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Tonic regulation of satiety by 5-HT_{1B} receptors in the mouse: converging evidence from behavioural and c-*fos* immunoreactivity studies?

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Abstract

Activation of 5-HT_{1B} receptors is thought to play an important role in the inhibitory influence of serotonin on feeding behaviour and body weight in mammals. Earlier studies have shown that 5-HT_{1B}-knockout (KO) mice eat more and are heavier than wild-type (WT) controls and that the selective 5-HT_{1B} receptor agonist CP-94,253 reduces food intake in food-deprived mice. Here we characterize the behavioural effects of both CP-94,253 and the selective 5-HT_{1B} receptor antagonist SB224289 on feeding and other behaviours within the behavioural satiety sequence, and also report a c-*fos* mapping study using CP-94,253. CP-94,253 produced a dose-dependent suppression of food intake with a profile consistent with a selective effect on feeding behaviour. These effects were absent or reduced in 5-HT_{1B}-KO mice and in WT mice pretreated with SB224289. SB224289 administered alone enhanced food intake consistent with impaired satiation; a similar effect was apparent in 5-HT_{1B}-KO mice compared to WT. CP-94,253 induced c-*fos* in a range of structures previously implicated in the expression of feeding behaviour. These results suggest that the activation of 5-HT_{1B} receptors is an important component of endogenous satiation mechanisms in the mouse.

Introduction

The importance of serotonin in the modulation of ingestive behaviour has been demonstrated in an extensive body of empirical data. Thus, agonists at several serotonin subtypes reduce food intake in both rat and mouse. These include the 5-$HT_{2C/1B}$ receptor agonist 1-(m-chlorophenyl)piperazine (*m*CPP) (Kitchener & Dourish, 1994; Hewitt *et al*., 2002), the 5-$HT_{2A/2B/2C}$ receptor agonist Ro 60–0175 (Clifton *et al*., 2000; Hewitt *et al*., 2002) and the 5-HT_{1B} receptor agonist CP-94,253 (Lee & Simansky, 1997; Clifton *et al*., 2003). In each case associated behavioural analysis excludes nonspecific behavioural effects, such as sedation or hyperactivity, as an explanation of the hypophagic response. Furthermore, serotonin receptor antagonists, under some behavioural conditions, enhance food intake in the rat (Fletcher, 1988; Dourish *et al*., 1989), which suggests a role for endogenous serotonin in the control of appetite.

The current classification of serotonin receptors recognizes 14 receptor subtypes (Barnes & Sharp, 1999). The 5-HT_{2C} and 5-HT_{1B} receptor subtypes are considered to play an especially prominent role in the modulation of feeding behaviour (Dourish, 1995; Simansky, 1996) and are expressed in areas that are associated with the modulation of feeding behaviour. 5-HT_{2C} receptors are widely expressed in the CNS (Barnes & Sharp, 1999), including structures within the limbic system and basal ganglia (e.g. hippocampus, amygdala, hypothalamus, nucleus accumbens, caudate nucleus and substantia nigra), several of which are also implicated in the modulation of feeding behaviour. Immunocytochemical studies in the rat have revealed 5-HT_{1B} receptor protein in various hypothalamic subregions (Makarenko *et al*., 2002). Appreciable 5-HT_{1B} receptor binding can also be measured in the nucleus of the solitary tract (Manaker & Verderame, 1990) and within the parabrachial complex (Lee *et al*., 1998) as well as in a range of forebrain structures. Indeed, 5-HT_{1B} receptors in the lateral parabrachial nucleus have been shown to mediate the hypophagic action of both the 5-HT_{1B} receptor agonist CP-93,129 (Lee *et al*., 1998) and of *d*-fenfluramine administered into this brain region (Simansky & Nicklous, 2002).

Although the 5-HT_{1B} receptor agonist CP-94,253 is known to reduce food intake in food-deprived mice (Clifton *et al*., 2003), the effects of 5-HT_{1B} receptor agonists on feeding behaviour patterns have only been characterized in the rat. Therefore, in the present study we describe the effects of CP-94,253 on the behavioural satiety sequence in mice. We confirm that the hypophagic action of CP-94,253 was mediated by 5-HT_{1B} receptors by comparing the effects of CP-94,253 in 5-HT_{1B} receptor-knockout (KO) mice and wild-type (WT) controls. We also challenged the action of the agonist with the selective 5-HT_{1B} receptor antagonist SB224289. The design of this study also allowed us to identify any intrinsic effects of SB224289 on food intake and feeding behaviour in the mouse. A possible additional effect of CP-94,253 at 5-HT_{1D} receptors was examined by challenging the action of the agonist with the 5-$HT_{1B/1D}$ receptor antagonist GR127,935. A number of recent reports have described the effects of nonselective serotonin agonists on the induction of c-*fos* immunoreactivity in the

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rat, in relation to both feeding (Rowland *et al*., 2001) and anxiety (Singewald & Sharp, 2000; Singewald *et al*., 2003). However, the effects of a selective 5-HT_{1B} receptor agonist on expression of an immediate early gene have not been reported in either rat or mouse. We therefore mapped the distribution of c-*fos* immunoreactivity in the WT mice using a dose of CP-94,253 that produced a hypophagic effect in our behavioural studies.

Materials and methods

Subjects

Breeding stocks of adult wild-type and 5-HT_{1B}-KO mice of the 129/Sv-ter strain (Saudou *et al*., 1994) were initially obtained from the New York State Psychiatric Institute animal facility. Offspring from breeding pairs of the two strains were maintained in groups of 2–4 until required. Animals were housed singly with *ad libitum* access to food and water 4 weeks prior to the two experiments in which the behavioural satiety sequence was measured. Solid-bottomed cages (North Kent Plastics, Type M2), were used with additional paper bedding in addition to sawdust. The feeding studies used within-subject designs with at least 2 days between treatments. All animals were maintained in a controlled environment held at 21 ± 1 °C and 50 ± 15% RH with a 12 : 12-h photoperiod (lights on at 05.30 h). The experiments were licensed under the UK Animals (Scientific Procedures) Act 1986 (Project License 70/5033), following approval by the University of Sussex Local Ethical Review Committee.

Drugs

CP-94,253 (3–1,2,5,6-tetrahydro-4pyridyl)-5-propoxypyrrolo[3,2-b]pyridine), a selective 5-HT_{1B} receptor agonist (Koe *et al*., 1992), and SB224289 (2,3,6,7-tetrahydro-1-methyl-5-{2-methyl-4[(5-methyl-1,2,4 oxadiazole-3-yl)biphenyl-4-yl]carbonyl}furo[2,3-f]indole-3-spiro-4-piperidine), a selective 5-HT_{1B} receptor antagonist (Selkirk *et al*., 1998), were synthesized in the Chemistry Department of Vernalis Research. GR127,935 (2′-methyl-4′(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-(5-methoxy-3–4-methyl-piperazin-1-yl)-phenyl]amide) was kindly donated by Glaxo plc (Ware, UK). The doses of CP-94,253, SB224289 and GR127,935 were chosen with reference to Clifton *et al*. (2003) and are expressed in terms of the weight of the salt.

