety earlier in the animals exposed to the pure CHO meal than in the animals exposed to the CHO/protein meal (pellets, which contained only 25% CHO), resulting in less food being consumed in the CHO group. Therefore, brain 5-HT levels may have been elevated in the CHO group enough to inhibit CHO consumption. However, if this is so, why weren't the animals in the CHO group inhibited in their sexual responding as an elevation in brain 5-HT would predict? It is possible that the elevation in brain 5-HT, while sufficient to inhibit CHO consumption, was not sufficient to inhibit sexual responding. However, the positive correlation established between the amount of food consumed and IL in the CHO group suggests that there may be some limited effect of CHO consumption on sexual activity in the male rat.

EXPERIMENT 3

It is possible that the differences in consumption of the two test diets in Experiment 2 may have been due to factors other than differences in brain 5-HT induced by the two diets. For example, test males were only exposed to the pure CHO diet once prior to testing, and this was in a competitive environment (i.e., in a group

housing situation), therefore all males may not have been exposed to the sugar diet. Further, the protein/CHO diet was the animals' regular pellet diet with which they were highly familiar. There is evidence that animals approach a novel food with caution, tasting only a small amount in order to determine whether or not it is poisonous (Barnett & Cowan, 1976; Kronenberger & Medioni, 1985). Therefore, a difference in novelty between one diet and another may have contributed to differences in ingestion in Experiment 2. Moreover, there were differences in the physical properties of the two diets: for example, sugar and pellets have a different texture and taste, factors that may influence ingestion. In the following experiment we controlled for these differences by exposing animals to two novel diets: CHO and protein/CHO, that had the same texture, consistency and taste. In order to familiarize animals with the diets, they were exposed to them three times prior to testing.

Animals

Fifteen, eight week old Long-Evans male rats (Charles River, Quebec) were used in this experiment. Males were singly housed in order that accurate diet consumption could be established for each male in his home cage. During the intervals between diet exposure, animals were given free access to Purina Rat Chow and water. Ovariectomized females were used to elicit sexual behaviour from the test males. Exogenous hormones were administered as outlined in the General Methods section.

Materials

The CHO diet was that employed by Fernstrom and Wurtman (1971). The CHO/protein diet was composed of 82% of the CHO diet and 18% powdered casein protein. Fernstrom & Wurtman (1972) have shown that this CHO/protein diet fails to affect brain 5-HT levels, while the CHO diet significantly elevates brain 5-HT levels. Both diets had the consistency of a thick paste, and were presented in glass petri dishes fixed to the bottom of each animal's home cage with a piece of duct tape.

Copulatory behaviour was tested with male rats in Plexiglas chambers and scored with a computer program as mentioned in the General Methods section.

Procedure

Animals were exposed to the diets on three separate occasions prior to commencement of the experiment. As well, males were exposed to stimulus females in the testing chambers on three occasions prior to the beginning of the experiment. A repeated measures design was employed such that animals were exposed to the CHO or the protein/CHO diet one week, and the opposite diet the next week. Animals were tested once per week. Males were deprived of food, but not water, 15 hours prior to diet exposure. Diet exposure lasted for 2-3 hours, with the amount consumed calculated after exposure. Two to three hours of food exposure has been shown to maximally elevate brain 5-HT levels in the male rat (Fernstrom & Wurtman, 1971). Males were then placed in Plexiglas testing chambers lined with a layer of San-i-cel and allowed 5 minutes to habituate. After the habituation period, a stimulus female was placed with each male in the testing chambers. Sexual behaviour and WDS were scored for 30 minutes as outlined in the General Methods section. WDS frequencies were scored by hand. After the 30 minute test session, males were returned to their home cages and allowed free access to food and water.

Data were analyzed using the student's t-test statistic and Bartlett's test for homogeneity of variance.

Results

Table 3 presents the means and standard deviations of the indices of sexual activity scored, the number of WDS displayed and the amount of food consumed by each of the diet groups. Dependent samples t-tests failed to reveal any significant differences between the two diet groups on any of the sexual behaviour measures scored (see Table 3). However, as found in Experiment 2, there was a significant difference in the amount of diet consumed between the CHO and the protein/CHO diets

(Table 3). Bartlett's test for homogeneity of variance on these data were not significant.

Table 3. A comparison of the effect of a pure carbohydrate or a carbohydrate/protein meal on the level of sexual responding, feeding behaviour and WDS (displayed during sexual behaviour testing). Data are presented as mean scores ± standard deviations. Student's t-test values and significance levels are cited in the final column of the table (NS=nonsignificant).

Comparison between a CHO and CHO/protein meal on Sexual Responding, Feeding Behaviour, and WDS.

Behavioural Measure	CHO	CHO/protein	T-TEST
Sexual Behaviour:			
ML	529.3 ± 753.8 s	577.9 ± 765.9 s	t(14)=0.32, NS
IL	667.1 ± 833.9 s	595.3 ± 765.5 s	t(14)=0.49, NS
EL	991.9 ± 666.5 s	896.2 ± 614.8 s	t(14)=0.68, NS
#WDS	3.93 ± 3.94	3.13 ± 3.52	t(14)=0.65, NS
Feeding Behaviour:			
Amount Consumed	6.46 ± 2.40 g	11.57 ± 3.69 g	t(12)=-4.25, $p<.01$
n	15	15	

Discussion

As in Experiment 2, this experiment failed to reveal any differences in sexual responding after animals had ingested a CHO or protein/CHO meal. These data suggest that the elevation in brain 5-HT induced by a pure CHO meal is not sufficient to influence sexual responding. When animals were exposed to the CHO diet, they consumed significantly less than when they were exposed to the protein/CHO diet. Since these diets differed only in their ratio of protein to CHO, this difference must be attributed to the components of the two diets. It should be noted that the caloric difference between these diets is only slight and is not likely to account for the large difference in amount ingested. As suggested in Experiment 2, it appears that the CHO diet increases brain 5-HT enough to inhibit CHO consumption but not enough to influence sexual responding.

5-HT_2 RECEPTORS AND SEXUAL BEHAVIOUR (EXPERIMENTS 4-6B)

The purpose of Experiments 4-6B is to investigate the effect of a 5-$HT_{2/1C}$ agonist (DOI) and a variety of selective 5-HT_2 antagonists (amperozide, SR46349B & RP62203) on sexual responding in the male rat. It has been established that DOI acts to inhibit male sexual behaviour, however, until recently, pharmacological probes have been unable to differentiate between 5-HT_2 and 5-HT_{1C} sites making it difficult to determine the relative contribution of these receptors to male sexual behaviour.

EXPERIMENT 4

This experiment was designed to investigate the effect of amperozide, a selective 5-HT_2 antagonist, to inhibit DOI-induced inhibition of male rat sexual behaviour. Pharmacological studies have established that amperozide displays a high affinity for 5-HT_2 receptors in the rat brain, and a low affinity for 5-HT_{1C} receptors

(Haskins, Muth & Andree, 1987). Given this binding profile, amperozide seems like an appropriate tool to determine whether DOI's effect on male sexual responding is due to it's effect at 5-HT_2 or 5-HT_{1C} receptors.

Subjects

Ten, 12 week old Long-Evans male rats (Charles River, Quebec) were employed as test males in this experiment. Twelve, 6 month old Long-Evans rats (Charles River, Quebec) who had been bilaterally ovariectomized and primed with exogenous hormones as described in the General Methods section, were employed as stimulus females. Animals were group housed in standard wire mesh cages. Males were housed 3-4 animals/cage, females were housed 5-6 animals/cage. Colonies were kept on a 12 hour reverse light cycle with lights off at 9 a.m., and maintained at a temperature of 21±1^{o}C.

Materials

Testing chambers, hormones and anesthetics were the same as those described in the General Methods section. (±)-2,5-Dimethoxy-4-iodoamphetamine hydrobromide [(±)-DOI hydrochloride] was purchased from Research Biochemicals International, Natick, MA. Amperozide hydrochloride was generously provided by Kabi-Pharmacia Therapeutics, Sweden. Both amperozide (2 mg/ml) and DOI (1 mg/ml) were dissolved in physiological saline and injected intraperitoneally in a volume of 1 ml/kg body weight.

Procedure

Prior to the beginning of the experiment, males were exposed to stimulus females in the testing chambers on at least three separate occasions. Baseline sexual behaviour was scored one week prior to commencement of testing such that males with low levels of sexual responding would not be included in the experiment. Sexual behaviour was scored as outlined in the General Methods section, except that the

number of ejaculations occurring within a treatment group (#E) was also calculated. Testing continued for four weeks, with one test conducted per week. A repeated measures design was employed such that each animal received each treatment once during the experiment. The four treatment groups employed were: amperozide/DOI, amperozide/saline, saline/DOI, and saline/saline. The order of treatment exposure was randomized to avoid any order effects of drug exposure. Forty minutes prior to testing, amperozide (2 mg/kg), dissolved in saline, or saline (1ml/kg) was injected intraperitoneally (IP) into the test males. Thirty minutes prior to testing, DOI (1mg/kg), dissolved in saline, or saline (1 ml/kg) was injected IP. Testing was conducted during the middle phase of the dark cycle. Data were analyzed using Analysis of Variance (ANOVA), Tukey's pairwise comparisons and Cochran Q analyses where appropriate.

