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SEROTONIN MEDIATED EFFECTS ON FOOD INTAKE, FEEDING BODY WEIGHT AND SUBJECTIVE VARIABLES

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PAGINATION ERRORS

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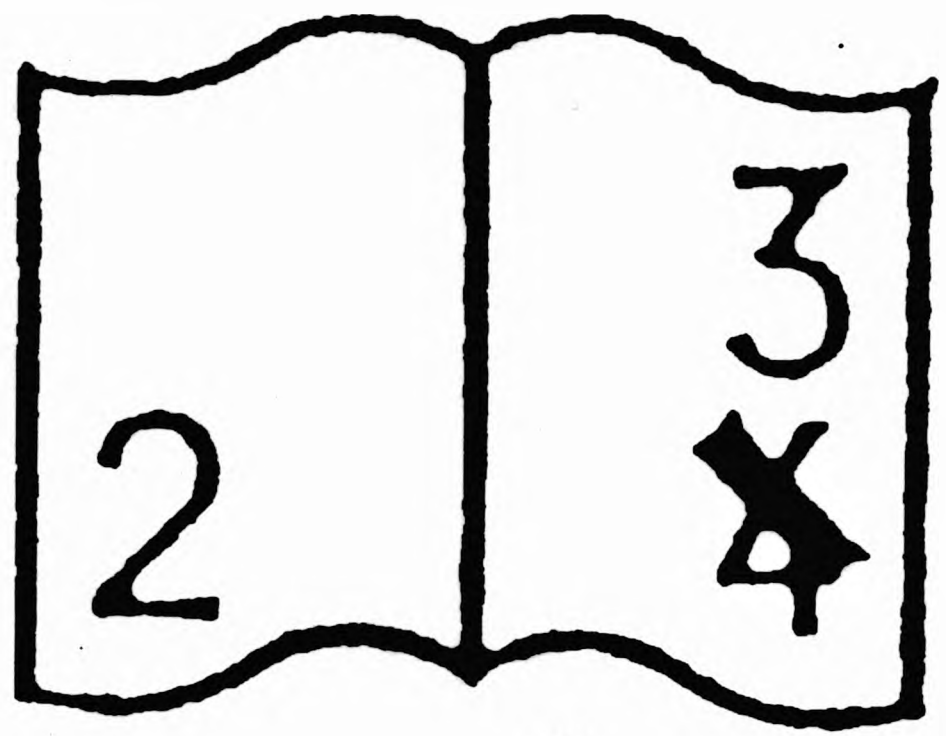

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ABSTRACT

The motivation for the main body of work presented in this thesis arose from three sources. Initially, the need to develop and test a reliable, portable and unobtrusive method of observing human feeding behaviour in a clinical setting. Secondly, curiosity concerning the serotonergic modulation of feeding. In practice, to examine the effects of the 5-HT reuptake inhibitor and anti-depressant fluoxetine on food intake and choice, body weight and subjective states in both non-depressed normal-weight and obese subjects. The third and final impetus arose from the observation that, in rats, the expression of meal-induced satiety offered a background against which to examine the possible mechanisms underlying the effects of acute and chronic administration of serotonergic agents on consummatory and behavioural functioning. Chapter context and main results are summarized below.

The observation methodology (Chapter 2), was devèloped and tested in a clinical (ward) and laboratory setting with both patient and volunteer populations and was proved feasible, reliable and acceptable to the different subject populations. An additional pharmacological study on food intake and feeding behaviour in normal volunteers tested the sensitivity of the methodology. D-amphetamine, and the isomers d- and l-fenfluramine were tested either alone or in combination, differences in the feeding behavioural profiles of d-amphetamine and d-fenfluramine were found.

The main findings from Chapter 2 were:

i) Observer reliability was substantiated relative to pilot work.

ii) D-amphetamine decreased meal duration, number of bites, and time spent in chewing and manipulation of food without reductions in eating rate. D-fenfluramine reduced eating rate, but not other parameters. L-fenfluramine did not alter feeding behaviour. Video-recording was used in all but the ward-based sessions.

An automated food dispenser (AFD), rating scales and measurements of physiological status were used to examine treatment effects in a normal-weight (Chapter 3) and obese (Chapter 4) populations in two fluoxetine (FXT) studies. Normal weight subjects received placebo controlled treatment for two weeks, obese subjects for four weeks. Obese subjects were offered foods varying in both macronutrient content and sweet and savoury components. In addition, obese subjects kept diary records of intake when not at the experimental unit. Video-recordings and behavioural observations were made of obese subjects during lunch meals. The main finding of Chapter 3 were:

i) Fluoxetine (FXT) reduced food intake and body weight in both subject groups.

ii) In obese subjects, FXT reduced selection of all three macronutrients. Diet diary records supported the laboratory-based intake suppressive effects of FXT.

iii) Evidence of some suppression of hunger and increases in ratings

of satiety was present in both subject groups. In obese subjects, FXT reduced eating rates on some days, but did not alter other feeding parameters, such as duration and latency.

In the acute animal study in Chapter 5, the comparable effects of five (hypophagic) serotonergic agents were examined via forty minute observation sessions, with sucrose intake and post-prandial behaviours acting as dependent variables. A summary of results from Chapter 5 are as follows:

i) FXT caused a dose-dependent reduction in sucrose intake with a concomitant early introduction of post-prandial resting. Acute fenfluramine reduced sucrose intake but appeared to suppress post-prandial resting.

ii) While the effects of femoxetine, paroxetine and eltoprazine differered somewhat to each other, reductions in sucrose intake and early introduction of post-prandial resting were present, lending support for serotonergic involvement in the mediation of satiety.

The animal study in Chapter 6 examined and compared the effects of a chronic three-week treatment regime with either fluoxetine, fenfluramine or vehicle. Rats were similarly observed as for the acute studies, with additional measurements of body weight and laboratory chow. These measurements continued on cessation of medication. Work carried out in Chapter 6 showed that:

i) Body weight loss occurred and was sustained with FXT treatment, whereas FF treated animals showed increases in body weight comparable to controls. Weight regain, in excess of starting baseline was evident in FXT treated animals on cessation of drug treatment.

ii) Intake of sucrose in the 40min tests was reduced consistently in the FXT group, and spasmodically in the FF treated animals. On cessation of treatment with FXT, increases in consumption relative to control were seen.

iii) There were noticeable differences in the behavioural profiles of chronically administered FXT and FF relative to control, and in comparison to the acute effects of these drugs in the previous study. Animals treated chronically with FXT exhibited increased levels of non-specific activity relative both to control and to FF treated animals, while post-prandial resting behaviour was slightly suppressed. In comparison, chronic treatment with FF resulted in increased resting relative to control, generally at the expense of grooming, which was suppressed.

A series of experiments was carried out in order to examine the hypophagic and consummatory behavioural effects of selected serotonergic agents in rats and humans. These studies have helped to clarify response variables in the form of anorectic potential and behavioural profiles for serotonergic agents, some of which may be clinically prescribed for long periods of time. In view of the neurochemical action of these drugs, the observed effects suggest that

activation of the serotonergic system by FXT and comparable agents is an adequate stimulus for these effects to occur. In addition, observational methods in the animal studies have helped to determine that these drugs are, on the whole, capable of enhancing the behavioural expression of meal-induced satiety in the form of post-prandial resting. The chronic animal study charted the development of differing effects which may manifest in response to either acute or chronic treatment.

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CHAPTER 1

SEROTONIN AND FEEDING

1.1 INTRODUCTION

Food intake, food choice, feeding behaviour and body weight regulation are essential components of species survival. Operative mechanisms in feeding are accepted as being multifactorial, comprising both central and peripheral influences (Smith & Gibbs, 1987; Oomura et al, 1990; Blundell, 1991), and controlled by psychological and cultural constructs (Rozin, 1976) The research area directed at operative mechanisms in feeding is motivated by questions relating to "normal" physiological feeding/body-weight regulation, and to systems which are classified as dysfunctional, such as anorexic, bulimic and obese states (Robinson, 1990; Garrow, 1991).

Endogenous substances that modulate feeding are numerous. They include peripheral peptides and brain peptides (Morley, 1989), amino-acids, and central monoaminergic substances (Leibowitz, 1986; Leibowitz, 1988). Initial research carried out in animal laboratories has contributed to scientific knowledge on some of the influential factors in feeding. Peripherally administered peptides, such as

cholecystokinin, glucagon, bombesin and somatostatin (Gibbs et al, 1973; Cooper, 1985) have been shown to suppress food intake and may play a role in termination of feeding and satiety.

Centrally, feeding is increased by site specific, particularly medial hypothalamic (paraventricular nucleus), administration of the neurotransmitters noradrenaline, neuropeptide Y, peptide YY, galanin, the amino acid GABA and the opioid peptides, β-endorphin and dynorphin (Stanley & Leibowitz, 1984; Leibowitz, 1988). Reductions in food intake have been described in response to centrally applied intraventricular glucagon, bombesin, and CCK (Cooper, 1985; Silver & Morley, 1991). In some cases, depending on site of injection, some substances will produce the opposite effect when injected into the lateral portion of the hypothalamus, particularly the perifornical region e.g GABA or noradrelanine injected into the PLH will suppress feeding behaviour (Leibowitz, 1986).

A possible role for serotonin in the modulation of food intake and feeding behaviour has been postulated for a number of years (Blundell, 1977; Blundell, 1984). A substantial number of studies in both animals and humans show that experimentally induced manipulations of serotonin result in changes in food-intake, feeding behaviour and body-weight, with both suppression and increases in intake described, although the latter are more difficult to demonstrate (Blundell, 1992).

1.1.1 Perspective on serotonin (5-HT)

The activities of the neurotransmitter serotonin (5-hydroxytryptamine) are discussed chiefly, in this thesis, in the context of modulation of food intake and feeding behaviour. 5-HT modulation of other vital systems is briefly acknowledged here, e.g. arousal, sexual behaviour, pain and thermoregulation (Glennon, 1991; Gorzalka et al., 1990). Serotonin, along with other neurotransmitters, may contribute to the modulation of affective state (Cowen, 1991), and appetite disturbances may be a feature of mental illness (Silverstone, 1983; Silverstone, 1987a).

Historically, understanding the structure and functioning of the serotonin system has been reciprocal with technical advances. The development of histochemical fluorescence techniques by Falck and Hillarp in 1962 facilitated mapping the neuroanatomical location of 5-HT neurons. More recently, developments in antibody, ligand and cloning technology and increasingly sophisticated autoradiographic techniques, all contribute to shape the information we accept about this system (Wang & Peroutka, 1988; Glennon & Dukat, 1991).

5-HT is produced both in the periphery and in the brain. The breakdown of 5-HT to 5-hydroxyindoleacetic acid is due to monoamine oxidase and 5-HT is removed from the synapse by an active pre-synaptic uptake mechanism. In the brain, most 5-HT cell bodies are found grouped in specific sites in the dorsal and median raphe nuclei with terminal projections to the forebrain areas. Nine groups of serotonergic cell bodies, B1 - B9, were described by Dahlstrom & Fuxe in 1964 (Molliver, 1987). Most of the 5-HT neurons and terminals relevant to food intake

are found in the medial hypothalamus and specifically in the paraventricular nucleus of the hypothalamus(PVN) (Leibowitz, 1986; Leibowitz, 1990). Surgical lesions of 5-HT raphe neurons and pathways result in increases in food intake and body weight (McDermott et al., 1977; Geyer et al., 1976), while similarly, depletion of 5-HT with selective neurotoxins, (e.g. intraventricular 5,6-dihydroxytryptamine) opposes fenfluramine induced anorexia (Clineschmidt, 1973).

Knowledge of central 5-HT receptor identification and classification is increasing rapidly. Currently, 5-HT receptors are classified into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_{2}, 5-HT_{3} and the more recently described, 5-HT_{4} (Glennon et al., 1991) . 5-HT_{1B} receptors are present in animals, while the 5-HT_{1D} receptor in humans is thought to be functionally similar (Hoyer et al, 1986). Receptors are classified, on the whole, by the affinity of 5-HT or agonists for the receptor type. The sub-types of specific receptors are described by reference to differences in antagonist profiles and second messenger systems. The second messenger systems are adenyl cyclase (5-HT_{1} receptors except 5-HT_{1C}), phospholipase C(5-HT_{1C} and 5-HT2) or ion gating(5-HT3). As classification according to affinity has resulted in the existence of some "orphan" receptors unable to fit the described criteria, it has been suggested that receptor classification should be defined by their second messenger systems, a classification which would place 5-HT_{1C} and 5-HT_{2} in the same family as, for both receptor activation is linked to the second messenger phospholipase C (Hartig, 1989). The current status of the above information has been aided by the advances in molecular genetics, with ligand-ligand studies used for cloning of 5-HT_{1A}, 5-HT_{1C} and 5-HT_{2} and 5-HT_{3} receptors; in particular molecular

genetic studies support the re-classification of the 5-HT_{1C} receptor as part of the 5-HT_2 receptor family (Shih et al., 1991; Westkaemper & Glennon, 1991).

1.2 Influence of 5-HT drugs on feeding in animals

The role of 5-HT in feeding has been examined in a continuous series of experiments in both animals and man. In animals, injections of 5-HT centrally into either the PVN or the third ventricle, suppresses feeding (Leibowitz & Shor-Posner, 1986). Weiss et al.(1986) have shown that the ability of centrally injected 5-HT to suppress feeding is present in food deprived rats and that 5-HT can inhibit noradrenaline induced feeding. Studies examining the influence of 5-hydroxytryptophan (5-HTP) the precursor of 5-HT, show that when administered peripherally to either animals (Blundell & Latham, 1978) or obese humans (Ceci et al., 1989), 5-HTP causes a reduction in food intake. Similarly, central administration of 5-HTP (Sugrue et al., 1978) reduces feeding in rats. Tryptophan the amino acid precursor of 5-HTP, reduces total food intake, and meal size without effecting meal frequency in freely feeding rats (Blundell et al., 1980; Grinker et al, 1982). In humans, tryptophan has been shown to reduce food intake at a dose of 2.0g (Silverstone & Goodall, 1984) and to reduce carbohydrate intake after a protein meal at a 1.0g dose (Blundell & Hill, 1987a).

1.2.1 Peripheral activity

Approximately 95% of endogenous 5-HT exists in the periphery, mostly in the gastrointestinal tract. Peripherally injected 5-HT causes a reduction in food intake (Fletcher & Burton, 1984) and delays gastric emptying. There is experimental evidence to suggest that peripheral 5-HT, possibly working together with central 5-HT, plays a mediating role in the process of satiety. Peripheral injections of 5-HT itself have been shown to produce a dose-dependent reduction in food intake in animal studies, and these effects are suggested to be independent of motor effects (Pollock & Rowland, 1981; Fletcher & Burton, 1985). The hypophagic effects of peripherally administered 5-HT, which does not cross the blood-brain barrier, (Olendorf, 1971), may be mediated by peripheral receptors.

The peripheral administration of fenfluramine (FF) which acts by both releasing and preventing the re-uptake of 5-HT, causes a delay in gastric emptying (Davies et al, 1983; Horowitz et al., 1985), increases both the release and utilization of lipid stores in adipose tissue (Nicolaidis & Even, 1986) and increases glucose uptake by peripheral tissue (Turner & Kirby, 1978). While there appears to be a peripherally mediated component to fenfluramine-induced anorexia, centrally acting antagonists effectively diminish the anorexic effect (Borsini et al., 1985), but the peripheral antagonist xylamidine does not (Borsini et al., 1982; Carruba et al., 1986).

1.2.2. Central activity

Destruction of central stores of 5-HT, leads to hyperphagia, suggesting an active role for central 5-HT in feeding. For example, Clineschmidt (1973) has described centrally administered neurotoxin-induced attenuation of FF anorexia, while central electrolytic lesions are similarly capable of weakening FF induced anorexia (Davies et al., 1983). Additionally, inhibition of central 5-HT synthesis with the 5-HT synthesis inhibitor p-chlorophenylalanine (p-CPA) increases feeding (Rowland & Carlton, 1986a).

Activation of central serotonergic sub-receptors have been linked to changes in feeding responses. As a general guide, 5-HT_{1A} agonists cause hyperphagia while 5-HT_{1B}, 5-HT_{1C} and 5-HT_{2} agonists have been shown to suppress food intake. However, 5-HT_{3} and 5-HT_{4} receptors do not appear to play a significant role in the modulation of feeding behaviour (Peroutka et al., 1990). Further indication of a modulatory role of serotonin in feeding comes from studies in which the activity of serotonin is blocked via pharmacological antagonism. Antagonists of 5-HT which are relatively non-selective for receptor sub-type e.g. the 5-HT_{1} antagonists metergoline and methysergide, and ritanserin, a relatively specific 5-HT2 antagonist, can increase feeding in non-deprived rats (Fletcher, 1988a; Neill & Cooper, 1989).

Centrally acting serotonergic agents can increase or decrease feeding through receptor activation (Angel, 1990). The 5-HT_{1A} agonist 8-OH-DPAT causes feeding in low doses, an effect thought to be mediated by activation of 5-HT_{1A} auto-receptors located on raphe cell bodies,

since injection of 8-OH-DPAT into the midbrain raphe increases feeding in free-feeding rats (Dourish et al., 1985; Dourish et al., 1986). The increased feeding seen in response to 8-OH-DPAT has been described as occuring separately from the marginal motor effects in either peripheral (Dourish et al., 1985) or central(raphe) (Hutson et al, 1986) administration paradigms. As 5-HT_{1A} autoreceptors are located on raphe cell bodies, the hyperphagia induced by 8-OH-DPAT may be a consequence of decreased levels of endogenous 5-HT which would normally serve to modulate and inhibit feeding. Other 5-HT_{1A} agonists, e.g. gepirone (Gilbert & Dourish, 1987), ipsapirone and buspirone (Dourish et al., 1986) also increase feeding in free-feeding rats.

Activation of 5-HT_{1B} receptors can suppress feeding (Angel, 1990). The direct application of the 5-HT_{1B} agonist, RU 24969 into the PVN causes hypophagia without inducing hyperlocomotion (Hutson et al., 1988). Additionally, the hypophagic effects of RU 24969 are seen in both food deprived and free-feeding rats (Kennett et al., 1987; Bendotti & Samanin, 1987; Kennett & Curzon, 1988). The 5-$HT_{1B/1C/5HT_2}$ mixed agonists, 1-(3-chlorophenyl)piperazine (m-CPP) and 1-[3-(trifuoromethyl)-phenyl]piperazine (TFMPP) also reduce feeding in both food deprived and free feeding rats (Kennett et al., 1987; Samanin et al., 1979). Curzon and colleagues carried out a series of experiments in an attempt to discover whether one particular receptor determined the expression of hypophagia with these two agonists (Curzon, 1990). They concluded that m-CPP and probably TFMPP- induced anorexia were dependent on the activation of both 5-HT_{1C} and 5-HT_{1B} receptors, with activation of 5-HT_{1C} receptors possibly leading to activation of 5-HT_{1B} receptors (Kennett & Curzon, 1988). Both m-CPP

and TFMPP cause hypolocomotion; however, injection of TFMPP into the PVN results in hypophagia without attendant hypolocomotion (Hutson et al., 1988).

Consistent with the anorectic effects of 5-HT agonists, drugs which increase endogenous levels of 5-HT also cause anorexia. These increases in 5-HT can occur through the activity of pharmacological releasing agents, synthesis enhancers and re-uptake inhibitors. P-chloroamphetamine and fenfluramine which release 5-HT from presynaptic storage pools, reduce food intake (Trulson & Jacobs, 1976; Garattini & Samanin, 1976). The inhibition of re-uptake by selective 5-HT re-uptake inhibitors such as fluoxetine (Goudie et al., 1976; Fuller & Wong, 1989), femoxetine (Blundell & Latham, 1978), and sertraline (Lucki et al., 1988), can cause anorexia. Angel et al. (1988) have shown that the ability of this class of drug to cause anorexia is correlated with in vivo inhibition of 5-HT uptake.

1.3 Influence of 5-HT drugs on feeding in humans

Examining the effects of serotonergic agents on feeding in humans has been important for two main reasons. Firstly, the research has identified serotonergic involvement in the modulation of human feeding and food intake. The second reason is clinically orientated, it has lead to safe, effective anorectic drugs which do not have stimulant/abuse potential, and which offer the obese individual help in reducing body weight without intolerable adverse effects.

One of the most frequently examined drugs with respect to both modulation of food intake/choice and body weight, is fenfluramine. Fenfluramine is capable of reducing food intake in humans (Kyriakides & Silverstone, 1979; Blundell & Rogers, 1980), d-fenfluramine has twice the anorectic potency of dl-fenfluramine (Silverstone et al., 1987). In addition, the d-isomer has been shown to possess greater anorectic potency than the l-isomer, in animals (Garattini et al., 1988) and in humans (Goodall et al., 1992a). D-fenfluramine has been shown to reduce hunger ratings and daily energy intake in healthy, normal weight women, an effect associated with a reduction in the number of snacks consumed (Hill & Blundell, 1986a). Metergoline, a non-selective 5-HT antagonist, has been shown to attenuate the anorectic effect of d-fenfluramine in normal weight non-dieting subjects (Goodall & Silverstone, 1988), recently, the fat suppressive effects of d-FF were blocked by ritanserin, a 5-HT_2 antagonist (Goodall et al., 1992b - personal communication). At present, the lack of selective 5-HT_1 antagonists available for use in human subjects, make clarification of the action of fenfluramine difficult at this time.

1.3.1 Influence of 5-HT drugs on hunger and satiety

Hunger represents a motivational state which is generally expressed via feelings of hunger and desire to eat; the normal behavioural expression of this motivational state is eating/feeding (Blundell, 1979; Silverstone, 1982). It is worthwhile acknowledging that the term "hunger" carries two meanings: one is the existence of a state of motivation assessed by reference to the observed expression of behaviour; alternatively, the term "hunger" can stand for conscious

sensations and feelings, the outcome of which may be eating (Blundell & Burley, 1987). Hunger ratings and food intake may show significant correlation (Goodall et al, 1987), but not always (Silverstone & Stunkard, 1968; Blundell & Rogers, 1980; Mattes, 1990) and may even be dissociated (Trenchard & Silverstone, 1983). Eating can occur in the absence of hunger, and may be more responsive to appetitive factors. The concept and expression of appetite may be seen to be associated more with the qualitative aspects of food such as perceived pleasantness and sensory characteristics, e.g. food preference has been shown to have differential effects on hunger and appetite before, during and after a meal (Hill & Blundell, 1984). The expression of appetitive eating does not necessarily need the presence of motivational hunger to occur but it is likely that they frequently co-orchestrate initiation and maintenance of food intake and choice.

The expression of satiation and satiety are the results of interacting and reciprocal process, identifiable in the "Satiety Cascade" postulated by Blundell et al., (1988a). Satiation refers to the move towards "end of play" within a feeding episode whereby eating stops, while satiety is a state of inhibition during which further eating does not occur (Blundell & Burley, 1987). A sensitive experimental design can demonstrate modulating influences on selective areas of feeding, e.g. increasing palatability strengthens hunger, but does not effect fullness (Hill et al., 1984). Manipulation of meal macronutrient composition with palatability held constant, has shown that fullness at the end of a meal can be increased through increasing the protein content of the meal (Hill & Blundell, 1986b).

The process of satiety, i.e. the food-abstinent inter-meal state, is complex. According to Blundell et al., (1989), the identification of four mediating processes; sensory, cognitive, postingestive and postabsorbptive, expressed through physiological, neurochemical and metabolic substrates, characterizes its expression. Sensory components are influential through the sight, taste, smell and texture of foods. Cognitive components are potent, mediated through beliefs/ideas concerning the foods' effects on the consumer. Physiologically, post-ingestive factors may exert their influence through the action of gut hormones, such as CCK, gastric distension, the rate of emptying, and vagally mediated signals (Russek, 1971; Gibbs et al., 1973).

Food possesses the capacity to induce satiety (Blundell et al., 1989), consequently, if a pharmacological agent is successful in reducing food intake, it reduces the intake of the natural commodity which causes satiation, possibly weakening the natural process. Identifying combinations of drugs and foods which are reciprocally supportive of enhancing satiation and satiety, is likely to be of value in the pharmacological treatment of obesity. The combination of d-FF and protein have been shown to possess greater satiating capacity than d-FF and CHO (Hill & Blundell, 1990), and consequently this combination could be used as a stratagem in assisting weight loss. before eating next occurs.

1.4 Modulation of food selection

Discussion of the intake and selection of macronutrients refers to selective choice and diet composition of fat, carbohydrate (CHO) and protein we consume. 5-HT has been thought to be influential in regulating the intake of protein (Anderson, 1979), influencing the relative proportions of protein and CHO (Fernstrom & Wurtman, 1973), and perhaps most controversially, modulating control over the intake of CHO (Wurtman & Wurtman, 1979). In recent years studies have examined the relationship between nutrition, neurotransmitters, behaviour and subjective states, with the debate focussing on the possible existence and functioning of a regulatory loop. The view has been put forward that dietary proportions of CHO and protein influence the plasma ratio of tryptophan (Trp) to other large neutral amino (LNAA) acids. This plasma Trp/LNAA ratio contributes to determining how much Trp enters the brain as access to the brain is via competition for active transport at the blood-brain barrier. Synthesis of 5-HT is influenced by the availability of the precursor Trp. Advocates of this view suggest that in response to dietary intake and selection, greater amounts of 5-HT are synthesized, released and act on serotonergic receptors. The observed behavioural manifestation of this regulatory loop in action would be selective food choice for the macronutrients of CHO or protein. A more detailed account of this hypothesis is given by Wurtman & Wurtman (1984).

1.4.1 Influence of 5-HT drugs on food choice

The macronutrient intake of freely feeding, drug-free rats varies during the diurnal cycle. In the early dark phase, CHO is the preferred macronutrient, while during the late dark phase, the intake of protein and fat increases, while the intake of CHO declines (Rogacki et al., 1989). Animal studies examining the putative effects of serotonergic drugs on food selection have involved direct application of 5-HT into the hypothalamus and systemic administration of serotonergic agonists and antagonists under varying experimental conditions (Leibowitz et al., 1988; Li & Anderson, 1984; Kanarek & Dushkin, 1988; Blundell 1984;1986).

Laboratory studies in rats allowed ad libitum access to diets of pure macronutrients, have shown that centrally administered injections of 5-HT into the paraventricular, ventro-medial, and suprachiasmatic nucleus of the hypothalamus, cause specific selective reductions in intake of CHO, particularly at the begining of the dark period. Total energy consumption is maintained, with trends towards augmentation of protein and fat intake (Leibowitz et al., 1988; 1989; 1990; Weiss & Leibowitz, 1988). The effect of centrally administrated d-norfenfluramine, the metabolite of d-fenfluramine, is similarly active in suppressing intake of CHO at the begining of the dark phase in freely feeding rats (Weiss et al. 1990). Both the central (PVN) and peripheral (see below), effects of fluoxetine were examined in rats given free access to pure macronutrient diets of CHO, fat and protein.

PVN administration of FXT resulted in reduction of CHO intake during the early dark phase only, intake of protein and fat were not altered (Weiss et al., 1991).

The peripheral administration of 5-HT (Leibowitz et al., 1989) has been shown to reduce the intake of CHO. Examination of the action of serotonergic agonists on food selectivity has demonstrated the presence of phase specific effects. 8-OH-DPAT and buspirone were shown to increase CHO intake in rats given simultaneous access to CHO and protein diets in a two hour test (Luo et al., 1990). In addition, the co-administration of 8-OH-DPAT and FXT in a separate experiment, demonstrated the ability of 8-OH-DPAT to block the CHO suppressive effects of FXT (Luo et al., 1990). Dl-fenfluramine has been shown to reduce intake of both high CHO and high fat diets in freely feeding rats given access to both diets, but with greater suppression of the high-fat diet. (Kanarek et al. 1991). In another study, D-fenfluramine, fluoxetine and RU 24969 reduced intake of a high CHO-low protein diet in rats, particularly during the first hour of feeding during a 12h observation period (Luo & Li, 1990). The same serotonergic drugs were examined in a six day study during which rats were allowed access, during the dark period, to a high CHO-low protein or a low CHO-high protein diet. Again the effects of these agonists were to reduce the intake of the high CHO-low protein diet. Peripheral administration of fluoxetine shows comparable results to that of central administration, with reductions in CHO intake during the early dark phase only (Weiss et al., 1991). Pharmacological challenge via the peripheral antagonists cyproheptadine and

metergoline, results in increases in feeding, particularly of CHO and fat, without concommitant increases in intake of protein (Leibowitz et al., 1988; Shor-Posner et al., 1986; Stallone & Nicolaidis, 1989).

It has been possible, by use of sensitive study design, to show that the selective effects of serotonergic agonists are not exclusively directed at CHO intake. Fenfluramine has been shown to reduce fat intake in a self-selection regime (Orthen-Gambill & Kanarek, 1982), and in the Weiss et al. (1991) study (above), peripheral FXT reduced both fat,(at 2.5mg/kg-10.0mg/kg) and protein (10.0mg/kg) intake in the "high dose" condition during the late dark period. In a study in which rats had been made obese by exposure to either a high-fat or high-CHO supplemented diet, d-FF showed comparable effects on suppression of intake of both these diets (Blundell & Hill, 1989). In addition, d-FF may reduce the intake of laboratory chow, but appears less able to reduce either sucrose and non-sweet polycose (Lawton & Blundell, 1989).

Alterations in macronutrient intake have also been described in response to manipulations of non-serotonergic systems, e.g. central administration of a_2-adrenoceptor agonists have been shown to cause increases in intake of CHO, in the early dark period. (Leibowitz & Shor-Posner, 1986).

There is no consistent evidence from human studies that serotonergic drugs exert a specific suppressive on dietary CHO in normal volunteers (Goodall & Silverstone, 1988; Goodall et al., 1992a; Hill & Blundell, 1986a). D-FF and fluoxetine-induced suppression of CHO has been reported for obese subjects (Wurtman et al., 1985; Pijl et al. 1991).

Some of the work reported above has been carried out to determine whether the dietary components and proportions of protein and CHO are related (via changes in plasma ratios of the 5-HT precursor, tryptophan), to other large neutral amino acids (LNAAs) (Wurtman, 1982). Of interest here is whether drugs acting on the serotonergic system, are able to effect macronutrient intake reliably, the theoretical and clinical significance of this relationship, and under what circumstances alteration in intake occurs. The important observation that d-fenfluramine suppressed non-sweet CHO while concurrently unable to suppress the intake of sweet CHO suggests that some variables which modulate intake, such as the hedonic aspect of foods, may be particularly potent (Goodall et al., 1992a). Recent research in the animal field has identified factors such as texture which act as sensory influences (Booth & Baker, 1990).