CP-94,253 was dissolved in distilled water. GR127,935 was dissolved in water with warming and sonication. SB224289 was initially dissolved in 20% of the final volume of PEG400, sonicated for 20 min and made up to volume with 10% (2-hydroxypropyl)-β-cyclodextrin (Fluka, Poole, UK) in water. SB224289, CP-94,253 and GR127,935 were administered i.p. in a volume of 10 mL/kg.

Behavioural procedures: behavioural satiety sequence

During an initial habituation period mice were presented for 40 min each day with a palatable wet mash consisting of 1 part powdered chow (Special Diet Services, UK) to 2.5 parts tap water until intake reached a stable level. The mash was presented in clear plastic Petri dishes (5 cm diameter) attached to rectangular pieces of aluminium (3×12 cm) which supported the dish on the surface of the sawdust bedding, thus minimizing contamination of the mash and also preventing it from being upturned. Food intake was measured by subtraction of initial and final weights and recorded to 0.01 g. A preweighed dish of mash was presented and the behavioural satiety sequence was observed for a period of 40 min beginning at 14.30–15.30 h. The sequence was recorded using the method described by Vickers *et al*. (1999). Behavioural categories were: *feed*, holding or ingesting of food, and drinking; *active*, moving, rearing and other behaviour patterns not defined elsewhere; *groom*, body care movements using mouth or forelimbs; and *inactive*, characterized as an absence of movement with a resting posture, with or without eye closure. Drug-induced stereotypy was rated, 5 min prior to food presentation, on a 4-point scale (0, no apparent stereotypy; 1, equivocal stereotyped movements; 2, clear evidence of stereotyped head movements and flat body posture; 3, pronounced stereotyped head movements and flat body posture; Vickers *et al*., 1999).

Each experiment used a separate naive group of 12 WT and 12 5-HT_{1B}-KO mice and each mouse received each drug dose randomised on an ascending Latin square design. The observer was blinded to the genotype of the mice until the conclusion of the experiment.

5-HT_{1B} receptor agonist-induced c-fos expression

Mice were held singly for at least 7 days, were handled daily and received at least four injections of 0.9% saline (10 mL/kg i.p.) prior to the experimental day. Separate groups of mice were used to evaluate the effects of CP-94,253 in the hindbrain ($N = 4$) and forebrain ($N = 5$). CP-94,253 20 mg/kg was administered i.p. and 90 min later the mice were deeply anaethetized (120 mg/kg sodium pentobarbital i.p.) and transcardially perfused with 50 mL phosphate-buffered (pH 7.4) saline (PBS) followed by 60 mL 4% paraformaldehyde. The brain was dissected from the skull and held in 4% paraformaldehyde in PBS (>36 h) followed by 30% sucrose in PBS (24 h) at 4 °C. The brains were blocked and cut at 40 μm on a freezing microtome. Individual sections were placed into wells and processed following a method adapted from Elmquist & Saper (1996). Every sixth section was Nissl-stained in the forebrain, and alternate sections were Nissl-stained in the hindbrain. The intensity of c-*fos* immunoreactivity was rated, by an observer blind to the drug treatment condition, on a 4-point scale (0, no *fos*; to 3, intense c-*fos* immunoreactivity in many cells) derived from Li & Rowland (1993)

Analysis

Behavioural data were analysed using ANOVA using Genstat 4.2 (Genstat, 1987). Subsequent paired comparisons used either Dunnett's method or *t*-tests with a Bonferroni correction. Means and SEM are given within the text. C-*fos* ratings for CP-94,253- and vehicle-treated groups were compared using *t*-tests.

Results

CP-94,253-induced hypophagia in 5-HT_{1B}-KO and WT mice

Intake of mash was significantly higher in 5-HT_{1B}-KO mice: $F_{1,22} = 9.1$, $P < 0.01$. The selective 5-HT_{1B} agonist CP-94,253 reduced intake of palatable mash in both 5-HT_{1B}-KO and WT mice: $F_{3,66} = 15.6$, $P < 0.001$ (Fig. 1). However, CP-94,253 (10 or 20 mg/kg), compared with vehicle, reduced intake in WT to 78 and 37% but only to 90 and 71% in 5-HT_{1B}-KO mice, reflected in a genotype × dose interaction for this analysis: $F_{3,66} = 5.16$, $P < 0.001$.

Within the satiety sequence, feeding was more protracted during the early part of the sequence in vehicle-treated 5-HT_{1B}-KO mice than in WT, and the KO mice also showed increased levels of active behaviour. Feeding behaviour was also reduced in a dose-dependent manner by CP-94,253: $F_{3,66} = 10.24$, $P < 0.001$. A significant dose × genotype × time interaction, $F_{21,462} = 5.41$, $P < 0.002$, reflected an increasing suppression of feeding in early time bins which was observed in WT but not 5-HT_{1B}-KO mice (10 mg/kg, Fig. 2; 20 mg/kg data not shown but see Fig. 4 for WT). Active behaviour was dose-dependently reduced by CP-94,253 ($F_{3,66} = 3.8$, $P < 0.05$) and was also higher in 5-HT_{1B}-KO mice ($F_{1,22} = 7.7$, $P < 0.01$). Inactive behaviour had an inverse relationship to active behaviour in both KO and WT mice, and