Results

Copulatory behaviour scores for all groups are presented in Table 4. ANOVA's revealed significant differences for ML [$F(3,27)=6.74$, $p<.01$], IL [$F(3,27)=12.34$, $p<.001$], EL [$F(3,27)=10.50$, $p<.001$], and PEI [$F(3,18)=4.38$, $p<.05$]. Tukey's pairwise comparisons revealed that the saline/DOI treatment acted to significantly lengthen ML, IL and EL relative to the saline/saline treatment, indicating an inhibition of sexual responding ($p<.05$; Table 4). Tukey's tests revealed that PEI was significantly longer in the amperozide/DOI group than in the saline/saline group indicating that the combination of DOI and amperozide influenced PEI, even though DOI alone and amperozide alone did not. Cochran Q analyses revealed a significant difference between groups on the number of animals ejaculating [$Q(3)=16.04$, $p<.01$]. Pairwise comparisons showed that the number of ejaculations displayed by the DOI group were less than any other group, while none of the other groups were significantly different from each other (see Table 4). In general, amperozide appeared to block the effect of DOI on ML, EL and #E as the amperozide/DOI group was not significantly different from the saline/saline group, and amperozide had no effect on its own.

Table 4. The effect of a selective 5-HT_2 antagonist (amperozide) and a 5-$HT_{2/1C}$ agonist (DOI), alone and combined, on sexual responding in the male rat. Except for the number of animals ejaculating, data are presented as means ± standard deviations. Sexual behaviours listed are ML=mount latency, IL=intromission latency, EL=ejaculation latency, and PEI=post-ejaculatory interval, definitions of these behavioural measures can be found in the General Methods section. [Amperozide=Amp; Saline=Sal].

Comparison between the effect of Amperozide and DOI (alone and in combination) on sexual behaviour in the male rat.

Behavioural Measure	Amp/DOI	Amp/Sal	Sal/DOI	Sal/Sal
ML	226.4 ± 477.6	190.4 ± 565.7	960.2 ± 893.8	6.2 ± 2.4
IL	261.4 ± 484.5	198.2 ± 563.1	262.2 ± 861.2	70.7 ± 195.5
EL	485.7 ± 509.4	359.8 ± 519.5	1319.9 ± 776.8	199.3 ± 147.6
PEI	398.6 ± 63.6	375.3 ± 89.7	350.7 ± 61.2	324.0 ± 50.9
# E	9	9	3	10
n	10	10	10	10

Discussion

The results of this experiment generally confirm those published by Klint, Lena Dahlgren and Larsson (1992) suggesting amperozide acts as a classic 5-HT_2 antagonist, blocking the inhibitory effect of DOI on male sexual responding while having no effect on its own. It is interesting to note that the conclusion made by Klint, et al. (1992) that amperozide is a classic 5-HT_2 antagonist does not necessarily reflect their actual findings. For example, several doses of amperozide alone (0.1, 1 and 5 mg/kg), significantly increased PEI in their study. In addition, the highest dose of amperozide also decreased the number of intromissions displayed by the animals. Further, when DOI (1 mg/kg, 30 minutes prior to testing) and amperozide (2 mg/kg, 40 minutes prior to testing) were injected simultaneously, intromission latency was significantly lengthened, however, PEI was unaffected. When considered with our finding that amperozide/DOI treatment acted to significantly lengthen PEI, one must question the selectivity of amperozide and it's usefulness in blocking 5-HT_2 receptors without having any other potentially confounding effects. Indeed, pharmacological studies have revealed that while amperozide binds with a high affinity to 5-HT_2 receptors, especially when compared to other 5-HT receptors, it also displays a moderate affinity for α_1 receptors (Svartengren & Simonsson, 1990) and acts to increase norepinephrine activity in the brain (Haskins, et al., 1987).

EXPERIMENT 5

This experiment was designed investigate the effect of RP62203 (RP) to block the DOI-induced inhibition of male sexual responding. Binding studies have revealed that RP has a high affinity for 5-HT_2 receptors in the rat brain (Doble, et al., 1992) and a low affinity for 5-HT_{1C} receptors (Malgouris, Flamand & Doble, 1993). This binding profile allowed for the speculation that RP would help establish whether DOI affected sexual behaviour through it's activity at 5-HT_2 or 5-HT_{1C} sites.

Subjects

Nine Wistar male rats (Animal Care Facility, UBC) were two months old at the start of the experiment. Twelve 6 month old Long-Evans female rats were employed to elicit sexual behaviour from the males. Females were primed with exogenous hormones as described in the General Methods section. Males were housed 3-4 animals/cage, females were housed 5-6 animals/cage. Colonies were kept on a 12 hour reverse light cycle with lights off at 9 a.m., and maintained at a temperature of 21± 1°C.

Materials

Testing chambers, hormones and anesthetics were the same as those described in the General Methods section. (±)-DOI hydrochloride was purchased from Research Biochemicals International, Natick, MA. RP was generously provided by Rhône-Poulenc-Rorer, France. DOI was dissolved in physiological saline to a dose of 1 mg/ml and injected IP in a volume of 1 ml/kg body weight. RP was suspended in a solution of 5% Tween 80 (T80) to a dose of 1 mg/ml and injected in a volume of 2 ml/kg body weight (2 mg/kg dose).

Procedure

Prior to the beginning of the experiment, males were exposed to stimulus females in the testing chambers on at least three separate occasions. Baseline sexual behaviour was scored one week prior to commencement of testing such that males with low levels of sexual responding would not be included in the experiment. Sexual behaviour was scored as outlined in the General Methods section, except that the number of ejaculations occurring within a treatment group (#E) was recorded. Testing continued for four weeks, with one test being conducted per week. A repeated measures design was employed such that each animal received each treatment once during the experiment. The four treatment groups employed were: RP/DOI, RP/saline,

T80/DOI, and T80/saline. The order of treatment exposure was randomized to avoid any order effects of drug exposure. Ninety minutes prior to testing, RP (2 mg/kg), or T80 (2ml/kg) was injected IP into the test males. Thirty minutes prior to testing, DOI (1mg/kg), dissolved in saline, or saline (1 ml/kg) was injected IP. Testing was conducted during the middle phase of the dark cycle. Data were analyzed using Analysis of Variance (ANOVA), Tukey's pairwise comparisons and Cochran Q tests where appropriate.

Results

Copulatory behaviour scores are presented in Table 5. ANOVA's revealed significant differences for ML [$F(3, 21)=75.34$, $p<.0001$], IL [$F(3,21)=99.38$, $p<.0001$], and EL [$F(3,21)=1093.13$, $p<.0001$]. No significant differences were established for PEI [$F(2,14)=0.45$, NS]. For ML, IL and EL, Tukey's pairwise comparisons revealed that the T80/DOI group was significantly different from all the other groups ($p<.001$), while none of the other groups differed from each other (see Table 5). Cochran Q analyses revealed a significant difference between groups on the number of animals ejaculating [$Q(3)=18.55$, $p<.001$]. Pairwise comparisons revealed that while DOI acted to significantly reduce the number of ejaculations when compared to controls [$Q(1)=6.0$, $p<.02$], the administration of RP prior to DOI acted to restore the number of ejaculations displayed to control levels [$Q(1)=1.0$, NS]. In addition, RP had no effect on the number of ejaculations displayed when administered on it's own [$Q(1)=1.0$, NS].

Table 5. The effect of a selective 5-HT_2 antagonist (RP 62203) and a 5-$HT_{2/1C}$ agonist (DOI), alone and combined, on sexual responding in the male rat. Except for the number of animals ejaculating (#E), data are presented as means ± standard deviations. Sexual behaviours listed are ML=mount latency, IL=intromission latency, EL=ejaculation latency, and PEI=post-ejaculatory interval, definitions of these behavioural measures can be found in the General Methods section. [RP=RP 62203, Saline=Sal, T80=5% Tween 80].

Comparison between the effect of RP 62203 and DOI (alone and in combination) on sexual behaviour in the male rat.