1.4.2 Influence of palatability on intake

The term palatability and its descriptive use has raised questions of definition (Rogers, 1990; Ramirez, 1990). It is suggested to be a hypothetical construct which may be experienced subjectively, or inferred from observed behaviour (Blundell & Rogers, 1991). Research with laboratory animals has shown that it is possible to increase energy intake and body weight by offering energy dense diets varying in palatability and variety (Grinker, 1988). Postingestional factors contribute to this effect, as conditioned flavour preference occurs when a flavour is paired with an intragastric nutrient infusion (Sclafani & Nissenbaum, 1988); alternatively, aversion to sucrose develops when paired with nausea (Booth, 1985). Offering animals

high-CHO, high-fat and mixed fat-sugar combination diets, will, depending on age, strain, sex and individual response pattern of the animal, increase intake and body weight (Sclafani, 1987; Sclafani, 1988, Grinker, 1988; Marks-Kaufman & Kanarek, 1988).

Obese subjects possess similar thresholds to normals for the detection of sucrose (Grinker et al., 1972), but unlike lean counterparts, do not exhibit a reduction in the rated pleasantness of sucrose after a glucose preload (Blundell & Hill, 1988). It has been suggested that degree of weight loss may effect ratings, with greater weight loss being associated with reduction in aversion to higher levels of sucrose (Rodin et al., 1976). More recently, Kleifield & Lowe (1991), relying on self-reported weight status, suggested that weight-loss history influenced hedonic ratings. Drewnowski et al. (1985), carried out a study in which the taste responsiveness of normal weight (NW), Obese (O) and formerly obese (FO) subjects was examined using 20 mixtures varying in sweetness, creaminess and fattiness. No differences were found with respect to ability to rate the intensities of the above mixtures, but there were population differences with respect to hedonic ratings of fat and sucrose content of the mixtures. NW subjects preferred stimuli containing 20% lipid and < 10% sucrose, obese (O) preferred >34% lipid and <5% sucrose, while subjects who had been obese previously, showed greater responsiveness to both fat and sucrose.

An animal paradigm in which it is possible to show enhanced energy intake, is useful as a means of testing the power of drug effects. Drugs which have been shown to suppress the intake of sweet substances include those acting to antagonize dopamine, e.g. pimozide reduces

sucrose sham-feeding (Geary & Smith, 1985) and palatability-induced ingestion (Leeb et al., 1991). Hoebel et al., (1989), in microdialysis studies, have shown that the taste of saccharin causes a release of dopamine in the nucleus accumbens. The endogenous opiate system has been suggested as being instrumental in modulating hedonic responses to sweet substances, with the opiate antagonist naloxone suppressing glucose and saccharin intake (Marks-Kaufman & Kanarek 1988). Both serotonergic agents fenfluramine and fluoxetine have been shown to reduce palatability-induced ingestion. Fenfluramine reduced consumption of sucrose in non-deprived rats, an effect antagonized by the centrally acting antagonist metergoline, but not by xylamidine, which has a peripheral locus of action (Borsini et al., 1985). Alternatively, fenfluramine treated rats reduced their intake of chow, but not sucrose when given access to both food sources (Orthen-Gambill, 1985). Leander (1987), using saccharin, which provides a sweet taste without being a concommitant energy source, demonstrated that FXT lead to a reduction in intake in freely-feeding rats.

In humans, it is possible to demonstrate a dissociation between subjective hunger ratings and hedonic expression. The administration of the opioid antagonist naloxone to normal volunteers reduced food intake without concurrent effects on hunger (Trenchard & Silverstone, 1983). More recently, the opioid antagonist, nalmefene reduced the rated pleasantness of both taste and smell in foods which had been rated as palatable (Yeomans & Wright, 1991). The serotonergic influence on the hedonic aspect of intake in humans is less clear than that seen in animals. Acute administration of d-fenfluramine suppressed the nonsweet component of energy intake, without suppressing

the intake of sweet foods (Goodall & Silverstone, 1988). In addition, the d-FF-induced suppression of hunger ratings can occur without altering the hedonic response to sweet taste (Blundell & Hill, 1988).

Grinker (1988), has pointed out the complex nature of the area under investigation and drawn attention to the fact that current experimental methods lack the sophistication to pin-point the exact nature of metabolic, catecholaminergic and receptor interactions.

Despite these cautions, the approach of using "cafeteria" diets to model the development of dietary-induced obesity in humans has been helpful in elucidating energy-enhancing components of species equivalent, readily available foods. Our knowledge on the effects of such diets has grown in recent years, for example, dietary fat may be more "efficient" in promoting weight gain due to poor oxidation, than either CHO or protein (Schutz et al., 1989).

1.5 Body-weight control

The complexity of the multifactorial regulation of food intake (Friedman, 1990) and body weight (Apfelbaum, 1987; Garrow, 1988; VanItallie & Kissileff, 1990), is difficult to represent in most experimental settings. Within an adequately functioning system we now recognize the contribution of central, peripheral, hepatic, hedonic and psychological variables (Stellar, 1990).

When it comes to examing systems in dysfunction, the task becomes even more difficult. McHugh (1990), assessing the interface between the approaches of research scientists and clinicians in this area,

describes an uneasy and disatisfying liaison. He points to the existence of two virtually separate lines of inquiry. While he indicates a possible productive union of these disperate approaches occurring in the future, at present, "both lines of investigation are still immature". Basic scientific experiments describe the "proximate". or immediate physiological substrates of food intake, (vital nerve tracts, describing oro-pharangeal, hepatic, humoral and neurotransmitter modulated components). Influential variables such as the gene-environment interaction and socio-cultural determinants are the long-term, pervasive and "ultimate" influences on food intake and body weight.

An example of recent collaboration between laboratory and clinical interests, has been the growth of research into cancer anorexia/cachexia. Rapidly occurring developments in this field of research have shown that the disease state of cancer can be accompanied by anorexia and weight loss, the latter in excess of what would be predicted from energy intake. Laboratory work has identified the area postrema in tumour bearing animals as important for effects on body weight and intake. The production of endogenous "cachectin", which acts ultimately to suppress lipogenesis and consequently contributes to tissue wasting. Laboratory experiments have also shown that a circulating factor contributes to the anorexia/cachexic state: a healthy animal joined parabiotically with a tumour bearing animal will exhibit weight loss without developing a tumour (Mc Hugh, 1990).

1.5.1 Disorder of eating and body weight control

Under the heading "Disorders of Eating", the Oxford Textbook of Psychiatry (Gelder et al., 1989), describes the conditions of psychogenic vomiting (not discussed here), anorexia nervosa, bulimia nervosa and obesity. Diagnosis of anorexic and bulimic conditions is made by reference to criteria in DSM-111-R, which is currently being updated, both conditions are complex and patients risk disordered physiological functioning relative to type and severity of the disorder (Robinson, 1990). A brief mention of anorexia and bulimia nervosa is made below, neither condition being addressed experimentally in this thesis.

Anorexic patients may have compromised neuroendocrine and metabolic systems (Robinson, 1990). Evidence of both reduced ability to monitor gastric contents and disordered interpretation of fullness and satiety, compared to controls, have been described in anorexia nervosa. Although a large number of pharmacological agents acting on the serotonergic system have been shown to cause hypophagia, increasing intake has been less easy to demonstrate (Blundell, 1987). Cyproheptadine has been reported to be clinically effective in nonbulimic anorexics relative to placebo or amitriptyline (Halmi et al., 1986). Drug treatment of anorexia nervosa which was directed at increasing food intake without attempts to address the possible attending cognitive factors in this condition, have not so far proved successful (Russell, 1985).

Patients with bulimia nervosa may fall within the normal weight category although may have been previously overweight, will engage in frequent episodes of bingeing and vomiting, possibly misuse laxatives and report mood disorder and consequently suffer disruption to both their own, and relatives, lives (Fairburn & Cooper, 1984). Russell et al., writing in 1986, described a fairly limited use for drugs in the treatment of eating disorders. Both fenfluramine (Robinson et al., 1985) and fluoxetine (Fava et al., 1989; Wilcox, 1990; Trygstad, 1990), have been reported as assisting in reducing episodes of bulimic behaviour. Recently, Marcus et al., (1990) have reported that fluoxetine is not particularly helpful in obese subjects who binge.

The unspecified sub-set of obese individuals who binge may soon be acknowledged in DSM-IV. Wilson & Walsh (1991) reported on recommended changes suggested by the Eating Disorders Work Group, for inclusion in the diagnostic criteria of these conditions for DSM-1V. The working group has attempted to establish diagnostic criteria for that subject population who binge without purging - Binge Eating Disorder. As mentioned above, Marcus et al. (1990) reported that binge eating occurred in 46% of of obese subjects in a trial they conducted, and this sub-set were more resistent to therpeutic intervention than obese non-bingers.

1.5.2 Animal models of obesity

A significant amount of experimental effort has been expended on developing animals models of obesity, and some clinicans suggest that the onus falls on laboratory-based scientists to provide, at least,

partial answers to this complex condition in humans (Garrow, 1988). The diverse areas of research on animal models of obesity have arisen from questions on, aetiology, physiological and behavioural substrates which operate to maintain obesity, and the mechanisms and effect of potential treatment regimes. Animal paradigms include; e.g. genetic obesity, surgically-induced obesity, diet-induced obesities, environmental (e.g., stress, isolation), and drug-induced obesitities.

The genetic expression of obesity results from; a single gene, dominant,(e.g. Yellow mouse), single gene, recessive (e.g. fatty rat), polygenic: spontaneously obese (e.g. Paul Bailey black mouse) and polygenic: obesity prone (e.g. Osborne-mendel rat) (Sclafani, 1984). There is wide variation in severity of obesity, neuroendocrine and metabolic dysfunction and chromosomal location of the genetic defect between genetically obese animals (Bray, 1984). Availability of genetically-expressed animal models of obesity has increased knowledge of, for example, the presence of impaired sympathetic function and poor innervation of brown adipose tissue in some animals. Bray (1990) reports that when brown adipose tissue of ob/ob mice is electrically stimulated, heat production is considerably less than that of lean litter controls. Many other differences, relative to strain under examination, have been described: e.g. endocrine: hypothermia, elevated corticosterone, elevated levels of insulin and glucose (ob/ob mouse), Cerebral neurotransmitter and peptide function can be different; increased levels of β-endorphin have been reported in the pituitary of ob/ob mouse, the opiate antagonist naloxone suppresses food intake in ob/ob mice (Shimomura et al., 1982).

It should be asked whether there are any tentitive analogies between genetic obesities in animals and the human condition? The obese Zucker rat may be considered as being a reasonable model for juvenile-onset obesity, the obesity is expressed in the Zucker rat via a single gene; increased fat cell number (hyperplasia) and fat cell size (hypertrophy) are both present. these animals defend a high body weight and will become hyperphagic when exposed to a palatable diet. The possibility of genetically expressed animal obesity being suitable for modelling human obesity can be acknowledged, although limitations on the human expression of this condition, and the weight of gene-environment interactions, make this difficult (Montgomery, 1991).

Obesity has been reliably produced in animals by localized cerebral lesions, particularly of the ventromedial hypothalamus (VMH), resulting in increased food intake and body weight gain (Sclafani, 1984, Bray, 1984). Hyperphagia and body weight gain are also seen with lesions of the paraventricular nucleus (Leibowitz et al., 1981) and ventral noradrenergic bundle, although the increase in intake and weight gain is not as dramatic as that seen with VMH lesions. VMH -lesioned animals are described as exhibiting immediate increases in food intake and body weight during a dynamic phase, followed by a static phase during which the gained weight is maintained and food intake relatively normal (Montgomery, 1991).

The importance of acknowledging the potential influence of environment on the development and maintenance of obesity, has been addressed in experiments in which external factors are manipulated. For example, over-feeding in weanling Zucker rats can result in hyperplasia in both

Zucker (obese) and their lean littermates (Johnson et al., 1973). Other examples of environmental manipulations include force-feeding to induce obesity (Rothwell & Stock, 1978) and tail pinch, which causes hyperphagia and weight gain (Rowland & Antelman, 1976).

An environmental manipulation which holds reasonable model validity for human obesity, is that of "cafeteria," or diet-induced obesity (Sclafani & Springer, 1976). Originally rats were offered the sorts of foods freely available to most Westerners e.g. cheese, chocolate-chip biscuits, peanut butter and sweetened condensed milk (Sclafani & Springer, 1976). The type of diet has been varied in experimental procedures with an available chow component and alternative high-fat, high-sugar options, a CHO solution option, 32% sucrose or Polycose, or self-select diets offering separate sources of fat, protein and CHO. The range of weight gain in these experiments was from 30% to 200% excess, with some diets causing considerably more weight gain compared to control than others (Sclafani, 1990). Both strain and individual differences in weight gain have been observed in rats. In one experiment, only approximately 50% of animals fed the high fat/high sugar combination gained significant amounts of weight compared to controls (Levin & Sullivan, 1987). Some metabolic and neurological differences have been established between animals likely to gain weight, and those which appear resistant. Levin & Sullivan were able to pinpoint a "biological marker" in the form of a plasma noradrelanine response to a gluocose load when rats were fed chow, this correlated with their later weight gain when exposed to the calorifically dense diet (Levin & Sullivan, 1987).

The induction of obesity in animals in response to drug treatment is another valid model for a perhaps, small, but vulnerable group of obese humans. In humans, both antidepressant- and neuroleptic-induced weight gain has been described (Paykel, 1977; Silverstone, 1987a; Silverstone et al., 1988). The 5-HT_{1A} agonist 8-OH-DPAT increases consumption in rats, although how specific this effect is has been the topic of some discussion (Montgomery et al., 1988). The anxiolytic gepirone causes a robust increase in feeding in freely feeding rats, while buspirone and ipsapirone do so marginally (Gilbert & Dourish, 1987).

Baptista et al. (1987) chronically administered the dopamine antagonist sulpiride to rats, resulting in hyperphagia and weight gain; these effects were blocked by pre-treatment with the dopamine agonist bromocriptine. Low doses of opiates have been shown to increase feeding in rats, an effect suppressed by opiate antagonists, although Zucker rats who have reached the obese state, do not appear to be as responsive to the anorectic effects of naloxone as they were before (McLaughlin & Baile, 1984). Shimomura et al. (1982) have shown that the effects of chronically administered naloxone in ob/ob mice and lean controls, is to increase feeding.

1.5.3 Definition of Obesity

Men and women who fall within the normal body weight range possess 15 - 18% and 20 - 25% body fat respectively (Gray & Bray, 1988). The state of obesity can be defined when body fat is greater than 25% of total body weight for men and greater than 30% for women. The accurate

measurement of body fat requires specialist techniques, e.g. isotopic/chemical dilution, conductivity/bioelectrical impedence, or alternatively, measurement of skin-fold thickness. However, as assessing body composition is not the main focus of most obesity clinicians, body weight may be the more likely measurement taken.

One of the most frequently used classification systems of body weight is the Quetelet or Body Mass Index, defined as weight divided by height squared (W/H^2). Garrow (1981) has defined grades of obesity by reference to BMI ranging from normal weight; grade 0 = 20.0 - 24.9, through to the upper range of obesity of grade III, W/H^2 >40.

The definition of obesity by reference to BMI is a simple guide and does not supply information on regional distribution of body fat, a risk factor for cardiovascular disease in men with a waist-hip ratio >0.95 (Larsson et al., 1984) and women with a waist-hip ration >0.85 (Lapidus et al., 1984). Bjorntorp (1990) has suggested that, in effect, obesity should be defined with respect to relative risk factor for disease, and consequently those with abdominal, as opposed to femoral or gluteal, fat depots would be defined as medically obese. Overweight in men tends to be abdominally situated, while in women overweight is more likely to gluteofemorally disposed. It is possible to find a high W/H ratio in nonobese individuals, but the presence of obesity makes a high W/H ratio a greater risk factor for metabolic abnormalities, e.g. hyperinsulaemia, diabetes and raised blood pressure (Landin et al., 1989).

Active obesity research areas in recent years have attempted to assess both the sole and interactive components of genetics, metabolism, environmental and psychological factors of the obese state.

Studies on the genetics of obesity have established a strong relationship between degree of fatness for biological mother and adult off-spring adopted during the first year of birth (Price et al., 1987). In another study, Sorensen et al. (1989) studied adoptees separated early in life and compared them with siblings and half-siblings reared by their natural parents. Strong relationships between Body Mass Index (BMI) were found across all weight categories, with fat adoptees having fat siblings in the original home. In studies of body weight status in twins, Stunkard et al., (1986) found high concordance rates (0.8) for degree of overweight with monozygotic twins having stronger correlations than dizygotic twins. Strong genetic expression for regional fat distribution, subcutaneous fat and fat-free mass have been established (Bouchard et al, 1988). Bray (1990) has argued for a contributory role for reduced sympathetic activity in obesity, with attendent less efficient responses to nutrient intake. The possibility of dysfunctional status in the autonomic system for both the sympathetic and the parasympathetic component in some obese individuals, has been suggested by a study by Peterson et al. (1988). Measurements of both noradrenaline and adrenaline concentrations in fifty-six men showed negative correlations between these variables and body fat in healthy obese men.

1.5.4 The treatment of obesity

The condition of obesity has been observed to be chronic and refractory and, despite an enormous range of previous and currently available treatments (Garrow, 1988; Bray, 1989), there are few completely convincing success stories (Blundell & Hill, 1986). Depending on the level of overweight, perceived aetiological source of obesity and treatment bias of the clinician, the obese patient may be offered either any one, or a combination of the following treatments.

(i) Dietary restriction, either conventional or very low calorie, support from a nutritionalist may be available.
(ii) Behavioural modification
(iii) Encouragement to exercise
(iv) Drug treatment
(v) Jaw wiring
(v) Surgical intervention

An evaluation of the benefits of drug treatment of obesity, would be expected to take into account the seriousness of side effects, and proof of long-term efficacy, and off-set these considerations against the continuing harmful effects of obesity, the unattractive proposition of weight-cycling and the effects the latter would have on both the physiological and psychological status of the obese individual. Drug treatment of obesity has been mainly towards the approach of reducing food intake through changes in levels of hunger and satiety. Other

drug approaches focus on the dynamics of thermogenesis (Cawthorne, 1990), and the possible use of fat synthesis inhibitors (Sepinwall & Sullivan, 1991).

Nearly twenty years ago, an evaluative study carried out by a working party under the aegis of the Federal Drug administration, examined 170 studies involving 1000+ subjects who had been treated in double-blind conditions, with either an anti-obesity drug or placebo. The additional loss seen as a result of drug treatment amounted to 200g per week (Scoville, 1973). While this supported the therapeutic value of anti-obesity medication, the repeatedly observed weight loss placebo effect seen in most drug trials should also be acknowledged.

1.5.4.1 Serotonergic agents in the treatment of obesity

As serotonergic agents do not possess stimulant properties, they lack abuse potential, and consequently provide clinicians with a reasonably efficient therpeutic tool to tackle obesity (Silverstone & Kyriakides, 1982). Fenfluramine reduces hunger ratings, (Silverstone et al., 1975); the current prescribing practice is towards the use of d-fenfluramine, which acts with greater selectivity on the serotonergic system and in suppressing food intake in animals (Garattini et al., 1988) and humans (Goodall et al., 1992a). D-fenfluramine has been described as having twice the anorectic potency as that of dl-fenfluramine in normal volunteers (Silverstone et al., 1987). In clinical trials with an obese population, d-fenfluramine has been used for periods of three months (Finer et al, 1985; Goodall et al., 1988), up to a year (Guy-Grand et al., 1989). Clinical prescribing guidelines recommend treatment periods of twelve to sixteen weeks duration, and

suggest concommitant use of exercise and changes in dietary habits. Slowing of weight loss in response to longer treatment periods has been reported (Finer et al., 1987), and after six months a stable weight level could be seen as a continuing treatment effect. The tendency for patients to regain weight on cessation of treatment appears almost inevitable when drug treatment is used as a sole treatment (Guy-Grand et al., 1990).

Antidepressants which act to inhibit the reuptake of 5-HT, have been shown to possess some weight reducing properties, stimulating interest with respect to their possible future use as anti-obesity agents. Abell et al., (1986), describe a study in which fluvoxamine maleate, initially at 50mg and ultimately at 100mg, twice daily, was given to 40 females with refractory obesity, in a placebo-controlled trial. Subjects received dietary advice to help them to adhere to approximately 1,000 kc/d, 29 subjects completed the 12 weeks, of whom 13 had received the drug. Subjects in both the placebo and the drug group lost weight, and significant differences between the two groups were not found. The placebo group had a higher starting BMI, falling just inside grade III at BMI of 40.7, while the starting weight of the drug group was 36.8, but according to the authors, this difference was not significant (Abell et al., 1986). This study is a good example of the value of non-pharmacological dietary treatment, as while the placebo group started heavier, their loss was 2.8kg, the loss with the drug group being 3.5kg.

1.6 Summary

The experimental studies described in this thesis are concerned primarily with the anorectic effect of the 5-HT re-uptake inhibitor and antidepressant fluoxetine. Placebo-controlled studies carried out and described in Chapters 3 (normal volunteers) and Chapter 4 (obese volunteers) examine both anorectic and subjective effects of this drug in response to two (Chapter 3) and four (Chapter 4) weeks treatment. The development of an observational methodology suitable for laboratory and field work with both volunteers and clinical populations is first described in Chapter 2; Chapter 4 describes the application of this methodology in obese patients treated with FXT. Subsequent studies examined the effects of fluoxetine on food intake, body weight and satiety behaviour in rats following acute (Chapter 5) and chronic (Chapter 6) administration and compared the effects of FXT with those of fenfluramine and other serotonergic agonists.

CHAPTER 2

DEVELOPMENT AND TESTING OF AN OBSERVATIONAL METHODOLOGY FOR STUDYING FEEDING BEHAVIOUR

2.1 METHODS OF EXPERIMENTAL INQUIRY INTO FEEDING

The development of methods to study feeding in animals and humans has been prompted by inquiry into both the determinants of functional feeding control systems, and of systems which appear to be dysfunctional for the host. For example, while population surveys may show that the selected population eat more fat than the current recommended 33% of total daily energy (Gregory et al., 1990), the majority of people do not appear to find food intake and body weight regulation a seriously intrusive consideration in daily life, which can be the case for those suffering from anorexia nervosa, bulimia nervosa (Robinson, 1990) or obesity (Garrow, 1991).

In general, investigation of influential factors in feeding have centred on (i) short-term meal-specific activity, laboratory based and frequently using "direct" objective measures. Efforts to evaluate measurement of (ii) longer-term feeding(24h and upwards) relied more on subjective measures and "indirect" field/observational work. Experimental methodologies have been developed to study feeding over a period of years (Hetherington & Rolls, 1987), At present, research methodology is advancing and is increasingly able to facilitate long-term objective

measurements via computerized techniques (Blundell & McArthur, 1981, Foltin et al., 1990). This continuing methodological refinement reflects the awareness and acceptance by researchers of the complex and interactive nature of their subject.

2.1.1 Methodologies in animals

The focus on component, or microstructural analysis of behaviour (Wiepkema 1971) encouraged the development of experimental methodologies with the capacity to dissect the structure and processes of behaviour. With respect to the effects of drugs on consummatory behaviour, this allowed researchers to examine qualitative and interactive as well as quantitative and absolute expressions of behaviour. In effect, this meant moving away from the analysis and presentation of data as absolutes (e.g. total grams), and towards analyzing and interpreting data in the context of interactive "behavioural flux" (Blundell & McArthur, 1981). The latter term refers to the concept of behaviour as linked, supported and modified by the continuously vital schema of the host.

Microstructural analysis in the pharmacological context was originally used to examine the effects on feeding of equipotent doses of amphetamine and fenfluramine, the former, at a dose of 1.0mg/kg producing an increased latency to eat, and rate of eating, while the latter (3.0mg/kg) reduced the rate of eating (Blundell & Latham, 1978; 1980). A refinement to the recording of intake methodology was the pellet detecting eatometer (Blundell & Leshem, 1975) which allowed continuous recording and consequently was able to elucidate differences in the profile of effects over 24 hours of amphetamine and fenfluramine in non-deprived rats. This approach demonstrated that fenfluramine

decreased meal size, while amphetamine resulted in a small increase in inter-meal interval (Blundell et al., 1976). The use of computerized continuous recording has helped to describe variables such as meal size and intra-meal eating rates (Blundell & Latham, 1980)

The observation that, on cessation of feeding, a rat will generally engage in a series of behaviours comprising grooming, being active and finally resting, which is considered to indicate the presence of satiety, has been demonstrated in freely feeding rats, but does not occur in sham-fed rats (Antin et al., 1975). This sequence of behaviours has provided a background on which to examine and characterize the effects of anorectic agents on consummatory behaviours. Drugs inducing anorexia were known to act mainly on either the catecholaminergic (amphetamine) or serotonergic (fenfluramine) systems, but there was little initial information on behaviourally relevant effects. Microstructural analysis of consummatory behavior has assisted in both the identification of the critical components in initiation, maintenance and termination of feeding (Rowland, 1987), and additionally, is usefully employed in clarifying differing mechanisms of action between anorectic agents.

A drug which is predicted to enhance satiety should not only induce a suppression in intake, but also enhance the behavioural correlates of satiety. A classic study, including video-taping, was carried out to examine and compare the effects of fenfluramine and amphetamine on energy intake, choice and consummatory behaviour in rats (Blundell & McArthur, 1981). Amphetamine-treated animals did not reduce energy intake but showed increases in grooming and activity, which were intrusive to the usually expressed sequence of eating, grooming,

activity and resting. The meal took longer, and it was suggested that there was no direct effect on eating. The effect of fenfluramine on energy intake over 24h period was to reduce total intake but maintain protein intake (offered from two sources). Fenfluramine increased the time spent eating, decreased the time spent grooming,and caused an early introduction of post-meal resting suggesting fenfluramine possessed satiety-mimetic qualities (Blundell & McArthur, 1981).

Greater sophistication in currently available recording methodology mainly through computerization, has helped to integrate the previous short-term observational studies with longer term field type studies. Measurement and observations that were primarily targeted on the discrete unit of a meal, can now be targeted both on the meal (pre-prandial, intra-prandial, post-prandial) and over longer periods of time (intra-meal, circadian, and longer-term regulation). The work of Foltin and colleagues, carried out with both primate and human populations, is a good example of experimental procedures which have been shown to be both flexible and sensitive to a variety of anorectic challenges. Recent developments include monitoring primates over period of 22 hours using continuous recording techniques to examine the effects of a selection of drugs (Foltin, 1989; Foltin & Fischman, 1988) and caloric manipulations (Foltin & Fischman, 1990).

2.1.2 Methodologies in humans - laboratory

a) Measurements using a liquid source of intake

In humans, the initial studies on energy intake and feeding behaviour tended to focus on attempts to establish differences between lean and obese subjects. For example, in a study by Price & Grinker, (1973), food was used as the main dependent variable, with level of deprivation (preload/deprived) as the independent variable.

The use of liquid energy sources allowed direct "on-line" measurement of nutrient intake, in a hidden reservoir, and connected by tubing which allowed the subject to "feed" (Jordan et al, 1966, Jordan, 1969). This methodology allowed covert manipulation of energy density and its effect on meal size (Speigel, 1973), a tactic now possible with solid foods through fat substitutes (Blundell & Burley, 1991). This method was used to monitor the development of satiation in obese subjects (Pudel & Oetting, 1977), and was used to test intakes in response to stress in normal and obese subjects (Meyer & Pudel, 1972). Kissileff & Kral (1986) used this method to examine eating behaviour and energy intake in 75 patients who were more than 100% overweight (morbid obesity). Obese subjects, and 41 controls consumed a standard 300 kcal breakfast and three hours later were given access to a milk-shake style liquid meal from an opaque container. No significant differences between obese and non-obese were found in either energy intake or duration of the "meal", eating rate was faster in the obese than the non-obese, and mean both

both consumed more than women, and had a higher eating rate. Concerns about the field validity of this method due to the absence of solid foods encouraged developments of more realistic methods.

b) Developments in Automatic monitoring of Intake

Kissileff et al., (1980) developed the Universal eating monitor (U.E.M), comprizing a continuously weighed (every 3 seconds) bowl which rests on an electronic scales set into a surface. The changing weight of which is used to plot intake across time, and hence to describe a cumulative intake curve (Kissileff et al., 1980). Either solid, (fruit, nuts and yogurt) or a semi-solid (liquid) version, could be offered to subjects, using a plate or bowl. Kissileff & Thornton, (1982) suggested that excitatory neural processes and their "cognitive" correlate of hunger were reflected in initial rates of eating, while state of neural inhibition, "cognitively" expressed as satiety, was reflected in a negatively accelerating intake curve. Deprivation (21h)(Kissileff et al., 1980) and the use of palatable food (Bobroff & Kissileff, 1986), were found to increase initial rate of eating, offering some support for this hypothesis. This method is sensitive enough to show drug effects: slow infusions of low doses of CCK in lean men resulted in subjects eating less and stopping eating sooner (Kissileff et al., 1981).

Kissileff has suggested that this method is also sensitive enough to demonstrate aberrant patterns of intake in bulimic patients. Interestingly, the type of instruction given to the patients played an influential part in the behavioural outcome, in that when ice-cream was offered with the instruction to eat as much as they could, bulimic patients ate both more rapidly and consumed greater amounts than

controls. When offered yogurt shake and asked to consume it as if it were a normal meal, there were no differences between normal and bulimic patients' intake or behaviour (Kissileff, 1988).

Westerterp et al. (1988) and Westerterp-Plantenga et al., (1990) have utilized the Universal Eating Monitor of Kissilef (1980) to examine the eating behaviour of both normal and obese subjects who are offered an acceptably palatable pasta dish. No consistent differences between normal and obese subjects were found in meal, meal length or changes in the slope of the cumulative intake curve. They suggest that deceleration or non-deceleration (linear) intake curves are related to cognitive state, as measured by Stunkard & Messick's (1985) Eating Restraint Scale. In normal weight subjects non-decelerated eating rate was related to cognitive restraint and disinhibition factors of the scale, suggesting that this group was partly restrained (Westerterp-Plantenga et al., 1990).

Another automatic device of a different nature, was the Bite-Indicating Telemetering Eatometer (BITE)(Moon, 1979). This device uses the contact of eating utensils with the subject's mouth to register the number of bites. Information on inter-bite interval and changing rates of eating, can be recorded. It has not been confirmed whether this device is suitable for a wide variety of food-stuffs, and it does not appear to be widely used (or referred to) in feeding research.

The Edogram, (Bellisle & Le Magnen, 1980), measured chewing and swallowing by monitoring movements of the Adams's apple and jaw, using this technique it was possible to show that palatable foods resulted in a bigger meal size; shorter amounts of time were spent chewing palatable

foods, and increases in inter-bite intervals occurred as the meal progressed, indicating a decrease in appetitive state and the development state of satiety (Bellisle et al., 1984).

c) Solid Food Dispensers

The concerns about validity of measurement using liquid sources of energy, prompted development of the more realistic Automated Solid Food Dispenser (AFD) by Silverstone et al. (1980). This method facilitated monitoring during a lunch meal in which subjects could choose from a selection of four items; (e.g. sandwich portions, crackers plus cheese, grapes, chocolate biscuits), by pushing a button (see Chapter 3) This method has proved sensitive enough to detect pharmacologically-induced changes in food intake and choice in healthy male subjects (Goodall & Silverstone, 1988). A more elaborate version of the AFD was later developed in which ten items of food varying in macronutrient composition and sweet/savoury components were available to subjects, thus allowing within-meal analysis of pharmacological effects on food intake (Goodall et al., 1992a).