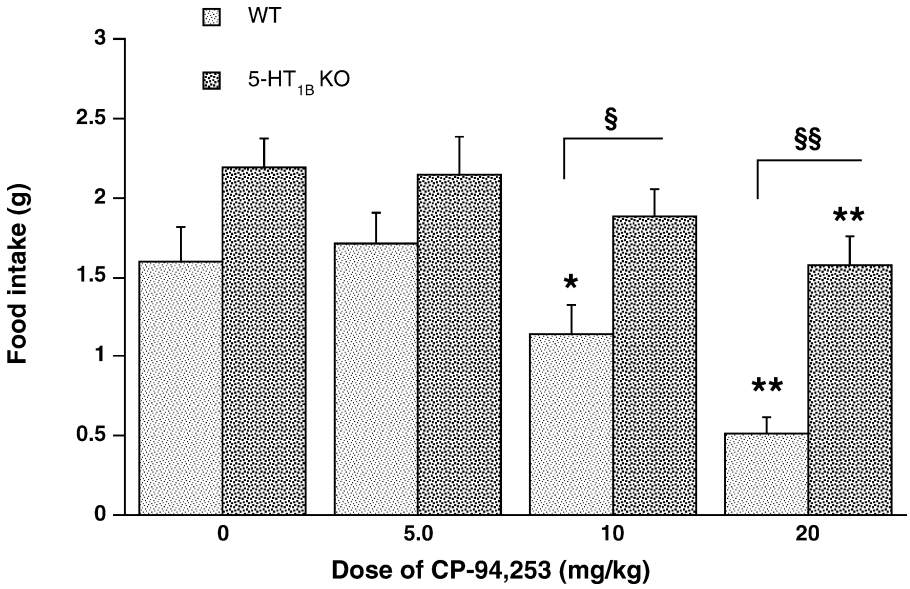

FIG. 1. Food intake (g + SEM) of 5-HT$_{1B}$-KO and WT mice following administration of CP-94,253 (0, 5, 10 or 20 mg/kg) given 30 min before the 40-min test session. $^{**}P < 0.01$, $^{*}P < 0.05$ compared with vehicle control; $P < 0.01$ comparing WT and 5-HT$_{1B}$-KO mice at that dose.

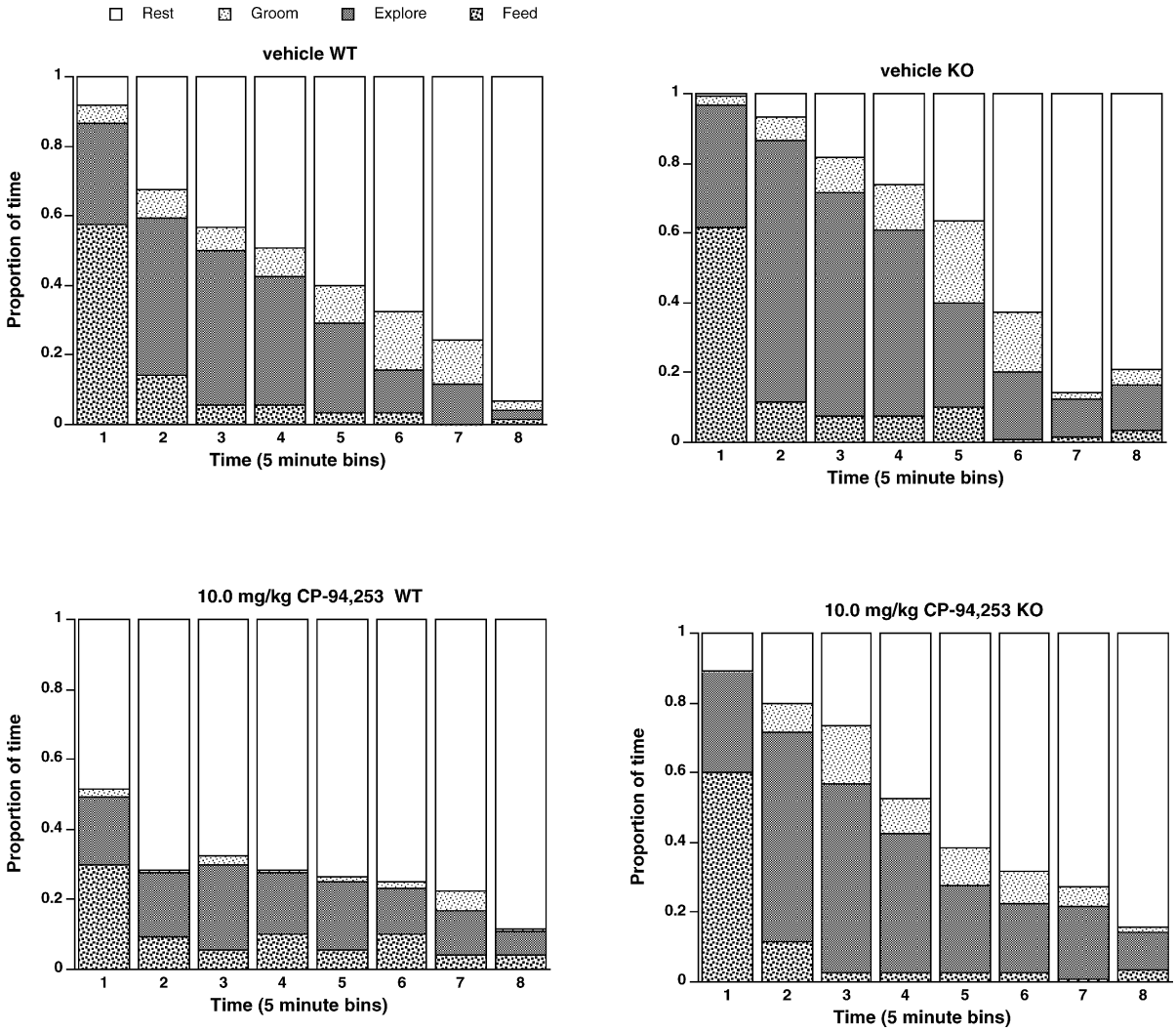

FIG. 2. Satiety sequences of 5-HT$_{1B}$-KO and WT mice following administration of either vehicle or CP-94,253 (10 mg/kg) given 30 min before the 40-min test session.

it was enhanced by CP-94,253 compared to vehicle ($F_{3,66} = 10.24$, $P < 0.001$) though to a lesser extent in 5-HT_{1B}-KO than in WT mice ($F_{1,22} = 13.4$, $P < 0.001$). Although levels of grooming behaviour were relatively low, there was a suppression by CP-94,253 ($F_{3,66} = 10.4$, $P < 0.001$) though to a lesser extent in 5-HT_{1B}-KO than in WT mice ($F_{1,22} = 5.7$, $P < 0.05$). The overall effect of increasing doses of CP-94,253 was disruption of the normal satiety sequence in WT mice following 10 mg/kg CP-94,253 whereas the sequence remained broadly intact in 5-HT_{1B}-KO mice until the highest dose of CP-94,253 (30 mg/kg).

Antagonism of CP-94,253-induced hypophagia by SB224289

The selective 5-HT_{1B} receptor agonist CP-94,253 produced a substantial reduction in food intake in WT mice pretreated with vehicle (Fig. 3). This decrease in food intake was abolished in animals pretreated with the selective 5-HT_{1B} receptor antagonist SB224289. As a consequence there was a pretreatment × treatment interaction: $F_{2,55} = 7.4$, $P < 0.001$. In addition, an analysis confined to those animals pretreated with the antagonist and then treated with vehicle confirmed a dose-dependent increase in food intake induced by SB224289: $F_{2,22} = 4.29$, $P = 0.027$.

