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Abecarnil and palatability: Taste reactivity in normal ingestion in male rats

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Abstract

Effects of the anxioselective anxiolytic abecarnil, a β-carboline benzodiazepine-receptor agonist, on initial licking responses for a 3% sucrose solution and on taste reactivity responses were evaluated in adult male rats. The rats' behaviour was video recorded, and analysed according to a frame-by-frame procedure to generate the durations of categorized fixed action patterns, and the number and rate of licking responses. The results indicated that abecarnil (0.3–3.0 mg/kg, i.p.) significantly increased the number of licks in the first continuous sample of licking, while significantly reducing the lick rate (licks/s). Additionally, abecarnil selectively enhanced positive ingestive responses, but had no effect on either neutral or aversive response categories in taste reactivity measures. On the basis on this pattern of results, we conclude that abecarnil can enhance taste palatability selectively, and that it may act as an agonist at $GABA_A$ benzodiazepine receptor subtypes which mediate drug effects on ingestive behaviour.

Keywords: Abecarnil; β-Carbolines; Benzodiazepines; Licking; Palatability; Taste reactivity

1. Introduction

Chlordiazepoxide and the classical 1,4-benzodiazepines, including diazepam and midazolam, act as full agonists at central benzodiazepine receptors (BZR) (Möhler and Okada, 1977; Squires and Braestrup, 1977) located at $GABA_A$ receptors (Barnard et al., 1998; Sigel and Buhr, 1997), and have therapeutically important effects (anxiolytic, anticonvulsant, hypnotic, myorelaxant) but also incur disadvantageous side effects including sedation, amnesia, ataxia and dependence. There is therefore considerable current interest in the continuing development of so-called *anxioselective anxiolytics*, newer compounds active at BZR which retain selected therapeutically relevant actions but which have reduced or absent side effects (Atack, 2003; Basile et al., 2004; Mehta and Ticku, 1999; Möhler et al., 2002). One of these compounds which has undergone preclinical and clinical testing is the β-carboline abecarnil (Basile et al., 2004), which shows high affinity for central BZRs, exhibits anxiolytic and anticonvulsant properties, is protective against benzodiazepine withdrawal effects, induces less dependence, less ataxia and muscle relaxation (Duka et al., 1993; Pinna et al., 1997; Stephens et al., 1990; Steppuhn et al., 1993; Turski et al., 1990). In view of the intense current interest in the mode of action of anxioselective anxiolytics in relation to the complex $GABA_A$ receptor molecular pharmacology (Barnard et al., 1998; Korpi et al., 2002; Mehta and Ticku, 1999; Rudolph and Möhler, 2004), there is a strong case for more extended behavioural characterization of abecarnil and other putatively selective drugs (Knoflach et al., 1993; Pribilla et al., 1993).

BZR receptor agonists (full and partial) exert pronounced effects to enhance food consumption (hyperphagia) in both food-deprived and non-deprived animals, across a wide range of mammalian species including human beings (Cooper, 1980, 1989a,b; Evans et al., 1999; Haney et al., 1997; Martin et al., 1993; Wise and Dawson, 1974). The hyperphagic effect leads to increases in meal size (Clifton and Cooper, 1996). Within the central nervous system, BZ-induced hyperphagia appears to be localized close to the IV ventricle (Higgs and

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Cooper, 1996a), most probably within the pontine parabrachial nucleus (Higgs and Cooper, 1996b; Söderpalm and Berridge, 2000), which is an important relay in the taste projection system (Norgren, 1995; Sewards, 2004).

We have shown that the β-carboline abecarnil significantly enhanced consumption of either a palatable sweetened mash or a 3% sucrose solution in non-deprived rats (Cooper and Greenwood, 1992). Abecarnil's hyperphagic effect has been confirmed (Chen et al., 1996), consistent with a computationally determined 3D pharmacophore developed for ligand recognition with benzodiazepine-induced hyperphagia as the endpoint (Filizola et al., 2000). More specifically, however, we have also demonstrated that abecarnil, over the dose-range 0.3–10.0 mg/kg, i.p., enhanced both sweet-taste preference (0.05% sodium saccharin) and salt-taste preference (0.9% sodium chloride) in a two-stimulus taste preference test (Cooper and Greenwood, 1992). This result has a direct bearing on recent proposals that BZR agonists act specifically to enhance taste palatability or hedonic evaluation, so promoting increased food consumption (Berridge, 1996; Berridge and Peciña, 1995; Cooper, 2004). In view of the current obesity epidemic (Lobstein et al., 2004; Silventoinen et al., 2004), and recent evidence that energy density (correlated with food palatability) and portion sizes in meals are significant determinants of human energy intake (Kral et al., 2004; Prentice and Jebb, 2003), it is increasingly important to improve our understanding of basic mechanisms of food/taste palatability and food consumption.