An analysis of the influential factors in food intake and feeding research constitutes an experimental paradox, in that validity, is invariably reduced in a laboratory setting (Rolls & Hetherington, 1990) . Field settings, while offering greater external validity (representing subjects eating habits), are difficult to control for extraneous influences/demand characteristics. Rolls & Hetherington (1990) make the point that a laboratory setting offers the opportunity to control and manipulate variables with far greater sensitivity than possible in a field setting. For example examination of the complex relationship between hedonics and energy content (Blundell et al., 1988b), could prove difficult to organize in a naturalistic setting. One of the aims of a

comprehensive research "model" could be to identify priority aspects in a laboratory setting, and then attempt to confirm this finding in a naturalistic setting. The use of diet diary records as an adjunct to an experimentally monitored meal, is an example of this approach.

Foltin et al., (1988; 1990) have used a residential laboratory setting to examine the effects of both caloric dilution (1988) and d-amphetamine (1990) on food intake in normal males for fourteen and fifteen days respectively. All behaviours (except dressing and toilet activities), were recorded via a computerized observation system. Although this type of experimental design is working towards the ideal of examining behaviour in a natural setting while being able to make accurate recordings, the lack of access to television, radio, mail or newpapers detracts from the field validity of an otherwise comprehensive design.

2.1.3 Field methodologies in Humans

The reasoning behind the organization of field, or naturalistic studies is that the observed behaviour is more likely to show external validity. However the researcher generally forfeits both accuracy and the likelihood of acceptably controlled manipulation. In the 1970's, a considerable number of field studies were carried out with a view to determining the existence of lean/obese differences in eating behaviour and intake. For example, the study of Stunkard et al (1980a) carried out in a fast-food restaurant, made attempts to control for portion size and in addition, to detect the presence of restraint in subjects. In this study, unobtrusive observers wearing dark glasses, purportedly reading

newspapers, recorded the feeding behaviour of an unsuspecting "subject" every 30s using a hand-held counter and by marking paper. Information on the possibility of restraint was gleaned by researchers, posing as market researchers, asking subjects "do you usually eat less at meals than you would like?" Subjects were 30 obese (visually determined) and 37 non-obese controls who were offered a free coupon "prize" meal. In this study, the eating style of the normal and obese subjects were similar, with comparable rates of eating. The obese subjects who chose to eat the large meal consumed more kilocalories per mouthful than controls, the opposite was true for the small meal. The point was made by the author of this study, that there was great variability in eating from subject to subject. It had not been possible to control for deprivation level. Attempts were made to control the energy content of the food by making two similar meals (hamburger/fries/soft drink) available. Subjects could choose a small meal comprising of one of each of the above and amounting to 985 kc, or a large meal, comprising of 1,800kc, comprizing two of the mentioned solid foods and a large soft drink. More normal weight (19) than obese (11) women ate the large meal. Another study examined over 5,000 episodes of food choice in nine different sites, to include four sites offering meals and five offering snack foods (Coll et al, 1979). Again, weight category of normal, overweight and obese, was assessed visually. At one snack site, obese people choose more calorifically dense foods than non-obese but, in general, food choice was dictated by type of site being observed, as opposed to weight category (Coll et al., 1979).

2.1.3.1 The use of diet diaries

Diet diary records and histories can provide useful insights into intake behaviour of selected sub-groups, although accuracy in reporting over long periods of time is unlikely. De Castro (1987) used diet diary intakes to examine the spontaneous intake of eight normal male and thirty female subjects over a nine day period. In this study daily rhythms were evident, with intake of CHO and fat varied with peaked intakes at meal time, protein intake was more consistent. Some suggestion, particularly in males, of greater energy consumption during the evening, De Castro felt that this may be in anticipation of the night fast, however, it may be just as likely to be in response to social context or response to television advertizing. Diet diary records have been used in an attempt to determine obese/lean differerences in intake. Lansky & Brownell (1982) carried out a study in which obese subjects self-reported consuming less kc than lean subjects. However, when intake was assessed by an experienced third party at interview, obese subjects were found to consume approximately 600kc more than non-obese. It is not unexpected to find that obese subjects in energy balance consume more than non-obese subjects. Prentice et al.(1986), have shown under-reporting in the obese using doubly-labelled water. Laessle et al. (1989) used information from 7 day diet diary records kept by restained and non-restrained young women to show the presence of a reduced intake of 400kc in the former compared to the latter. Protein intake was found to be higher in restrained subjects, records also suggested that restrained subjects consumed less high-fat/CHO energy dense foods. Diet diary records can certainly provide indications of intake behaviour but accuracy in recording intake is unlikely to persist over long periods of time, and

the recording of consumption of foods will in effect, become an independent variable. More recently diet diary intake has been used to examine the effects of serotonergic drugs on diet selection (Pijl et al., 1991). Perhaps one of more valid uses of diet diaries can be directed towards establishing the presence of drug/placebo differences in response to drug treatment (see Chapter 4).

2.2 An observation methodology for studying feeding behaviour

The act of observing behaviour as an experimental methodology has ethological roots; being used in field studies with animals in the 1920's and 1930's. Analysis of behavioural sequences in animals has become increasingly sophisticated and therefore allows a more comprehensive approach to examining behaviour (Douglas & Tweed, 1979). In psychological research, observational techniques are not widely used. Hutt and Hutt (1970) suggest such studies only represent a small proportion(approximately 8%) of total experimental work. In observational studies the observed behaviour is likely to occur naturally, be recorded at a molar level with fewer subjects but for a longer period of time (Fassnacht, 1982). In addition, observers need training and must work together until acceptable levels of inter-rater reliability have been achieved, and periodically record further behaviour together for a recommended 20% of observations (Sackett, 1978). Observers are likely to record large amounts of data, thus making high agreement levels more difficult to achieve. The latter requirement is invariably expensive in time as the research staff are needed for both training and experimental work. Examples of observational studies in human subjects focussed on children in the form of mother/infant interaction studies (Olvera et al., 1990), pre-school play and hyperkinetic (Hutt & Hutt, 1970); as well

as mentally retarded and autistic children (Goodall & Corbett, 1982). In adults, observational techniques have been used for example, in an attempt to determine obese/lean differences in feeding behaviour, an issue which has previously demonstrated inconsistent experimental findings (Stunkard & Kaplan, 1977).

The main aim of the present three part study was to establish a reliable and portable observational method suitable for studying feeding behaviour in varied sub-populations of humans. Suitable target areas for application of this methodology would be monitoring changes in feeding behaviour during the course of depressive illness and detecting changes in feeding behaviour under specific drug conditions. A continuous, as opposed to a sampling method of observation was choosen, as the latter (both whole-interval and partial interval recording) can underestimate or overestimate the true percentage of observed behaviour (Murphy & Goodall, 1980). In continuous recording each occurrence of all specified behaviours is recorded with exact information on total time, latency, frequency and the start and finish of each behaviour (Martin & Bateson, 1988). In an initial pilot study (Mc Guirk, 1985), observer agreement was determined using Cohen's Kappa Statistic, a stringent measure of inter-rater reliability which analyses areas of both agreement and disagreement (Hollenbeck, 1978). Observer agreement levels using Kappa in the video-recorded conditions were .78 to .96 and .72 to .91 in the live conditions. Percentage agreement was choosen for subsequent studies as an acceptable agreement measure for observers who had previously demonstrated satisfactory agreement levels under stringent conditions. The first experiment had two purposes: firstly, to establish acceptable

levels of observer inter-rater reliability; secondly, to examine the feasibility of live recording of feeding behaviour in a psychiatric setting.

Subsequently, the observational methodology was applied in the context of a drug trial. A study examining the differential anorectic, psychomotor, and hormonal effects of the d- and l- isomers of FF and amphetamine (Goodall et al.,1992a; Feeney et al., in prep.) provided a suitable basis for testing the method. This had the advantage of testing the methodology against previously observed drug effects (Rogers & Blundell, 1979; Kyriakides & Silverstone, 1979), with the additional inclusion of l-FF.

The gross measurement of amount of food consumed during an experiment may conceal subtle differences in the behavioural mechanisms of anorcetic drug action. Drugs that reduce food intake may do so by bringing meals to a premature end, by decreasing the rate of food ingestion, or by a variety of other means. For example, a difference in the behavioural mechanism of action between catecholaminergic and serotonergic anorectics is now well established. Thus the prototypical catecholaminergic drug, d-amphetamine, has been found to decrease food intake, the duration of meals and feeding bouts within meals, although, as reported in some studies, has increased the rate of food intake within bouts. In contrast, the prototypical serotonergic anorectic, dl-fenfluramine, decreases food intake and the rate of eating, but does not alter meal duration (Blundell & Latham, 1978, 1980; Cooper & Francis, 1979; Blundell & McArthur, 1981; Burton et al., 1981; Towell et al., 1988; Foltin & Fischman, 1988).

When this drug-pair has been tested in human subjects under laboratory conditions, the difference in action of d-amphetamine and dl-fenfluramine has been confirmed, but not always identical (Blundell et al., 1979; Rogers & Blundell, 1979; Kyriakides & Silverstone, 1979), The questions remaining on the differential effects of these drugs surround their capacity to sustain effect in a fairly wide food choice situation, and as similar as possible to buffet-style foods available in public eating venues or at home.

In effect, there were three main objectives in setting-up this experiment: the development of a suitable methodology for studying human eating behaviour in a variety of settings; to determine the effects of d- and l-fenfluramine on eating behaviour; and to examine the interaction between fenfluramine and amphetamine. On the basis of the available literature, it was predicted that both d-amphetamine and d-fenfluramine would reduce food intake, but with evidence of differential effects in feeding behaviour, and that l-fenfluramine would be largely ineffective.

2.3 METHODS

2.3.1 Subjects:

Experiment 1: Fifteen subjects in total volunteered. These were mixed male and female, comprizing of seven psychiatric patients and eight normal volunteers, subjects were matched as far as possible in the circumstances, for age and body mass index. The normal volunteers were recruited from the hospital staff, the incentive being a free hospital lunch. On recommendation from the nursing staff, patients who were well enough to attend Occupational Therapy sessions were asked if they would be willing to eat their lunch while being observed. The research staff were introduced to individual patients and answered questions concerning the intended project. Patients suffering from florid psychosis or disturbed behaviour were excluded. The patient population were of mixed diagnoses, comprizing schizophrenic(n=3), schizo-affective(n=2), and affective disorded(n=2) patients. The need to establish observer agreement was given as the reason for the study to all subjects.

Experiment 2: Six psychiatric patients made up the live ward-based subject population of schizophrenic(n=4) and affective disordered(n=2)patients. As in Experiment 1, the nursing staff made the initial recommendation as to which patients would be suitable for observation on the ward. Observer agreement was again given as the rationale for the study.

Experiment 3: Twelve healthy male subjects participated in the pharmacological study. Their mean age was 28.4 years, s.d. 2.9(range 24 to 32). They underwent a medical and had no history of psychiatric illness.

2.3.2 Observational methodology:

In order to gain an applicable behavioural taxonomy prior to the experiment, video-tapes of normal volunteers eating a meal were made and viewed by departmental staff. The specific acts of manipulation(food and utensils), biting and chewing of food were classified as eating behaviours and specified as follows:

Manipulation: Contact with food by fingers or eating utensils.
Biting: Food enters mouth.
Chewing: Visible jaw movement while food is in mouth.
Similiar behavioural taxonomy has been used in an attempt to establish obese/normal differences(Stunkard et al.,1980a).

Recording of behaviour: Each observer used a portable Epson HX20 "lap" computer to make recordings of the lunch meal. A customized verson of D Felce's "lunch" program enabled the two observers to record the start and finish of each bout of feeding behaviour, e.e. manipulating, biting and chewing of food, using keyboard input. The summary data on print-out gave duration of the total meal, duration of each feeding behaviour, frequency per minute and percentage of total time occupied by each eating behaviour.

2.3.3 Experimental settings and procedures:

Experiment 1: Subjects ate their lunch in a twelve foot by sixteen foot room in the Occupational Therapy Department. This setting was chosen for the recording site as the patients attended there daily and prepared and ate lunch there once a week as a part of rehabilitation therapy. In addition, the patients were familiar with the use of a video camera in this setting as filmed sessions formed part of the social skills training programme. The video camera was positioned in a corner of the room. It was mounted on a tripod and directed at the dining table in the opposite corner of the room. The television monitor and recorder were on a trolly positioned outside the room and out of view of the subjects. Participants were asked to arrive at the specified room in the Occupational Therapy Department at twelve noon. Two participants, either two psychiatric patients or two controls ate together. While both participants were filmed, only one was a subject for live observation. Subjects were seated at the table and served their choice of lunch. The observer using the portable computer was seated beside the tripod and in the opposite corner of the room to the subjects. The video-recorded was activated just before the subjects were served their lunch. Psychiatric patients ate a standard hospital lunch. They were offered a choice of two different meals being served on the ward that day. Normal volunteer subjects ate a hospital lunch. They selected from a choice of two lunches being served in the canteen. Eating behaviour, as previously defined, was recorded using the portable computer. When both subjects had finished their meal they were asked if they would like to view the tape of their meal on the monitor.

Experiment 2: The dining area of the acute admission ward was the setting for the live observation. Each observer used an Epson laptop microcomputer to record feeding behaviour as before. The dining area consisted of a rectangular room with approximately six circular dining tables. The observers sat in two of the easy chairs normally placed against the walls of the ward. The usual lunch-time routine was maintained in which patients went to a serving hatch, collected their own lunch, returned to a table and ate there. Patients taking part in the observation session sat at a table near the seated observers. On cessation of the observation session, the patients were thanked for their co-operation and the observers left the ward.

Experiment 3: The pharmacological study was a within subjects double-blind and counter-balanced design, each subject received all six treatments conditions as follows: 30mg d-FF & amphetamine placebo; 30mg L-FF & amphetamine placebo; FF placebo & 15mg amphetamine; 30mg d-FF & 15mg amphetamine; FF placebo & amphetamine placebo. Subjects attended the laboratory weekly on six occasions and were tested individually, there was a one week interval between treatments. Subjects fasted overnight and arrived at approximately 0845h. At 0900h(approx) visual analogue scales(VAS) were completed, followed by tests of psychomotor function, and a blood sample was taken. VAS were administered hourly thererafter and blood withdrawn at 2h intervals. At 0930h, the subject took two identical capsules, containing l-fenfluramine (2 X 15mg), d-fenfluramine (2 x 15mg), or placebo; at 1100h a further capsule was given, containing either placebo or d-amphetamine(15mg).

At 1400h subjects ate lunch in a room approximately 14' 6" by 6' 6". The room contained a table with eating implements, a chair, a wall mounted sony video camera and a Wittenborg food dispensing machine adapted for experimental purposes(Wittenborg UK Ltd, Horley, Surrey). This was similar in appearance to most modern automatic food dispensers seen, for example, in airports or hospital night-staff dining areas (Fig 2.1). This ten-channel automated food dispenser(AFD) allowed subjects to input a code number and help themselves to a small portion of either one individual food or a selection of different foods. Table 2.1 describes the available foods with energy contents. A specific program(A Leach-Compular) recorded details of food intake on an Apricot computer linked to the Wittenborg and housed in an adjacent room. The T.V. monitor and video recorder were also in an adjacent room which allowed observers to record the meal and make observations of feeding behaviour using an Epson laptop microcomputer as described in the previous study(Fig 2.2).

Fig 2.1 Ten channel Automated Food Dispenser (AFD), eating area, and subject

Fig 2.2

Table 2.1 Foods available to subjects from the ten channel Automated Food Dispenser(AFD).

	Description	Portion weight	portion energy(kj)
Tray			
1	Cream cracker	7.5	139
2	Bread roll	46.0	473
3	Apple	104.0	157
4	Strawberry dessert	128.0	628
5	Margarine	10.0	299
6	Cheddar cheese	20.0	336
7	Chocolate biscuits	21.0	431
8	Tomato	78.0	47
9	Chicken	20.0	124
10	Ham	20.0	143

2.3.4 Analysis

Experiments 1 and 2: Intra- and inter-rater reliability was assessed as follows; approximately one week after the video recording both observers made behavioural recordings from the video-tapes each using a portable computer. Percentage levels of agreement were determined based upon a 2 second tolerance for observer agreement on the start and finish of described behaviours.

Experiment 3: One experimenter viewed all video-tapes and two observers viewed 27% of tapes to determine percentage agreement levels for inter-rater reliability(IRR)(Sackett, 1978). Feeding behaviours were analyzed by 2-way within-subjects analysis of variance supplemented by tests of simple main effects and planned comparisons.

2.4 RESULTS

2.4.1 Experiments 1 and 2:

Figures 2.3 and 2.4 show mean per cent agreement between observers in the laboratory(n=15) and in the ward setting(n=6). Bars represent the range of percent agreement. Histograms represent percent agreement for observers, with ranges, as they move from viewing video in a laboratory setting to live ward-based recording without video. Figure 2.3 shows mean overall percent agreement levels and Figure 2.4 shows mean agreement levels for the start and finish of acts of biting, chewing and manipulation of food. Observer agreement levels were the same when observers made the ward based observation as in the Occupational therapy setting(Table 2.2) . The discrete act of biting produced the highest agreement scores between observers in both video and live settings. Per cent agreement for the start of biting in the video and live vs. video conditions were 88% and 90.9% respectively; agreement on the finish of biting was 87% and 87.7%. In the live conditions agreement levels were higher: 96% and 90% for the start of biting and 97% and 89% for the finish. Agreement scores for the start and finish of all behaviours were over 80% with the exception of the finish of manipulation and chewing.

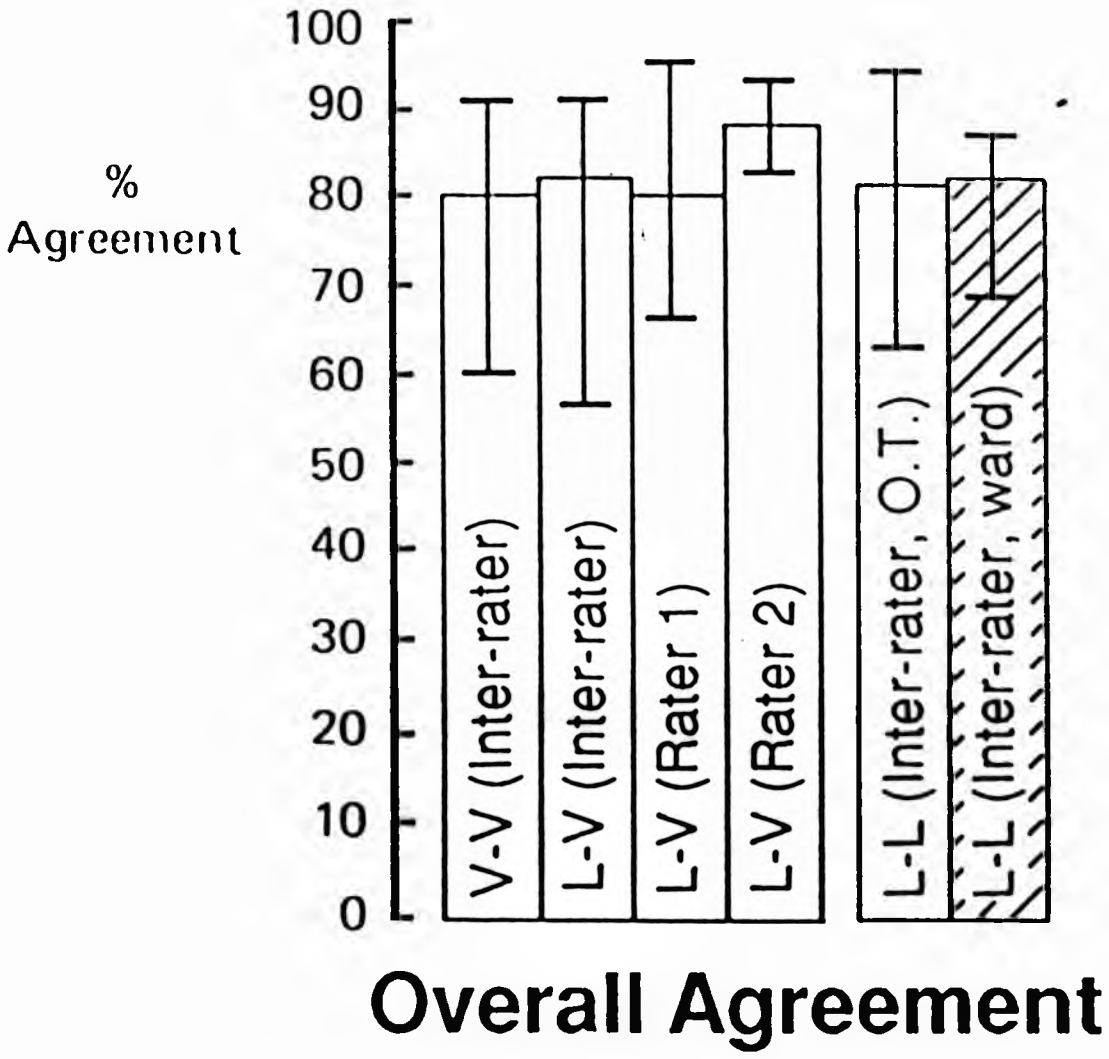


Fig 2.3 Mean percent overall agreement (2 observers). Bars represent range of percent agreement.

Reading from left to right histograms show progression from two observers viewing a video twice (V-V); through two observers making a live observation and later viewing the video, called live versus Video (L-V); (L-V) with rater number given in brackets, refers to within rater agreement (observer test-re-test reliability. (L-L) refers to two observers making live observations; and the final histogram on the right represents the field based live observations on the ward (L-L). (L-L) is hatched to indicate the final setting of a live environment

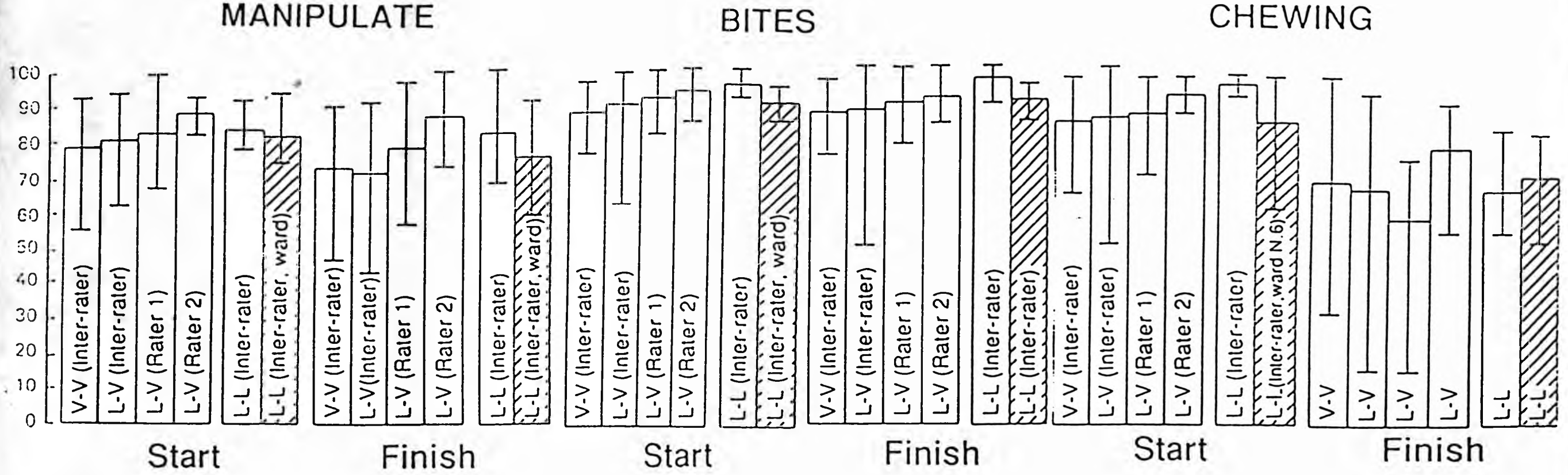


Fig 2.4 Mean percent agreement for the start and finish of the behaviours of manipulating, biting and chewing. Bars represent ranges of percent agreement

Table 2.2 Reliability of observations of eating behaviour[1,2,3,4]

	Overall	Manipulation		Biting		Chewing	
Method[3]		**Start**	**Finish**	**Start**	**Finish**	**Start**	**Finish**
V-V[1]	.80(2.4)	.79(2.9)	.73(3.5)	.88(2.0)	.87(1.8)	.85(2.8)	.68(5.3)
L-V	82(2.8)	81(2.9)	72(3.8)	91(3.0)	88(3.8)	85(3.9)	65(5.4)
L-L(lab)	81(4.2)	85(1.7)	82(4.1)	96(0.8)	97(0.6)	95(0.7)	65(3.8)
L-L(ward)	82(2.6)	82(2.8)	75(5.1)	90(1.5)	89(1.7)	84(5.4)	69(4.5)
V-V[2]	92(0.5)	84(2.1)	76(2.5)	98(0.6)	97(0.6)	92(2.0)	75(3.2)

1 The first four sets of values are mean(with s.d.) of the kappa statistic for overall agreement between the two observers, and for the start and finish of individual behavious, from the preliminary validation study.

2 The final set of figures show percentage agreement between the observers(mean and s.d.) in the pharmacological study.

3 Inter-rater reliability for live versus live(L-L) and video versus video(V-V) sessions; and intra-rater reliability between live and video sessions(L-V).

4 Laboratory setting, n=15; ward setting, n=6.

Observer agreement on the finish of chewing in video and live conditions ranged from 65% to 68%; agreement scores on the finish of manipulation ranged from 71.8% to 82% (Fig 2.4).

Observed differences between patients and controls: Fifteen subjects(8 control and 7 seven patients) formed the subject population of the laboratory based observations. Differences in eating behaviour between the two populations are shown in Fig 2.5. Figures are the means and standard deviations. Patients took more bites than controls which tended towards significance[$t=-2.1$; $p<0.06$, significant at 2.3), although their meal course time was slightly shorter (Fig 2.5). Control subjects had a longer inter-bite interval, possibly attributable to higher levels of conversation absent from the predominantly schizophrenic patient population. In the initial pilot study, not reported here, control subjects spent approximately 28% of meal-time in conversation compared to 1% in the patient population (Mc Guirk, 1985); there was significant subject variability due to differing levels of conversation.

2.4.2 Experiment 3

2.4.2.1 Food intake: The effects of amphetamine and fenfluramine on food intake and eating behaviour are summarized in Table 2.3, and the statistical analysis of these data are summarized in Table 2.4. Both amphetamine and d-fenfluramine significantly reduced food intake(g) and energy intake (kj). The amphetamine/fenfluramine interaction terms were non-significant, indicating that the effects of amphetamine and d-fenfluramine were additive(Table 2.3, see Table 2.4 and note). L-fenfluramine was essentially inactive. The effect of l-fenfluramine on food intake was non-significant; and while l-fenfluramine caused an 8%

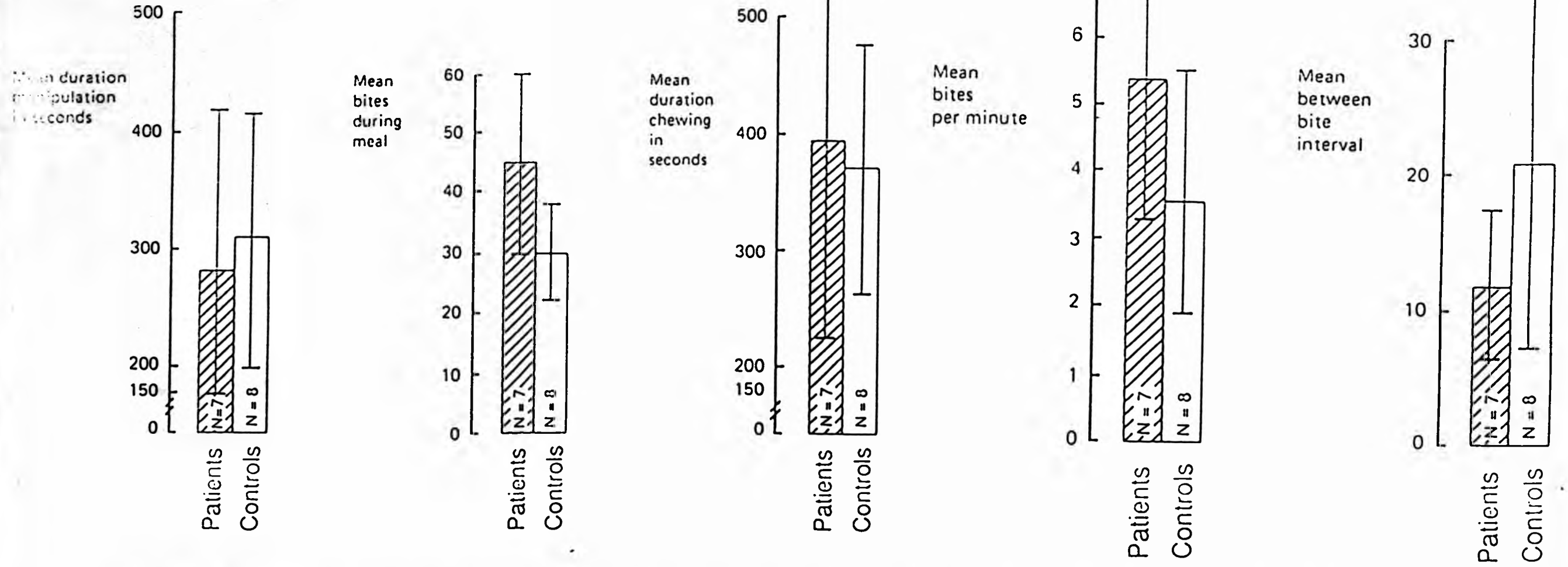


Fig 2.5 Differences observed between subjects and patients during the observation methodology development

Table 2.3 Effects of amphetamine and fenfluramine on eating behaviour[1,2]

	PLACEBO			AMPHETAMINE		
	PLC	L-FF	d-FF	PLC	L-FF	d-FF
Intake:						
Grams	583(52)	556(49)	494(44)**	452(56)***	434(36)	402(58)
kjoules	4644(453)	4251(387)	3908(431)**	3492(439)**	3221(395)	2985(486)
Duration(sec):						
Total meal	1433(74)	1343(69)	1342(74)	1197(96)*	1101(103)	1155(138)
Manipulation	1025(60)	952(55)	941(56)	819(66)**	790(82)	815(93)
chewing	890(43)	852(44)	826(31)	727(62)*	662(59)	794(76)
Bites(number)	72(6)	68(5)	63(5)	58(6)*	59(5)	53(5)
Eating rate:						
Local(g/min)	39.5(3.7)	40.2(0.9)	36.2(3.3)*	37.6(3.4)	40.4(2.5)	31(3.3)
Local(ki/min)	318(34)	309(31)	275(30)*	288(28)	300(25)	277(34)

1 Values are means(with s.d.). PLC=placebo; AMP=d-amphetamine; L-FF=fenfluramine; d-FF=d-fenfluramine.

2 Significance levels are shown for differences from the PLC/PLC condition for AMP alone(simple main effect) and for each of the FF isomers (pairwise comparisons, given a significant simple main effect). Significance levels with respect to PLC are not shown for the AMP/FF combinations: these comparisons are not possible within a factorial design.