Data from the behavioural observations confirmed the clear antagonism of the effect of CP-94,253 by SB224289. In the analysis of feeding behaviour, in addition to main effects of time, pretreatment and treatment (each $P < 0.001$), there was a three-way interaction, $F_{14,154} = 5.72$, $P < 0.001$, reflecting the substantial reduction in feeding that was only apparent in the vehicle–agonist condition (Fig. 4). A similar three-way interaction was found for inactive behaviour ($F_{14,154} = 3.07$, $P < 0.001$), reflecting the inverse relationship of these two behaviour patterns. However, although grooming behaviour was substantially reduced by CP-94,253 ($F_{1,11} = 22.2$, $P < 0.001$), there was only a minimal, non dose-related and nonsignificant attenuation by pretreatment with SB224289.

Antagonism of CP-94,253-induced hypophagia by GR127,935

The selective 5-HT_{1B} receptor agonist CP-94,253 again produced a substantial reduction in food intake in WT mice pretreated with vehicle (Fig. 5). This decrease in food intake was reduced in animals pretreated with the 5-$HT_{1B/1D}$ receptor antagonist GR127,935, resulting in a pretreatment × treatment interaction: $F_{2,55} = 6.89$, $P < 0.01$.

Pretreatment with GR127,935 reversed many of the changes in the satiety sequence induced by CP-94,253. In the analysis of feeding behaviour, in addition to main effects of time, pretreatment and treatment (each $P < 0.01$), there was a three-way interaction, $F_{14,154} = 1.78$, $P < 0.05$, reflecting a reduction in feeding that was only apparent in the vehicle–agonist condition (Fig. 6). Grooming behaviour was again reduced by CP-94,253 ($F_{1,11} = 61.73$, $P < 0.001$), with no significant attenuation following pretreatment with GR127,935.

*Distribution of c-*fos *immunoreactivity induced by CP-94,253*

Treatment with CP-94,253 (20 mg/kg) induced c-*fos* immunoreactivity in a variety of brain areas whereas vehicle treatment was associated with low levels of c-*fos* immunoreactivity (Table 1). A clear but localized response was present in the nucleus of the solitary tract (Fig. 7a) and extended from medial to more lateral subregions. However, comparisons with adjacent Nissl-stained sections indicated that the response excluded both the hypoglossal nucleus and dorsal vagal nucleus. A clear immunoreactive response was observed within the parabrachial complex (Fig. 7b) and was restricted to the external lateral and dorsal regions of this structure. c-*Fos*-immunoreactive cells were observed throughout the locus coeruleus (Fig. 7c). In general the responses within hindbrain and midbrain were restricted to these structures. For example, within the periaqueductal grey there was a slight-to-moderate amount of c-*fos* staining scattered over both dorsal and ventral areas throughout the rostral–caudal extent of this structure. However, this staining showed no difference in intensity between control and experimental groups. There was moderate staining in the nicotinamide adenine dinucleotide phosphate (NADPH)-positive laterodorsal tegmental nucleus which lies below and lateral to the cerebral aqueduct in the mouse but again with no difference between control and experimental animals. Within the forebrain distinct c-*fos* immunoreactivity was especially marked in the paraventricular nucleus of the hypothalamus (Fig. 7d) and the central nucleus of the amygdala (Fig. 7e) in mice treated with CP-94,253.

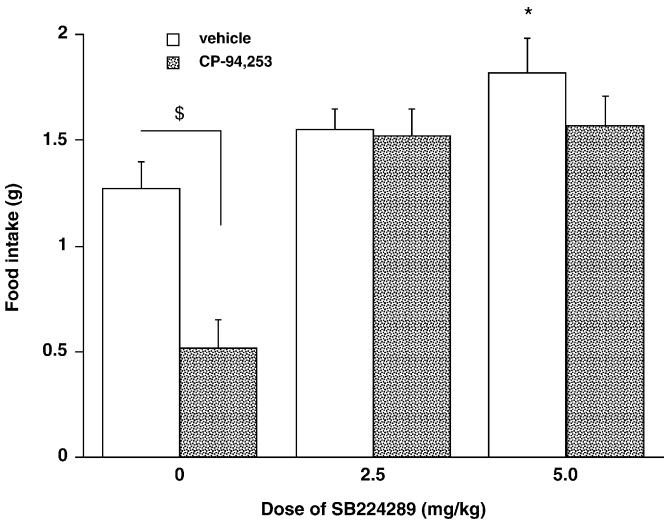

FIG. 3. Food intake (g + SEM) of WT mice treated with either vehicle or SB224289 (2.5 or 5 mg/kg) 60 min, and either vehicle or CP-94,253 (20 mg/kg) 30 min prior to the presentation of palatable mash for 40 min. $^{*}P < 0.01$ compared with vehicle control, $^{\$}P < 0.01$ comparing vehicle and antagonist pretreatment.

Discussion

Our behavioural data have demonstrated that the 5-HT_{1B} receptor agonist CP-94,253 both reduces food intake and, at moderate doses, advances the behavioural satiety sequence in mice. Higher doses of this agonist produced some nonspecific disruption of the sequence. These effects were greatly reduced in 5-HT_{1B} receptor-KO mice, were absent in WT mice pretreated with the selective 5-HT_{1B} receptor antagonist SB224289 and were attenuated in WT mice pretreated with the 5-$HT_{1B/1D}$ antagonist GR127,935. Pretreatment with SB224289, in the absence of agonist treatment, was associated with a dose-related enhancement of food intake and delay in the behavioural satiety sequence; a similar behavioural profile was evident in 5-HT_{1B}-KO mice. In a final experiment we observed that c-*fos* immunoreactivity was stimulated by CP-94,253 in a number of brain regions associated with the expression of motivated behaviour.

In the rat several recent studies using the selective 5-HT_{1B} receptor agonist CP-94,253 have demonstrated a role for the 5-HT_{1B} receptor in the modulation of feeding behaviour. Thus CP-94,253 advances the satiety sequence in rats (Halford & Blundell, 1996) and this effect is blocked by pretreatment with the selective 5-$HT_{1B/1D}$ receptor antagonist GR127,935 (Lee *et al*., 2002). In meal-pattern studies, CP-94,253 reduces meal size without affecting the rate of eating or intermeal