Behavioural Measure	RP/DOI	RP/Sal	T80/DOI	T80/Sal
ML	8.0 ± 8.4	5.2 ± 3.2	1121.6 ± 759.7	5.4 ± 2.7
IL	86.3 ± 204.9	11.9 ± 13.1	1221.2 ± 778.9	19.1 ± 27.2
EL	208.9 ± 88.5	190.3 ± 52.4	1428.9 ± 736.7	219.8 ± 91.3
PEI	323.1 ± 38.9	335.0 ± 61.1	326.5 ± 8.0	344.5 ± 26.1
# E	9	9	2	8
n	9	9	9	9

Discussion

The results from this experiment suggest that the inhibitory effect of DOI on male rat sexual behaviour is due to its activity at 5-HT_2 receptors as RP62203, a drug which has a high affinity for 5-HT_2 receptors and a low affinity for 5-HT_{1C} receptors (Malgouris, et al., 1993), was able to block the effect of DOI. In addition, RP62203 had no effect on it's own, which is characteristic of selective 5-HT_2 antagonism.

EXPERIMENT 6A

This experiment was designed to examine the effect of SR46349B (SR) on the DOI-induced inhibition of male rat sexual behaviour. Pharmacological studies have shown that SR is selective for the 5-HT_2 receptor, having a lower affinity for the 5-HT_{1C} receptor and a very low affinity for other 5-HT_1, dopamine, adrenergic or histamine (H_1) receptors (Rinaldi-Carmona, et al., 1992). This pharmacological profile suggested that SR would be a useful tool in investigating the relative importance of 5-HT_2 or 5-HT_{1C} receptors in the DOI-induced inhibition of sexual responding.

Subjects

Ten Wistar male rats (Animal Care Facility, UBC) were two months old at the start of the experiment. Twelve 6 month old Long-Evans female rats were employed to elicit sexual behaviour from the males. Females were primed with exogenous hormones as described in the General Methods section. Males were housed 3-4 animals/cage, females were housed 5-6 animals/cage. Colonies were kept on a 12 hour reverse light cycle with lights off at 9 a.m., and maintained at a temperature of 21± 1°C.

Materials

Testing chambers, hormones and anesthetics were the same as those described in the General Methods section. (±)-DOI hydrochloride was purchased from Research

Biochemicals International, Natick, MA. SR was generously provided by Sanofi Recherche, France. DOI was dissolved in physiological saline to a dose of 1 mg/ml and injected IP in a volume of 1 ml/kg body weight. SR was suspended in a solution of 5% Tragacanth Gum (Tgum) to a dose of 0.4 mg/ml and injected IP in a volume of 1 ml/kg body weight.

Procedure

Prior to the beginning of the experiment, males were exposed to stimulus females in the testing chambers on at least three separate occasions. Baseline sexual behaviour was scored one week prior to commencement of testing such that males with low levels of sexual responding would not be included in the experiment. Sexual behaviour was scored as outlined in the General Methods section, and the number of ejaculations occurring within a treatment group (#E) was also recorded. Testing continued for four weeks, with one test being conducted per week. A repeated measures design was employed such that each animal received each treatment once during the experiment. The four treatment groups employed were: SR/DOI, SR/saline, Tgum/DOI, and Tgum/saline. The order of treatment exposure was randomized to avoid any order effects of drug exposure. Sixty minutes prior to testing, SR (0.4 mg/kg), or Tgum (1ml/kg) was injected IP into the test males. Thirty minutes prior to testing, DOI (1mg/kg), dissolved in saline, or saline (1 ml/kg) was injected IP. Testing was conducted during the middle phase of the dark cycle. Data were analyzed using Analysis of Variance (ANOVA), Tukey's pairwise comparisons and Cochran Q tests where appropriate.

Results

Copulatory behaviour scores are presented in Table 6. ANOVA's revealed significant group differences for ML [$F(3, 27)=17.83$, $p<.0001$], IL [$F(3,27)=29.01$, $p<.0001$], and EL [$F(3,27)=7.53$, $p<.001$]. No significant differences were obtained for

PEI [F(3,10)=2.15, NS]. Tukey's pairwise comparisons revealed that the Tgum/DOI group had significantly longer ML's and IL's than all the other groups ($p<.01$), while none of the other groups differed from each other (see Table 6). Tukey's tests performed on EL measures, while showing significant differences between the Tgum/DOI group and both the SR/saline and the Tgum/saline groups ($p<.01$), failed to reveal any differences between the Tgum/DOI and the SR/DOI groups. Further, Cochran Q analyses revealed significant group differences in the number of ejaculations displayed [Q(3)=17.25, $p<.001$]. Pairwise comparisons showed that DOI acted to significantly decrease the number of ejaculations relative to controls [Q(1)=81.0, $p<.001$], and SR administered alone had no effect on the number of ejaculations displayed [Q(1)=1.8, NS]. While the administration of SR acted to attenuate the inhibitory effect of DOI [SR/DOI vs. Tgum/DOI: Q(1)=4.0, $p<.05$], it did not restore the number of ejaculations to control levels [SR/DOI vs Tgum/Sal: Q(1)=5.0, $p<.05$].

Table 6. The effect of a selective 5-HT_2 antagonist (SR46349B) and a 5-$HT_{2/1C}$ agonist (DOI), alone and combined, on sexual responding in the male rat. Except for the number of animals ejaculating (#E), data are presented as means ± standard deviations. Sexual behaviours listed are ML=mount latency, IL=intromission latency, EL=ejaculation latency, and PEI=post-ejaculatory interval, definitions of these behavioural measures can be found in the General Methods section. [SR=0.4 mg/kg SR46349B, Saline=Sal, Tgum=5% Tragacanth gum].

Comparison between the effect of SR46349B and DOI (alone and in combination) on sexual behaviour in the male rat.

Behavioural Measure	SR/DOI	SR/Sal	Tgum/DOI	Tgum/Sal
ML	546.0 ± 865.4	14.5 ± 16.1	1429.3 ± 638.8	8.5 ± 4.5
IL	546.0 ± 865.4	19.0 ± 26.5	1631.8 ± 356.3	8.8 ± 4.5
EL	1290.0 ± 613.5	984.2 ± 510.2	1684.7 ± 364.6	795.8 ± 525.7
PEI	374.6 ± 30.3	368.6 ± 68.6	270.0 ± 0	391.6 ± 58.6
# E	5	8	1	10
n	10	10	10	10

Discussion

Overall, the results of this experiment complement those of Experiment 5, and suggest that the effect of DOI on sexual behaviour appears to be due to its activity at 5-HT_2, and not 5-HT_{1C}, receptors since the selective 5-HT_2 antagonist SR (Rinaldi-Carmona, et al., 1992) generally blocked the effect of DOI on sexual responding. SR 46349B has been shown to bind with a high affinity to 5-HT_2 receptors and with moderate affinity to 5-HT_{1C} receptors (Rinaldi-Carmona, et al., 1992). Like other 5-HT_2 antagonists, SR had no effect on sexual behaviour when administered alone. Although SR partially attenuated the inhibitory effects of DOI on ejaculation latency and the number of ejaculations, the fact that it did not completely block DOI's effects may have been due to an insufficient SR dose.

EXPERIMENT 6B

This experiment was conducted in order to determine whether the failure of SR46349B (SR) to completely block DOI's effect on ejaculation latency and the number of ejaculations per group in Experiment 6A was due to the relatively low dose of SR employed. It was hypothesized that a larger dose of SR would significantly block the inhibitory effect of DOI on sexual behaviour.

Subjects

Fifteen Long-Evans male rats (Charles River, Quebec) were eight months old at the start of the experiment. Twelve 6 month old Long-Evans female rats were employed to elicit sexual behaviour from the males. Females were primed with exogenous hormones as described in the General Methods section. Males were housed 3-4 animals/cage, females were housed 5-6 animals/cage. Colonies were kept on a 12 hour reverse light cycle with lights off at 9 a.m., and maintained at a temperature of 21±1^{o}C.

Materials

Testing chambers, hormones and anesthetics were the same as those described in the General Methods section. (±)-2,5-Dimethoxy-4-iodoamphetamine hydrobromide [(±)-DOI hydrochloride] was purchased from Research Biochemicals International, Natick, MA. SR 46349B was generously provided by Sanofi Recherche, France. DOI was dissolved in physiological saline to a dose of 1 mg/ml and injected IP in a volume of 1 ml/kg body weight. SR 46349B (SR) was suspended in a solution of 5% Tragacanth Gum (Tgum) to a dose of 1 mg/ml and injected in a volume of 2 ml/kg body weight (2 mg/kg dose).