*=$p<0.05$;

**=$p<0.01$; ***=$p<0.001$.

Table 2.4 F-values for analysis of energy intake and feeding behaviour[1]

Treatment	L-FF	d-FF	AMP	A X F
Degrees of freedom	(1,21)	(1,21)	(1,11)	(2.22)
Intake:				
Grams	1.2	11.3**	20.9***	0.5
Kioules	4.9*	17.2***	23.0***	0.2
Duration:				
Total meal	3.1	1.6	10.7**	0.1
Manipulation	6.2*	1.8	13.6**	0.8
Chewing	2.6	0.0	6.9*	2.2
Bites	0.2	4.2	6.3*	0.5
Eating rate:				
Local(g/min)	1.1	8.0**	1.4	2.5
Local(kj/min)	0.0	22.7***	3.6	1.3

1 The table shows F-values for main effects of drugs on the various measures. Values for amphetamine (AMP) are the main effect of amphetamine; values for l-fenfluramine (L-FF) and d-fenfluramine (d-FF) are pairwise comparisons of l-FF and d-FF versus placebo within the fenfluramine main effect; A X F is the amphetamine/fenfluramine interaction term. *=$p<0.05$; **=$p<0.01$; ***=$p<0.001$.

decrease in energy intake, which was marginally significant in the overall analysis, comparisons of l-fenfluramine with placebo[F(1,44)=2.9] and of the l-fenfluramine/amphetamine combination with amphetamine alone [F(1,44)=1.4] did not show significant differences.

2.4.2.2 Microstructural analysis: Microstructural analysis of eating behaviour showed that amphetamine and d-fenfluramine reduced food intake by different and independent behavioural mechanisms. Amphetamine significantly decreased both the total meal duration, and that of all of the individual behaviours; the time spent manipulating food, number of bites and chewing time(Tables 2.3/.4). However, d-fenfluramine had no significant effect on any duration measure, and the amphetamine/fenfluramine interaction terms were also non-significant.

By contrast, d-fenfluramine reduced the rate of eating. The measures presented in Table 2.3 are the local eating rates(total intake or total energy divided by chewing duration), which describe the rate at which food is disposed of once inside the mouth; both measures were significantly decreased by d-fenfluramine(Table 2.4). Howèver, amphetamine had no significant effect on either rate measure, and the amphetamine/fenfluramine interaction terms were again non-significant.

The only significant effect of l-fenfluramine on the behavioural measures was a 9% reduction in the time spent manipulating food. However, this was derived from a non-significant main effect [F(2,22)=3.1], and comparisions of l-fenfluramine with placebo [F(1,44)=2.0] and of the l-fenfluramine/amphetamine combination with amphetamine alone [F(1.44)=3.0] were also non-significant. A number of behavioural

parameters were unaffected by any of the three drugs, including the latency to commence eating(time to first bite; overall mean = 125 seconds) the rate at which food was loaded into the mouth(total intake/manipulation time: overall mean = 32.2 g/min), and the size of each bite(total intake/number of bites: overall mean = 7.8 g/bite).

2.5 DISCUSSION

2.5.1 Methodology development:

The aim of this study was to develop an observational methodology specific to the recording of feeding behaviour and suitable for both laboratory and field settings for a variety of subject populations. It was a further aim to demonstrate acceptable levels of intra- and inter-observer reliability in both settings. We demonstrated that this method of behavioural observation was suitable for an environment as unpredictable as an acute psychiatric ward where experimental work with psychiatric patients is acknowledged as being a difficult exercise (Hutt & Hutt, 1970). With a psychiatric patient population it is more common to use subjective, e.g. visual analogue scales (Robinson et al., 1975), than objective measures.

While the pharmacological study was carried out in the laboratory the initial methodology was established and tested in the less controlled environment of a psychiatric ward and therefore is suitable for recording behaviour in a field setting (cf Stunkard & Kaplan, 1977; Foltin et al., 1990). Inter-rater reliabilities were constant irrespective of whether the data were derived from observations of pre-recorded laboratory videotapes, from live observations in the laboratory, or from live observations in the dining room of a busy hospital ward. The absence of a decrease in the reliability of live observations, relative to video, is of particular importance, since there are many non-laboratory situations in which a video camera would be unacceptably obstrusive but a small lap-top computer can pass unnoticed.

Martin & Bateson(1988) raise the question of feasibility of observational methodology as a separate consideration to reliability and validity. In effect, observers need to consider the practical application of their methodology, is it a worthwhile experimental exercise; are we likely to detect changes from which our knowledge of an area is(even marginally) increased? The present results suggest that it is possible to detect differences in behavioural status within or between groups using this observation methodology.

In this study, observer agreement levels were not consistently uniform. There are a variety of reasons why observers may not agree(assuming that the training period and behavioural taxonomy are not at fault with respect to identification of a behaviour); protocol alignment(starting together, Sellitiz et al, 1976), or inconsistencies in volume and frequencies of behaviour. Caro et al (1979) report that changes in reliability of observation will occur as the frequency of behaviour increases, or conversely, decreases to very low levels. In this study we found that with simultaneous occuring behaviours e.g. manipulating and chewing, inter-rater reliability on the finish of chewing decreased. Of these two feeding behaviours(manipulating and chewing) manipulating food which involves hand movements, appeared to be more"successful" at drawing attention. The effect of attentional competition was to decrease observation efficiency.

2.5.2 Pharmacological study

The pharmacological data confirm d-fenfluramine as the active isomer, in humans as in animals (Garattini et al., 1988). Although the use of single drug dose limits the generality of these conclusions, these results also represent the first demonstration that the anorectic effects of d-fenfluramine and d-amphetamine appear additive. The analysis of variance interaction terms were non-significant.

However, it is clear from the behavioural analysis that these two agents achieve their anorectic effect by different behavioural mechanisms. The results confirm the earlier report of Rogers and Blundell (1979), using racemic dl-fenfluramine, that the anorectic effect of d-fenfluramine is mediated by a decrease in eating rate, whereas the anorectic efffect of d-amphetamine is mediated by a decrease in eating time, exactly as observed in animal studies(see Introduction). Both of these effects were present following co-administration of d-amphetamine and d-fenfluramine, as indicated by the absence of statistically singificant interactions in any of the analysis of variance. Although most of the effects of d-amphetamine or d-fenfluramine alone on the structure of eating behaviour were only significant at the $p<0.05$ level, all were exactly as predicted a priori on the basis of the animal literature(see Introduction) and the results of Rogers and Blundell (1979). That differences in the behavioural mechanisms underlying d-amphetamine and

d-fenfluramine anorexia were apparent in a subject group of healthy males in a varied free-choice feeding condition demonstrates the potency of these effects.

These results are relevant to the theoretical interpretation of fenfluramine anorexia. On the basis of observations studies of postprandial behaviour in rats, it has been hypothesized that fenfluramine suppresses food intake by hastening the onset of postprandial satiety (Thurlby et al., 1983; Blundell, 1986; Kirkham & Blundell, 1986; Blundell & Hill, 1987b). However, other studies of postprandial behaviour have produced data incompatible with the satiety hypothesis (Neill & Cooper, 1988; Montgomery & Willner, 1988; Willner et al., 1990). The results of the present study are consistent with previous work in showing that in human subjects, d-fenfluramine reduces the rate of eating, but does not decrease the duration of the meal (Rogers & Blundell, 1979). The same pattern of effects was observed in animal studies (Blundell et al, 1979: Cooper & Francis, 1979; Blundell & McArthur, 1981; Burton et al., 1981; Blundell, 1986; Foltin & Fischman, 1988), provided that a solid food diet is used (Montgomery & Willner, 1988).

The satiety hypothesis predicts strongly that fenfluramine should hasten the cessation of eating, as has been observed, for example, with the 5-HT re-uptake inhibitor fluoxetine (Clifton et al., 1989; Willner et al., 1990). The failure of fenfluramine to reduce meal duration argues against a potentiation of intra-meal satiety as the mechanism of fenfluramine anorexia in humans, and suggests that some other process may be responsible. uch as a subtle motor impairment (Montgomery & Willner, 1988; Fletcher, 1988b), a decrease in food palatability (Cooper & Neill, 1987; Samanin, 1989). We return to this question in the animal studies in Chapter 5 and Chapter 6.

In a chapter entitled "The Value and Validity of Laboratory Studies of Human Eating Behavior", Kissileff (1988) concludes that 25 years of laboratory research has confirmed for him the complexity of control mechanisms in human feeding, and the necessity of exercising constraint when tempted to generalize from one set of experimental results to another. Yet, like Rolls & Hetherington (1990), similarly experienced researchers, he is convinced of the value of laboratory-based research as an effective tool to delineate both functional and dysfunctional factors in human feeding.

CHAPTER 3

ANORECTIC ACTION OF FLUOXETINE IN NORMAL VOLUNTEERS

3.1 INTRODUCTION

There has been an increasingly active research focus in recent years, on the effects of serotonergic agents on food intake and body weight. This interest is motivated both by scientific and practical applications, with some degree of overlap. Alteration of food intake and body weight in animal experiments generate hypotheses on the role of serotonin in feeding (Blundell, 1977). The general practitioner(G.P), sitting opposite a markedly obese patient, may be more concerned with the ability of a pharmacological agent to reduce weight without unpleasant side-effects and, equally as important, to hold no abuse potential (Silverstone, 1982; Garrow, 1988).

Serotonergic agents which demonstrate specificity, such as fluoxetine (Fuller & Wong, 1977, Fuller, 1982) generate focussed experimental questions concerning their action and possible therapeutic use. Both this chapter and the next attempt to examine the activity of fluoxetine, a specific 5-HT re-uptake inhibitor in both normal weight and obese human subjects. Our initial enquiry is centered on the activity of fluoxetine in normal weight volunteers.

Central 5-hydroxytryptamine (5-HT) pathways have been implicated in the regulation of feeding behavior (Blundell,1977; Blundell, 1984) and in the pathogenesis of mood disorders (Asberg-Wistedt, 1989). Factors which influence 5-HT neurotransmission can affect mood, appetite and food intake (Goodall & Silverstone, 1988). Fluoxetine (FXT) is a highly selective pre-synaptic 5-HT re-uptake inhibitor (Fuller, 1982) which specifically inhibits 5-HT uptake in rat brain synaptosomes (Fuller & Wong, 1977). In clinical trials it has been shown to be an effective antidepressant (Cohn & Wilcox, 1985; Lader, 1988; Noguera et al., 1991) which does not cause weight gain (Chouinard, 1985). Overweight patients lose weight on FXT, an effect reported to be related to initial weight with little or no weight loss for normal weight and underweight patients (Cooper, 1988; Orzack et al., 1990).

When given to rats, FXT suppresses food intake (Goudie et al.,1976) particularly of carbohydrate (Wurtman & Wurtman, 1977; Kim & Wurtman, 1988). In obese subjects FXT promotes weight loss (Ferguson & Feighner, 1987; Levine et al., 1989; Pijl et al., 1991) and has been investigated as a possible treatment for bulimia (Freeman & Hampson, 1987; Wilcox, 1990). Thus FXT would appear to have anorectic properties, although this has not presently been tested directly in normal volunteers.

The present study was designed to examine the effects on appetite, food intake and body weight of FXT in normal subjects. Normal weight male subjects were chosen for this study as male subjects were thought less likely than females to confound the results by being overconcerned with food intake and body weight (Wardle & Beales, 1986). Our hypothesis was

that the weight loss reported in the clinical literature would also be present in non-depressed and non-obese subjects and therefore could be ascribed to the effect of fluoxetine as opposed to the effects of either depression (in patients) or linked to strong initial levels of motivation to reduce food intake, as possibly present in obese subjects. The likelihood of changes in subjective state in response to FXT in normal subjects was, in addition, a focus of examination.

3.2. METHODS

3.2.1. Subjects:

Eleven healthy male volunteers, age 18-40 years with no history of psychiatric illness participated. Subjects were recruited via hospital notice-board posters and were paid expenses on completion. Their mean weight was 73.1kg (range 58.4 - 95.3kg), their mean body mass index(BMI) was 23.5. Subjects underwent a medical examination including an electrocardiogram and haematology and biochemistry screening. The study was approved by the local ethical committee and all subjects gave written informed consent. Twelve subjects were recruited originally for this trial, but one subject had a car accident while on placebo; the partially completed trial data for this subject was excluded.

3.2.2. Design:

The trial was of a randomised, double-blind, crossover design. The pharmacokinetics of FXT are such that it takes approximately two to three weeks to reach plasma steady state; it has a half-life of 1 - 3 days and NorFXT the active metabolite, has a half-life of 7 - 15 days (Lemberger, 1988). Each subject was given either 60mg of FXT or matching placebo daily for two weeks followed by a minimum one month drug free period, after which the alternative treatment was given for a further two weeks. Subjects attended on Day 1, 8 and 15 of each treatment phase for detailed measurements of appetite, food intake and body weight.

3.2.3 Measurements

The measurements taken were of: body weight, food intake, subjective states, plasma levels of fluoxetine and norfluoxetine, pupil diameter, blood pressure and pulse.

Food intake was measured using a four channel automated solid food dispenser(AFD)(Silverstone, et al., 1980). Each of the four channels contained a different food with the choice of foods presented to each subject being based on the answers given to a food preference questionnaire completed before the trial began. This questionnaire contained twenty-two food items allowing a wide choice among sandwiches, salad, fruit, sweet desserts and cake items. On the food preference questionnaire subjects rated foods from 1, - highly preferred through to 5 - intensely disliked. Foods offered to subjects were taken, if possible, from the middle preference area numbered 2, 3 and 4. The foods on offer represented the equivalent of a cafeteria type snack meal; a typical example being beef sandwiches, small sausage rolls, tomatoes, and small cartons of custard dessert. Table 3.1 shows the energy content per 100g, the energy content per average portion and the average weight per portion of foods finally offered to subjects. Foods used in the experiment were purchased from a major food chain store and nutritional information on items was available both on wrapping and on information sheets supplied by the marketing department. Table 3.2 gives some examples of the type of foods choosen by three subjects for their lunch meal.

Table 3.1 Foods offered in the Automated Food Dispenser(AFD)

	Number of subjects offered this choice	kj(kc) per 100g	Kj(kc) per average portion	Wt. per average portion
Roast Beef sandwich	5	624(149)	249(60)	40g
Chicken sandwich	2	875(209)	350(84)	40g
Prawn sandwich	1	1046(250)	314(75)	30g
Salmon sandwich	3	854(204)	256(61)	30g
Sausage roll	5	1712(409)	548(131)	32g
Slice of Quiche	1	1256(300)	565(135)	45g
Chicken leg	5	904(216)	588(140)	65g
Tomato	7	59(14)	35(8)	60g
Raw carrot	1	42(10)	10(3)	25g
Cucumber	3	42(10)	19(5)	35g
Apple(fruit)	1	147(35)	168(40)	115g
Orange(fruit)	1	167(40)	75(18)	45g
Trifle dessert	2	691(165)	877(210)	127g
Chocolate dessert	5	532(127)	553(132)	104g
Apple tart	2	1352(323)	757(181)	56g

Table 3.2 Foods available to three subjects from four channels of the Automated Food Dispenser.

	CHANNELS			
	1	2	3	4
Subject				
6	sandwich(beef)	sausage roll	cucumber	apple tart
7	" (chicken)	sausage roll	tomato	dessert(choc)
9	" (salmon)	drum-sticks	tomato	trifle

Subjective measurements: Visual Analogue Scales (VAS) for hunger, arousal, mood, thirst, and satiety were completed hourly. Visual Analogue Scales comprise of 100mm lines with subjective statements at either end; "I do not feel at all hungry"- I feel as hungry as I have ever felt"; "I feel very empty"- "I feel extremely full". Each hourly VAS scale for the above were presented on one page, previous scales were not visible. Ratings for nausea and gastric discomfort were presented at the begining and end of the experimental session. VAS have been shown to be reliable and valid for quantifying a variety of subjective states including hunger (Silverstone & Stunkard, 1968; Goodall et al,. 1987).

Other measurements: Blood pressure, pulse, pupil diameter and body weight in indoor clothing were measured on assessment days. Fluid intake was measured by determining how much water or low calorie cordial subjects consumed from a measuring jug which was available throughout the experimental day. A blood sample(5-10ml) was taken for plasma levels of FXT and NorFXT. Plasma levels were measured by gas chromatography with electron-capture detection (Nash et al., 1982).

3.2.4 Procedure

Before the trial proper, subjects spent two hours becoming familiar with the experimental surroundings, including using the VAS and the automated food dispenser. On day 1, 8 and 15 of each two week experimental phase subjects arrived at the Unit at 0850h having fasted from 2100h the previous evening. Subjects were weighed, and their blood pressure and pulse recorded. At 0900h subjects were given that day's capsule to take in the presence of the experimenter. Hourly VAS were completed throughout the day until 1500h. At 1200h a 10ml sample of blood was taken and pupil diameter measured using a plastic pupil gauge. Subjects spent the morning in a lounge with comfortable chairs where, according to inclination, they could watch television, read, listen to the radio, or do paper-work. From 1300h to 1500h subjects had access to the AFD which was in a separate room with a table and chairs. Eating implements were provided. Aliquots of food were obtained by pressing one the four buttons on the AFD. This activated a pen recorder such that the time and nature of the food selected was recorded. Subjects were asked to select one item of food at a time and finish eating that before replenishing their plates. At 1500h after a brief interview to determine

the incidence of any adverse experiences, subjects were given enough medication to last until the next visit, with instructions to take a capsule with fluid at 0900h daily from day 2 to 7, and day 9 to 14 of each treatment phase. They were then free to go.

3.2.5 Data analysis

Food intake: Cumulative food intake in kilojoules(kJ) was measured at ten minute intervals throughout the 2 hour feeding period on day 1, 8 and 15 of each treatment phase. As some subjects ate considerably more than others in the 10 minute intervals the data did not have a normal distribution. Wilcoxon tests, appropriate for such a distribution, were performed at specific intervals to test for differences in intake between drug and placebo conditions. Analysis was carried out at 10, 20, 30, 40, 50, 60, 70, 90, and 120 minutes. A Wilcoxon test was performed on the difference in the area under the curve for drug and placebo conditions, after transformation of the data by trapezoidal rule.

Subjective VAS ratings: Non-parametric statistics were used as subjective ratings did not adhere to a normal distribution. Hourly changes from baseline in VAS ratings of hunger, mood, arousal, satiety and thirst were calculated, as were the change in VAS ratings from 0900h to 1500h in nausea and gastric discomfort. Wilcoxon tests were performed on the differences in the change from baseline between placebo and drug conditions. Differences in total area under the curve for placebo and drug conditions were also analyzed, after transformation by trapezoidal rule, and a Wilcoxon test was performed on the differences between the two conditions. To allow for the possibility that initial starting

scores on the subjective rating scales may have been different, a Wilcoxon test was performed on the incremental area under the curve for both treatment conditions.

Body weight: Having confirmed that there was no significant difference in the starting weight in each phase, analysis of variance(ANOVA) was used with subject, treatment and time(day 1, 8 and 15) being the main factors.

Correlation between food intake/plasma level of FXT and between body weight/plasma level of FXT: Pearson's correlation coefficients were calculated between food intake and plasma levels of FXT at 10, 20, 30 and 120 minutes after access to the AFD. Correlations between body weight and mean plasma levels of FXT/NorFXT were calculated combining all measurement days, and on days 8 and 15 separately. Changes in body weight were correlated with changes in plasma levels of FXT/NorFXT and additionally percentage changes in body weight were correlated with percentage changes in plasma levels of FXT/NorFXT.

3.3 RESULTS

3.3.1 Body weight:

At the end of the two weeks' treatment, the mean weight change on placebo was a gain of 0.15kg(± 0.35kg) while treatment with FXT resulted in a mean weight loss of 1.07kg(±0.25kg). Nine of the eleven subjects(81%) lost weight during FXT treatment. Of the two remaining subjects, one maintained his weight on FXT while gaining 0.3kg on placebo; the other gained 0.25kg on FXT and 0.4kg on placebo. **Fig 3.1** shows the mean weight changes(kg) observed during the first and second week of each treatment phase. Analysis of Variance(ANOVA) yielded a drug-time interaction [$F(2,10)=3.78$, $p<0.05$].

3.3.2 Food intake and fluid intake:

There was a modest decrease in food intake following FXT on days 1($W=7.0$, $p<.0.04$) and 8($W=7.0$, $p<0.02$), which reached significance at one time point on each day(**Fig 3.2 Table 3.3**). There was no difference between the two groups on Day 15.

Table 3.3 Median, minimum and maximum kilojoule(kj) intake at lunch

		DAY 1	DAY 8	DAY 15
PLACEBO	Median	5400	4877	4914
	min	3031	3658	3043
	max	8435	6291	7091
FLUOXETINE	Median	4550*	4261*	5048
	min	2126	2332	2068
	max	6861	6815	7464

*=p<0.04(day 1), *=p<0.02(day 8)

Subjects were less thirsty on FXT at hourly measured time points on days 1, 8 and 15, significant differences in change scores from 0900h to 1500h were present on day 1[W=9.0, p<0.03], and significant differences in incremental area under the curve between drug and placebo were present on day 8[W=8.0, p<0.05](**Fig 3.3**). An increase in fluid intake was seen on day 8(**Fig 3.4**), but this was mainly due to one subject drinking 1700ml, and the difference was non-significant[W=33.5, p=-0.575].

3.3.3 Subjective ratings

a) Feelings of hunger: No significant differences were found between FXT and placebo at hourly time points on Day 1(**Fig 3.5**). On Day 8 subjects

tended to be less hungry on FXT [W=53.5, p<0.07], while on Day 15 subjects started the experimental day more hungry on FXT[W=42.0, p<0.02], scores on hunger ratings were similar by lunch-time(**Table 3.4**).

b) Ratings of fulness: There were no significant differences between FXT and placebo in the subjective ratings of fulness [day 1; W=14.0, p<0.1; day 8; W=19.0, p<0.23; day 15; W=25.0, p<0.81]. Change scores (**Fig 3.6**), and actual ratings are shown (**Table 3.4**)

c) Ratings of mood: Subjects did not show discernable changes in ratings of mood in response to two weeks treatment with FXT. **Table 3.5** shows hourly median ratings throughout the experimental days.

d) Arousal, Nausea and Gastric discomfort: With respect to arousal scores, subjects on FXT rated themselves as more drowsy during the morning on experimental day 8 [W=7.5, p<0.026] and day 15 [W=43.5, p<0.01]. This drowsiness was not present at the pre-lunch rating at 1300h. Analyses of rating scores(change from 0900 to 1500h) for nausea and gastric discomfort on day 1, and 8 showed no significant differences between placebo and FXT treatment. Subjects taking FXT started day 15 feeling more gastric discomfort on FXT compared to placebo [W=2.0, p<0.03]. Change scores from 0900h to 1500h were not significantly differenct from placebo for either nausea [W=8.0, p<0.35] or gastric discomfort [W=27.0, p<0.23].

Table 3.4 Hourly VAS ratings for hunger and (below) fulness

	HOURS					
	0900h	1000h	1100h	1200h	1300h	1400h
Placebo:						
Day 1 median	65.0	70.0	71.0	79.0	80.0	11.0
Day 8 median	56.0	63.0	70.0	73.0	80.0	14.0
Day 15 median	56.0	59.0	71.0	75.0	76.0	9.0
Fluoxetine:						
Day 1 median	61.0	74.0	78.0	78.0	82.0	19.0
Day 8 median	67.0	70.0	72.0	70.0	80.0	20.0
Day 15 median	61.0	68.0	70.0	78.0	80.0	16.0

Values are median absolute scores; 0=not hungry, 100=very hungry

Hourly VAS ratings for fulness during the experimental day

	0900h	1000h	1100h	1200h	1300h	1400h
Placebo:						
Day 1 median	28.0	22.0	20.0	12.0	18.0	80.0
Day 8 median	27.0	23.0	27.0	20.0	15.0	72.0
Day 15 median	31.0	26.0	26.0	15.0	17.0	74.0
Fluoxetine:						
Day 1 median	28.0	21.0	16.0	24.0	20.0	75.0
Day 8 median	32.0	26.0	29.0	18.0	20.0	71.0
Day 15 median	28.0	25.0	23.0	14.0	17.0	71.0

Values are median absolute scores; 0=very empty, 100=extremely full.

Table 3.5 Hourly VAS ratings for mood during the experimental day

	HOURS					
	0900h	1000h	1100h	1200h	1300h	1400h
Placebo:						
Day 1 median	52.0	57.0	54.0	57.0	51.0	58.0
Day 8 median	60.0	60.0	54.0	57.0	61.0	60.0
Day 15 median	54.4	48.5	59.0	54.0	60.0	51.0
Fluoxetine:						
Day 1 median	49.0	54.0	58.0	53.0	53.0	53.0
Day 8 median	55.0	53.0	59.0	57.0	58.0	59.0
Day 15 median	55.0	55.5	52.0	58.0	59.0	59.0

Values are median absolute scores; 0=very depressed, 100=very elated.

3.3.4 Plasma levels

Mean plasma levels for FXT and NorFXT on Day 1, 8 and 15 are shown in **Figure 3.7**. Statistically significant negative correlations were found between food intake and FXT plasma levels on Day 1 at 20 minutes ($r=-0.6$), at 30 minutes ($r=-0.7$), at 60 minutes ($r=-0.8$) and at 120 minutes ($r=-0.8$) [$t=-4.18$, $p<0.01$]. On Day 1 plasma levels of FXT were low, mean 23.4ng/ml,range 9 - 54ng/ml. When measurements for Day 1, 8 and 15 were combined the correlation disappeared as plasma levels of FXT increased.

Plasma levels of FXT/norFXT and body weight: Mean plasma levels of FXT/NorFXT tended to correlate inversely with body weight for all three measurement days. The low correlation on Day 1 ($r=-0.2$) reflects the low plasma levels that day. Correlations between plasma levels of FXT and body weight on Days 8 and 15 approach significance($r= -0.59$) for both days, with a lower body weight tending to be associated with a higher plasma level of FXT. There was also a trend towards a decrease in mean body weight to be associated with an increase in mean plasma levels of FXT on day 8($r=-0.39$). Percentage mean body weight change and percentage mean plasma levels of FXT also showed a slight negative correlation on Day 8 ($r=-0.31$) and Day 15($r=-0.38$).

3.3.5 Blood pressure, pulse and pupil diameter

No changes in either systolic or diastolic blood pressure were observed. Treatment with FXT resulted in a reduction in pulse rate of approximately three beats per minute, this was present on day 8 [$W=3.0$, $p<0.04$] and

day 15 [W=8.0, p<0.05]. Subjects taking FXT showed an increase in pupil size of approximately 1mm by the end of treatment(Fig 3.8). This difference from placebo was significant on day 8 [W=56.5, p<0.04] and day 15 [W=44.5, p<0.01].

3.3.6 Adverse experiences

Table 3.6 gives a summary of adverse experiences reported by subjects at the end of experimental Days 8 and 15. Drowsiness was reported by the greatest number of subjects (4 on Day 8) compared to 2 subjects reporting drowsiness on Day 8 while on placebo. Stomach discomfort, reported by the next largest number of subjects(3) was reported on Day 8 and 15 of FXT treatment.

Table 3.6 Subjects self-reported adverse experiences during treatment

	PLACEBO			FLUOXETINE		
	DAY 1	DAY 8	DAY 15	DAY 1	DAY 8	DAY 15
Drowsiness		2			4	2
Headache		3				
Stomach discomfort		1	1		3	3
Anorexia		1	1		1	1
Concentration loss					1	1
Insominia					1	1
Tiredness						1
Reduced desire to eat CHO						1
Stiff jaw						1
Awakening early	1	2	1		1	1
Feeling low			1			
Anxiety					1	

3.4 DISCUSSION

The primary aim of this study was to quantify the putative anorectic activity of FXT in a normal, non-dieting population. For this purpose changes in subjective hunger, food intake and body weight were recorded on three occasions during two weeks treatment with FXT and with matching placebo.

The most robust finding was weight loss, in what was basically a non-overweight healthy population. Reductions in food intake on experimental days were present, although kilojoule intake on day 15 was too near placebo levels to be significantly different. The subjective VAS ratings show that while FXT reduces feelings of hunger on day 8 of treatment, it does not appear to effect this variable strongly. This might suggest that FXT does not influence weight primarily through affecting subjective hunger, a not too distressing finding considering the possibility that sensations of hunger may not always correlate to energy intake (Mattes, 1990).

While FXT showed an anorectic effect on Day 1 and Day 8 when subjects ate less and stopped eating sooner, by Day 15 of treatment, food intake had returned to placebo levels. Tolerance is not an appropriate explanation as weight loss accelerated in response to continued FXT treatment suggesting FXT is continuing to have an effect. A study of chronic fluoxetine treatment in rats, described in Chapter 5 supports this view.

Serotonergic drugs can act both centrally and peripherally (Carruba et al, 1986), and it is possible that fluoxetine may act in part, like fenfluramine, by slowing the rate of gastric emptying (Horowitz et

al., 1985) and reducing the frequency of some meals (Hill & Blundell, 1986a). A metabolic effect such as increased glucose transport into muscle (Turner et al., 1982) would contribute to weight loss and has been cited as a contributory cause for the weight loss in rats which continues after the anorectic effect of fenfluramine wears off (Burton et al., 1981). However we have no evidence to suggest that FXT was causing an increase in metabolic rate, and the lowered pulse rate would argue against such an explanation. Furthermore, FXT did not cause increased thermogenesis in a small number of mice (Yen et al., 1987).

FXT may be having a specific effect on food choice. In recent years the dynamics of 5-HT in macronutrient choice and dietary self selection has received considerable critical attention (Fernstrom, 1987;Blundell & Hill, 1987b; Wurtman, 1987; Leathwood, 1987a; Peters & Harpers, 1987). In animals, serotonergic drugs may selectively reduce CHO intake and spare or increase protein intake depending on the proffered diet (Wurtman & Wurtman, 1977; 1979). However research increasingly suggests that a selection of factors such as test-time within the light-dark cycle (Leibowitz et al.,1990; Luo & Li, 1990) and texture of offered foods (Booth & Baker, 1990) can exert influence on choice.

In humans, Wurtman et al (1985) have reported that d-fenfluramine which enhances 5-HT neurotransmission by increasing release from pre-synaptic neurones as well as blocking re-uptake (Garattini & Samanin, 1976), reduced intake of CHO but not of protein. However, this effect on CHO intake has been shown to be differential in that d-fenfluramine reduced nonsweet kilojoule intake, but did not have a suppressive effect on sweet foods (Goodall & Silverstone, 1988). While we did not examine in detail the preferential effect of FXT in reducing CHO-containing foods, it is

interesting to note that one subject reported a decreased urge to eat bread and other starchy foods while on FXT. This point warrants further examination.