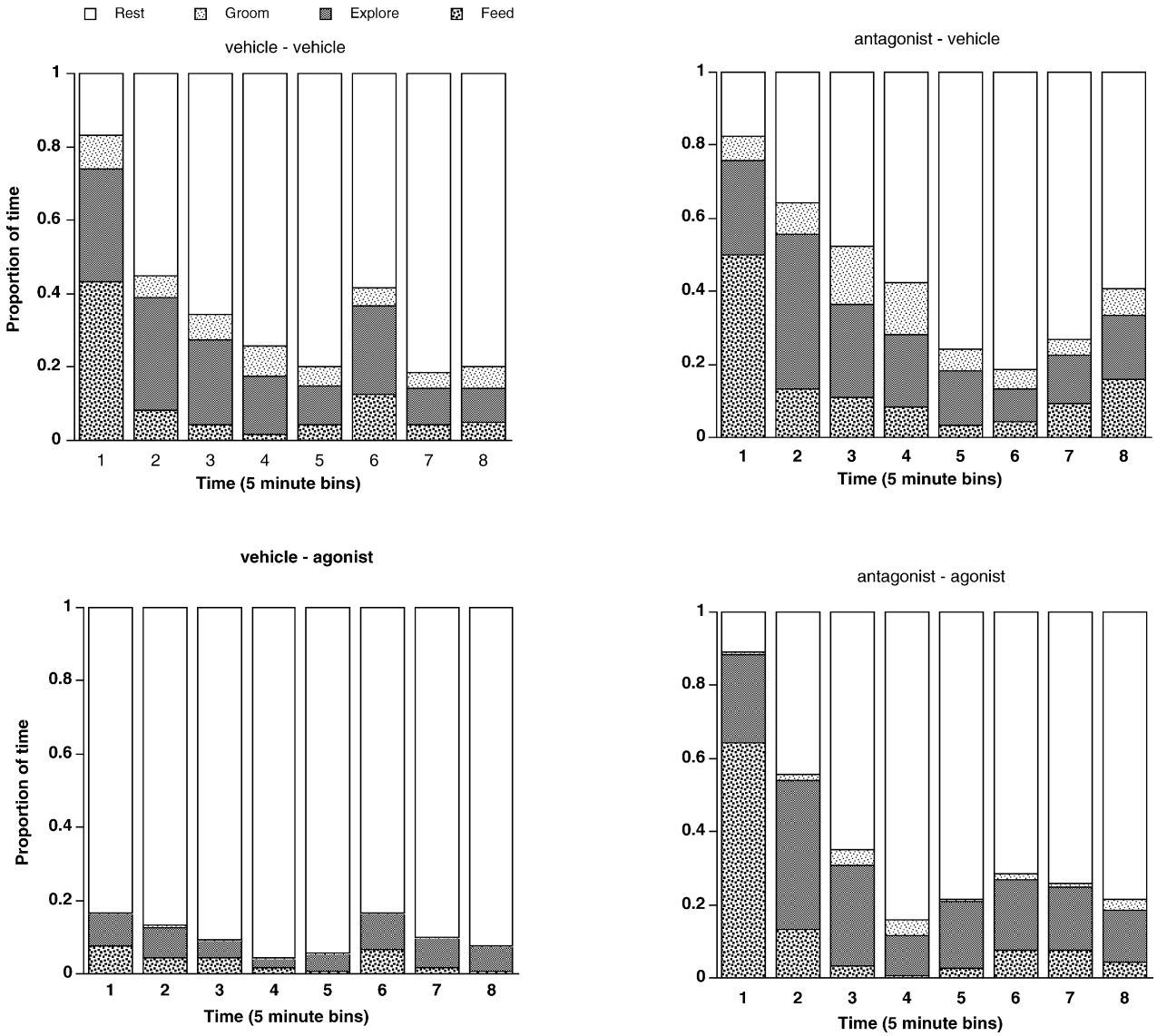

FIG. 4. Satiety sequences of WT mice following administration of either vehicle or the 5-HT$_{1B}$ receptor antagonist SB224289 (5 mg/kg) 60 min, and either vehicle or the 5-HT$_{1B}$ receptor agonist CP-94 253 (20 mg/kg) 30 min prior to the presentation of palatable mash for 40 min.

interval, but this study also revealed a separate hypodipsic action of CP-94,253 that reduced the likelihood of drinking being initiated (Lee *et al*., 2002). Although 5-HT$_{1B}$ receptors are widely distributed within the rat nervous system, one probable site of the action of CP-94,253 on feeding behaviour is the lateral parabrachial nucleus. Infusions of the related selective 5-HT$_{1B}$ receptor agonist CP-93,129 into the lateral parabrachial nucleus reduce feeding behaviour in the rat (Lee *et al*., 1998).

In the rat a detailed analysis of the satiety sequence induced by CP-94,253 revealed considerable similarity to the changes induced by a short period of prefeeding (Halford & Blundell, 1996). Sequences recorded in the rat using an identical time-sampling protocol to the present study revealed a similar dose-related advance in the satiety sequence following CP-94,253 treatment (Lee *et al*., 2002). These effects were attenuated by pretreatment with a selective 5-HT$_{1B}$ antagonist. In addition this study demonstrated that meal size and duration were reduced by CP-94,253. All of these effects of CP-94,253 are consistent with the hypothesis that 5-HT$_{1B}$ receptor activation is one component of a system that allows serotonin to act as a satiety signal in the rat. The present data are consistent with a similar role for 5-HT$_{1B}$ receptor activation in the mouse. The behavioural profile obtained with CP-94,253 (10 mg/kg) was consistent with an advancement of satiety onset, although higher doses produced nonspecific effects and it was less easy than in the rat to obtain a dose range over which progressive but selective behavioural effects were observed.

It is likely that the effects of CP-94,253 on feeding in our behavioural experiments reflect an action at 5-HT$_{1B}$ rather than 5-HT$_{1D}$ receptors because the 5-HT$_{1B}$ antagonist SB224289 blocked the effects of CP-94,253 on feeding. GR127,935, which also acts at 5-HT$_{1D}$ receptors, also attenuated the effects of CP-94,253. The incomplete blockade observed may reflect a partial agonist action of GR127,935 at 5-HT$_{1B}$ receptors or its appreciable affinity for the 5-HT$_{2C}$ and 5-HT$_{2A}$ serotonin receptor subtypes (Roberts *et al*., 2001), which are both implicated in the modulation of feeding. The residual effects of a high dose of CP-94,253 in 5-HT$_{1B}$-KO mice may reflect an action at non5-HT$_{1B}$ receptors, and may also have been responsible for the reduction in grooming which was not attenuated by either GR127,935 or by SB224289 in WT mice. However, it is also possible that grooming is