The taste reactivity paradigm, which determines patterns of stereotyped orofacial responses elicited by positively and negatively evaluated taste stimuli, respectively, has become an indispensable methodological tool to evaluate positive taste palatability, or *liking* for tastes (Berridge, 1996, 2003; Grill and Berridge, 1985; Steiner et al., 2001). Pertinently, benzodiazepines are pharmacologically distinctive in that they specifically enhance the elicited response to hedonically positive taste (*liked*) stimuli (Berridge, 1988; Berridge and Peciña, 1995; Berridge and Treit, 1986; Gray and Cooper, 1995). They provide, therefore, a key approach to investigating basic mechanisms which mediate food-associated taste palatability. In the present study, our aim was to characterize further the behavioural profile of abecarnil using a taste reactivity approach. The method followed that previously used by us to investigate the effects of midazolam on taste reactivity (Gray and Cooper, 1995).

2. Materials and methods

2.1. Subjects

Sixteen adult, male, blackhooded rats (General strain, bred in the University of Birmingham laboratory) were housed in pairs with free access to standard food pellets and water, except as described below. The animal holding room was maintained under a 12-h light/12-h dark cycle (lights on at 07:00 h), at a constant room temperature of 23 ± 2 °C. The animals weighed between 230 g and 360 g at the start of the experiment. All procedures were conducted to comply with UK Home Office licence requirements.

2.2. Drug

Abecarnil (isopropyl 6-benzyloxy-4-methoxymethyl-β-carboline-3-carboxylate) was administered in doses of 0.3, 1.0 and 3.0 mg/kg, based on data for effective doses in tests of ingestive behaviour (Cooper and Greenwood, 1992). Due to its insolubility in water, abecarnil was ultrasonically dispersed in distilled water to which 0.3% Tween had been added. The vehicle condition consisted of distilled water with 0.3% Tween added. All injections were administered by intraperitoneal (i.p.) route, 30 min before the start of each test session.

2.3. Apparatus

The experimental chamber consisted of a rectangular tank of transparent Perspex (220 mm wide * 210 mm high * 370 mm long), placed on a larger sheet of transparent Perspex, mounted on a portable trolley. A mirror was suspended below the tank at 45° to reflect the ventral aspect of the subject's head. A feeder bottle (50 ml), fitted with angled stainless steel tube and ball-valve spout, was mounted at one end of the tank. At this end, a field of activity was defined within the tank (170 mm long), beyond which the subject was regarded as out of view. A video camera (Panasonic F10 CCD) was set up to record the mirror image, and was connected with a timer unit (FOR-A VTG-33), which added on-screen digital timing to one-hundredth of a second accuracy. The video signal was recorded on conventional VHS tape (Konica Super SR E180) at 50 frames per second, using a high-quality VCR (Panasonic NV-F758B VHS), and was monitored live (Sony KX-14CP1). The camera shot was fixed, and taste reactivity (TR) analyses were based on those responses produced in the immediate vicinity of the feeder spout.

2.4. Procedure

The subjects were first habituated to frequent handling and weighing and were trained to drink voluntarily from unlimited 3% sucrose solution in the apparatus. The training period was extended to 14 days, first to habituate the animals to the test conditions, and also to attain stable levels of sucrose drinking. Subjects were injected with vehicle 30 min prior to the last two training sessions. Within the testing period, each animal served as its own control, and also received each of the three doses of abecarnil. The order of drug administration was counterbalanced across subjects, and at least 48 h separated consecutive injections to minimise any possible drug-carryover effects.

Table 1
Taste reactivity categories observed following voluntary ingestion

Category	Activity
Ingestive	(i) L—additional drinking during the test period ('licking')
	(ii) MM—rapid, rhythmic mouth movements
	(iii) TP—explicit tongue protrusions
	(iv) FL—floor licking (drinking of spilled test solution)
Neutral	(v) LO—locomotion
	(vi) OS—subject temporarily out of camera shot
Aversive	(vii) FW—face washing
	(viii) R—rearing
	(ix) OF—subject outside defined test field in apparatus
	(x) G—gaping
	(xi) CR—chin rubbing (on floor of apparatus)
	(xii) FF—forelimb flailing
	(xiii) HS—head shaking
	(xiv) SB—spout biting (distinguishable from drinking)