On all experimental days, subjects felt less thirsty on FXT compared to placebo although fluid intake was not affected. One interpretation of this finding supports the role of learning and conditioned responses in feeding and drinking behaviour (Booth, 1981). Subjects drank approximately the same amount and would only alter intake if they became aware of physiological signals for dehydration. Alternatively, FXT may be exerting an unspecified physiological effect on the thirst mechanisms. The observed FXT-induced effects on subjectively reported thirst may be in keeping with the general "tonic suppression" effect of serotonin on selected brain functions, such as temperature, pain and feeding (Leathwood, 1987b).

This experiment has shown that FXT possesses weight altering and some anorectic properties as evident in a normal weight non-dieting male subject group. The next line of enquiry therefore centres on the activity of FXT in an obese group of subjects, including women, and with a more detailed analysis of food intake throughout the experimental period.

CHAPTER 4

FLUOXETINE IN OBESITY

4.1 INTRODUCTION

Some of the literature discussed so far strongly supports the contention that serotonin is one of the neurotransmitters implicated in both the modulation of food intake (Blundell, 1977) and affective states (Asberg-Wistedt, 1989) 1986) It is not surprising therefore, that the anti-depressant fluoxetine was found to cause weight loss in some depressed patients (Cohn & Wilcox, 1985; Harto et al., 1988). Corne & Hall, (1989) carried out a double-blind six week study on depressed outpatients who lost weight while taking FXT (-0.874kg); however, no mention is made of starting weight status in these patients. In the previous chapter, an experiment involving normal weight non-depressed male volunteers was described which found a greater reduction in food intake and body weight during two weeks' treatment with FXT compared to placebo (McGuirk & Silverstone, 1990). This experiment confirmed that subjects whose physiological and psychological status is free from the effects of a depressive episode respond to fluoxetine, with weight loss.

The "side effect" of weight loss during clinical testing spawned experiments designed to examine the possible anorexic action of this drug in obese subjects. Work so far suggests that FXT is more effective than placebo in helping patients lose weight (Ferguson & Feighner 1987; Levine et al., 1989 Pijl et al., 1991). The changes in body weight and reported changes in food intake and choice (Pijl et al., 1991) indicated

a possible selective effect of FXT on these variables. The "selective" influence of serotonergic drugs on food choice has been a focus of debate in recent years. In animal studies, some experiments have supported the view that modulation of 5-HT results in reduction of carbohydrate while the intake of protein is maintained (Wurtman & Wurtman, 1977; Kim & Wurtman), However, critics of this view point out that such findings are influenced by experimental settings and conclusions need to be set within this context (Blundell, 1983; Booth, 1987).

More recently, research has demonstrated that while food choice may be influenced by the modulation of 5-HT, the response is not a simplistic reduction(or non-reduction) in macronutrients. D-FF does not result in a homogenous overall reduction in carbohydrate, but is capable of reducing intake of non-sweet food, while having less of an effect on sweet food (Goodall & Silverstone, 1988). In addition, it is becoming clear that other factors, such as the texture of available foods can modify drug effects and food choice (Booth & Baker, 1990).

An obese population qualifies as a long-range target for drugs which have been shown to modify appetite and body weight. Charting the body weight and dietary response to fluoxetine should provide information to add to current knowledge of response systems in obese subjects. Hill & Blundell (1990) have shown that obese subjects respond, like lean subjects, to the potent effects of a d-FF/protein pre-load challenge and it is of interest to chart the effects of FXT in obese subjects, as they will be a target group for treatment.

Obese subjects taking part in the Pijl et al (1991) study did not receive any instructions to change their diet yet all subjects receiving FXT lost weight, reduced kj intake for both meal and intra-meal consumption and were reported to consume less carbohydrate during "snack" consumption. However, these findings were based upon records of diary intakes and were not based on objective measures of food intake.

The previous chapter established that fluoxetine has the capacity to reduce body weight in normal weight subjects, and consequently, it makes sense to focus on the effects of this drug in a possible target population - obese subjects. The present study was designed to address questions concerning weight, food intake and choice, mood status and feeding behaviour in obese subjects. Comparisons were made on the effects of one month treatment with fluoxetine to that of placebo in male and female subjects. The study uses objective measures of food intake and feeding behaviour established in Chapter 2.

4.2 METHODS

4.2.1 Subjects:

Twenty clinically obese subjects(10 men and 10 women) with a body mass index(BMI) over 30 were recruited through hospital staff notice boards. An initial comprehensive medical examination including E.C.G., biochemical and haematological tests was carried out. The mean weight of the whole group was 97.4kg(S.D. 11.9kg, range 78.5-121.5kg and the mean BMI 34(S.D. 2.5, range 30.9-38.8). The mean age was 33.4 years(S.D. 7.9 years, range 23-48 years). The mean BMI for the ten men and ten women was 34.1 and 33.9 respectively. All subjects gave written informed consent and the study had the approval of the local ethical committee.

4.2.2 Design:

The trial was of a randomized, double-blind cross-over design. Subjects received either 60mg FXT or matching placebo for a one month period, followed by a minimum of one month wash-out, followed by the alternative treatment.

4.2.3 Measurements:

These included objective measurements of food intake, choice and feeding behaviour, body weight, pulse, blood pressure, pupil diameter, plasma levels of FXT and assessment of the subjective

variables of hunger, satiety, mood, arousal, thirst, nausea and gastric discomfort, using visual analogue scales(VAS). Subjects were asked to keep daily diet diary records. Side effect profiles were compiled by interview at the end of each experimental day. Approximately one year after the trial had finished, subjects received a follow-up letter and stamped addressed envelope asking them to record their current weight in indoor clothing.

4.2.3.1 Food intake - experimental Unit

A choice of foods was available from a ten-channel automated food dispenser (AFD) adapted for experimental purposes and as described in chapter 2 (Fig 2.1). In the adjacent room, a micro-computer (Apricot F1, Apricot, Halesowen, West Midlands) recorded food intake by means of a programme (A Leach, Compular Ltd, Ewell, Surrey). Food composition tables (Paul & Southgate, 1978) and manufacturers information were used to determine energy and macronutrient content. The aim was to be able to offer subjects varied familiar food-stuffs from which they could choose either a variety of foods in small or large amounts (e.g. bread, meat or cheese, tomatoes and dessert) or foods such as crackers, cheese, chocolate biscuits, dessert (Fig 4.1).

Table 4.1 shows that foods offered were, for example non-sweet (cream crackers and hovis rolls) two foods in which carbohydrate had a higher percentage representation than other macronutrients(58.8% and 64.1% respectively). Two other non-sweet foods, chicken and ham, contained a high percentage of protein(67.8% and 71.1%) compared to other macronutrients. Flora is mainly fat(99.1%), the available cheddar

Fig 4.1

cheese also provided more fat(73.7%) than other macronutrients. The offered sweet foods, chocolate biscuits and strawberry desserts, contained both carbohydrate and fat, with a slight greater percentage of carbodyrate available in each (Table 4.1) Dessert apples(Golden Delicious) provided nearly all sweet carbohydrate(97%) while tomatoes provided 74.5% carbohydrate and 25.5% protein.

4.2.3.2 Food intake - diet diaries

Subjects were supplied with records to fill-in for daily intake other than at the experimental unit. Records were filled in every day for the duration of the trial in both conditions. Subjects were asked to supply information on amount e.g. teaspoons, cups, grams/ozs and supply brand names of convenience foods consumed. Diet diaries were examined(blind to condition) with a view to analyzing those which supplied adequate quantities and descriptions of foods. The diet diaries of ten subjects, six men and four women were considered fit for analysis in that amounts, such as grams, cupfuls, etc were supplied. Food manufacturers of brand name products were approached by letter with requests for nutritional information. Significant amounts of time were spent in supermarkets noting down nutritional information in notebooks. Asking subjects to bring in information on convenience foods they had consumed was considered unwise as this may have drawn undue attention to both energy and constitutent components of food.

The information from product manufacturers was used to build an additional data-base to a food analysis programme (Lifeline Nutritional Services Ltd). The data-base of the nutritional analysis program

"comp-eat" was based on nutritional information published in Paul & Southgate, 4th edition (1978). In case of subjects supplying no measured portion size, reference was made to Crawley (1988) where "Food Portion Sizes" guidelines are supplied.

Food intake for week one and week four of each phase was selected for analysis in order to determine initial onset of drug effect on food intake and to determine if the effect remained for the duration of the trial. While still blind to condition, information in the form of descriptions and quantities of foods consumed in each phase was analyzed for each day of the selected weeks. The summary print-out supplied information on total kj, grams and percentages of fat protein and carbohydrates. These summaries were later used for purposes of analysis once the trial was completed and subject codes broken.

4.2.3.3 Feeding behaviour

The observational methodology for feeding behaviour as described in chapter 2 was carried out for the duration of the trial. On each experimental day during the lunch meal, feeding behaviour was recorded using the portable Epson HX20 microcomputer and measurements of meal parameters taken as described. The meal was recorded via a discrete wall-mounted camera, onto video-tape, which was later viewed by the trained experimenter (Chapter 2, Figure 2.3). 12.5% of the video tapes were again viewed at a later date by two observers to establish levels of inter-rater reliability. 8.5% of tapes were also again viewed by the experimenter to determine test-re-test reliability (Table 4.2). Overall percentage levels of agreement were 88.3% and 88.4% respectively. The

recommended goal of two observers viewing 20% of behaviours (Sackett, 1978) could not be achieved: however, the two observers did view 20% of tapes over a period of years while the methodology was being established. As in the earlier studies (Ch 2), high levels of agreement were obtained for all of the individual means with the exception of the end of chewing episodes.

Table 4.2 Inter-rater and test-retest reliability: percent agreement levels

	MANIPULATE		BITE		CHEWING		OVERALL %
a) IRR	**start**	**finish**	**start**	**finish**	**start**	**finish**	
x(s.d)	84.9(11.9)	75.6(14.9)	96.5(2.8)	94.8(4.3)	86.8(12.1)	56.5(16.6)	88.3(5.2
b) Test Re-test							
x(s.d)	84.0(12.8)	67.7(22.5)	93.7(4.3)	91.7(4.7)	88.5(15.7)	52.8(22.2)	88.4(4.8

Values are: a) percent agreement between two observers viewing 12.5% of tapes
b) percent agreement for main observer on 8.5% of tapes.(reviewed)

4.2.3.4 Other measurements

Visual Analogue Scales(VAS) (100mm lines) were used to assess hunger(not at all hungry - hungry as I have ever felt); satiety(very empty - extremely full); mood(very depresed - very elated); arousal(very alert - very sleepy); thirst(not at all thirsty - as thirsty as I have ever felt); nausea(very nauseous - not at all nauseous) and gastric discomfort(do not feel any gastric discomfort - feel extreme gastric

discomfort). Body weight in indoor clothing, pulse, blood pressure and pupil diameter were recorded on experimental days. A 5-10ml fasting sample of blood was taken to assess plasma levels of FXT and NorFXT, which was measured by gas chromatography with electron-capture detection (Nash et al, 1982).

4.2.4 Procedure

Subjects underwent a familiarization session prior to the start of the trial which involved using the AFD and becoming aware of what an experimental day would involve(no drug was administered or blood taken during familiarization). During the trial proper subjects attended on days 1, 8, 15, 22 and 29 of each treatment phase, arriving at approximately 0845h having fasted from 2100h the previous evening. At 0900h a fasting blood sample(10ml) was taken, the medication administered, and weight, blood pressure and pulse recorded. VAS for hunger, satiety, mood, arousal, and thirst were completed hourly; VAS for nausea and gastric discomfort were completed at the begining and end of each experimental day. Pupil diameter was measured at 1200h using a simple pupil gauge. The subjects spent most of the experimental day in the library of the unit which had comfortable chairs, a radio and T.V.

Lunch was provided in a separate room containing the AFD. Subjects helped themselves to food from the ten-channel AFD by pressing their code number(previously given) and then removing the food from the machine. Subjects were asked to remove only the foods they wanted to eat immediately; they were permitted to return to the AFD for further food as often as required during the meal. The time allowed for lunch

was one hour, with subjects asked to spend a minimum of thirty minutes in the feeding room. If they had finished by then they could return to the library in the knowledge that they had further access to the AFD until the hour was up. At approximately 1430h, subjects completed the final VAS, were given a brief interview for the purpose of assessing the incidence of side effects, received the following weeks' medication, which they were requested to take each morning with water, and were supplied with new diet diary sheets. They then left the unit.

Recording of feeding behaviour: The observational methodology described in chapter 2 was employed to examine the effect of FXT on feeding behaviour. Video-recordings were taken via a discrete wall-mounted camera in the experimental laboratory and the behavioural recordings were made in the adjacent room using an Epson HX20 and monitor(Chapter 2, Fig 2.3). Feeding behaviours recorded included: latency to manipulate, latency to bite, duration of meal, duration of chewing and chewing rates. The observational methodology is described in detail in Chapter 2. Subjects were asked to allow the meal to be video-taped in order to build files of control values for a possible future study of appetite in depressed patients. It was made very clear to subjects that this was not a compulsory part of the trial and they were free to say no. All agreed to be filmed.

4.2.5 Data Treatment

Food intake-experimental days. Normal probability plots were drawn for kj data and all other variables. Using standard tables, total food intake kilojoules(kj), showed no significant departure from normality.

Tests for period/carry over effects proved negative. Data were analyzed by analysis of variance, supplemented by tests of simple main effects and planned comparisons. T-tests were performed on the difference in incremental area under the curve between drug and placebo conditions on this variable, and those described below.

Diet diary data: Data from the diet diaries was logged and analyzed as for the above.

Feeding behaviour: Observational data were tested for normality of distribution, following which analysis of variance was performed on all measures with the exception of latency to manipulate on which Wilcoxon tests were performed.

VAS ratings. T-tests were performed on the differences between the drug and placebo conditions both at selected time points and on the overall changes from 0900h to 1300h.

Body weight. Subjects who had received FXT as the first treatment, lost weight which was not regained by the start of their second treatment phase (placebo), consequently these subjects still sustained a treatment effect (Pocock, 1985). Weight data were therefore analysed as for a between subjects design in which the ten subjects who had fluoxetine first were compared to the ten subjects who had placebo first. Analysis of variance supplemented by tests of simple main effects and planned comparisions were carried out.

4.3 RESULTS

4.3.1 Body weight:

One month's treatment with fluoxetine resulted in all subjects losing weight; mean weight loss was -3.26kg(s.d.±2.1). Seven of the ten subjects in the placebo group also lost weight; the total mean weight loss during placebo treatment was -0.66kg (s.d.±1.38). The difference between the two treatments with respect to weight loss was -2.60kg(-2.98%). There were significant main effects of drug [$F(1,18)=10.94, p<0.005$]; days [$F(3,54)=8.04, p<0.005$] and drug x days interaction [$F(3,54)=5.27, p<0.005$]. Significant differences between treatment conditions were present on measurement days 15 [$F(1,72)=10.24, p<0.005$]; day 22 [$F(1,72)=12.17, p<0.005$] and 29 [$F(1,72)=15.45, p<0.005$](**Fig 4.2**). The difference between drug and placebo in incremental area under the curve was significant [$t=-3.26$, d.f.=18.0, $p<0.01$]. In addition, drug/placebo differences in area under the curve for percent body weight change were also significant [$t=-3.12$, d.f. 18.0, $p<0.01$]. There was a significant correlation (Pearson's correlation coefficient) between decreases in body weight throughout the treatment period and increases in plasma levels of FXT [$r=-0.725$, $t=-2.78$, $p<0.05$].

Analysis of weight change with respect to gender (n=5), showed greater weight loss for FXT treated male subjects (n=5) who lost -3.98kg (-3.69%) compared to the -2.175kg (-2.27%) lost by FXT treated female subjects. Weight loss in the placebo condition was -0.44kg (-0.44%) for

males, and -0.8kg (-0.93%) for females. A similar analysis with respect to percent body weight change between male and female subjects was non-significant.

Eleven subjects (55%), responded to the query on post-trial weight status. These comprized seven men and four women, all subjects had returned to approximate starting weight, except one woman who reported herself as 1.5kg below starting weight.

4.3.2 Food intake and choice:

Effects of FXT on total energy intake and on each of the three macronutrients in the test meal are shown in **Figures 4.3/4.4** and **Table 4.3.** FXT significantly reduced both overall kj intake [$F(1,19)=7.22$, $p<0.05$], and intake of all three macronutrients; fat [$F(1,19)=4.81$, $p<0.05$]; carbohydrate; [$F(1,19)=9.50$, $p<0.01$] and protein [$F(1,19)=4.91$, $p<0.05$]. None of the drug x day interactions were significant [maximum F-value found for total kj for fat, [$F(4,76)=1.14$, N.S]. However, tests of simple main effects showed that the reduction caused by FXT on day 1 was not significant; [maximum [$F(1,95)=1.46$, N.S]. Total energy intake was significantly reduced on day 8 [$F(1,95)=5.44$, $p<0.05$]; day 15 [$F(1,95)=5.54$, $p<0.05$] and day 22 [$F(1,95)=6.21$, $p<0.05$]. A t-test on the difference in incremental area under the curve between drug and placebo was non-significant [$t=-0.66$, $p<0.52$]. FXT caused significant reductions in total grams consumed relative to placebo, main drug effects [$F(1,19)=20.07$, $p<0.001$], with significant reduction present on all days with the exception of day 1.

Table 4.3 Total and macronutrient intake on experimental days

	Day 1	Day 8	Day 15	Day 22	Day 29
Total ki (x ± s.e)					
Placebo	3446(311)	3457(309)	3690(302)	3904(304)	3754(305)
Fluoxetine	3196(272)	2899(264)	3128(238)	3309(280)	3416(290)
Difference	-250(308)	-557(209)*	-562(221)*	-595(208)*	-338(249)
% reduction	-7.2%	-16.1%	-15.2%	-15.2%	-9.0%
Fat:					
Placebo	1470(177)	1537(158)	1659(162)	1773(145)	1694(150)
Fluoxetine	1437(139)	1291(134)	1391(131)	1503(148)	1501(147)
Difference	-33(165)	-246(100)*	-268(112)*	-270(98)*	-193(134)
% reduction	-2.2%	-16.0%	-16.2%	-15.2%	-11.4
Carbohydrate:					
Placebo	1283(108)	1283(105)	1396(116)	1440(112)	1375(113)
Fluoxetine	1204(102)	1115.3(100)	1170(78)	1210(101)	1224(112)
Difference	-79(94)	-167.7(81)	-226(92)**	-230(79)**	-151(90)
% reduction	-6.2%	-13.1%	-16.2%	-15.9%	-9.8%
Protein:					
Placebo	629(58)	652(61)	655(70)	698(76)	688(78)
Fluoxetine	557(47)	494(45)	568(49)	596(52)	597(47)
Difference	-69(65)	-158(40)**	-87(57)	-102(55)	-91(70)
	-11.4%	-24.2%	-13.2%	-14.6%	-13.2%

Values are means(±s.e.) with percentage reduction. *=p<0.05; **=p<0.01

Significant reductions were present in fat intake on day 8 [F(1,95)=3.9, p<0.05]; day 15 [F(1,95)=4.65, p<0.05]; and day 22 [F(1,95)=4.71, p<0.05]. CHO kj intake was significantly reduced on day 15 [F(1,95)=7.01, p<0.01] and day 22 [F(1,95)=7.22, p<0.01]; and approached significance on day 8 [F(1,95)=3.85, p<0.06]. Significant reduction in intake of protein kj was present on day 8 [F(1,95)=7.61, p<0.01]. **Table 4.3** shows the effects of FXT expressed as percent reduction from the control baseline. The mean reductions, calculated across the final four test days, were; fat, -14.7%; CHO, -13.8% and protein -16.3%. Intake of the three macronutrients, expressed as percent of total kj intake was reasonably consistent across both days and conditions(**Table 4.4**). Differences in incremental areas under the curve were non-significant [t=-1.12, p<0.28; t=-0.67, p<0.51; t=-0.64, p<0.53 respectively].

Table 4.4 Macronutrient intake expressed as percent of total intake

	Placebo			Fluoxetine		
	Fat	CHO	Protein	Fat	CHO	Protein
DAY 1	42.6%	37.2%	18.2%	44.9%	37.6%	17.4%
DAY 8	44.4%	37.1%	18.8%	44.5%	38.4%	17.0%
DAY 15	44.9%	37.8%	17.7%	44.5%	37.4%	18.1%
DAY 22	45.4%	36.8%	17.8%	45.4%	36.5%	18.0%
DAY 29	45.1%	36.6%	18.3%	43.9%	35.8%	17.5%

The division of total kj intake into savoury and sweet components showed that FXT had comparable effects with a tendency towards reduction of savoury kj, [F(1,19)=2.82. N.S], and a significant reduction in sweet kj [F(1,19)=4.22, p<0.05]. Sweet kj intake was significantly reduced on day 15 [F(1,95)=6.46,p<0.05] and day 22 [F(1,95)=4.99, p<0.05](Fig 4.5). Further breakdown of savoury and sweet kj into macronutrient components showed changes similar to the overall picture(Fig 4.6). Drug/placebo difference in incremental area under the curve for savoury [t=0.21, p<0.84] and sweet intake [t=-0.50, p<0.62], was non-significant.

Intakes (total intake, and sweet and savoury components) were analyzed with respect to sex (Fig 4.7). Main effects of sex [F(1,18)=6.14, p<0.02], day [F(4,72)=3.43, p<0.01] and a trend in drug effects were present [F(1,18)=3.06, p<0.09]. FXT reduced the intake of savoury kj in male [F(1,18)=5.08, p<0.05], but not females [F(1,18)=0.05, N.S.] Subjects. Male and females had similar intakes of sweet calories, and the effects of FXT were also similar (Fig 4.7).

4.3.3 Food diary intake and choice

Total kilojoule(kj) intake was analyzed for week one and week four. Diet diary macronutrient intake was analyzed for week one only. There were significant main effects of FXT on total kj intake[F(1,9)=35.5, P<0.001] and a significant drug by day interaction[F(6,54)=2.76, P<0.05]. Percent reduction in mean overall intake in week one was -21.3% (78.7% of control). In week one, FXT reduced kj intake on day 5 [F(1,9)=12.02,

P<0.001], day 6 [F(1,9)=14.98, P<0.001] and day 7 [F(1,9)=6.14, P<0.05](**Fig 4.8**). Overall percent reduction in kj intake in week four was -16.1% (83.9% of control). Significant reductions in kilojoule intake was present on days 26[F(1,9)=9.03, P<0.01], day 27 [F(1,9)=7.59, P<0.01] and day 28 [F(1,9)=8.66, P<0.01]. **Table 4.5** shows daily energy intake [day 1 includes intake at the experimental unit]: reductions in intake were present from day 1, and significantly different from day 5 onwards. T-tests performed on the differences in incremental area under the curve for logged placebo and drug data were significant in week one [t=10.55, p<0.001) and week four [t=9.28, p<0.001).

Analysis of macronutrient intake for week one showed that FXT significantly reduced diet diary intake of all three macronutrients: [fat:F (1,9)=21.88, p<0.001; CHO: F(1,9)=18.27, p<0.002; protein: F(1,9)=15.36, p<004]. The drug by day interaction terms approached significance for fat [F(6,54)=2.04, p<0.07] and protein [F(6,54)=2.09, p<0.07], but not for CHO [F(6,54)=1.82, p<0.11]. Significant reductions were seen in fat intake on day 5 [F(1,9)=12.55, P>0.01], day 6 [F(1,9)=12.84, P<0.01] and day 7 [F(1,63)=7.50, p<0.05] (**Fig 4.9**). **Table 4.5** shows the kj energy intake of fat, CHO and protein with percent reductions from placebo. CHO was reduced on day 5 [F(1,9)=4.66, P<0.05], and day 6 [F(1,9)=15.76, P<0.001], and FXT reduced protein on days 5 [F(1,9)=7.30, P<0.05], day 6 [F(1,9)=8.81, P<0.05] and day 7 [F(1,9)=7.50, P<0.05] (**Fig 4.9**). **Table 4.6** gives reported intakes of macronutrients expressed as percentages of total

Table 4.5 Daily reported intake(kj) in diet diaries(n=10)

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
Total kj(x̄±s.e.)							
Pl	8536(1181)	6685(534)	7332(735)	8167(721)	8401(1047)	9088(1092)	7695(601)
FXT	7527(961)	6222(680)	6469(784)	6948(831)	5448(670)	5791(636)	5584(601)
diff	-1010(576)	-464(722)	-863(457)	-1212(1019)	-2953(916)***	-3296(1105)***	-2111(381)*
% red.	-11.8%	-6.9%	-11.7%	-14.9%	-35.1%	-36.3%	-24.4%
Fat:							
Pl	3802(529)	3038(313)	3079(380)	4140(462)	3931(652)	4354(579)	3655(298)
FXT	3378(317)	2863(304)	3005(492)	3495(530)	2096(242)	2468(287)	2494(238)
diff.	-424(361)	-176(398)	-74(536)	-613(784)	-1834(595)**	-1886(632)**	-1076(264)*
% red	-11.2%	-5.7%	-2.4%	-15.6%	-46.6%	-43.5%	-31.7%
Carbohydrate:							
Pl	3516(614)	2452(191)	2886(357)	2545(300)	2929(322)	3821(632)	2926(348)
FXT	3007(374)	2467(307)	2170(253)	2484(449)	2105(306)	2305(308)	2216(251)
diff.	-509(345)	+15(336)	-717(238)	-61(411)	-672(309)*	-1516(623)***	-668(301)
% red.	-14.5%	+0.6%	-24.8%	-2.3%	-28.1%	-39.7%	-24.2%
Protein:							
Pl	1173(112)	1053(128)	1103(159)	1467(151)	1276(180)	1322(224)	1255(139)
FXT	1154(101)	1029(142)	1152(159)	1141(154)	869(148)	825(141)	842(120)
diff.	-19(85)	-24(162)	+45(155)	-325(158)	-555(234)*	-493(250)*	-459(99)*
% red.	-1.6%	-2.3%	+3.9%	-22.2%	-31.8%	-37.6%	-32.9%

	DAY 22	DAY 23	DAY 24	DAY 25	DAY 26	Day 27	Day 28
Total kj only(x̄±s.e.)							
PL	8435(682)	7563(989)	6873(928)	7189(637)	8249(926)	7952(1051)	9599(1569)
FXT	7083(666)	7473(966)	6171(803)	8037(1510)	5689(727)	5606(735)	7093(825)
diff.	-1158(399)	-189(1105)	-839(985)	+987(1716)	-2917(817)***	-2541(1051)***	-2309(168)**
% red.	-16%	-1.2%	-10.2%	+10.5%	-31%	-29.5%	-26.1%

Values are means (±s.e.). * =p<0.05; ** p<=0.01; *** =p<0.001.

intakes. As in the food intake data recorded in the experimental unit (Table 4.4), there is no evidence that FXT had differential effects on macronutrient choice.

Table 4.6 Macronutrient intake expressed as percentage of total intake

	PLACEBO			FLUOXETINE		
	Fat	CHO	protein	Fat	CHO	Protein
DAY 1	44.5%	42.0%	13.7%	44.8%	40.0%	15.3%
Day 2	45.4%	36.7%	15.7%	46.0%	39.6%	16.5%
Day 3	42.0%	39.3%	15.1%	46.4%	33.5%	17.8%
Day 4	50.6%	31.2%	17.9%	50.3%	35.7%	16.4%
Day 5	46.7%	34.8%	15.2%	38.4%	38.6%	15.9%
Day 6	47.9%	42.0%	14.5%	42.6%	39.8%	14.2%
Day 7	47.5%	38.0%	16.3%	44.6%	39.6%	15.1%

Values are mean percent(n=10).

Day 22 to day 26 are not significantly different from placebo [maximum $F(1,9)=2.52$, N.S.]. Significant reductions were present on day 26 [$F(1,90)=9.03$, $p<0.01$], day 27 [$F(1,9)=7.59$, $p<0.01$] and day 28 [$F(1.9)=8.66$, $p<0.01$].(**Fig 4.8**).

4.3.4 Feeding Behaviour

Feeding rates expressed either as kj/min or grams/min were decreased by FXT, main effects [F(1,19)=11.03, p<0.004] and [F(1,19)=10.46, p<0.004] respectively. Examination of the data for individual days showed significant reductions in chewing rate(kj/min) on day 1 [F[1,19)=4.39, p<0.05], day 15 [F(1,19)=10.97, p<0.001]. **(Fig 4.10)**. Chewing rate grams/min was significantly decreased on day 15 only [F(1,95)=9.32, p<0.01], other days were non-significant; e.g. day 1 [F(1,95)=2.96, N.S.]. FXT significantly reduced number of bites taken on day 15 [F(1,19)=6.36, p<0.02] and day 29 [F(1,19)=7.10, p<0.02]. Other measured variables: latency to start manipulation, latency to bite, meal length, kj/per mouthful and grams/per mouthful were not significantly different from placebo **(Table 4.7)**.

Local eating rates (grams/min) for male/female subjects were differentially effected by FXT. Main effects of drug [F(1,18)=21.7, p<0.005], and a drug x sex interaction [F(1.18)=4.44, p<0.04]. FXT slowed chewing (gram/min) in female subjects [F(1,18)=22.89, p>0.005], but effects for male subjects were non-significant [F(1,18)=3.26, N.S.]. **Table 4.8** shows eating rates for grams on experimental days.