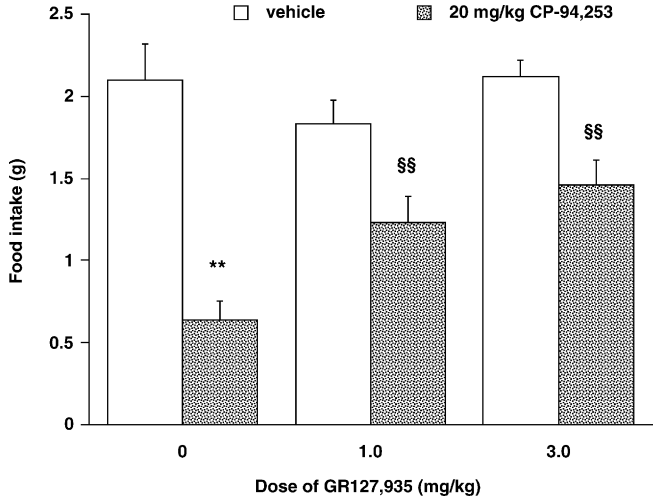

FIG. 5. Food intake (g + SEM) of WT mice treated with either vehicle or GR127,935 (1 or 3 mg/kg) 60 min, and either vehicle or CP-94,253 (20 mg/kg) 30 min prior to the presentation of palatable mash for 40 min. $^{*}P < 0.01$ compared with vehicle control, $^{\$}P < 0.01$ comparing vehicle and antagonist pretreatment.

more sensitive to the effects of CP-94,253 and that effective antagonism would have been observed in the presence of a lower dose of the agonist.

Our data also strengthen the evidence for a role of endogenous serotonin in the modulation of feeding in the mouse. 5-HT_{1B} receptor-KO mice are characterized by increased body weight and food intake (Bouwknecht *et al*., 2001), although these animals show no increase in the proportion of body fat or other features of an obesity syndrome. Results from our first experiment demonstrate that the increase in food intake is associated with a slower termination of feeding during the satiety sequence. The data from our second experiment also show a clear increase in food intake and feeding behaviour following administration of the selective 5-HT_{1B} receptor antagonist SB224289. These data are consistent with a tonic control of feeding behaviour through activation of 5-HT_{1B} receptors, blockade of which lead to release of this behaviour, and also with reports that SB224289 can increase food intake in the rat (Vickers *et al*., 2001). By contrast, the tonic control of feeding behaviour by 5-HT_{2C} receptors is less clear. 5-HT_{2C}-KO mice develop an obesity syndrome which includes enhanced body weight, increased food intake (Tecott *et al*., 1995) and the endocrine characteristics of Type 2 diabetes (Nonogaki *et al*., 1998). Despite attempts using a variety of feeding paradigms, several studies have shown that selective 5-HT_{2C} receptor antagonists fail to induce hyperphagia in

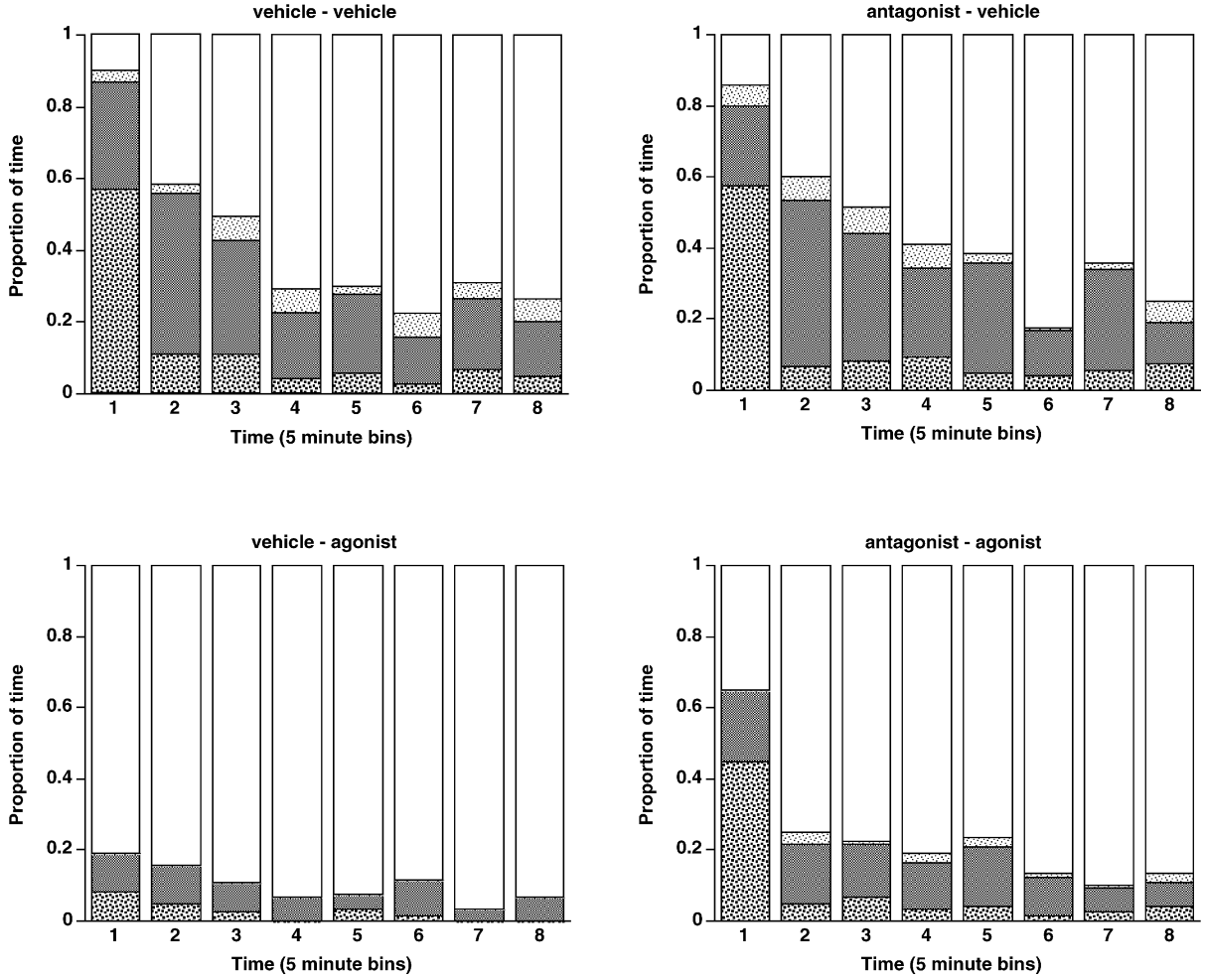

FIG. 6. Satiety sequences of WT mice following administration of either vehicle or the 5-HT_{1B} receptor antagonist GR127,935 (3 mg/kg) 60 min, and either vehicle or the 5-HT_{1B} receptor agonist CP-94,253 (20 mg/kg) 30 min prior to the presentation of palatable mash for 40 min.