Procedure

Prior to the beginning of the experiment, males were exposed to stimulus females in the testing chambers on at least three separate occasions. Baseline sexual behaviour was scored one week prior to commencement of testing such that males with low levels of sexual responding would not be included in the experiment. Sexual behaviour was scored as in the previous experiment. Testing continued for two weeks, with one test being conducted per week. A between-within design was employed such that animals were assigned to receive saline or DOI and all animals received SR or Tgum once during the experiment. The four treatment groups employed were: SR 46349B/DOI, SR 46349B/saline, 5% tragacanth gum/DOI, and 5% tragacanth gum/saline. The order of treatment exposure was randomized to avoid any order effects of drug exposure. Sixty minutes prior to testing, SR 46349B (2 mg/kg), or 5% tragacanth gum (2 ml/kg) was injected IP into the test males. Thirty minutes prior to testing, DOI (1mg/kg), dissolved in saline, or saline (1 ml/kg) was injected IP. Testing was conducted during the middle phase of the dark cycle. Data were analyzed using Analysis of Variance (ANOVA) and the Cochran Q test was employed to analyze data collected on the number of ejaculations (#E). Due to the between-within design

employed in this experiment, data were analyzed with between and within subjects ANOVA's, and simple effects ANOVA's to determine specific group differences.

Results

Copulatory behaviour means are presented in Table 7. Statistical analyses conducted on the data collected revealed a significant interaction between DOI and SR for ML [$F(1,13)=5.79$, $p<.05$], IL [$F(1,13)=7.76$, $p<.05$], and EL [$F(1,13)=5.93$, $p<.05$]. Simple effects ANOVA's revealed that the DOI group was significantly different from the SR/DOI group for ML [$F(1,13)=12.827$, $p<.01$], IL [$F(1,13)=17.730$, $p<.01$] and EL [$F(1,13)=11.067$, $p<.01$], however the SR/DOI group was not different from controls in any of these measures. Further, the DOI group had significantly longer ML's [$F(1,13)=17.73$, $p<.01$], IL's [$F(1,13)=27.85$, $p<.001$] and EL's [$F(1,13)=38.21$, $p<.0001$] than control group animals. SR had no significant effect on any sexual behaviour scored when given alone. A Cochran Q analysis on the number of ejaculations displayed revealed significant group differences [$Q(3)=10.2$, $p<.02$]. Pairwise comparisons revealed significant differences between the DOI group when compared to the control group [$Q(3)=5.0$, $p<.05$] and the SR/DOI group [$Q(3)=4.0$, $p<.05$]. None of the other groups were significantly different from one another on the number of ejaculations displayed (see Table 7).

Table 7. The effect of a selective 5-HT_2 antagonist (SR 46349B) and a 5-$HT_{2/1C}$ agonist (DOI), alone and combined, on sexual responding in the male rat. Except for the total number of animals ejaculating (#E), data are represented as means ± standard deviations. Sexual behaviours listed are ML=mount latency, IL=intromission latency, EL=ejaculation latency, and PEI=post-ejaculatory interval, definitions of these behavioural measures can be found in the General Methods section. [SR=2 mg/kg SR 46349B, Saline=Sal, Tgum=5% Tragacanth gum].

Comparison between the effect of SR 46349B and DOI (alone and in combination) on sexual behaviour in the male rat.

Behavioural Measure	SR/DOI	SR/Sal	Tgum/DOI	Tgum/Sal
ML	458.8 ± 827.9	7.1 ± 3.1	1351.5 ± 830.5	22.1 ± 40.7
IL	464.3 ± 824.6	8.0 ± 4.5	1435.6 ± 693.4	38.9 ± 74.8
EL	727.8 ± 733.6	215.0 ± 104.5	1495.6 ± 566.9	159.9 ± 53.0
PEI	358.8 ± 150.0	304.3 ± 58.0	393.5 ± 51.6	247.1 ± 73.0
#E	6	7	2	7
n	8	8	7	7

Discussion

In this experiment, DOI acted to significantly inhibit sexual responding on all measures, and SR was able to block this effect. Further, SR acted as a pure 5-HT_2 antagonist, having no observable effect on behaviour when administered alone. These results lend further support to the hypothesis that DOI's effect on sexual responding is due to it's influence at 5-HT_2 receptors as opposed to 5-HT_{1C} receptors. This experiment confirmed that the dose of SR employed in the previous experiment was insufficient to reverse the inhibitory effect of DOI. It should be noted that while there were no significant differences between the SR/DOI group and controls, Table 7 suggests that SR did not completely restore sexual responding to control levels. Although it is possible that a higher dose of SR would act to fully block the effect of DOI on sexual responding, the dose of SR selected (2 mg/kg) has been shown to occupy 95-100% of central 5-HT_2 sites (Rinaldi-Carmona, et al., 1992). This calls into question whether SR is a highly effective tool for determining the 5-HT_2 activity of compounds.

Discussion: Experiments 4-6B

The results of these experiments suggest that the effect of the 5-$HT_{2/1C}$ agonist, DOI, to inhibit sexual responding may be due to it's effect on 5-HT_2 receptors. Experiments 4-6B reveal that the selective 5-HT_2 antagonists employed were able to block the effect of DOI on male sexual responding. However, it appears that the relative effectiveness of these antagonists to block the DOI effect on sexual behaviour is not uniform. Both SR 46349B and amperozide appear to be less effective than RP 62203 in attenuating the DOI-induced inhibition of male rat sexual behaviour, perhaps reflecting a relative difference in 5-HT2 receptor occupation between these compounds. It should be noted that the antagonists employed, while being highly selective for 5-HT_2 receptors over other 5-HT receptors, have differential binding to other types of receptors in the brain which may influence sexual behaviour. Perhaps the additional

receptors bound by amperozide and SR 46349B act to inhibit sexual responding resulting in what appears to be a reduced effectiveness to antagonize DOI's effect.

5-HT2 AND FEEDING BEHAVIOUR

(EXPERIMENTS 7-9)

The purpose of Experiments 7-9 is to investigate the effect of DOI and a variety of selective 5-HT_2 antagonists (amperozide, RP 62203 & SR 46349B) on feeding behaviour in the male rat. Research has established that DOI inhibits feeding in males, however, as with sexual behaviour, the relative contribution of 5-HT_2 and 5-HT_{1C} receptors in feeding remains to be established.

A further purpose of Experiments 7-9 is to determine whether similar activation and blockade 5-HT_2 receptors elicits comparable effects on feeding to those established with male sexual behaviour in Experiments 4-6B. For example, does the same dose of DOI inhibit both sexual behaviour and feeding behaviour? Do the antagonists employed act to block the effect of DOI in a comparable manner? This information may help to determine whether or not feeding and sexual behaviour rely on the same level on 5-HT_2 activation.

Pilot data for this thesis revealed that the dose of DOI necessary to consistently inhibit feeding behaviour is higher than that necessary to inhibit sexual behaviour in the male rat. Due to these data, the following feeding behaviour experiments employ a 1.5 mg/kg dose of DOI rather than the 1 mg/kg dose used in the sexual behaviour experiments. It should be noted that this dose of DOI, while reliably inhibiting feeding, failed to influence the animal's overall motor activity. In addition, review of pharmacological data suggests that the doses of antagonists employed in the sexual behaviour experiments were sufficient to block most, if not all, 5-HT_2 receptors, therefore, these doses were employed in the following feeding behaviour experiments.

EXPERIMENT 7

This experiment was designed to investigate the effect of amperozide, a selective 5-HT_2 antagonist, to block the DOI-induced inhibition of feeding behaviour in the male rat. As mentioned previously, pharmacological studies have established that amperozide displays a high affinity for 5-HT_2 receptors in the rat brain, and a low affinity for 5-HT_{1C} receptors (Haskins, et al., 1987). Further, amperozide has been shown to act as a 5-HT_2 antagonist in sexual behaviour studies (Experiment 4; Klint, et al., 1992). Given these data, amperozide seems like an appropriate tool for determining whether DOI's effect on feeding behaviour is due to it's effect at 5-HT_2 or 5-HT_{1C} receptors. It was hypothesized that if DOI's inhibitory effect on feeding behaviour was due to it's effect at 5-HT_2 receptors, amperozide would act to block the effect of DOI while having no effect on feeding itself.

Subjects

Eighteen male Wistar rats (Animal Care Unit, UBC) were 5 months old at the beginning of the experiment.

Materials

The milk diet and drinking tubes employed are outlined in the General Methods: Feeding Behaviour section. DOI was purchased from Research Biochemicals International, Natick, MA. Amperozide hydrochloride was generously provided by Kabi-Pharmacia Therapeutics, Sweden. Both amperozide (2 mg/ml) and DOI (1.5 mg/ml) were dissolved in physiological saline and injected IP in a volume of 1 ml/kg.

Procedure

The experimental design and testing procedures are outlined in the General Methods section. The four treatment groups employed were: amperozide/DOI, amperozide/saline, saline/DOI, and saline/saline. Testing was conducted over a two week period, with one test per week. Animals were randomly assigned to receive either

DOI or saline on both test days, and all animals received amperozide on one test day, and no amperozide on the other. The order of treatment exposure was randomized to avoid any order effects of drug exposure. Forty minutes prior to testing, amperozide (2 mg/kg), dissolved in saline, or saline (1ml/kg) was injected intraperitoneally (IP) into the test males. Thirty minutes prior to testing, DOI (1.5 mg/kg), dissolved in saline, or saline (1 ml/kg) was injected IP. Animals were given a predetermined amount of milk diet at the beginning of the dark cycle (11 a.m.; 30 minutes after the DOI injection), and the amount consumed was measured 30 minutes later (11:30 a.m.). Data were analyzed using Analysis of Variance (ANOVA) for overall significance and simple effects analyses where appropriate.