Table 4.7 Observed feeding behaviour in obese subjects(n=20) at a lunch meal

		DAY 1	DAY 8	DAY 15	DAY 22	DAY 29
Latency to manipulate(seconds: range)						
Pl	median	60.0(37-93)	59.0(37-173)	59.0(31-191)	51.0(33-87)	47.0(9-95)
Fxt	median	69.5(18-167)	57.5(25-134)	56.5(7-122)	56.0(26-201)	56.5(18-148)
Latency to bite(secs)						
Pl	X(s.e.)	128(9.8)	130(12.5)	118(12.8)	121(13.1)	111(13.9)
Fxt	X(s.e.)	126(11.9)	133(11.8)	128(13.1)	122(11.7)	119(11.7)
Length of meal(min)						
Pl	X(s.e.)	26.9(8.8)	24.1(7.3)	26.0(5.3)	28.4(10.4)	24.4(6.7)
Fxt	X(s.e.)	28.1(10.3)	28.4(12.1)	26.9(9.7)	25.7(8.8)	23.8(6.8)
Number of bites						
Pl	X(s.e.)	56(3.8)	54(4.2)	63(4.2)	60(3.9)	63(5.3)
Fxt	X(s.e.)	56(4.9)	54(5.3)	52(4.8)*	51(3.7)	51(4.5)*
Kj/mouthful						
Pl	X(s.e.)	60.7(4.8)	65.9(6.4)	63.4(4.6)	66.9(5.1)	62.3(4.9)
Fxt	X(s.e.)	58.2(3.6)	57.2(4.6)	63.9(4.3)	65.5(4.7)	73.9(6.3)
Grams/mouthful						
Pl	X(s.e.)	7.4(0.5)	7.4(0.6)	7.4(0.5)	7.2(0.6)	7.2(0.5)
Fxt	X(s.e.)	6.8(0.3)	6.1(0.5)	6.6(0.5)	6.8(0.5)	7.5(0.5)
Chewing rates(kj/chewing time)						
Pl	X(s.e.)	233.4(17.1)	249.4(19.5)	245.0(15.6)	240.5(15.7)	246.3(17.5)
Fxt	X(s.e.)	204.6(12.8)	222.4(27.9)	199.5(13.2)	221.2(17.4)	246.4(17.3)
difference(s.e.)		-28.8(13.8)*	-26.3(22.6)	-44.7(10.6)***	18.4(11.2)	-5.4(11.4)
Chewing rate(grams/chewing time)						
Pl	X(s.e.)	29.0(2.3)	28.5(2.4)	28.7(2.4)	26.9(2.4)	28.6(2.3)
Fxt	X(s.e.)	24.6(1.8)	25.7(3.4)	21.3(1.7)	23.8(2.5)	26.3(2.5)
difference(s.e.)		-4.3(1.5)	-3.4(3.3)	-6.5(2.58)**	-2.5(1.4)	-2.9(1.2)

*=p<0.05; *=p<0.02.(no. of bites); **=p<0.01; ***=p<0.001

Table 4.8 Chewing rates grams/min for male and female subjects

		DAY 1	DAY 8	DAY 15	DAY 22	DAY 29
Chewing rate(grams/chewing time) for all subjects						
Pl	X(s.e.)	29.0(2.3)	28.5(2.4)	28.7(2.4)	26.9(2.4)	28.6(2.3)
Fxt	X(s.e.)	24.6(1.8)	25.7(3.4)	21.3(1.7)**	23.8(2.5)	26.3(2.5)
Female chewing rates						
Pl	X(s.e.)	27.1(9.6)	27.9(11.3)	27.3(10.8)	23.9(10.6)	26.3(9.5)
Fxt	X(s.e.)	22.0(7.6)	21.1(15.9)	18.4(7.4)	19.8(10.6)	23.3(10.6)
Male chewing rates						
Pl	X(s.e.)	30.8(11.0)	29.2(10.3)	29.9(9.4)	29.6(10.2)	31.0(9.7)
Fxt	X(s.e.)	27.2(7.8)	30.4(11.9)	24.5(6.1)	27.7(9.9)	28.8(9.7)

**= $p<0.005$.

No differential drug effects between male/female subjects were found for kj/min chewing rates.

4.3.5 Feelings of hunger and fulness

Subjects rated themselves as less hungry on day 8, [$t=-3.01$, $p<0.03$], and day 22 [$t=-3.03$, $p<0.03$], but incremental area under the curve was not statistically different, maximum $t=1.25$, $p<0.23$(**Fig 4.11**). On day 1 [$t=0.46$, $p<0.46$], day 15 [$t=1.25$, $p<0.23$], and day 29 of FXT

[t=-0.06, p<0.95] hunger ratings were not statistically different from placebo. There was no strong or consistent association between feelings of hunger and kj consumed for either placebo [r=0.27] or FXT [r=0.28].

Subjective ratings of fullness were also affected by FXT. Data from day 8 and day 22 are shown in **Figure 4.11**; but differences(area under the curve) were not statistically different from placebo, day 8: [t=0.98, p<0.34; day 22 [t=-1.04, p<0.31]. The trend towards higher subjective ratings of satiety was also present on day 29 [t=2.35, p<0.06]. If the satiety data are displayed as change(within condition) from baselines at 0900h to pre-meal ratings at 1300h, subjects showed a greater drop from baseline in feelings of fullness as the morning wore on(**Fig 4.12**). The exception to this is day 8, when subjects showed less of a move towards feelings of emptiness.

4.3.6 Other measurements:

There were no significant differences in mood ratings in response to continuing treatment with FXT [maximum t=-1.72, p<0.10] (**Fig 4.13**). Change scores from 0900h to 1300h in subjective ratings of arousal(sleepy-alert) showed non-significant increases in levels of drowsiness [maximum t=1.94, p<0.07], which tended to occur within 2-3 hours of taking the medication [area under the curve: t=0.93, p<0.37, n.s.]. On day 1, subjects on FXT rated themselves as more thirsty compared to placebo [t-1.82, p<0.08]. On day 22 the opposite occurred with subjects rating themselves as less thirsty [change scores 0900h to 1300h :t=-1.96, p<0.06]. No differences were present on the other days

[e.g. day 8: t=0.47, p<0.64]. Subjects did not rate themselves as feeling either nauseated [t=-1.32, p<0.20] or suffering from gastric discomfort [t=0.83, p<0.42].

Blood pressure, pulse and pupil diameter: Mydriasis occurred in response to FXT treatment, pupil size increased by 0.87mm by day 29 [t=2.54, p<0.02]. A slowing in pulse rate was evident by day 22 [mean -3.06, t=-2.39, p<0.02]. FXT did not affect systolic or diastolic blood pressure. Plasma levels of FXT and norFXT are shown in **Table 4.9**

Table 4.9 Plasma levels of FXT and norFXT (ng/ml)

	DAY 1	DAY 8	DAY 15	DAY 22	DAY 29
FXT	0(0.0)	111.6(±55.5)	190.3(±59.8)	246.6(±73.9)	263.1(±87.2)
NorFXT	0(0.0)	73.3(±33.4)	131.3(±34.1)	167.4(±51.0)	186.7(±52.6)

Values are means (±s.d.)

Adverse experiences: The incidence of reported adverse experiences/side effects was low. The most frequently reported effects related to sleep and comprised of early awakening and/or early pm drowsiness.

4.4 DISCUSSION

Consistent with previous studies in obese animals (Yen et al., 1987) and humans (Levine et al, 1987; Pijl et al, 1991), FXT significantly reduced body weight during a one month treatment period. Strong support for the involvement of serotonin in body weight control has been demonstated in studies with other 5-HT agonists, noticeably d-fenfluramine, with which clinical efficacy has been maintained for periods of three months (Goodall et al, 1988, Finer et al, 1985) or a year (Guy-Grand et al., 1989). Long-term studies should be viewed as successful if weight loss, which can plateau at about six months of treatment, is maintained under medication (Guy-Grand et al., 1989). The reporting of weight gain on cessation of medication (Guy-Grand et al., 1990) is perhaps an indication that chronic treatment may be considered as a possbility for patients whose health is affected by excess weight and who need help in weight loss maintenance (Turner, 1990). Subjects who responded to requests for information on weight status had regained weight, and it is likely that those who did not respond were also at pre-trial level.

The ideal of avoiding weight gain has been suggested as a goal of preventative medicine by Rissanen and colleagues, who have identified overweight(BMI >25.0) as a risk factor for early disabilities, mainly related to the cardiovascular and musculoskeletal systems (Rissanen et al., 1991). It may become acceptable to treat patients in order to avoid obesity developing as it is generally accepted that rates of recidivism are dishearteningly high in obese patients.

In this study, objectively measured reductions in total food intake on experimental days confirms the anorectic potential of FXT in an obese population (Pijl et al., 1991). The anorectic response of normal weight subjects to FXT appeared to diminish over a two week period(Chapter 3), although weight loss continued: in obese subjects reductions in food intake on FXT were present on all experimental days although the effects were less potent on day 1 and day 29. On day 29, subjects on placebo reduced their kj intake, thus decreasing the difference between the two conditions. As the experiment progressed, it is possible to see an increase in energy intake in both placebo and FXT conditions during the experimental meals. As this occurs in both conditions, it may represent a disinhibitory effect on intake over time. Irrespective of familiarization prior to the start of the trial, subjects gradually consumed more as the month progressed. This raises the issue of how representative laboratory-based studies are of food intake in the field. A study which addressed this question was that of Obarzanek & Levitsky (1985) who asked eight subjects (4 male, 4 female) to keep diaries externally for four days, Monday to Thursday, and then eat their food in a laboratory. Whereas significant between-subject differences were found, there was no significant within-subject differences between energy intake at home and in the laboratory setting.

FXT induced changes in macronutrient intake on experimental days show that firstly, all three macronutrients were reduced by FXT; secondly, there was no evidence that CHO intake is preferentially reduced compared to reductions in fat and protein; and thirdly, the observed reduction in savoury intake is largely generated by the male subjects. As

mentioned in the Introduction, the issue of serotonergic modulation of macronutrient choice has been controversial for a number of years. Animal studies have demonstrated that there is a complex interation between serotonergic function and the regulation of protein/CHO intake (Fernstrom & Wurtman, 1973). The modulatory role of 5-HT in macronutrient choice has been examined via pharmacological challenges. Animal studies showing a selective suppressive effect of FXT and fenfluramine on CHO, with the concomitant sparing of protein (Wurtman & Wurtman, 1977), have not been clearly reproducible in human studies. The present data provide no support for the protein-sparing hypothesis.

D-fenfluramine reduces total food intake with differential effects on nonsweet/sweet components showing a more potent suppressive effect on nonsweet foods than sweet (Goodall & Silverstone, 1988); the intake suppressive effects of d-fenfluramine occur both within and between meals (Hill & Blundell, 1986a). In addition, d-fenfluramine appears to enhance the satiating capacity of food, with the combination of d-FF and a high protein content (=54% of total energy), capable of suppressing later energy intake in obese subjects (Hill & Blundell, 1990). The general suppressive effects of FXT on all macronutrients including fat may be noteworthy, fat being both more calorific per gram than either CHO or protein and more capable than CHO at promoting storage of energy (Boozer, 1991). Short-term reductions or increases in total energy intake have been achieved by manipulating the amount of fat in the diet (Lissner et al, 1987). Deliberate reductions in fat intake may result in preference for lower levels of fat in the diet (Mattes, 1991), and FXT could be used as an adjunct to dietary advice for individuals susceptible to weight gain.

Considerable effort is being expended in finding an acceptable fat-mimetic which has minimal energy but comparable quality of taste (Drewnowski, 1991), and there is evidence that fat substitutes may be beneficial in reducing amount of fat consumed in the short term (Blundell & Burley, 1991).

Irrespective of whether or not it is possible to demonstrate a clear relationship between macronutrient choice and neurotransmitter synthesis in humans, there are myriad extraneous influences on food choice, with culture (Rozin, 1976), exposure to food-stuffs in childhood (Birch & Marlin, 1982) and mood state (Geliebter & Aversa, 1991), being strong influences.

The effects of FXT on savoury kilojoule intake shows this effect was mainly male generated. Serotonergic drugs like d-FF have been shown to exert anorectic effects on nonsweet foods without being capable of reducing sweet foods in animals (Orthen-Gambill, 1985; Blundell & Hill, 1989; Lawton & Blundell, 1989) and humans (Goodall & Silverstone, 1988). FXT appeared to exert some marginally suppressive effect on sweet kj as the trial continues: whereas kj intakes for sweet foods were similar on day 1, by day 29 intake in the placebo condition had risen, while intake of sweet kj in the FXT condition remained stable. This effect is slight however, and overall, the pictures for sweet and savoury kj appear very similar (Fig 4.5). FXT has been shown to reduce palatability-induced drinking in rats (Leander, 1987), and this aspect of possible functioning of FXT in humans deserves examination, possibly looking at highly preferred items or non-caloric palatable foods. Another 5-HT agonist, d-FF did not reduce the perceived pleasantness of

a sweet taste (Blundell & Hill, 1988). There is a stronger line of argument for the role of endogenous opiates in palatability-induced eating, with experimental evidence that the opioid receptor antagonist nalmefene reduced the pleasantness of both the smell and taste of palatable foods in human (Yeomans & Wright, 1991). In addition, dopaminergic mechanisms may play a role in palatability-induced ingestion; dopamine is known to be involved in reward mechanisms (Wise, 1982).

The subjectively reported data from the Diet diary records showed energy intake, subjectively reported, is supportive of laboratory findings of reduced intakes with FXT. The data suggested that effective reductions in intake are present from about day five onwards. The less consistent pattern of reduction in week four may be due to "diary fatigue" as by the start of week four, subjects had been keeping diaries for three weeks. With hindsight, it would have been more advantageous to ask subjects to keep only weeks one and four. Diet diary record keeping is reported as being very variable with respect to accuracy in both obese and normal weight subjects (Lansky & Brownell, 1982; Prentice et al., 1986; Lissner et al., 1989). Hulten et al(1990) examined reported intakes of overweight and obese women and using a urine nitrogen test for validation, found significant underreporting of intakes. They suggest that dietary surveys should have an independent validation test as described, although as our obese subjects acted as their own controls, the main focus of interest here concerned differences in intakes between conditions, rather than absolute intake. Errors of reporting in both conditions may not have remained stable however, and

diet diary records should really only be viewed as corroborative evidence, although some studies have relied on them rather more conclusively (Pijl et al. 1991).

Diet diary reports of macronutrient intakes in response to treatment with FXT are supportive of the unit based findings, with all three macronutrients being reduced. As with the objectively measured food intakes, there is no evidence from these subjectively reported data that FXT acts specifically to reduce CHO or spare protein as reported elsewhere (Wurtman & Wurtman, 1977; 1984). Once again, these diet diary findings need to be considered with respect to differences between drug and placebo conditions, as opposed to absolute measures of accurate intakes.

Changes in Feeding behaviour in response to FXT were similar to those seen with the 5-HT agonist fenfluramine described in Chapter 2, with reductions in meal size and eating rates, without changes in either latency or meal duration (Rogers & Blundell, 1979). The portable observer methodology described in Chapter 2 has been suitable for analysis of data from this particular subject population. The existence of a particular eating style in the obese has never been conclusively established (Stunkard & Kaplan, 1977), and it is more likely that there is a wide range of eating styles among individuals, with aspects such as deceleration of rate being present in some individuals and not others, irrespective of weight category (Westerterp et al., 1990).

The differential effects of FXT on male/female feeding rate for gram/min may suggest a greater sensitivity in females to manipulation of the serotonergic system in response to FXT. Serotonergic system status has been shown to be differentially effected by weight loss in male and female subjects. Weight loss in both depressed female patients (Cowen & Charig, 1987) and normal dieting female volunteers (Goodwin et al., 1987), results in an increased prolactin response to an infusion of the 5-HT precursor L-tryptophan. The authors suggest that the increased prolactin response, which was not present in male dieting volunteers, indicated that dieting caused changes in the serotonergic system in females, but not in males. A later study in dieting females, in which a dopamine antagonist did not elicit the above increased prolactin response, offered support for serotonergic modulation of this effect (Anderson et al., 1989). Reductions in total plasma pryptophan and the TRP/LNAA ratio were also found.

4.5 CONCLUSIONS

There is some evidence from this study that FXT is capable of modulating food intake and feeding behaviour in humans. The effects on food intake and choice are relatively non-specific and do not support either the animal (Wurtman & Wurtman, 1977; Kim & Wurtman, 1988) or human literature (Pijl et al., 1991) that suggests that fluoxetine has a selective CHO suppressive effect, while the intake of protein is maintained or increased.. It has been possible to establish that FXT, like d-FF reduces the rate of eating, which offers some support for serotonergic modulation of the expression of feeding behaviour. The

measured changes in subjective ratings are not robust, or alternatively, it is possible that the instruments used, VAS, are not sensitive enough to detect chronic change. It may be that the subjects become tired of the task and although being asked to consider each scale carefully, may become jaded. The reductions in ratings of hunger and increases in satiety are marginal, yet cannot be ignored. Years of experimentation with the more potently anorectic qualities of amphetamine may have obscured our perspective on the role of marginal changes.

In order to elucidate some possible mechanisms whereby FXT exerts the changes in body weight and food intake, the next step was to examine the activity of this drug in animals and make comparisons with other serotonergic agents, with specific reference to feeding and related behaviours.

CHAPTER 5

ACUTE EFFECTS OF 5-HT AGONISTS ON CONSUMMATORY BEHAVIOUR IN RATS

5.1 INTRODUCTION

It has been suggested that the capacity to enhance satiety may be a feature of serotonergic agents that contributes to their ability to reduce food intake (Blundell, 1986). The indirect serotonergic agonist, d-fenfluramine is similarly capable of reducing sensations of hunger (Hill & Blundell, 1988) and has been reported to increase sensations of fullness both before and during a meal (Hill & Blundell, 1986a). However, FF may not noticeably effect ratings of satiety (Goodall & Silverstone, 1988); similarly, fluoxetine does not result in significantly increased levels of satiety ratings. Observational studies with animals have led to the hypothesis that fenfluramine may suppress food intake by hastening the onset of post-prandial satiety (Thurlby et al., 1983; Blundell, 1986; Kirkham & Blundell, 1986; Blundell & Hill, 1987). However, other studies of fenfluramine in animals suggest, in fact, that this drug might alter satiety processes (Montgomery & Willner, 1988; Neill & Cooper, 1988; Willner et al., 1990).

The studies described in the present chapter examined the effects of FF and FXT(as well as other serotonergic anorectics) on satiety in rats, using the characteristic sequence of post-prandial behaviours known as the "behavioural satiety sequence" (Antin et al., 1975).

In the rat the offset of eating is followed by a short period of active behaviours such as locomotion, sniffing, and grooming, followed in turn by a longer period of resting (Antin et al., 1975; Smith & Gibbs, 1979). This sequence of behaviours, initially described by Richter in 1922, is normally seen only in animals that have consumed a caloric diet such as food pellets (Richter, 1922), a balanced liquid diet (Antin et al., 1975, Smith & Gibbs, 1979), or a sucrose solution (Montgomery & Willner, 1988; Willner et al.,1990). The presence of the resting component of the sequence is not seen following the consumption of saccharin or weak sucrose solutions, or following sham feeding (Antin et al., 1975; Kushner & Mook, 1984; Montgomery & Willner, 1988). The satiety sequence, and in particular, the occurrence of post-prandial resting, therefore seems to be a reliable behavioural correlate of a state of post-ingestive satiety.

Animal work reported so far has suggested that fluoxetine is able to reduce energy intake in the form of lab chow without changing feeding behaviour, although an early introduction of resting behaviour has been reported (Clifton et al.,1989). Studies on the effects of fenfluramine on satiety have produced variable findings; dl-fenfluramine has been reported to have no effect on resting (Blundell & Latham, 1980); or to enhance resting(in non-deprived

animals) (Blundell & Mc Arthur, 1981). Other studies report that dl-fenfluramine, on the other hand, produce a more disturbed profile of post-prandial behaviour; which includes suppressing post-prandial resting after a meal of wet mash (Montgomery & Willner, 1988). This effect was present with d-fenfluramine, the active isomer (Hudson et al., 1989) as well as with racemic dl-fenfluramine (Montgomery & Willner, 1988). There is, at present, no direct comparison between the effects of fenfluramine and fluoxetine on behavioural satiety.

In the present study, five serotonergic agents were examined in order to compare the effects of FXT, FF and other serotonergic anorectics within the same experimental paradigm. In addition to FF and FXT, three other drugs studied: paroxetine, femoxetine and eltoprazine. Two of these drugs paroxetine (Hrdina et al., 1990) and femoxetine (Blundell & Latham, 1978) are specific 5-HT uptake inhibitors which, like FXT reduce food intake on acute administration to rats (Rasmussen et al, 1990). The hypophagic actions of fenfluramine (Neill & Cooper, 1989; Samanin et al, 1989) and of another 5-HT uptake inhibitor, sertraline (Lucki et al., 1988) are known to be mediated by $5HT_1$ receptors, and food intake is reliably decreased by directly acting $5HT_1$ agonists, such as m-chlorophenylpiperazine(m-CPP), trifluoromethylphenylpiperazine (TFMPP), quipazine, and RU24969 (Fuller et al.,1981; Kennet et al, 1987; Samanin et al., 1979). We have therefore also examined the effects on satiety behaviour of a $5HT_1$ agonist, eltoprazine (Schipper et al., 1990).

In a series of three experiments an observational technique was used to examine and compare the effects of dl-fenfluramine and fluoxetine(experiments 1 and 2), paroxetine, femoxetine and eltoprazine(experiment 3), on intake and feeding parameters.

5.2 METHODS

5.2.1 Subjects:

Male Lister hooded rats(National Institure for Medical Research, Mill Hill), weighing approximately 300g were maintained on a 12h light-dark cycle (lights on at 0800h). Animals were housed singly, and except as described below, deprivation times excluded, lab chow(Lillico, Herts.) and water were freely available. The three experiments were conducted throughout the months of spring.

5.2.2 Design: Experiments 1 & 2:

In experiment 1, two groups of rats(n=12 per group) were tested under either fluoxetine(0, 1.5, 3 or 6mg/kg) or dl-fenfluramine(0, 0.75, 1.5 or 3mg/kg)conditions. Treatments were administered in a counter-balanced order under single-blind conditions, and there was a two-day interval between treatments. At the end of experiment 1 the 24 animals were again divided into groups of twelve, matched for performance and previous drug treatment. A drug free period of one week followed after which Experiment 2 commenced. Fluoxetine(0 and 6mg/kg) was administered to the two groups of animals, in a counter-balanced order and single-blind conditions, one group receiving a 35% sucrose solution, while the other received water.

Experiment 3: Three groups of animals (n=12) were tested following acute treatment with paroxetine (0, 1, 2 or 4mg/kg), femoxetine (0, 2.5, 5 or 10mg/kg) or eltoprazine (0, 0.5, 1 or 2mg/kg),

respectively. The four doses of each drug were administered in a single-blind condition and in counterbalanced order, with a minimum of 2 drug-free days between tests.

5.2.3 Procedure

Testing procedure was similar for all three experiments with the exception of the starting time of experiments 1 and 2(approx. 1400h) compared to between 1600h and 1800h for experiment 3. Animals were deprived of food and water for 4hour prior to testing. At the start of the test, animals were presented with a a pre-weighed bottle containing a 35% sucrose solution, which was available for 40 min. The animals were observed for the duration of the test in a compact experimental room which housed the observer, the animals and the observational equipment. The observer sat approximately four feet away from the cages and using the keyboard of a BBC microcomputer scored a behaviour every 15 seconds. The recorded behaviours were defined as: drinking, active, grooming, standing motionless, or lying down. These behaviours were mutually exclusive. For the purpose of analysis the categories of standing motionless and lying down were combined to form the category 'resting behaviour'. The category 'active', which includes sniffing, rearing and locomotion, was used when none of the other categories was applicable; thus the use of this category does not in itself carry any implication of a stimulant effect.

5.2.4 Drugs:

Fluoxetine HCL(Lilly, Indianapolis, USA) and dl-fenfluramine HCL(Sigma, Poole, U.K.), paroxetine HCL, femoxetine HCL(Ferrosan, Sebory, Denmark), and eltoprazine HCL(Duphar, Weesp, The Netherlands), were dissolved in physiological saline, and injected i.p., 45 minutes prior to testing in a volume of 1ml/kg. Doses were calculated as the salts. Physiological saline was used for the control injections.

5.2.5 Analysis:

Data were analyzed by analysis of variance, supplemented by tests of simple main effects and planned comparisons (Winer, 1971). Separate analyses were performed for each drug and for each behavioural observation category.

5.3 RESULTS

5.3.1 Experiment 1:

On consuming a 35% sucrose solution, animals in the vehicle condition behaved in the expected fashion, that is they first engaged in drinking, were active, grooming behaviour, for a time and then rested for the main part of the observation session(**Fig 5.1**).

Fenfluramine reduced sucrose intake, but only significantly at 3mg/kg[F=(3,33)=75.5, p<0.001](**Fig 5.1**). In keeping with the reduction in intake, fenfluramine caused a reduction in drinking behaviour frequency[F(3,33)=5.9, P<0.01], which was significant at both the 1.5 and 3mg/kg doses(Fig 5.1). Significant increases in grooming[F(3,33=14.4, 29.9, P<0.001] and activity were evident, while resting was suppressed by all doses of fenfluramine, with the highest dose of 3mg/kg causing great alteration [F(3,33=35.7, P<0.001] (**Fig 5.1**). The resting profile may have been related to increased grooming.

FXT, on the other hand, caused a dose dependent decrease in sucrose intake [F(3,33)=10.3, P<0.001] and resulted in an early introduction of resting behaviour, causing a leftward shift in the satiety sequence(Fig 5.2). These effects are mainly dose dependent in the drinking, grooming, activity(not shown) and resting behaviours(**Fig 5.2**).

5.3.2 Experiment 2:

As in experiment 1, vehicle treated animals consuming the 35% sucrose solution, displayed the behavioural satiety sequence of drinking, active behaviour, grooming and resting(**Fig 5.1**) FXT at 6mg/kg suppressed sucrose consumption [$F(1,11)=8.7$, $P<0.01$], and advanced and enhanced post-prandial resting in animals drinking sucrose (**Fig 5.3**). FXT caused a non-significant decrease in water-drinking which was followed by initial significant [$F(1,176)=11.0$, $P<0.001$] increases in grooming: grooming then decreased. FXT(water-intake) animals showed a significant increase in resting behaviour, evident from 25 minutes onwards. Animals in the water only condition did not rest(Fig **5.3**, right panel).

5.3.3 Experiment 3:

Paroxetine, femoxetine and eltoprazine all significantly reduced sucrose intake [paroxetine: $F(3,33)=3.73$, $P<0.02$; femoxetine: $F(3,33)=3.25$, $p<0.05$; eltoprazine, $F(3,33)=12.48$, $p<0.001$](**Figs 5.4, 5.5, 5.6,** left panels). The effects of femoxetine and paroxetine were slightly greater at the intermediate than at the highest dose, though in no case were the differences between effective doses significant (**Figs 5.5, 5.4,** left panels).

Drug effects on the structure of feeding behaviour are shown in Table **5.1** and Figs. **5.4-5.6**(middle and right panels). As in experiments 1 and 2, in the vehicle condition, drinking behaviour constituted the

Table 5.1 Behavioural effects of paroxetine, femoxetine and eltoprazine

	Dose(mg/kg)	Drinking	Activity	Grooming	Resting
Drug					
Paroxetine	0.0	19.1	9.0	12.8	119.2
	1.0	14.0	7.8	21.2	116.8
	2.0	10.2**	10.8	12.6	124.0
	4.0	12.6*	12.0	16.0	120.0
Femoxetine	0.0	16.0	9.5	16.2	118.4
	2.5	13.6	6.0*	10.5	131.2**
	5.0	13.4	4.6**	9.5*	132.0**
	10.0	16.0	6.7*	10.7	124.2*
Eltoprazine	0.0	14.1	10.6	18.4	117.2
	0.5	8.5*	12.6	9.1*	130.4
	1.0	5.1**	16.0	5.0**	133.9*
	2.0	5.7**	26.6***	5.7*	121.4

Values are the mean number of observations of each type of behaviour(max=160). *=$p<0.05$; **=$p<0.01$; ***=,$p<0.001$, significance of difference from vehicle.

main observed behaviour in the early part(evident up to about fifteen minutes into the session). (Fig **5.4-5.6**, middle panels). Drinking was followed by a period of active behaviours (not shown) and grooming (not shown). These behaviours were followed by resting, the increasingly predominant behaviour in the second half of the session(Fig **5.4-5.6**, right panel).

Paroxetine dose-dependently reduced drinking time [$F(3,33)=3.04$, $p<0.05$], leading to an early termination of drinking [dose X time interaction, $F(21,231)=4.37$, $p<0.001$]. These effects were significant at the lowest dose tested, which did not significantly reduce intake. Paroxetine did not significantly alter activity or grooming [$F(3,33)=1.51$, 1.38, N.S.]. The main effect of paroxetine on resting was also nonsignificant [$F(3,33)=0.4$, N.S.], but there was a significant dose X time interaction [$F(21,231)=2.4$, $p<0.001$]: all doses increased resting in the second 5-min time period (**Fig 5.4**).

Femoxetine also tended to reduce drinking early in the session (Fig.5.5). In this case, the overall effect of the drug was nonsignificant [$F(3,33)=1.3$, N.S.], but there was a significant simple main effect of drug in the first 5min time period [$F(3,264)=5.36$, $p<0.05$]. Femoxetine reduced activity [$F(3,33)=4.53$, $p<0.01$] and advanced the offset of grooming [dose X time interaction: $F(21,231)=1.69$, $p<0.05$; results not shown]. However, the most striking effect was an increase in resting behaviour [$F(3,33)=3.97$, $p=0.02$] in the second, third and fourth 5-min time periods (**Fig 5.5**).

Eltoprazine, like paroxetine, caused a dose-dependent reduction in drinking [$F(3,33)=7.95$, $p<0.001$], which was significant at all doses, including the lowest, which did not significantly reduce intake (**Fig 5.6**). The highest dose of eltoprazine significantly increased activity [$F(3,33)=5.25$, $p<0.01$] at the expense of grooming [$F(3,33)=5.29$, $p<0.01$]. From informal observations it was clear that the highest dose of eltoprazine caused a marked stimulation of locomotor behaviour. As with paroxetine, the main effect of eltoprazine on resting was nonsignificant [$F(3,33)=2.54$, N.S.], but there was a significant dose X time interaction [$F(21,231)=2.11$, $p<0.01$]: all doses increased resting early in the session (**Fig 5.6**).

5.4 DISCUSSION

In this chapter it has been possible to examine and compare the acute effects of fluoxetine and fenfluramine in experiments 1 and 2, and in addition, to examine the comparative profile of three other 5-HT agonists on energy intake and on the satiety sequence (Expt. 3).

Experiment 1 shows that fluoxetine appears to differ markedly compared to fenfluramine with respect to the expression of satiety.

Firstly, FXT treated animals showed a quite clear dose-dependent reduction in sucrose intake, and this was attended by an early introduction of the resting component of the behavioural satiety sequence. Clifton et al.,(1989) have also reported that 10mg/kg of fluoxetine, given to free-feeding rats reduced meal size(chow) and resulted in an earlier introduction of resting compared to vehicle. In the study reported here, the enhancement of the satiety sequence is evident in all aspects, from the earlier off-set of drinking through to the early introduction of resting behaviour. In a further study not reported here, FXT caused a dose-dependent suppression of random interval operant responding and had a greater effect in a reinforcement lean schedule relative to a reinforcement-rich schedule (Willner et al. 1990). These effects are similar to those seen with a decrease in food deprivation time.

Similarly, the effects evident during the behavioural satiety sequence have been argued to be similar to those seen with prefeeding or as a result of a reduction in the duration of food deprivation Montgomery & Willner, unpublished observation). These findings are supportive of the suggestion that 5-HT plays a significant role in post-prandial satiety (Blundell, 1986; Blundell & Hill, 1987). The effect of FXT in experiment 2, where animals were offered water as a non-caloric alternative to sucrose, is interesting, as FXT treated animals responded with a reduction in intake, an enhanced profile of grooming which parallels the response in the vehicle-sucrose condition, and a resting response which is reduced compared to sucrose. The net effect is suggestive of partial satiation and offers further support for the role of serotonin in post-prandial satiety (Blundell, 1986, Blundell & Hill, 1987). However, the findings in Experiment 1 suggest that the effects of acute fenfluramine alter the process of post-prandial satiety, the observed alterations in resting may be related to increased grooming. While the 3.0mg/kg dose of FF clearly reduces sucrose intake, and causes an early introduction of resting, the resting component stays suppressed compared to vehicle levels.