TABLE 1. Rating of c-*fos* immunoreactivity induced by CP-94,253 or vehicle in WT mice

Brain area	Vehicle	CP-94,253 (20 mg/kg)
Nucleus of the solitary tract	1.2 ± 0.25	$4.0 \pm 0.0^{**}$
Locus coeruleus	1.7 ± 0.25	$3.7 \pm 0.25^{**}$
Lateral parabrachial nucleus	1.2 ± 0.25	$3.2 \pm 0.25^{**}$
Bed nucleus of the stria terminalis	0.2 ± 0.20	$2.6 \pm 0.24^{**}$
Hypothalamus, paraventricular nucleus	1.2 ± 0.37	$3.8 \pm 0.20^{**}$
Lateral hypothalamus	1.4 ± 0.40	1.6 ± 0.24
Hypothalamus, arcuate nucleus	1.6 ± 0.40	1.8 ± 0.37
Hypothalamus, ventromedial nucleus	0.6 ± 0.20	$2.0 \pm 0.55^{*}$
Hypothalamus, dorsomedial nucleus	1.4 ± 0.40	2.0 ± 0.0
Basolateral nucleus of the amygdala	0.8 ± 0.20	0.8 ± 0.20
Central nucleus of the amygdala	0.8 ± 0.20	$2.8 \pm 0.20^{**}$

C-*fos* immunoreactivity was rated on a 0–3 scale (0, no c-*fos*; to 3, intense c-*fos*). Table entries show the mean ± SEM. $N = 5$/group (forebrain areas), $N = 4$/group (hindbrain areas). $^{*}P < 0.05$, $^{**}P < 0.01$.

either rats or mice (Kennett *et al*., 1997; Hewitt *et al*., 2002). However, the selective 5-HT$_{2C}$ antagonist RS102221 was reported to increase daily food intake in the rat, although it failed to reduce *m*CPP-induced hypolocomotion in the same study (Bonhaus *et al*., 1997). Thus, further studies are required to establish whether stimulation of 5-HT$_{2C}$ receptors by endogenous serotonin has a critical role in the short-term modulation of feeding behaviour.

The pattern of c-*fos* immunoreactivity produced by CP-94,253 or other selective 5-HT$_{1B}$ receptor agonists has not been previously described in either the rat or mouse. We observed a discrete pattern of response, especially within the hindbrain. The nucleus of the solitary tract, lateral parabrachial nucleus and locus coeruleus each showed dense c-*fos* immunoreactivity that was not seen in adjacent areas. For example, we observed no enhancement within the hypoglossal nucleus, adjacent to the nucleus of the solitary tract, despite a report that 5-HT$_{1B}$ binding sites are found at higher density in the hypoglossal nucleus than in the nucleus of the solitary tract (Manaker & Verderame, 1990). In the forebrain a marked c-*fos* response was noted in the paraventricular nucleus of the hypothalamus and the central nucleus of the amygdala amongst other areas (Table 1). These areas were previously reported to show substantial c-*fos* immunoreactivity in response to the mixed 5-HT$_{1A/1B}$ receptor agonist RU24969 (Lucas *et al*., 1998)

The 5-HT$_{1B}$ receptor is negatively linked to adenylate cyclase and generally functions as either an autoreceptor on serotonergic terminal fibres or as a heteroreceptor on a variety of nonserotonergic neurons (Barnes & Sharp, 1999). Stimulation of such heteroreceptors may modulate the release of neurotransmitters such as dopamine, acetylcholine or GABA (Barnes & Sharp, 1999). Activation of 5-HT$_{1B}$ autoreceptors is unlikely to evoke a c-*fos* response, because the cell body is distal to these terminal autoreceptors, but may produce such activation of other cells through indirect mechanisms. For example, there is a strong innervation of the locus coeruleus by fibres arising in the dorsal raphe (Kaehler *et al*., 1999). Electrophysiological studies have shown that the firing rate of locus coeruleus cells is increased by the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (Szabo & Blier, 2001). Conversely, antagonism of cell body autoreceptors by the 5-HT$_{1A}$ receptor antagonist WAY 100 635 indirectly decreases the firing rate of locus coeruleus neurons via stimulation of postsynaptic 5-HT$_{2A}$ receptors (Szabo & Blier, 2001). These findings are consistent with the reported enhancement of c-*fos* immunoreactivity within the locus coeruleus by the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT (Hajos-Korcsok & Sharp, 1999). Stimulation of 5-HT$_{1B}$ terminal autoreceptors would also be expected to decrease serotonergic inhibition of the locus coeruleus and lead to an increase in c-*fos* immunoreactivity in these cells. Similar indirect mechanisms may be responsible for the activation that we observed in some other structures. For example, there is a substantial level of 5-HT$_{1B}$ receptor binding in the central nucleus of the amygdala (Bruinvels *et al*., 1994) and a projection from the dorsal