Results

The amount of milk ingested for each treatment group is shown in Table 8. A between-within ANOVA revealed a significant effect of DOI on food consumption [$F(1,16)=7.75$, $p<.05$], a significant amperozide effect [$F(1,16)=39.26$, $p<.0001$], and a significant interaction between amperozide and DOI [$F(1,16)=16.24$, $p<.01$]. Simple effects analyses revealed that the saline/DOI group was significantly different from the saline/saline group [$F(1,16)=17.53$, $p<.001$], however the amperozide/DOI group was not significantly different from the saline/DOI group [$F(1,16)=2.50$, NS]. Inspection of Table 8 reveals that while DOI acted to significantly decrease feeding, amperozide failed to block this affect. Further, the amperozide/saline group ingested significantly less milk than saline treated animals [$F(1,16)=53.01$, $p<.0001$, Table 8], suggesting that amperozide has an inhibitory effect on feeding when administered alone.

Table 8. The effect of a selective 5-HT_2 antagonist (amperozide) and a 5-$HT_{2/1C}$ agonist (DOI), alone and combined, on feeding behaviour in the male rat. Data are presented as means ± standard deviations.

Comparison of the effect of DOI and Amperozide, alone and in combination, on feeding behaviour

Treatment Group	Amount of milk ingested (ml)	Number of animals tested
Amperozide/DOI	6.56 ± 4.33	9
Saline/DOI	10.22 ± 4.76	9
Amperozide/Saline	6.22 ± 6.00	9
Saline/Saline	23.11 ± 7.91	9

Discussion

This experiment revealed that while DOI acted to significantly inhibit feeding, amperozide failed to block this effect as would be predicted if DOI's effect on feeding was due to it's activity at 5-HT_2 receptors. Further, amperozide acted to significantly inhibit feeding when administered on it's own. These data challenge the classification of amperozide as a selective 5-HT_2 antagonist as one would expect such a compound, if it had any effect at all, to elevate feeding since activating 5-HT_2 receptors inhibits feeding. Therefore, it seems likely that amperozide is acting on some other receptor type to influence feeding. Indeed, as mentioned earlier, amperozide has a low affinity for 5-HT_{1C} receptors, a moderate affinity for α_1 receptors (Svartengren & Simonsson, 1990) and increases norepinephrine activity in the brain (Haskins, et al., 1987). Elevated central norepinephrine activity is associated with decreased food consumption (Booth, 1968; Grossman, 1962; Leibowitz, 1978; Leibowitz, Hammer & Chang, 1983). Therefore, the failure of amperozide to block the effect of DOI on feeding can be explained in several ways. Amperozide may be acting through a non-5-HT_2 mechanism. Alternatively, DOI may be acting through a 5-HT_{1C} rather than a 5-HT_2 mechanism. Additional experiments may differentiate between these alternatives.

EXPERIMENT 8

This experiment was designed to investigate the effect of RP62203 (RP) to block the DOI-induced inhibition of feeding behaviour. As discussed earlier, binding studies have revealed that RP has a high affinity for 5-HT_2 receptors in the rat brain, however, it also shows moderate affinity for α_1 receptors (Doble, et al., 1992). RP has a low affinity for dopamine (D_2) receptors, histamine (H_1) receptors (Doble, et al., 1992) and 5-HT_{1C} receptors (Malgouris, et al., 1993). The purpose of this study is to use RP and DOI to differentiate between 5-HT_2 and 5-HT_{1C} receptor involvement in feeding activity.

Subjects

Eighteen male Wistar rats (Animal Care Unit, UBC) were 5 months old at the beginning of the experiment.

Materials

The milk diet and drinking tubes employed are outlined in the General Methods section. DOI was purchased from Research Biochemicals International, Natick, MA. RP was generously provided by Rhône-Poulenc-Rorer, France. DOI was dissolved in physiological saline to a dose of 1.5 mg/ml and injected IP in a volume of 1 ml/kg body weight. RP was suspended in a 5% Tween 80 (T80) solution (2 mg RP /2 ml T80) and injected IP in a volume of 2 ml/kg body weight.

Procedure

The experimental design and testing procedures are outlined in the General Methods section. The four treatment groups employed were: RP/DOI, RP/saline, T80/DOI, and T80/saline. Testing was conducted over a two week period, with one test per week. Animals were randomly assigned to receive either DOI or saline on both test days, all animals received RP on one test day, and T80 on the other. The order of treatment exposure was randomized to avoid any order effects of drug exposure. Ninety minutes prior to testing, RP (2 mg/kg), or T80 (2ml/kg) were injected IP into the test males. Thirty minutes prior to testing, DOI (1.5 mg/kg), or saline (1 ml/kg) were injected IP. At the beginning of the testing session (11 a.m.; 30 minutes after the DOI injection), animals were given a predetermined amount of milk diet. Testing ended when the animals had been exposed to the diet for 30 minutes (11:30 a.m.) and the amount consumed was recorded at this time. Data were analyzed using Analysis of Variance (ANOVA) for overall significance and simple effects analyses where appropriate.

Results

The amount of milk ingested for each treatment group is shown in Table 9. A between-within ANOVA revealed a significant effect of DOI on food consumption [$F(1,16)=14.84$, $p<.01$], and a significant RP effect [$F(1,16)=4.88$, $p<.05$]. However, a significant interaction between RP and DOI was not established [$F(1,16)=0.96$, NS]. Simple effects analyses revealed that the saline/DOI group was significantly different from the saline/saline group [$F(1,16)=11.30$, $p<.01$], and the RP/DOI group was significantly different from the saline/DOI group [$F(1,16)=5.08$, $p<.05$]. Further, while the RP/saline group was not significantly different from the T80/saline group [$F(1,16)=0.76$, NS], it appears that RP didn't fully block the effect of DOI on feeding (see Table 9; RP/DOI versus T80/DOI and T80/sal) which may be the source of the significant RP effect.

Table 9. The effect of a selective 5-HT_2 antagonist (RP62203) and a 5-$HT_{2/1C}$ agonist (DOI), alone and combined, on feeding behaviour in the male rat. Data are presented as means ± standard deviations.

Comparison of the effect of DOI and RP62203, alone and in combination, on feeding behaviour

Treatment Group	Amount of milk ingested (ml)	Number of animals tested
RP/DOI	13.11 ± 5.44	9
T80/DOI	8.22 ± 4.68	9
RP/Saline	17.89 ± 3.52	9
T80/Saline	16.00 ± 5.12	9

Discussion

This study revealed that RP62203 blocked the inhibitory effect of DOI on feeding while having no effect on its own. These results support the hypothesis that DOI acts to inhibit feeding via its effect at 5-HT_2 receptors as opposed to 5-HT_{1C} receptors since RP62203 is a selective 5-HT_2 antagonist with low affinity for 5-HT_{1C} sites.

EXPERIMENT 9

This experiment was designed to examine the effect of SR 46349B (SR) on DOI's inhibitory effect on feeding behaviour in the male rat. Pharmacological studies have shown that SR is selective for the 5-HT_2 receptor, having a lower affinity for the 5-HT_{1C} receptor and a very low affinity for other 5-HT_1, dopamine, adrenergic or histamine (H_1) receptors (Rinaldi-Carmona, et al., 1992). The purpose of this study is to use SR and DOI to differentiate between 5-HT_2 and 5-HT_{1C} receptor involvement in feeding activity.

Subjects

Twenty male Wistar rats (Animal Care Unit, UBC) were 5 months old at the beginning of the experiment.

Materials

The milk diet and drinking tubes employed are outlined in the General Methods section. DOI was purchased from Research Biochemicals International, Natick, MA. SR was generously provided by Sanofi Recherche, France. DOI was dissolved in physiological saline to a dose of 1.5 mg/ml and injected IP in a volume of 1 ml/kg body weight. SR was suspended in a 5% Tragacanth gum (Tgum) solution (2 mg SR/2 ml Tgum) and injected IP in a volume of 2 ml/kg body weight.