In other studies fenfluramine has been reported to increase the resting component of the post-prandial satiety sequence (Blundell & McArthur 1978; Blundell & Latham, 1980). It is difficult to reconcile the findings of Experiment 1, i.e. altered resting, with enhanced satiety. Some authors have shown that the l-isomer of dl-fenfluramine preferentially affects catecholamines and may have anti-dopaminergic

effects (Invernizzi et al., 1986), and the observation that the increased locomotor activity seen with some serotonergic agents may be associated with interactions of 5-HT/DA systems (Green & Grahame-Smith, 1976).

In experiment 3, although the effects varied slightly between drugs, the acute actions of all three agents in suppressing consumption time and advancing the onset of resting are again compatible with an enhancement of postprandial satiety (Antin et al., 1975; Smith & Gibbs, 1979). In the case of paroxetine and eltoprazine, the behavioural changes were present at doses that did not significantly reduce the quantity of sucrose consumed. This pattern of effects is very similar to that seen with fluoxetine (expts. 1 & 2) (Clifton et al., 1989). The effects of femoxetine in particular, which significantly advanced grooming as well as resting, were very similar to those of fluoxetine. As demonstrated in this chapter, fenfluramine is capable of altering the satiety sequence (Montgomery & Willner, 1988). The results of experiment 3 suggest that fenfluramine is atypical, with an effect dissimilar to other serotonergic anorectics.

Experiments which have examined the anorectic profile of fenfluramine have established that it is possible to antagonize the anorectic effect of fenfluramine by nonspecific 5-HT antagonists, or by a $5\text{-HT}_{1A/1B}$ antagonist, but not by selective 5-HT_2 or 5-HT_3 antagonists, leading to the conclusion that the effects were most likely mediated by 5-HT_1 receptors (O'Neill & Cooper, 1989; Samanin et al, 1989). The same conclusion was reached in a similar study of the 5-HT uptake inhibitor sertraline (Lucki, et al., 1988). At this stage the subtype of 5-HT_1 receptor involved in the anorectic action of endogenous 5-HT has not

yet been determined. Most of the directly acting 5-HT_1 agonists known to reduce food intake, such as m-CPP (Samanin et al, 1979; Fuller, 1981) TFMPP, quipazine, and RU24969 (Fuller, 1981; Hutson et al, 1989; Kennet, et al., 1987; Samanin, 1989) are active, to varying degrees, at 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} receptors. However, their 5-HT_{1A} activity cannot be responsible for the hypophagic effect, since specific 5-HT_{1A} agonists reduce food intake only at high doses, which elicit the 5-HT syndrome. At lower doses, 5-HT_{1A} agonists increase food intake (Dourish et al, 1986; Samanin, 1989) These findings implicate 5-HT_{1B} and 5-HT_{1C} receptors in 5-HT-mediated hypophagia (Hutson et al, 1989; Samanin, 1989).

The finding that eltoprazine enhanced satiety behaviour is of considerable interest in this context. In contrast to other 5-HT_1 agonists, eltoprazine is an agonist at 5-HT_{1A} and 5-HT_{1B} receptors, but a 5-HT_{1C} antagonist (Schipper et al., 1990) This implies that a selective stimulation of 5-HT_{1B} receptors may be sufficient to elicit the behavioural satiety sequence. A further implication is that 5-HT_{1B} receptors, possibly localized within the paraventricular nucleus of the hypothalamus (Hutson et al, 1989) may be primarily responsible for mediating the hypophagic effects of 5-HT uptake inhibitors.

In summary, these experiments have examined the effects of serotonergic drugs on sucrose intakes and the attendant effects on the behavioural satiety sequence. The results are consistent with the suggestion that fluoxetine may cause an early introduction of, and enhancement of, satiety processes. This effect is shared by paroxetine, femoxetine and eltoprazine. However, fenfluramine does

not appear to be conducive to enhanced satiety, and can apparently alter this process. As fenfluramine has been used chronically for a number of years in clinically therapeutic settings, and a similar role for fluoxetine is possible, it was a pressing question to examine the effects of fenfluramine and fluoxetine in a chronic paradigm, and this work in described in Chapter 6.

CHAPTER 6

CHRONIC EFFECTS OF FLUOXETINE AND FENFLURAMINE ON CONSUMMATORY BEHAVIOUR AND BODY WEIGHT IN RATS

6.1 INTRODUCTION

Acute modulation of feeding parameters by serotonergic drugs allows us to examine initial drug effects, and consequently to single out areas for greater focus. The combination of certain findings, namely, the ability of acute fluoxetine to advance behavioural satiety; the contrasting ability of acute fenfluramine to alter behavioural satiety and the knowledge that a successful anorectic will be used chronically, leads to the question of measurable long-term effects of these drugs.

The advantage of the serotonergic drugs as potential anorectics is that, compared to anorectics available in the 1970's, serotonergic drugs are not reported to possess stimulant properties (Silverstone, 1982; 1987b). The focus of research on these agents has been geared towards determining the mechanism for their anorexic effect, with objective measures of food intake and food-related subjective measurements as variables (Goodall & Silverstone, 1988; Hill & Blundell, 1986a). Yet, it is equally important to examine the behavioural effects of chronically administered serotonergic agents, as observed changes in energy intake related behaviour may be found to contribute to the total effect.

Compared to fluoxetine, fenfluramine has been available as a prescription anorectic for a number of years (Munro & Ford, 1982) and fairly extensive trials are being conducted to assess the effects of long-term use (Guy-Grand et al., 1989, 1990). More recently, fluoxetine has also been shown to decrease body weight in normal weight (McGuirk & Silverstone, 1990; in Chapter 3) and obese (Levine et al., 1987; Pilj et al., 1991; Chapter 4) human subjects. In the previous chapter, evidence was presented that on acute administration, fenfluramine and fluoxetine differ in their behavioural mechanisms of acute anorectic action. However, as the clinical usage of anorectics necessarily involves chronic drug administration, the effects of chronic treatment in animals are likely to be of greater relevance than acute drug effects. The study described in this chapter therefore examined the effects of fenfluramine and fluoxetine on food intake, body weight and the behavioural satiety sequence in rats, during three weeks of daily treatment, and also, following withdrawal.

6.2 METHODS

6.2.1 Subjects:

Male Lister hooded rats(National Institute for Medical Research, Mill Hill), weighing approximately 350g were maintained on a 12h light-dark cycle(lights on at 0800h). Except as described below, they were housed singly, with lab chow(Lillico, Herts.) and water freely available.

6.2.2 Procedure

Behavioural testing was carried out between 1600h and 1800h, following deprivation of food and water for four hours. The test consisted of the presentation of a bottle containing a 35% sucrose solution, which was available for forty minutes. Sucrose consumption was measured by weighing the bottle before and after the test. Animals were observed for the forty minutes of the test session, using the method described in the previous Chapter, for observing the satiety sequence: briefly, the animals(housed separately) were observed by the experimenter who used the keyboard of a BBC microcomputer to score a behaviour every 15 seconds. Observed behaviours were: drinking, active, grooming and resting.

Drug treatment commenced after sucrose intakes in twice-weekly tests had stabliized(six sessions). Three groups of animals(n=12) were tested following injections of fluoxetine (6mg/kg), fenfluramine(3mg/kg) or saline. Doses were chosen, on the basis of previous studies in this laboratory, to produce a comparable suppression of sucrose drinking(40%

approximately) on acute administration. Starting on the following day(day 2), all animals received twenty further daily injections, each animal receiving the same drug throughout, at the same time each day. Five further intake and observation sessions were recorded as described above, on days 4, 7 14, 17 and 21. Body weight and 24h chow consumption were measured daily. Fluoxetine treatment terminated on day 21; fenfluramine was administered for a further three days at double the previous dose(at 6mg/kg).

"Off-drug" measurements: Following the termination of drug administration, chow consumption was measured daily until day 32, and daily body weight records were maintained until day 29. Three further sucrose tests and sessions of behavioural observation were carried out blind after drug withdrawal, on days 24, 28 and 33.

6.2.3 Drugs

Dl-fluoxetine HCl(Lilly, Indianapolis, U.S.A.) and dl-fenfluramine HCl(Sigma, Poole, U.K.) were dissolved in physiological saline, and injected i.p., 45 minutes prior to testing, in a volume of 1 ml/kg. Physiological saline was used for control injections.

6.2.4 Analysis

Data were analyzed by analysis of variance, supplemented by tests of simple main effects. Separate analyses were performed on data obtained during drug treatment(days 1 - 21) and data obtained following fluoxetine withdrawal (days 22 onwards). Observational data were

analyzed separately for each behavioural category. On day 24, sucrose intake data were lost from half of the animals in each group(however, complete behavioural records were obtained); the remaining day 24 data were analyzed using Mann-Whitney U-tests.

6.3 RESULTS

6.3.1 Observations during chronic drug treatment

Analysis of body weight data over the course of chronic drug treatment(**Fig 6.1**) showed a significant main days effect [$F(20,660)=3.84$, $P<0.001$] and a treatment x days interaction[$F(40,660)=4.0$, $P<0.001$]. While body weight increased steadily in the control and fenfluramine groups, body weight decreased, over the first ten days of treatment, in the fluoxetine group. Over the second half of the treatment period, the fluoxetine group maintained a constant weight differential, relative to the other two groups, of approximatley 15g, equivalent to around 4% of body weight.

The two drugs also had different effects on chow intake(**Fig 6.2**) Fenfluramine significantly reduced chow intake on day 1 [$F(2,33)=6.4$, $p<0.05$], but had no significant effect thereafter. By contrast, fluoxetine did not reduce chow intake significantly on day 1, but did cause significant reductions on subsequent days, particularly during the first two weeks of treatment[treatment main effect: $F(2,33)=37.8$, $p<0.001$]. When the data are expressed as the difference in chow intake between control and drug treatment groups(**Fig 6.2**, lower panel), some tolerance to the hypophagic effect of fluoxetine is evident towards the end of the 3-week treatment period, and there is a treatment x days interaction: [$F(40,660)=3.2$, $P<0.001$].

Analysis of the of sucrose intake data across the 6 tests(Fig 6.3), revealed a significant effect of treatment [$F(2,33)=24.1$, $P<0.001$], and a significant treatment X days interaction [$F(10,165)=24.1$, $P<0.001$]. Further analysis of simple main effects showed that there was no significant effect of days in either the vehicle or fluoxetine groups [$F(5,165)=1.5$, 1.7, N.S., respectively), but a highly significant effect of days in the fenfluramine groups [$F(5,165)=6.8$, $P<0.001$]. On the first day fluoxetine and fenfluramine suppressed sucrose intake to a comparable extent, the effect of fenfluramine being slightly, but not significantly, greater. The effect of fluoxetine remained highly significant on subsequent tests, with the exception of the final test(day 21). By contrast, the effect of fenfluramine was non-significant on the second and third test(days 4 and 7), and while significant effects of fenfluramine were observed on the fourth and fifth tests(days 14 and 17), these were greatly reduced relative to day 1. In the fenfluramine group, intakes on test days 4, 7,14, 17 and 21 were significantly higher than day 1[Minimum $F(1,165)=5.9$, $P<0.025$]. On the last day of fenfluramine treatment, when the dose was increased to 6mg/kg, suppression of sucrose intake was evident, but the effect was small and only marginally significant(Fig 6.3).

Differences were also apparent in the behavioural effects of the two drugs. Fig 6.4 summarizes the total observations of each of the four behaviours, across the six test days, and Fig 6.5 provides full time courses(five minute bins for forty minutes) for day 1(acute administration) and for day 14; the day 14 data are typical of those obtained in the 5(fluoxetine) or 6(fenfluramine) tests carried out

during chronic drug administration. In all tests, control animals showed the characteristic pattern of prandial behaviour: drinking was the predominant behaviour at the start of the session, followed by a brief period of grooming and other active behaviours, superceded by resting, which was the predominant behaviour in the second half of the session(**Fig 6.5**). These conclusions are supported by significant linear trends, over the course of the forty minute sessions, for drinking and resting, and significant quadratic trends for activity and grooming(minimum $F(1,231)=14.9$, $P<0.001$).

On acute administration(day1) fenfluramine reduced drinking, but increased activity and decreased resting(**Fig 6.4**). The full time course(**Fig 6.5**, left panels) shows a decrease in drinking at the start of the session, followed by a brief period of decreased grooming and increased resting, superceded by increases, relative to controls, in grooming and other active behaviour, at the expense of resting. After chronic treatment, fenfluramine no longer decreased drinking; indeed, a small increase was apparent in the early part of the session, which was significant in each of tests 3(day7) to 7(day 24). **Fig 6.5**, top right panel for test 5(day 14) shows this increased drinking). In contrast to the late increase in grooming and decrease in resting seen with acute treatment, after chronic treatment, fenfluramine decreased grooming and increased resting(**Fig 6.4**). The time course data (**Fig 6.5** , right panels) now reveal a sequence in which increased drinking is followed by near control levels of activity, but early off-set of grooming and increased levels of resting.

In accordance with the sucrose intake data, <u>fluoxetine</u> decreased drinking time; this effect was significant overall [$F(1,33)=7.62$, $p<0.01$), and within the forty minute sessions, FXT continued to suppress drinking behaviour on all test days(e.g. **Fig 6.5**, top right is typical) Fluoxetine also suppressed grooming on all tests [$F(1,33)=14.8$, $p<0.001$)(**Fig 6.4**, **Fig 6.5**, lower right]. The incidence of active behaviours was slightly decreased by fluoxetine on day 1(**Fig 6.5**, upper left panel). This reduction occurred approximately ten minutes into the session; the increases in resting are simultaneous. Levels of activity were increased on all other test days(**Fig 6.4**; **Fig 6.5**, upper right panel). Fluoxetine increased resting on day 1(**Fig 6.4**), by advancing the onset(**Fig 6.5**, bottom left panel). These effects were not seen on any of the subsequent tests: indeed, during chronic treatment, fluoxetine tended to decrease resting behaviour(**Fig 6.5**, bottom right panel).

<u>6.3.2 Observations after withdrawal of drug treatments</u>

As noted, <u>fenfluramine</u> did not alter chow intake(other than on Day 1, or body weight. Consequently, withdrawal of fenfluramine had no significant effects on either of these measures(**Figs 6.1/ 2**). Similarly, no significant effects on sucrose intake or feeding behaviour were observed in tests carried out 4 and 9 days following fenfluramine withdrawal(**Figs 6.3, 6.4**).

Following withdrawal from fluoxetine, body weight recovered rapidly, 80% of lost weight being recovered within four days (**Fig 6.1:** treatment X days interaction, [F(14,231)=13.5, p>0.001]. During those first four days of withdrawal, chow intake in animals withdrawn from fluoxetine did not differ from that of control animals. However, on day five after withdrawal, chow intake increased significantly above control level in fluoxetine-withdrawn animals, and this elevation was sustained until the end of the experiment(**Fig 6.2**).

Sucrose intake was non-significantly increased above control levels three days after fluoxetine withdrawal, but large increases were recorded after seven and twelve days of withdrawal(**Fig 6.3**). On the three day withdrawal test(day 24 from the start), drinking was increased early in the session(not shown) but the overall increase was not siginficant (**Fig 6.4**). Otherwise, the behaviour of fluoxetine-withdrawn animals was very similar to that observed during chronic treatment. Activity was increased, while grooming and resting were decreased(Fig. 6. 4). On subsequent tests(days 7 and 12) drinking behaviour was increased relative to controls(consonant with the increase in sucrose intake), but other behaviours were not significantly altered(**Fig 6.4**).

6.4 DISCUSSION

The results in this study have supplied us with an acute "window" on drug effects comparable to the data reported in Chapter 5 and a follow-on analysis of chronic treatment effects. The findings support the value of continued measurement and observation past the point of drug withdrawal, particularly with a drug such as fluoxetine which has a long half-life and a potent metabolite.

In this study it was confirmed that tolerance to the effects of fenfluramine developed fairly rapidly, as described in other studies (Mennini et al., 1981; Rowland et al., 1982; Rowland, 1986; Rowland & Carlton, 1986b). On acute administration, fenfluramine reduced sucrose intake by approximately 45% and caused a small (15%) decrease in overnight chow intake. These effects did not persist although fenfluramine continued to show some marginal suppression of sucrose intake. The introduction of a hundred percent increase in dosing on the final day of treatment, while decreasing sucrose intake compared to vehicle, also had a modest effect. However, the observation that fenfluramine did suppress 35% sucrose intake to some extent following chronic treatment is worthy of note. Withdrawal of fenfluramine did not result in any noticeable effect on any measured variable.

The administration of fluoxetine to either animals or humans and the measurement of the effects, must be set within the context of the pharmacokinetics. Although the half life of fluoxetine and nor-fluoxetine is considerably shorter in rodents than in humans (Caccia

et al., 1990), effects of a single 10mg/kg dose are detectable up to 48h later (Leander, 1987). In the light of this observation, some potentiation of the effect of fluoxetine would be expected with repeated administration, related to an increase in steady state plasma levels (Rowland et al., 1982). In the present study, suppression of sucrose intake by fluoxetine was relatively constant over the first two weeks of treatment, but the suppression of overnight chow intake increased up until day 6. It is often stated that tolerance does not develop to the hypophagic effect of fluoxetine(e.g. Rowland et al., 1982; Clifton et al., 1989). However, it is clear in the present data that, from day 6 onwards, tolerance did develop to the suppression of overnight chow intake. Fluoxetine continued to exert a significant hypophagic effect throughout the three week treatment period, but in the final week, the size of the effect was less than half that observed at 3 - 6 days. Exactly comparable data were reported in an earlier study, in which the hypophagic effects of fluoxetine peaked at 5 - 8 days and decreased thereafter (Wong et al., 1985). The early increase in response to fluoxetine, which presumably reflects an increase in plasma levels rather than a genuine pharmacological sensitization(cf. Willner, 1989), tends to obscure the later development of tolerance. However, it is clear that a mode of tolerance does develop to the hypophagic effect of fluoxetine, provided that the assessment of tolerance begins from a point at which steady state plasma levels can be assumed.

The loss of body weight in FXT-treated animals increased until approximately ten days into medication, when an undulating plateau of body weight was reached, which persisted until fluoxetine withdrawal on day 21. A study with mice (Morley & Flood, 1987), showed a similar

effect on body weight. In another study in ad lib fed lean and obese mice, Dubuc & Peterson (1990) found that initial suppressions in food intake and body weight in response to three weeks treatment with subcutaneous FXT did not persist.

Animals in the present study lost approximatly 4% of body weight, relative to controls. This figure was greater than that observed in lean humans following two weeks treatment with fluoxetine(described in Chapter 3. Obese subjects taking FXT for one month lost near to 4% (in absolute, not drug minus placebo terms), of body weight(Chapter 4). Weight loss in depressed patients treated with FXT vary; an overview of some studies suggests about 2% (Kinney-Parker et al., 1989), and weight loss has been reported as being relative to starting weight with overweight patients losing the most, underweight patients showing no consistent trend, while normal weight patients have been reported to gain weight (Orzack et al., 1990). Depressed patients in these trials were taking doses of FXT ranging from 20mg to 80mg daily.

In this currently described animal study, withdrawal of fluoxetine resulted in a dynamic weight change, with rapid re-gain over four days. There is a suggestion, from the chow intake data, that fluoxetine treated animals were gradually showing a "claw-back" in intake, while still being significantly below vehicle and day 1 values. Five days after release from fluoxetine, animals were consuming significantly greater amounts of chow.

It is possible that the weight gain following withdrawal from fluoxetine results from an increase in metabolic efficiency. The fact that in some circumstances fluoxetine can reduce body weight in the absence of a decrease in food intake (Morley & Flood, 1987) suggests that fluoxetine may decrease metabolic efficiency, in addition to its hypophagic effect. It is also important to consider the role that homeostatic mechanisms will play in any sudden loss of either weight or appetite. Daily weight-records of fluoxetine treated animals may show that there is a step-wide response; with weight-loss, then plateau and recovery, then further weight loss.

The advantage of continuing measurements is apparent in that five days after fluoxetine withdrawal, a prolonged hyperphagia was observed. This effect followed the period of recovery from weight loss, and continued to the end of the experiment seven days later. Rebound hyperphagia has not been reported in previous studies of chronic fluoxetine administration (Rowland et al., 1982; Clifton et al., 1989), which used shorter withdrawal periods. Rebound hyperphagia could constitute a problem if it occurred in patients treated with fluoxetine. Obese patients have been shown to initially regain more weight compared to placebo, when d-fenfluramine has been stopped after one year of treatment (Guy-Grand et al., 1990).

The behavioural satiety sequence

Observation of acute drug effects, as previously reported (Montgomery & Willner, 1988; Willner et al., 1990, chapter 5), and this currently described study, have pin-pointed some integral differences in the effects of fenfluramine and fluoxetine.

Acute administration of fenfluramine caused an early termination of sucrose drinking, but altered the behavioural satiety sequence, causing a marked suppression of resting behaviour, with concommitant increases in grooming and other active behaviours towards the end of the session(**Fig 6.5**, left panels). By contrast, after chronic treatment, fenfluramine markedly increased resting behaviour. In these tests, an orderly sequence of behavioural changes was observed. The offset of drinking was followed by a brief period of grooming and an early onset of resting. However, it is unclear that these changes are properly described as an advancement of the behavioural satiety sequence, as drinking time early in the session was actually increased(**Fig 6.5**, top right panel). The method used in this study does not directly provide a measure of drinking rate. However, the increase in drinking time early in the session, in the context of a small decrease in intake, suggests that fenfluramine decreased the rate of consumption, an effect that has frequently been reported in other studies using solid diets (Cooper & Francis, 1979; Burton et al., 1981; Blundell, 1986; Foltin & Fischman, 1988;) d-FF induced reductions in feeding rates are described in humans in Chapter 2.

Thus, the overall pattern could reflect two independent effects; a decrease in drinking rate and an enhancement of satiety. On the other hand, both a rate decrease and an increase in resting could equally reflect non-specific sedative effects. In either case, the pronounced increases in resting in the context of non-significant or marginally significant decreases in intake suggest that the presumed relationship between post-prandial resting and post-prandial satiety should be treated with caution.

The behavioural effects of fluoxetine also point in this direction. On acute administration, fluoxetine slightly decreased drinking time, altered grooming and advanced the onset of resting. These results, which are consistent with a potentiation of the post-prandial satiety, are similar to those previously obtained following acute administration of fluoxetine (Clifton et al., 1989; Willner et al., 1990), and were also seen with femoxetine, paroxetine, and the 5-$HT_{1A/1B}$ agonist, eltoprazine (Chapter 5). However, following chronic administration of FXT, there was no longer any evidence of an increase in resting. If anything, there was a tendency for resting to be decreased or delayed. The major behavioural effect of chronic fluoxetine, relative to control animals, was an increase in 'other active behaviours', largely at the expense of grooming. Thus, in contrast to the effect of acute treatment, following chronic administration of fluoxetine there was no evidence of potentiation of the behavioural satiety sequence, despite a persistent decrease in sucrose intake.

These results illustrate an important principle; that the use of the behavioural satiety sequence as a marker for post-prandial satiety is inherently unreliable. Consider first the case of fluoxetine. The effects of acute fluoxetine are compatible with enhanced satiety, but those of chronic fluoxetine are not. However, there is no evidence that any tolerance to fluoxetine develops between day 1 and day 7(when the tendency for chronic fluoxetine to decrease post-prandial resting was clearly present; see **Fig 6.4**); on the contrary, the effects on intake intensify during this period. It follows that the disappearance of a satiety-enhancing effect of fluoxetine during the course of the first week of treatment is unlikely to result from tolerance, and more probably reflects the emergence of some new effect that masks the expression of postprandial resting behaviour.

A similar argument applies, in reverse, to fenfluramine. The effects of chronic fenfluramine may be compatible with an enhancement of satiety(subject to the reservations expressed above), but the effects of acute fenfluramine certainly are not. However, the effects of fenfluramine undergo extensive tolerance with repeated treatment. Therefore, it is unlikely that the enhancement of post-prandial resting following chronic treatment represents the emergence of a new effect; more likely, the effect on resting was present all along, but was masked on acute administration by some other action of fenfluramine, to which tolerance must develop before an increase in resting can be observed. In other words, when the behavioural data are considered in the context of known tolerance and sensitization effects, it is apparent that both drugs could enhance post-prandial satiety behaviour, both acutely and

chronically, but that in some circumstances(acute fenfluramine; chronic fluoxetine), other, as yet unkown processes, interfere with the expression of the satiety sequence.

Clearly, these conclusions require further investigation, particularly in relation to the identity of the putative 'masking effects'. A starting point may be provided by the observation that systemic administration of the $5\text{-HT}_{1A/B}$ agonist RU24969 causes both hypophagia and locomoter hyperactivity, but these two effects are mediated by different receptor mechanisms and at different neuroanatomical locations (Kennett et al., 1987; Hutson et al., 1988).

The purpose of presenting this analysis in detail is not primarily to draw conclusions about the actions of fenfluramine and fluoxetine, but rather to emphasize the general point that the absence of a temporal shift in the behavioural satiety sequence cannot be used to infer the absence of drug effects on satiety processes. The presence of a temporal shift would appear to provide stronger evidence in favour of an effect on satiety, though clearly, inferences drawn from such data should also be treated as tentative until supported by evidence of extensive similarities between the effects of the drug and those of non-pharmacological manipulations such as pre-feeding. The inability to draw reliable conclusions from studies in which drug effects are not expressed simply as temporal shifts in the sequence of post-prandial behaviours seriously limits the usefulness of this technique.

CHAPTER 7

CONCLUSIONS

7.1 Introduction

The principal focus of this thesis has been upon the effects of pharmacological manipulation of the serotonergic system on feeding in rats and humans. Observational methods, objective data and, in the human studies, subjective ratings were used to elucidate possible mechanisms of action of serotonergic anorectics in both acute and chronic paradigms. Part of the overall methodological approach has been to establish and validate experimental tools useful in this area of research and sensitive enough to detect drug-induced changes in the variables being measured. In each experiment it has been possible to identify aspects of change in food-intake and related variables brought about by pharmacological manipulation. Additionally, it was expected that these experiments would help elucidate the mechanism of action of specific serotonergic agents on food intake and consummatory behaviour in rats and humans. The main clinical approach of this thesis was directed towards obesity, with a comprehensive examination of the effects of a 5-HT reuptake inhibitor on consumption and related variables.

7.2 Considerations of experimental methodology

It was considered important to take observer measurement methods out of the laboratory and into field settings for a variety of reasons. Firstly, behaviours, particularly feeding, are less likely to be subject to demand characteristics if occurring in naturalistic settings (Rolls & Heatherington, 1990). Secondly, by developing a portable methodology, it becomes feasible to consider research of a more complex nature, e.g. patients suffering from neuroleptic-induced obesity (Silverstone, 1983; Silverstone et al., 1988) may show gradual changes which would be evident under longer-term observation. Alternatively, the reported changes in appetite and body weight which may occur in patients suffering from major depression have been viewed largely as diagnostic criteria (DSM-III-R) as opposed to valuable indices in themselves. Yet changes in appetite and body weight have been shown to be associated with greater severity of depression (Paykel, 1977) and associated symptoms of anxiety, retardation and cognitive impairment (Casper et al., 1985). Additionally, Nelson et al. (1984) found that post treatment appetite symptom severity (rated with the Hamilton Depression Rating Scale), correlated with treatment response to the antidepressant desimipramine during a three-week treatment period. A prospective study, using a combination of ward-based observer methodology and interview/rating scales, may elucidate the nature of the relationship between changes in mood and appetite and clarify the extent to which improvement in appetite may be used to predict improvement in affective state, and how these changes are expressed.

Observer methodology has found valued uses in varied clinical settings, such as categorizing self-injurious behaviour in mentally-handicapped and autistic (Goodall & Corbett, 1982), or hyperkinetic (Hutt & Hutt, 1970), children. Additionally, mother-child interactions have been the focus of observation (Olvera et al., 1990). The experimental questions in this thesis resulted in work being carried out in a variety of settings, and upon different subject groups. Of the total of 64 subjects who took part in the complete series of studies, 53 subjects were observed during lunch, comprizing 20 (37.7%) normal, 20 (37.7%) obese, and 13 (24.5%) psychiatric patients, thus establishing the flexibility of the methodology.

The initial validation of the observational methodology confirmed previously reported differences between the actions of d-fenfluramine and d-amphetamine (Rogers & Blundell, 1979). The behavioural and anorectic effects of fenfluramine were also confirmed as residing in the d-isomer (Garattini et al., 1986). As previously reported, d-fenfluramine reduced eating rate, while d-amphetamine reduced the time spent eating. In addition, FXT also reduced eating rate selectively. The action of d-FF and FXT in slowing eating rate, while holding the structure of the meal intact, may possibly be considered of therapeutic advantage, as the strategy of slowing rate of consumption is part of behavioural modification treatment in obesity. In keeping with this, it could be beneficial to let female patients know that FXT will reduce the amount consumed by slowing eating rate, thus acting as an adjunct to behavioural modification.

Additional support for using field data to confirm laboratory-based findings comes from diet diary data. Findings reported here (Chapter 4), and by others (Obarzanek et al., 1985) suggest field data collected in this way parallel laboratory-based intake. However, diet dairy data from Chapter 4 suggest that asking subjects to keep records for long periods of time is counter-productive. Apparent massive swings in intake are suggested by records for week 4 of diet diary intakes (**Fig 4.8**), yet perhaps this is more likely to be evidence of diary-keeping fatigue as opposed to real changes in intake.

By examining placebo data <u>only</u> from normal weight and obese male subjects from Chapter 2, Chapter 3 and Chapter 4, some comparison of both intake and feeding behaviour data is possible. There is no indication from the lunch-meal food intake or feeding behaviour data recorded on experimental days, that the obese male subjects (Chapter 4) either consume more than their lean counterparts (Chapter 2, Chapter 3), or exhibit faster eating rates than normal volunteers (Chapter 2). Table **7.1** shows both food intake and feeding rate data from obese and normal weight male subjects recorded under placebo conditions.

Table 7.1 Eating rates in normal weight (Ch 2) and obese (Ch 4) men[1]

	Kilojoules	Grams	g/min	kj/min
Placebo condition				
Normal (n=12)	4644(453)	582(52)	39.5(3.7)	318(34)
Obese (n=10)	4074(281)	473(49)	30.8(3.5)	260(19)

[1]Values are means (±s.e.). Values for normal subjects represent one observation. Values for obese are the mean (±s.e) across the five test days.

Normal weight subjects taking part in the FXT study in Chapter 3 were not video-taped, so eating rate could not be calculated, but their median energy intake on the three placebo test days was 5400kj, 4877kj and 4914kj respectively [mean; 5064]: some 20% higher than in the obese subjects.