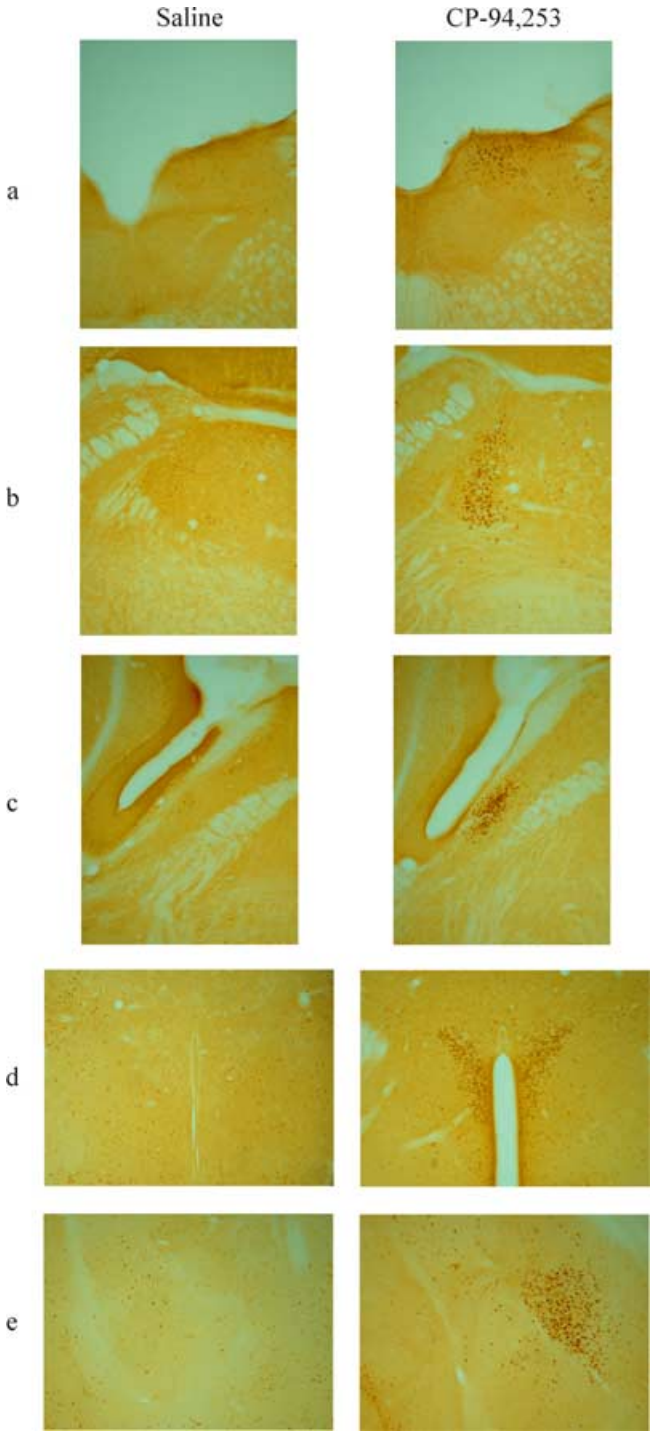

FIG. 7. Representative sections at the level of (a) the nucleus of the solitary tract, (b) locus coeruleus, (c) lateral parabrachial nucleus, (d) paraventricular nucleus of the hypothalamus and (e) central nucleus of the amygdala. The left panels show the effects of vehicle treatment and the right panels show the effects of CP-94,253 (10 mg/kg i.p.).

raphe to this area (Ma *et al*., 1991). Antagonism of 5-HT_{1B} terminal autoreceptors might also lead to disinhibition of neural activity within the amygdala and hence to a positive c-*fos* response.

Although increases in c-*fos* immunoreactivity within specific cell groups may occur as a consequence of several different mechanisms, it is striking that the overall set of structures identified in this study are closely associated with feeding behaviour. Thus, the nucleus of the solitary tract is an important sensory relay for gustatory information and the lateral parabrachial nucleus is a second-order relay for such information (Saper, 2002). Hence one possible explanation of the pattern of c-*fos* activation might be in terms of a broad modulation of the neural circuitry for feeding in such a way as to mimic the neural changes that occur towards the end of a meal, leading to the decrease in feeding that we observed in our behavioural experiments. c-*Fos* studies of other substances with a selective hypophagic action would support this interpretation. For example, the gut peptides amylin and GLP-1 reduce food intake and also increase c-*fos* immunoreactivity in a similar set of structures including nucleus of the solitary tract, lateral parabrachial nucleus and central nucleus of the amygdala (Rowland *et al*., 1997).

One issue with this interpretation of our c-*fos* data is that activation of an overlapping but not identical set of structures has been associated with other motivational or emotional states. For example, it has been shown that five neurochemically diverse drugs, known to be anxiogenic in both human and rat, also increase c-*fos* immunoreactivity in the nucleus of the solitary tract, locus coeruleus, lateral parabrachial nucleus and additionally in the ventrolateral and dorsolateral periaqueductal grey (Singewald & Sharp, 2000). A study using a similar series of drugs demonstrated increases in c-*fos* immunoreactivity in the central nucleus of the amygdala, bed nucleus of the stria terminalis, the paraventricular nucleus of the hypothalamus and several other forebrain structures (Singewald *et al*., 2003). These studies were interpreted in terms of a substantial literature implicating these structures in stress, fear and anxiety-related behaviour. However, it is also clear that structures such as the central and basolateral nuclei of the amygdala play a critical role in appetitive learning. Lesions of the central nucleus disrupt the development of orienting responses to stimuli that predict food (Gallagher *et al*., 1990) and connections between the basolateral amygdala and lateral hypothalamus are critical to the influence of food-associated cues on feeding behaviour in sated rats (Petrovich *et al*., 2002).

Thus the fact that similar circuitry is activated by a variety of drug treatments complicates the interpretation of the data presented here although it should also be emphasized that the sets of structures activated only partially overlap. Notably, we did not find a CP-94,253-induced increase in c-*fos* immunoreactivity in the periaqueductal grey. It might be suggested that some anorectic drugs reduce food intake by inducing anxiety- or fear-like states. For example, administration of the 5-HT_{2C} receptor agonist *m*CPP may be anxiogenic in humans (Charney *et al*., 1987) and also has an anxiogenic profile in mouse plus maze (Rodgers *et al*., 1992). However, in humans, *m*CPP reduces food intake and body weight without causing anxiety (Walsh *et al*., 1994; Sargent *et al*., 1997). The evidence in relation to 5-HT_{1B} receptor agonists is also ambiguous. Brain-penetrant 5-HT_{1B} receptor agonists are now in widespread use as antimigraine agents in humans and are not reported to either induce or exacerbate anxiety. However, rats treated with CP-94,253 show decreased exploration time on the open arms of a plus maze (Lin & Parsons, 2002) at doses similar to those that reduce food intake (Lee *et al*., 2002). In contrast, although the 5-HT_{1B} receptor agonist CGS 12066B stimulated closed arm entries in the mouse, it did not increase risk assessment behaviour (Rodgers *et al*., 1992). Further studies are required to elucidate the relationship of hypophagic and anxiogenic effects of serotonergic agents, particularly those acting at 5-HT_{2C} and 5-HT_{1B} receptors.

In summary, the enhancement of food intake and feeding behaviour by the 5-HT_{1B} receptor antagonist SB224289 strongly suggests a tonic role for 5-HT_{1B} receptors in the modulation of ingestive behaviour in the mouse. The similar profile of feeding behaviour observed in 5-HT_{1B}-KO mice confirms this interpretation. The reduction of food intake induced by the 5-HT_{1B} receptor agonist CP-94,253 is also consistent with this role, although the behavioural data also provide evidence for some behaviourally nonspecific effects at higher drug doses. In addition, CP-94,253 produces an intense but localized c-*fos* response in a number of brain areas already implicated in the control of ingestive behaviour as well as other aspects of motivated behaviour. These include the nucleus of the solitary tract and the lateral parabrachial nucleus in the hindbrain, and the paraventricular nucleus of the hypothalamus. Taken together these results suggest an important role for the activation of central 5-HT_{1B} receptors in the endogenous control of ingestive behaviour in the mouse.

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Abbreviations

5-HT, 5-hydroxytryptamine or serotonin; CP-94,253, 3–1,2,5,6-tetrahydro-4pyridyl)-5-propoxypyrrolo[3,2-b]pyridine; GR127,935, 2′-methyl-4′(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-(5-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide; KO, knockout; *m*CPP, 1-(m-chlorophenyl)piperazine; PBS, phosphate-buffered saline; SB224289, 2,3,6,7-tetrahydro-1-methyl-5-{2-methyl-4[(5-methyl-1,2,4 oxadiazole-3-yl)biphenyl-4-yl]carbonyl}furo[2,3-f]indole-3-spiro-4-piperidine; WT, wild-type.

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