Procedure

The experimental design and testing procedures are outlined in the General Methods section. The four treatment groups employed were: SR/DOI, SR/saline, Tgum/DOI, and Tgum/saline. Testing was conducted over a two week period, with one test per week. Animals were randomly assigned to receive either DOI or saline treatment on both test days, all animals received SR on one test day, and Tgum on the other. The order of treatment exposure was randomized to avoid any order effects of drug exposure. Sixty minutes prior to testing, SR (2 mg/kg), or Tgum (2ml/kg) was injected IP into the test males. Thirty minutes prior to testing, DOI (1.5 mg/kg), or saline (1 ml/kg) was injected IP. At the beginning of the testing session (11 a.m.; 30 minutes after the DOI injection), animals were given a predetermined amount of milk diet. The amount of diet consumed was measured 30 minutes later, at the end of the test session (11:30 a.m.). Data were analyzed using Analysis of Variance (ANOVA) for overall significance and simple effects analyses where appropriate.

Results

The amount of milk ingested for each treatment group is shown in Table 10. A between-within ANOVA revealed a significant effect of DOI on food consumption [$F(1,17)=113.08$, $p<.01$], while SR failed to influence feeding [$F(1,17)=0.27$, NS]. Further, a significant interaction between SR and DOI was not established [$F(1,17)=0.30$, NS]. Simple effects analyses revealed that the Tgum/DOI group consumed significantly less than the Tgum/saline group [$F(1,17)= 9.72$, $p<.01$], however, the SR/DOI group was not significantly different from the Tgum/DOI group [$F(1,17)=2.60$, NS]. Further, the SR/saline group consumed significantly more than the SR/DOI group [$F(1,17)=7.39$, $p<.05$] but did not differ from the Tgum/saline group [$F(1,17)=0.003$, NS].

Table 10. The effect of a selective 5-HT_2 antagonist (SR46349B) and a 5-$HT_{2/1C}$ agonist (DOI), alone and combined, on feeding behaviour in the male rat. Data are presented as means ± standard deviations.

Comparison of the effect of DOI and SR46349B, alone and in combination, on feeding behaviour

Treatment Group	Amount of milk ingested (ml)	Number of animals tested
SR/DOI	12.70 ± 4.67	10
Tgum/DOI	9.30 ± 5.21	10
SR/Saline	18.60 ± 2.84	10
Tgum/Saline	17.78 ± 7.79	9

Discussion

The results of this study suggest that while DOI acts to inhibit feeding, SR46349B failed to block this affect. These results suggest that the effect of DOI on feeding behaviour may be due to its influence at receptors other than 5-HT_2.

Discussion: Experiments 7-9

The results of these experiments suggest that the effect of DOI to inhibit feeding may involve it's activity at 5-HT_{1C} receptors. The 5-HT_2 antagonists employed in Experiments 7-9 were unable to reliably block DOI's effect on feeding behaviour. While 5-HT_2 receptor activity may have some role in the DOI-induced inhibition of feeding behaviour, the influence of DOI on 5-HT_{1C} receptors may be more important for this effect. As in the sexual behaviour experiments, the effectiveness of the antagonists to block DOI's effect was not uniform. Indeed, RP 62203 was the only antagonist to significantly attenuate the DOI effect. SR 46349B decreased the effect of DOI, however not significantly, and amperozide, not only failed to block the DOI-induced inhibition of feeding, it decreased feeding when administered alone, an effect inconsistent with 5-HT_2 antagonism. It may be that the inability of SR 46349B and amperozide to attenuate the DOI-induced inhibiton of feeding behaviour is due to the activity of these compounds at receptors other than 5-HT_2.

GENERAL DISCUSSION

The present experiments were an investigation of the relationship between feeding, sexual activity and serotonergic mechanisms in the male rat. Serotonin (5-HT) has been shown to be important in both feeding (Samanin & Garattini, 1989) and sexual behaviour (Gorzalka, et al., 1990). However, the role of 5-HT and 5-HT_2 subtypes in the relationship between these two behaviours has never before been investigated. Three different experimental approaches were employed in order to determine a possible relationship between sexual responding and feeding, and whether

5-HT plays a role in this relationship. In Experiment 1, natural observation of feeding, sexual responding and WDS (recall that WDS is a behavioural indicator of 5-HT_2 receptor activation) in male rats was conducted. This experiment was designed to determine whether level of sexual responding and feeding are naturally related in male rats. Further, through the observation of WDS, the results of Experiment 1 were intended to determine whether 5-HT_2 receptor activation is involved in the relationship between sexual behaviour and feeding. Experiments 2 and 3 employed test diets that have been shown to modify brain 5-HT levels. Sexual responding and WDS's were scored after exposure to these diets in order to determine whether modifications in brain 5-HT due to variation in nutrient components had an effect on sexual behaviour and WDS. Experiments 4 through 9 employed pharmacological tools to determine the importance of 5-HT_2 (and to some extent 5-HT_{1C}) receptors in sexual behaviour and feeding in the male rat, since pharmacological tools to date have been unable to differentiate between 5-HT_2 and 5-HT_{1C} receptors. The results of the three sets of experiments are reviewed and discussed separately in the three following sections of the Discussion. The fourth section of the Discussion is an integration of the findings from Experiments 4-6B and Experiments 7-9. The last section of the Discussion provides an integration of the results of all of the experiments and makes some general conclusions as to the relation between sexual activity and feeding in the male rat, the importance of 5-HT_2 activation in each of these behaviours and generalizability to humans. Implications for future research are also considered.

1. General Discussion: Experiments 1-3

These studies suggest that while there may be some natural relationship between sexual behaviour and feeding behaviour (Experiment 1) which can be explained through serotonergic mechanisms, diet manipulations that elevate brain 5-HT in sexually active rats fail to influence sexual responding (Experiments 2 & 3). Experiment 1 revealed that duds (males that failed to ejaculate) ate less than studs

(males that ejaculated). These results are consistent with the hypothesis that duds have a higher level of natural 5-HT which acts to inhibit sexual responding and feeding. Another potential explanation for the results of Experiment 1 is that animals who engage in copulatory behaviour expend more energy than those who don't, which would act to increase their food consumption. However, these animals were only exposed to females once per week, and, while the duds did not ejaculate, they did engage in copulatory activity, investigated the female and moved around the chamber quite vigourously. Experiment 1 also revealed that duds displayed more WDS during sexual behaviour testing than studs, suggesting that duds have a higher level of 5-HT_2 activity than studs. It should be noted that few WDS were displayed in either group during feeding tests. It may be that 5-HT_2 activity is higher in situations where animals engage in sexual behaviour than when in a feeding situation, or that WDS are only elicited in a sexual behaviour situation. Indeed, previous research in our laboratory (Watson & Gorzalka, 1990) suggests that the differential display of WDS in duds and studs is selective to situations where copulation is likely (i.e., pairing with a non-receptive female or another male failed to produce a difference in WDS between groups).

Experiments 2 and 3 involved exposure of sexually active male rats to two different diets, one with elevated brain 5-HT (CHO) and another with no apparent effect on brain 5-HT levels (protein/CHO). These experiments were conducted in order to determine whether diet modifications that act to increase brain 5-HT would modify sexual responding. While no differences were found between diet groups, a positive correlation was established between amount of CHO diet consumed and IL in Experiment 2 . This correlation supports the hypothesis that higher brain 5-HT levels (due to increased CHO consumption) result in decreased sexual responding. However, such a relationship was not obtained in Experiment 3, therefore this result must be considered tentative and requires replication. In both Experiments 2 and 3, animals in the CHO group ingested significantly less than animals in the CHO/protein group,

suggesting that brain 5-HT may have been elevated enough to inhibit feeding in the CHO animals, but not enough to modify sexual responding. It is possible that the effect of increasing brain 5-HT to inhibit further CHO consumption occurs before 5-HT levels have increased sufficiently to influence sexual responding. Further experiments could employ a drug that inhibits 5-HT metabolism in the synapse (e.g., carbidopa) in conjunction with a CHO meal, such that 5-HT levels remain elevated for a longer period of time, resulting in a larger 5-HT effect due to CHO. It is possible that such a design will reveal an influence of CHO on sexual responding in the male rat.

Collectively, while it may be that the difference between the ingestion behaviour of "studs" and "duds" is due to differences in serotonergic activity (studs consuming more than duds due to lower natural brain 5-HT levels), an elevation in brain 5-HT in studs through acute diet modifications, while influencing diet consumption, is generally ineffective in influencing sexual responding. Therefore, it appears that a high carbohydrate meal is able to elevate brain 5-HT enough to inhibit feeding but not enough to influence sexual responding. It may be that the feeding regulation system in the brain is more sensitive to 5-HT level changes than the system that modifies sexual responding in the male rat. On the other hand, Experiment 1 revealed that there may be a natural difference in 5-HT_2 activity in studs versus duds due to the difference in the amount of WDS displayed (duds greater than studs). It is possible that the lack of effect of diet modification on sexual responding in Experiments 2 and 3 is due to insufficient activation of 5-HT_2 receptors. It may be that it is not the level of brain 5-HT that differentiates studs from duds, but the amount of activity at 5-HT_2 receptor sites. The final experiments of this thesis investigated the effects of 5-HT_2 activation on sexual behaviour and feeding in the male rat.