It appears from these placebo data that the obese male subjects may actually consume less than lean subjects in these trials. However, obese male subjects increase their intake across each experimental day (as do obese female subjects), with a final test day intake of 4378kj on day 29. This amounts to an increase of 13.5% (589kj) relative to day 1 intake (3789kj), but even so, these final intakes remain lower than those recorded in normal-weight subjects. Consequently, the indication from the data is that the intake of the

obese males may actually be less than that of the lean males. Comparison of diary intake is not possible in this instance. As discussed in Chapter 1, laboratory studies of single meals have not consistently demonstrated that obese subjects either consume more, or possess different eating behaviour, than lean controls. (Spitzer & Rodin, 1981; Kissileff, 1989). There is some indication that obese subjects actually require more energy relative to lean counter-parts, in order to maintain their greater weight/lean tissue (Lean & James, 1988; Welle et al. 1992).

Interestingly, the intake data for both day 22 and day 29 begin to approach placebo intakes for normal males (Table 7.1) in Chapter 2, and the mean of three day intakes of 5064kj in Chapter 3. Despite a familiarization day, obese subjects may start the trial with a suppressed intake due to inhibition, from which a gradual release occurs as the trial progresses. Support for this comes from the observation that increases in intake across experimental days also occured in the FXT condition.

7.3 Serotonin - mediated effects on food intake and body weight

The objective of the fluoxetine study in normal volunteers (Chapter 3), was to examine the anorectic potential of this 5-HT reputake inhibitor in a healthy, non-depressed non-dieting group. Our findings of marginal reductions in food intake, and small unsustained alterations in ratings of hunger and satiety over a two week FXT treatment period were accompanied by reasonable reductions in body weight of approximately 1.5%($\pm$0.37%).

The obese group of subjects (Chapter 4), treated with FXT for a period of one month, responded with a more clear-cut anorectic effect, showing reductions in food intake, relative to placebo on most treatment days. Again, the measured ratings of both hunger and satiety in response to FXT and relative to placebo, were marginally changed and showed some evidence of reduced levels of hunger and increased levels of satiety, mainly on day 8 and day 22 (Fig 4.11, Chapter 4).

However, significant reductions in body weight were occurring throughout the treatment period while concurrent subjective ratings of hunger and satiety appeared marginally changed. This may indicate that there are disassociable aspects to the effects of FXT on body weight and feelings of hunger and satiety. Experiments in Chapter 3 and Chapter 4 show that fluoxetine is capable of reducing body weight and may reduce food intake by mechanisms which do not primarily effect the above ratings. By the end of one month's treatment with FXT both the experimental data from day 29, (-9.0% relative to placebo) and the mean diet diary intake from week four (-16.1% relative to placebo), suggest that food intake is still being reduced by FXT (although reservations about accuracy during the last week of diet diary records need to be borne in mind).

One potential explanation of these discrepancies is that FXT may be capable of altering body-weight set point (Keesey, 1986; 1989). In this case, the observed reductions in food intake would constitute secondary mechanisms brought into play to support the new lowered

body weight (Stunkard, 1982). There is clear evidence of continued weight loss (**Fig 4.2**) running concurrently with less potent changes in intake (**Fig 4.3**) and subjective ratings (**Fig 4.11**), evidence of both a dissociation, and the possible emergence of some tolerance to the anorectic effect of FXT. However, it may be necessary to consider the possibility that Visual Analogue Scales, while sensitive enough to detect absolute changes in subjective ratings, are not sensitive enough to detect more subtle changes which may be occurring within either of these areas of feelings of hunger and satiety.

Nevertheless, the animal data in Chapter 6 are supportive of the view that FXT may induce alterations in body weight set-point. Body weight in FXT treated animals remains suppressed relative to both FF treated animals and controls (**Fig 6.1**), while chow intake, although still below control, starts to climb towards control levels from day seven onwards (**Fig 6.2**). On cessation of FXT treatment, body weight increased several days prior to increases in chow intake, showing a possible dissociation of the effects of FXT on body weight and intake.

Cessation of treatment with FXT resulted in a rapid weight regain in animals, and most likely weight regain also occurred in most FXT-treated obese humans. It may be advisable to consider long-term drug treatment to sustain a reduced set-point, and unfortunately, the gradual increases in food intake seen in both the placebo and the FXT conditions may indicate attempts to defend original body

weight. It has also been noted that long- term treatment with FXT does not appear to result in sustained body weight loss (Darga et al., 1991).

Of course, critics of set-point theory would prefer greater clarification about the mechanisms which purportedly regulate body weight, body fat and lean tissue. VanItallie & Kissileff (1990) suggest that with the enormous variation in adipose fuel supply and reserves, there is no biological cause for set-point regulation. It is also argued that bodily demand for fuel, on which survival may depend, should take precedence over a regulatory set-point mechanism, which could perhaps be counter-regulatory for survival. These arguments are countered by the suggestion, by Nicolaidis, of a conceptually more flexible regulatory mechanism which is termed "homeorhetic", allowing for dynamic components of both ascending and descending movements within the regulated system (Stallone & Stunkard, 1991).

The examination of the effects of FXT on food selection in the obese, is not supportive of the work of others (Pijl et al, 1991) which reports a specific carbohydrate suppressive effect for this drug. In the study of Pijl et al. (1991), unfortunately only subjects' self reported intake was assessed as opposed to the objective data gained while subjects attended for lunch. Analysis of the diet diary data in Chapter 4 was based on total intake and macronutrient intakes, and did not differentiate between snacks and meals, as these may be determined either by manufacturer criteria or subjective preference. The reduction in intake of protein is not

supportive of a protein-sparing effect for FXT, this may not be advantageous, as the combination of protein and d-fenfluramine have been shown to possess greater anorectic potency relative to d-fenfluramine when paired with CHO (Hill & Blundell, 1990). If, in addition to the generally suppressive effects of FXT on other macronutrients, the effects FXT on suppression of fat intake are validated elsewhere, this could be seen as a valuable attribute for this drug, for reasons mentioned before, e.g. energy density of fat, and the metabolic efficiency with which fat consumed is stored as body fat (Danforth, 1985). It was interesting to see a robust suppressive effect of FXT on the consumption of savoury kj in male subjects (**Fig 4.7**).

The availability of an antidepressant which has anorectic potential is advantageous on three main counts; firstly, it means there is an available treatment which, unlike tricyclics, does not cause unwanted weight gain and jeopardize compliance (Russ & Ackerman, 1988). Secondly, depressed patients who are already overweight may benefit from a treatment which addresses both their clinical depressed state and their obesity. Thirdly, FXT may be used to treat obesity itself. From the subjective ratings of mood there does not appear to be a risk of inducing mania. Despite the administration of three times the clinical therapeutic dose, there was no evidence of mood alteration (Fig **4.13**).

However, considering both the chronicity and refractory nature of obesity, monitoring long term effects on body weight is essential. Darga et al. (1991), reported on FXT treated obese subjects who,

having attained maximum weight loss at week 29 of a 52 week trial, started to regain weight (Fig 7.1). The gradual weight "claw-back" seen in these patients is similar to the body-weight regain seen in the chronic FXT study in rats in Chapter 6 (Fig 6.1), and is strongly suggestive of partial tolerance to the effects of FXT. In addition, on cessation of medication, and after wash-out had occurred, FXT treated animals exhibited weight regain prior to the increases in intake of sucrose and chow. Taking body weight on day 0 as a baseline, and looking at percentage weight change which had occurred by day 29 (approximately one week after treatment stopped), control animals had gained 3.1%, FXT animals 3.1% and FF animals 4.0%.

Effectively, body weight changes in control and FXT treated animals did not differ markedly when observations continued well beyond treatment cessation. Again, these findings do appear to offer some support for the operation of a set-point mechanism. For a clinically obese population, weight regain may be an unwelcome and disheartening prospect on cessation of medication (Guy-Grand et al., 1990).

Drugs which reduce body-weight may be seen as continuing to be therapeutic if, despite a weight-loss plateau, weight regain is avoided. For example, d-fenfluramine has been reported as being succesful at keeping weight regain at bay compared to placebo on cessation of a very low calorie diet (Finer et al., 1987). The use of d-fenfluramine in addition to an initial (approx. 4 months) very low calorie diet during a one year trial caused greater

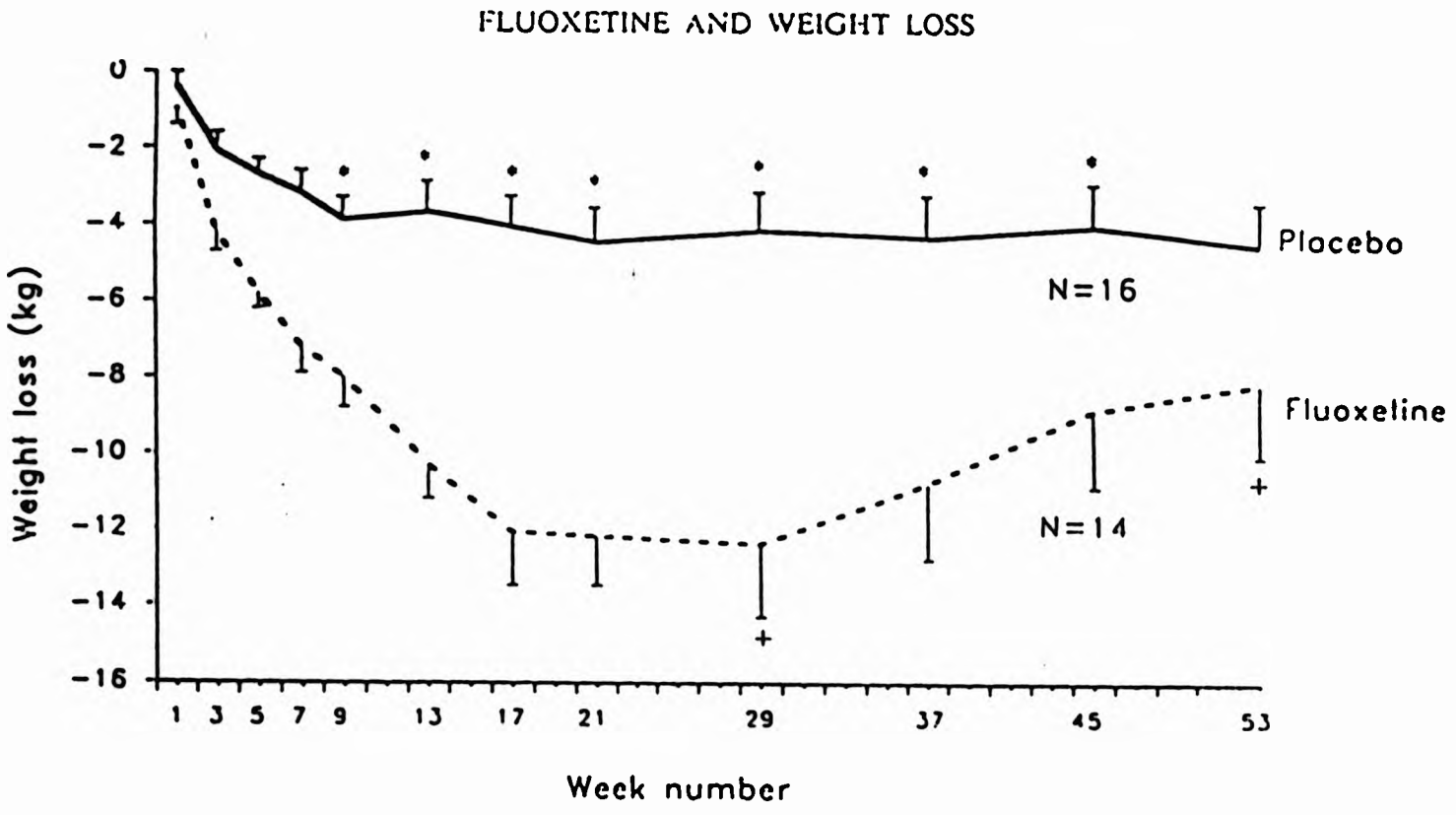


Fig 7.1 Progress of weight-loss during a one year trial in obese subjects. Taken from Darga et al. (1991). American Journal of Clinical Nutrition, 54, 321-25

percentage weight loss relative to a very low calorie diet alone. However, in this trial, weight regain began to occur after six months (Andersen et al., 1992).

Of course, the more objective question to ask is whether there is a place for anorectic and weight reducing agents in the long-term treatment of obesity. Practitioners with considerable experience in obesity research suggest that perhaps by viewing the treatment of obesity as a long-term regime, needing a skilful eclectic treatment approach, using alternavive/mixed treatment approaches, it may be possible to sustain weight loss (Silverstone & Goodall, 1992). A study completed over a decade ago in which Stunkard et al., (1980b) compared six months of either behaviour therapy, pharmacotherapy (FF) or a combination of the two, found that although FF and the combined treatments caused greater weight loss than behavioural modification during the trial, a one year fóllow-up showed small weight regain levels in behavioural modification group compared to the other treatment. However, analysis of weight status five years after a study in which individuals used either low calorie diets, behavioural modification, or a combination, showed disappointing results, with weight regain the usual pattern (Wadden et al., 1989). These studies emphasize the importance of a long-term treatment perspective, with regular monitoring when weight loss had actually occurred.

7.4 Serotonergic drug effects on consummatory behaviour

Acute administration of fluoxetine, dl-fenfluramine, paroxetine, femoxetine and eltoprazine, resulted in reduced intake and alterations in the behavioural satiety sequence (BSS). These 5-HT agonists, with the exception of fenfluramine, all appear to result in an early introduction of resting during the BSS. The effect of acute FF on the BSS may be considered atypical relative to the other serotonergic drugs being examined, in that the resting component is suppressed is a dose-dependent manner (**Fig 5.1**). Looking at this initial effect of fenfluramine on the BSS, it would have been difficult to predict the long-term effects seen on resting.

It has been of some value to examine the anorectic and consummatory behavioural effects of the $5\text{-HT}_{1A/1B}$ agonist, eltoprazine. Eltoprazine was found to possess a satiety-enhancing effect with reductions in sucrose intake and increases in resting behaviour (**Fig 5.6**). While eltoprazine acts at both $5\text{-HTA}_{1A/1B}$ receptors, it is also a 5-HT_{1C} antagonist. Evidence that stimulation of 5-HT_{1A} autoreceptors results in hypophagia has been seen with the 5-HT_{1A} agonist, 8-OH-DPAT (Dourish et al., 1986). Decreases in intake in response to administration of 8-OH-DPAT occur at high doses, which also elicits the 5-HT behavioural syndrome. This analysis suggests that drugs which act as agonists at 5-HT_{1B} receptors may advance the behavioural satiety sequence.

However, the results from both the acute and the chronic studies (below) show the limitations inherent in assessing the significance of drug-induced changes in consummatory behaviour, in the context of the behavioural satiety sequence (BSS). The effects of acutely administered FXT appear consistent with enhanced satiety while the acute effects of fenfluramine appear to alter it, and yet the reverse is seen in response to chronic administration of either drug. It becomes necessary to consider that in both the acute FF and in the chronic FXT regimes, the satiety-enhancing capacity of each drug may be masked by competing responses.

The effects of chronically administered FF further highlight the weaknesses in using the BSS as an index of satiety. Animals treated chronically with FF exhibited an enhanced BSS, in the components of suppression of grooming and a very clear early introduction of resting. However, as behavioural observations on day 14 show (Fig 6.5), drinking behaviour was above control levels, despite intake of sucrose being reduced. There is a possible explanation for these sequence of events which would suggest that FF acts more to induce sedation as opposed to enhancing satiety. As early introduction of resting behaviour is present from observations by Day 4. Animals may "learn" that their drinking time of sucrose will be cut short due to encroaching sedation, and consequently attempt to consume as much sucrose as possible before this occurs.

The increase in non-specific activity seen in response to chronic treatment with FXT (**Fig 6.4**), may be similar to the effects of other serotonergic drugs on the motor component of behaviour, and points to possible interaction between 5-HT and DA systems. Like eltoprazine, at the highest dose tested (**Table 5.1**), the 5-HT_{1B} agonist RU 24969 also induces hyperlocomotion, an effect suggested as being the result of reduced 5-HT inhibitory influences on dopaminergic neurons (Bendotti & Samanin, 1987). Pretreatment with the DA antagonist haloperidol has been shown to block the hyperlocomotive effect of RU 24969 without effecting the hypophagia (Bendotti & Samanin, 1987). Serotonergic antagonists do not block the hyperlocomotion induced by RU 24969 (Green et al. 1984; Kennet et al. 1987).

Although acute FXT did not reportedly alter psychomotor function in normal volunteers (Hindmarch, 1987), some psychiatric patients treated with FXT, are described as having extrapyramidal symptoms such as restlessness (Lipinski et al. 1989). "Restlessness" could have been an appropriate descriptive category in the chronic rat study, as the observed behaviour comprized continual movement, without a notably stimulant aspect.

The likelihood of being able to draw a comprehensive conclusion to explain the emergence of dissimilar effects of d-FF and FXT in the chronic rat study is limited on two counts. Firstly, the observation methodology used in this study was designed to examine drug effects on four related consummatory behaviours of drinking,

grooming, activity and resting. This maximally engaged the attention span of the observer and while the end result was a good general description of overall behavioural occurrence, it was not possible to "fine tune" to any particular behaviour. Secondly, the observation periods were limited to 40 minutes on test days, and behaviours occurring outside of this period were not observed. This made it impossible to establish whether any of the behavioural differences, from control values, were present at other times. It is likely that the alterations in non-consummatory activity, grooming and resting were present at times outside the observation period, but this cannot be stated conclusively.

There are at least two possible developments in an experimental methodology which would clarify the picture of results here: A methodology of continuous recording (Blundell & McArthur, 1981) would allow measurements over longer periods of time. Additionally, as initial observations highlighted signnificant deviations from control values in. for example, levels of non-specific activity in FXT treated animals, particular focus could be trained on selected behaviour using categories with more descriptive levels. Because of the attention span described above, some behavioural categories were condensed for descriptive purposes. The category of behaviour scored as 'active' (activity) actually included sniffing, rearing and locomotion, while the category of behaviours scored as resting included both standing motionless and lying down. An observer methodology which focussed on a particular behaviour at several levels, and benefited from computer-assisted measurement could refine the descriptive categories and reduce the ambiguity of the

more general categorization used in the study here. For example, Chagraoui et al., (1990), examined the effects of acute antidepressant and chronic dopamine agonist treatment on motor responses in mice using a computerized Digiscan actimeter which measured horizontal and vertical activity, and the number of small movements occurring.

In addition, further examination of the possible mechanisms behind the effects of FXT may be possible by using drugs to determine that the observed effects are definitely specific to 5-HT receptors, as opposed to acting on another system, e.g. histamine antagonists should clarify that the anorectic effect is not histaminergic. Later development may see clarification of the source of FXT-induced effects. The anorectic effect of FXT may largely reside in one isomer, as with FF (Garattini et al., 1986), and the dopaminergic effects may be largely present in the other. Wong et al (1988), have shown that both the R and S-enantiomers of fluoxetine lower food intake in meal-fed and in rats made hyperphagic by 2-deoxyglucose, they report that the S-enantiomer is slightly more potent at suppressing food intake than the R-enantiomer.

7.5 Final Comment

Stellar (1989), acknowledging himself as one of the "oldest living members" in the field of feeding research, suggested that while there have been advances in knowledge concerning the physiology of feeding, there are few success stories in the search for sound treatment,(since his entry into research in 1938), for the

conditions of anorexia nervosa, bulimia nervosa or obesity. Whether the condition of obesity should be placed in DSM-111-R alongside the other eating disorders is a contentious point. More recently, there are reports of suggested reclassification of diagnosis, to include identification of obese people who binge (Wilson & Walsh, 1991). At present, it is not determined what percentage of obese people are eating disordered.

A prophylactic approach to the avoidance of obesity is one way of acknowledging the gene-environment interaction mentioned in Chapter 1 (Ward, 1990), it is clear that obesity arises from a variety of sources (Blundell, 1990). Some researchers are attempting to identify early risk factors. For example, a study which divided children into either high risk (with one or both parents overweight) or low risk (normal weight parents) groups, showed that the high risk group consumed more fat throughout the course of a year, and weighed more than the low risk group, by the end of the year (Eck et al., 1992). Another study which examined the relationship between temperament status and the development of obesity in children, found correlations between weight gain and parent-rated difficult behavioural style. A cross-sectional section of the same study found obese children to be less predictable and persistent than matched controls (Carey et al., 1988). Obviously behaviourists and geneticists would hold differing views on what is occurring in these children, and would consequently recommend differing treatment regimes. As both childhood asthma and hyperkinesia are treated with drugs, perhaps there is a role for pharmacological intervention when the alternative would be the development of obesity. Alternatively,

of course, alterations in dietary intake are recommended by experts in countries, such as Japan, in which large increases in childhood obesities are occurring (Kinugasa & Kusunoki, 1990).

For adult obesities, classification and treatment continues to be randomly dependent on persuasion of the clinican, yet there is every possibility that refining diagnostic criteria and using this as a basis for a treatment plan, would assist in treatment success. Classification of obesity based on weight or BMI is useful, but too simplistic (Bjorntorp, 1990). Some attempts are being made to develop a more sophisticated typology of obesity which would help determine treatment approaches. For example, Allison & Heshka (1991) identified component aspects of either lifestyle, or physiology (gynoid/android), which appeared to contribute to the presenting obesity. Their suggestion was that lifestyle obesities may benefit from, for example, a behavioural modification approach, while gynoid obesity would, perhaps, merit a pharmacological intervention.

The likelihood of successful treatment for obesity is additionally confounded by the probability that obese patients, presenting themselves for treatment, are a heterogeneous group with respect to aetiology. Figure 7.2 shows how the gene-environment interaction may be expressed for the development and maintenance of obesity to occur

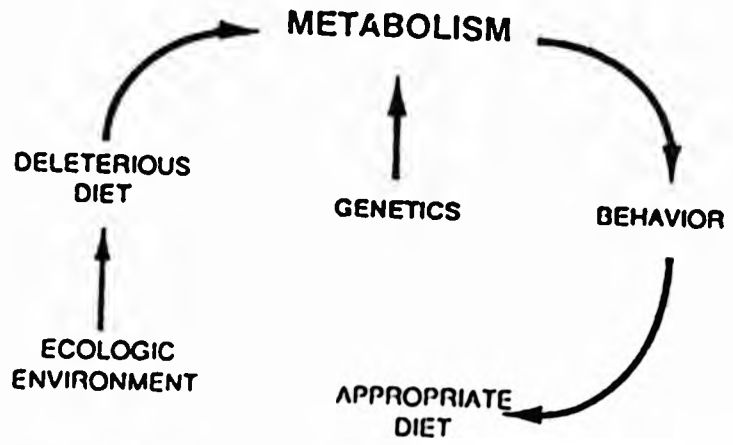


Diagram of a negative feedback loop with beneficial effect. In this particular paradigm, genetic differences in metabolism lead to modification of behavior that culminates in avoidance of deleterious foods. Examples of such phenomena include the alcohol flush reaction and lactose intolerance.

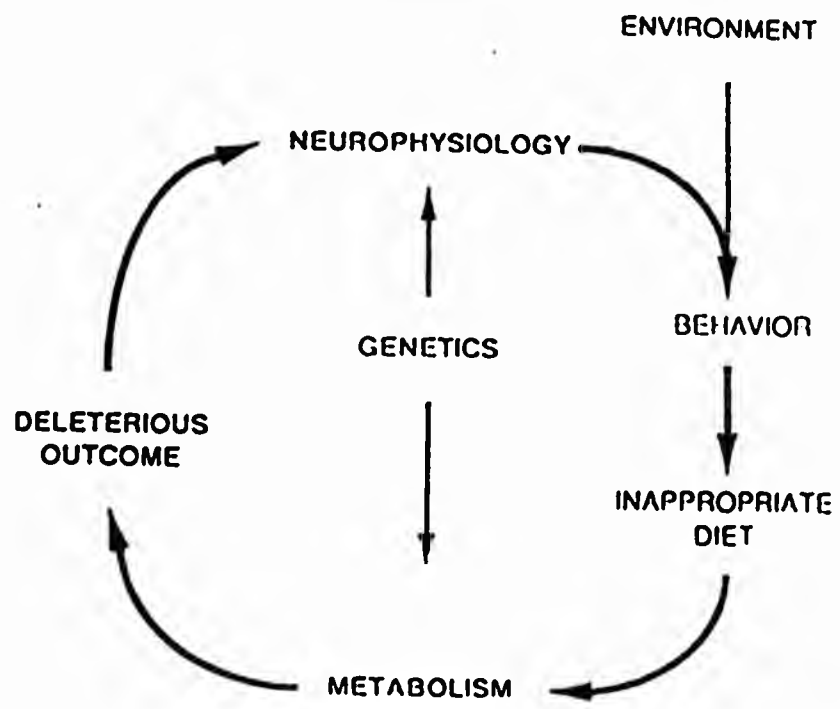
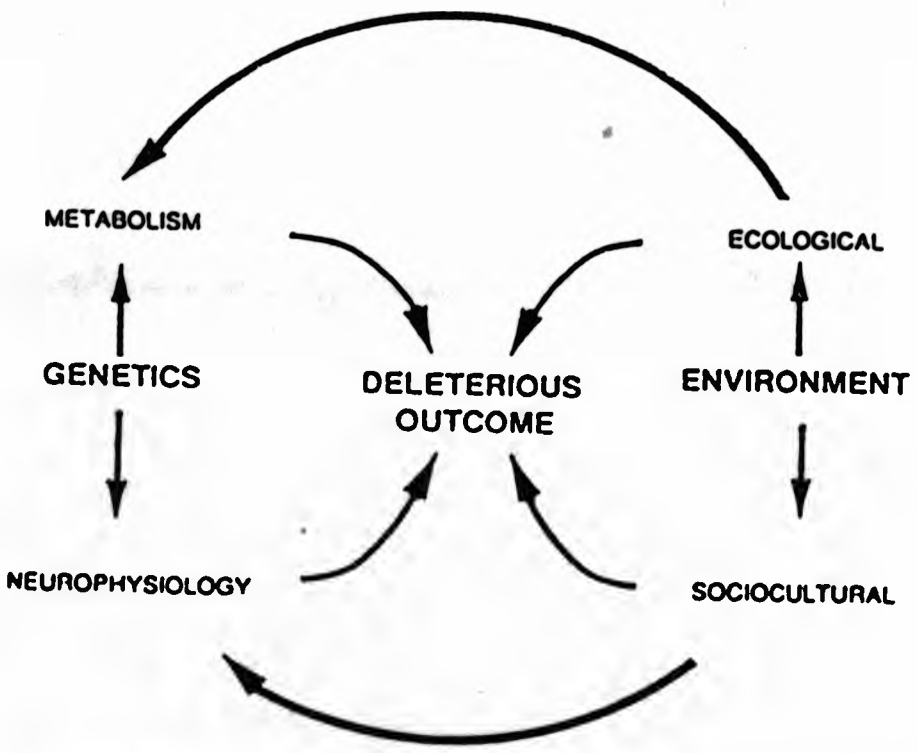


Diagram of a positive feedback loop with deleterious effects. The paradigm illustrated is appropriate for complex situations like alcohol abuse, where genetic differences in both metabolism and neurophysiology can exacerbate the deleterious effects of alcohol.



Example of the complex interactions leading to obesity, and similarly complex outcomes. Note that all four causal pathways (metabolic, neurophysiologic, ecologic and sociocultural) are involved, each establishing its own positive feedback loop contributing to a deleterious effect over all.

Fig 7.2 Feedback loops with beneficial(top) and deleterious(middle, bottom)outcome

(bottom panel); examples of both beneficial (alcohol avoidance) and another deleterious interaction (alcohol abuse) are shown (top and middle panels respectively)(Ward, 1990).

Psychological assessment, prior to treatment for obesity, may be beneficial in determining either suitable treatment for that individual, or likelihood of success as predicted by psychological profile. Clark et al, (1991), have been attempting to identify psychological self-efficacy constructs present in the obese before and after treatment. Self efficacy (Bandura, 1977), defined as a person's judgement of their own ability to efficiently manage a situation, has been a useful construct in predicting outcome in studies of addictive behaviour e.g. drinking, smoking. Clark et al (1991), who developed and validated a Weight Efficacy Life-Style Questionnaire, suggest that this questionnaire is used to identify specific aspects; e.g. negative emotions, positive activities, which may contribute to dieting outcome. Use of such questionnaires could also pinpoint areas of vulnerability which would necessarily differ between individuals. Blair et al., (1990), reported that a reduction in levels of "emotional" eating was associated with greater success in weight loss, again pin-pointing on the crucial role of psychological variables for weight control.

Individual reports and spontaneous comments from the obese subjects in the present study suggest that perhaps long-term treatment regimes will need to be comprehensively tailored to the need and assessed status of that particular individual. For example, some subjects reported eating whether they felt hungry or not,

particularly sweet foods (female). Single male subjects reported eating take-away foods and drinking beer frequently, while married men consumed home-cooked meals, the content of which was largely determined by their wives, and also consumed beer. This intake behaviour is not aberrant, but was obviously contributing to the static obese state. Alcohol consumption has been suggested as playing a contributory role in the development and maintenance of obesity in males (Seidell et al, 1988). It can be seen that drastic and possibly unattractive changes of lifestyle are necessary if weight loss is to occur.

At present obese patients presenting for treatment are assessed for degree of overweight and general health status. The treatment offered will depend on the persuasion of the clinician and this treatment will most likely be unsuccessful in the long-run. The possible combination of energy intake restriction, behaviour therapy and exercise would, no doubt, be successful while it lasted, but would require tremendous motivation for patients themselves, support from relatives, and a sound environmental context (Ward, 1990). The singular most hopeful aspect for those for whom obesity is an affliction, is in the diverse research areas which are consistently active and likely to continue so.

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Publications resulting from work presented in this thesis

Sections of the work described in this thesis have been presented as conference papers, and to date most of the work has been published. These publications are listed below:

Chapter 2

Mc Guirk, J., Goodall, E., Silverstone, T. & Willner, P. (1991). Differential effects of d-fenfluramine, l-fenfluramine and d-amphetamine on the microstructure of human eating behaviour. Behavioural Pharmacology, **2**, 113-119.

Chapter 3

Mc Guirk, J., & Silverstone, T (1990). The effect of the 5-HT re-uptake inhibitor fluoxetine on food intake and body weight in healthy male subjects. International Journal of Obesity, **14**, 361-372

Chapter 4

Mc Guirk, J., & Silverstone, T. The effects of fluoxetine on food intake and choice, body weight and subjective variables in obese subjects. Submitted

Chapter 5

Mc Guirk, J., Muscat, R., & Willner, P. (1992). Effects of the 5-HT uptake inhibitors, femoxetine and paroxetine, and a 5-HT_{1A} agonist, eltoprazine, on the behavioural satiety sequence. Pharmacology, Biochemistry & Behavior, **41**, 801-805

Chapter 6

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