2. General Discussion: Experiments 4-6B

In all four experiments, the 5-$HT_{2/1C}$ agonist, DOI (1 mg/kg), consistently inhibited sexual responding in the male rat, while the selective 5-HT_2 antagonists

employed generally blocked this effect. Further, all of the antagonists generally failed to influence sexual behaviour when administered alone, suggesting that these antagonists did not have any intrinsic activity when binding to 5-HT_2 receptors.

In Experiment 4, amperozide acted to block the inhibitory effect of DOI on ML, IL, and EL, however, the amperozide/DOI group showed a longer PEI than the control group. Neither amperozide nor DOI alone significantly affected PEI, suggesting that it is the interaction between amperozide and DOI that is important for this effect. Further, while amperozide failed to influence PEI significantly, it produced a nonsignificant trend to elevate PEI. Further support for this conclusion come from data obtained by Klint, et al. (1992). These researchers showed that amperozide has effects of its own to lengthen PEI. Recall that amperozide shows moderate affinity for α_1 receptors and increases norepinephrine (NE) activity in the brain (Haskins, et al., 1987), and peripheral NE acts to inhibit sexual responding in the male rat (Malmnas, 1973). It is possible that amperozide influences sexual responding through adrenergic activity. These results suggest that amperozide may not be a useful tool for investigating 5-HT_2 selective behaviours.

Experiment 5 was designed to investigate the effect of the selective 5-HT_2 antagonist, RP62203 on the DOI-induced inhibition of sexual behaviour. Pharmacological data have shown that RP62203 binds with high affinity to 5-HT_2 receptors, and with moderate affinity to α_1 receptors (Doble, et al., 1992). This experiment revealed that while RP62203 acted to significantly block the effect of DOI on sexual responding, it had no effect on its own. The fact that RP62203 also binds to α_1 receptors allows for the cautious speculation that amperozide's effect on PEI is not due to it's influence at α_1 receptors.

Experiments 6A and 6B were designed to investigate the effect of SR46349B on the DOI-induced inhibition of sexual responding. Experiment 6A employed a low dose of SR46349B (0.4 mg/kg), which was not sufficient to block the effect of DOI on sexual behaviour. However, the 2 mg/kg dose of SR46349B employed in Experiment 6B did

block the DOI-induced inhibition of sexual behaviour. It is interesting to note that the 0.4 mg/kg dose of SR46349B employed in Experiment 6A has been shown to occupy approximately 85-90% of 5-HT_2 sites in the brain while 2 mg/kg occupies 95-100% of the 5-HT_2 sites (Rinaldi-Carmona, et al., 1992), suggesting that only a small number of 5-HT_2 receptors need to be activated by DOI in order to inhibit sexual responding in the male rat. Such a slight difference in receptor occupancy of the two doses of SR46349B employed in these two experiments and the large difference observed in the ability of the two doses to block DOI's effect suggest that the 5-HT_2 receptor may be of prime importance in male rat sexual behaviour.

Overall, the results of these experiments lend further support to the hypothesis that 5-HT_2 receptors are of prime importance in male rat copulatory behaviour, and the DOI-induced inhibition of sexual responding is due to it's activity at 5-HT_2, not 5-HT_{1C}, receptors.

3. General Discussion: Experiments 7-9

The 5-$HT_{2/1C}$ agonist, DOI (1.5 mg/kg), consistently inhibited feeding in the male rat, however, the 5-HT_2 antagonists employed failed to completely block this effect. In Experiment 7, amperozide not only failed to block the effect of DOI on feeding, but it acted to inhibit feeding when administered alone, an effect inconsistent with 5-HT_2 antagonism. The 5-HT_2 antagonist, RP62203, employed in Experiment 8 attenuated the effect of DOI on feeding in the male rat, but failed to bring food consumption back to control levels. In Experiment 9, SR46349B attenuated the effect of DOI on feeding, but not significantly. These results allow for the speculation that 5-HT_{1C} receptors are involved in DOI's effect on feeding. Indeed, Gibson, Kennedy and Curzon (1993) found that 5-HT_{1C} receptors are more important than 5-HT_2 receptors in d-norfenfluramine's (the major metabolite of the anorectic, fenfluramine) anorectic effect on feeding, and chronic treatment with pCPA (5-HT depletor, leading to

hypersensitivity of 5-HT receptors) elevated the anorectic effect of mCPP (a 5-HT_{1C} agonist) and d-norfenfluramine.

Comparisons of the antagonists used in all three experiments, indicate that RP62203 and SR46349B had no effect on feeding alone, while amperozide acted to inhibit feeding. It is possible that amperozide's effect on feeding may be due to its influence on nonserotonergic receptors. Pharmacological data show that amperozide influences the adrenergic system (Haskins, et al., 1987; Svartergren & Simonsson, 1990), and this system is known to be of prime importance in feeding regulation (see Raible, in press). It may be that amperozide's influence on the adrenergic system is more important than its effect on 5-HT_2 receptors. Although amperozide has been classified as a selective 5-HT_2 antagonist (Experiment 4; Klint, et al., 1992), our feeding experiments suggest that this classification is not accurate, as amperozide has its own effect on feeding and in a mannner not consistent with 5-HT_2 antagonism.

It is important that the above results be considered in the context of the diet regime employed. Experimental animals were put on a diet of sweetened condensed milk for a total of 17 days for each experiment. This diet is quite different from the normal laboratory rat diet of dry pellets. In particular, the milk diet is high in CHO (approximately 44%, Western Families, personal communication), while pellets are approximately 20% CHO. Since different nutrient ratios have been shown to modify brain tryptophan and 5-HT levels (Fernstrom & Wurtman, 1972), it is possible that chronic exposure to the milk diet affected the animals' in some way such that their 5-HT and/or 5-HT receptor levels were no longer normal. It is interesting to note that this is the diet employed by Schechter & Simansky (1988) who were able to establish a DOI effect to inhibit feeding at a dose of 1 mg/kg. Indeed, experiments employing more "normal" diet regimes have used considerably higher doses of DOI (e.g., 2 mg/kg DOI, Hewson, et al., 1989; 2.86 mg/kg, Lawton & Blundell, 1993b). These facts seem to magnify the conclusion that 5-HT_2 receptors do not appear to play a large role in feeding behaviour in the male rat.

4. General Discussion: Integration of Experiments 4-6B & 7-9

Comparison between sexual behaviour (Experiments 4-6B) and feeding behaviour experiments (Experiments 7-9) suggest that 5-HT_2 receptor activity may not be as important in feeding as in sexual behaviour. The dose of DOI necessary to consistently inhibit feeding is somewhat higher (1.5 mg/kg) than that required to inhibit sexual responding (1 mg/kg). As well, most other studies investigating the effect of DOI on feeding have employed doses at least as high as that utilized in our experiments (Hewson, et al., 1989; Lawton & Blundell, 1993a; Lawton & Blundell, 1993b). However, some studies found an effect of DOI to inhibit feeding at a lower dose (Lawton & Blundell, 1993a; Schechter & Simansky, 1988), but, this was only under very specific conditions. For example, Schechter and Simansky (1988) injected DOI 6 minutes prior to diet exposure. Indeed, pilot studies in our laboratory found an inhibitory effect of 1 mg/kg DOI if injected 6 minutes prior to diet exposure. However, both control and drugged animals ingested much less than animals that were allowed to rest for 30 minutes between injections and diet exposure. It is possible that after only 6 minutes, animals are sufficiently influenced by injection stress that their feeding is inhibited, resulting in a greater magnification of the difference between drug treatment groups. Lawton and Blundell (1993a) were able to find an effect of DOI to decrease CHO (polycose, a tasteless CHO) and regular rat chow consumption when polycose was presented in dry form and rat chow was mixed with water. These researchers state that the DOI induced inhibition of diet intake appears to be manifest only under very specific experimental conditions (Lawton & Blundell, 1993a).

Further comparison of the results from the sexual and feeding behaviour experiments reveals that while the all antagonists blocked the effect of DOI on sexual responding, they failed to do so with feeding behaviour, lending further support for the conclusion that 5-HT_2 receptors are more important in sexual behaviour than in feeding. This statement must be qualified with the comment that the dose of

antagonists employed was kept constant across feeding and sexual behaviour experiments, while the dose of DOI was not. It may be that the antagonist dose was not sufficient to block the effect of the higher dose of DOI employed in the feeding experiments. However, pharmacological data show that the antagonist doses selected bind to 95-100% of the 5-HT_2 receptors for an extended period of time. Further research with higher doses of antagonists is necessary to determine whether the DOI effect on feeding can be blocked by selective 5-HT_2 antagonists.

Given the limitations of these data, I would like to continue with the tentative conclusion that 5-HT_2 activation is less important in feeding regulation than in sexual responding in the male rat. Research has shown that 5-HT exerts a tonic modulatory influence on its targets (Jacobs & Azmitia, 1992), and 5-HT acts to inhibit norepinephrine (NE) release (e.g., Leibowitz & Papdakos, 1978; Weiss, et al., 1986). Central NE has been shown to increase feeding in rats (e.g., Leibowitz & Papadakos, 1978; de Rooy & Coscina, 1990; Weiss, et al., 1986). Perhaps it is 5-HT's inhibitory influence on NE release that is important in the 5-HT inhibition of feeding behaviour, while binding to serotonergic postsynaptic receptors is less important. Indeed, a variety of researchers have discussed the idea that 5-HT and NE act in an antagonistic fashion to influence feeding behaviour (e.g., Leibowitz & Papadakos, 1978; de Rooy & Coscina, 1990; Weiss, et al., 1986). Perhaps this antagonism is due to 5-HT's modulatory influence on NE and not due to its own activity, in isolation from the NE system. Some support for this hypothesis comes from the experiments conducted with amperozide. Amperozide failed to block the effect of DOI on feeding behaviour at a dose which was sufficient to influence sexual responding (Experiments 4 & 7) and amperozide inhibited feeding when administered on its own. This effect is not consistent with 5-HT_2 antagonism, but can be explained through amperozide's effect to increase NE activity. Elevated peripheral NE acts to decrease feeding (opposite to NE's central effect), but has less clear effects on sexual behaviour. It may be that amperozide's influence on

the adrenergic system dominated its effect on feeding, while having little effect on sexual responding.

This thesis provides the first evidence that 5-HT_2 activation does not act to inhibit motivated behaviour in general. In so far as feeding and sexual behaviour may be considered motivated behaviours, the fact that the same dose of DOI failed to inhibit both behaviours suggests that activation of 5-HT_2 receptors does not act to similarly inhibit any motivated behaviour. Instead, it appears that, while 5-HT_2 receptors may be inhibitory in nature, their relative importance in behavioural inhibition seems to vary from one behaviour to another.

5. Conclusions, speculations, and implications for future research

The main objective of this thesis was to determine whether there is a relationship between feeding and sexual behaviour in the male rat through a serotonergic mechanism, and whether 5-HT_2 receptors are important in this relationship. Results obtained in Experiment 1 suggest that there is a natural relationship between feeding and sexual behaviour, however diet modification (Experiments 2 and 3) and pharmacological studies (Experiments 4-9) failed to reconstruct this relationship. It may be that the difference between duds and studs in their feeding (Experiment 1) involves systems that were not manipulated in the other experiments of this thesis. Perhaps there are differences in hormone (and/or receptor) levels between animals that differ in their sexual activity; the 5-HT or NE systems in each group of animals may respond differently to food presentation; and there could well be metabolism differences. There are likely to be many factors that cause duds and studs to be different and, while differences in the serotonergic system may be one such factor, manipulating this system alone may not be sufficient to modify feeding behaviour in sexually active rats (Experiments 2 and 3).

Beyond the dud/stud phenomenon, there may still be some serotonergic link between feeding and sexual behaviour in the male rat. However, the paradigms

employed in this thesis may not have been appropriate for revealing this relationship. For example, perhaps a 5-HT-mediated feeding/sexual behaviour relationship cannot be revealed in experiments that influence the short-term regulation of food intake only. Indeed, Raible (in press) discusses three different systems of energy regulation: emergency, short-term, and long-term. It appears that each system involves a different combination of biological factors (Riable, in press). It may be that 5-HT is more important in one mechanism than the others. This thesis, and the majority of feeding research to date, have investigated the short-term regulation of feeding only. Perhaps the feeding/sexual behaviour relation is important in long-term feeding regulatory mechanisms, an idea consistent with the dud/stud difference in food intake.

It is not uncommon for neurotransmitter systems to interact with one another, and the relation between 5-HT and NE appears to be an important consideration when investigating the effect of 5-HT on feeding. For example, research has shown that exogenous NE has clear effects on feeding and 5-HT acts to attenuate this effect (Leibowitz, et al., 1988; Weiss, et al., 1986). However, the importance of NE in sexual responding is unclear, while 5-HT appears to be of considerable importance. I would like to suggest that while 5-HT seems to play a role in both feeding and sexual behaviour in the male rat, it's relative importance in the control of each of these behaviours is different. Further, while 5-HT seems to have effects on sexual behaviour due, at least in part, to its effect at 5-HT postsynaptic receptors, 5-HT's influence on feeding may be due to its interaction with the adrenergic system. It is possible that 5-HT is the primary neurotransmitter for regulating sexual behaviour and NE modulates this effect, while NE is the primary neurotransmitter regulating feeding and 5-HT is modulatory. Further research on the relationship between these neurotransmitters in sexual responding and feeding is warranted in order to determine whether such receptor interactions are important in food intake and copulation and whether the primary neurotransmitters are reversed between these two behaviours.

This dissertation suggests that while 5-HT_2 receptors appear to be of significant importance in the display of sexual behaviour, they are of less importance in feeding behaviour. In Experiment 1, WDS's (indicating 5-HT_2 activation) were only displayed in sexual behaviour tests which further emphasizes the greater importance of 5-HT_2 in sexual as opposed to feeding behaviour. Feeding and sexual behaviour experiments employing DOI (a 5-HT_2 agonist) revealed that a higher dose of DOI was necessary to inhibit feeding than was required to inhibit sexual behaviour, suggesting that sexual behaviour is more sensitive to 5-HT_2 activation than feeding behaviour. Further, the selective 5-HT_2 antagonists employed were able to block the effect of DOI on sexual responding, while having a weaker effect on feeding. These results suggest that DOI's activity at 5-HT_2 receptors is dominant for sexual behaviour, while it's effect on feeding may be due to 5-HT_{1C} activity. It is possible, however, that future research will determine that the antagonists used in these experiments are not as selective to 5-HT_2 receptors as presently believed. Indeed, our data suggest that amperozide is not a highly selective 5-HT_2 antagonist (Experiment 7). Also, as research on serotonergic receptor subtypes continues, more and more 5-HT receptor subtypes are being discovered. For example, 5-HT_{2A} and 5-HT_{2B} receptors have been labelled (McKenna & Peroutka, 1989), and it is not known to what extent the 5-HT_2 antagonists employed in this thesis bind to each of these "subsubtypes", or to what extent each subsubtype is involved in 5-HT_2 mediated behaviours.

It remains to be determined whether the data obtained in these experiments have implications for a relationship between sexual behaviour and food ingestion in the human. The fact that 5-HT agonists (e.g., fenfluramine) that have been used to treat obesity also decrease sexual desire (Pinder, Brogden, Sawyer, Speight & Avery, 1979) suggests that there is some serotonergic link between feeding and sexual behaviour in humans. However, research on human eating behaviour has revealed some inconsistencies with animal data. For example, research investigating CHO intake and it's effects on later food intake show that CHO ingestion in humans acts to increase the

amount of food eaten during the next meal, particularly elevating protein consumption (Huon & Wootton, 1991). While the increased appetite for protein does fit with animal data, the general increase in appetite does not, suggesting other factors are involved in human food consumption that are not important in the rat. Further, the CHO inhibition of further CHO consumption that is displayed in rats is not seen in humans, as CHO consumption after a CHO meal or a protein/CHO meal are not different (Huon & Wootton, 1991). These data are confounding in light of the large body of evidence showing the effect of CHO on brain typtophan and 5-HT results in an inhibition of CHO and food intake in the rat (Fernstrom & Wurtman, 1971; 1972; Garatinni, et al., 1986). There could be important biochemical differences between the rat and human. Furthermore, there are social factors that have effects on human behaviour that are of minimal importance in the rat.

As stated in the introduction, communication between systems regulating energy intake and copulatory activity is necessary for the survival of the individual, as well as the species. However, the extent to which this relationship can be illuminated remains questionable. Perhaps such a relation is only strongly evident in situations of extreme deprivation, so that in order for the individual to survive, copulatory behaviour must be sacrificed. This dissertation investigated some of the neurochemical mechanisms that the system may use to communicate the level of food consumption to brain regions regulating copulaton. It appears that 5-HT may play some role in this relation, however, the extent to which 5-HT is involved still requires some clarification. It appears that other neurotransmitters, particularly NE, may interact with 5-HT to relate food intake information to brain areas regulating mating, and vice versa. Further, the mechanism that relays information to sexual behaviour and feeding areas in the brain may use at least some of the same neurotransmitters, however the relative importance of each may not be the same for both behaviours. While these are only speculations at this point, future research in this area may help to clarify the role of 5-HT in the relation between feeding and sexual behaviour in the male rat.

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