The aims of the video analysis were (i) to provide a measure of sucrose ingestion, in the form of the number of licking responses; (ii) to measure the local rate of licking within the first continuous bout of licking for sucrose; (iii) to generate a measure of taste palatability, determined by taste reactivity (TR) analysis. All these behavioural analyses were derived from meticulous consecutive frame-by-frame video playback, using the superimposed times to resolve durations to an accuracy of 1/50 s. For each test trial, the total number of licks in the first continuous bout of sucrose drinking was counted. The bout criterion used was that the bout terminated with the first interlick interval of 1 s or greater (Gray and Cooper, 1995). This provided an accurate measure of the sucrose ingestion. The local rate of licking (licks/s) was determined as the number of licks in the first bout divided by the duration of the first bout of licking (in seconds). For the subsequent cumulative non-licking period of 20 s, a number of defined *fixed action patterns* were measured (see Table 1 for a list of the categories used) for the TR analysis. They were divided into *ingestive*, *neutral* and *aversive* categories. The duration of each event was determined, and then accumulated within each category to provide a cumulative duration (seconds), sampled over the course of the 20 s measurement period. Further details are available in Gray and Cooper (1995).

2.5. Statistical analyses

All data were analysed using a one-factor, repeated-measures (ANOVA), followed by a Dunnett's *t*-test to compare individual dose treatments against the vehicle. The results are shown as group means and S.E.M.s ($N=16$).

3. Results

3.1. Ingestive behaviour

Abecarnil (0.3–3.0 mg/kg, i.p.) produce a marked dose-related increase in the number of licking responses emitted to consume the sucrose solution, during the first continuous bout of drinking [$F(3,45)=4.35$, $p<0.01$]. Fig. 1 indicates that the increases in sucrose licking responses were significantly greater than control values at 1.0 mg/kg [$p<0.05$] and 3.0 mg/kg [$p<0.01$], respectively. The magnitude of the increases was impressive: with a 483% increase over the control level of licking at 1.0 mg/kg, and 567% increase over the control value at 3.0 mg/kg. Importantly, these large increases occurred within the first bout of licking responses. The duration of the first bout of licking responses also showed similar dose-dependent increases, $F(3,45)=3.63$, $p<0.05$ (data not shown). During this same period, abecarnil produced a significant dose-related decrease in the mean rate of licking (licks/s), [$F(3,45)=15.6$, $p<0.005$]. As Fig. 2 indicates, abecarnil (1.0 and 3.0 mg/kg) reduced the mean rate of licking from 7.35 licks/s to around 6.6 licks/s. This represents about a 10.2% reduction in the within-bout rate of licking.

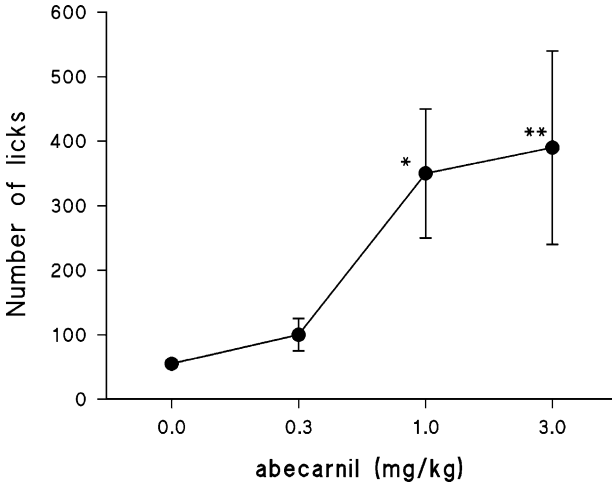

Fig. 1. Effect of abecarnil (0.3–3.0 mg/kg, i.p.) on the number of licks generated in the first continuous episode of 3% sucrose drinking. The results are show as mean±S.E.M. ($N=16$).

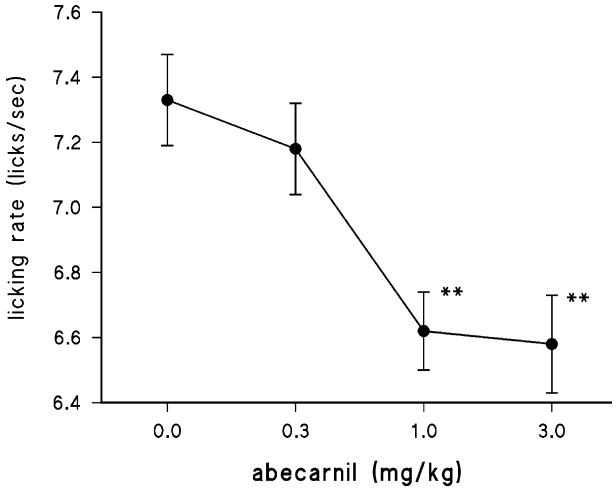

Fig. 2. Effect of abecarnil (0.3–3.0 mg/kg, i.p.) on the local rate of licking (licks/s) in the first continuous episode of 3% sucrose drinking. The results are shown as mean±S.E.M. ($N=16$).

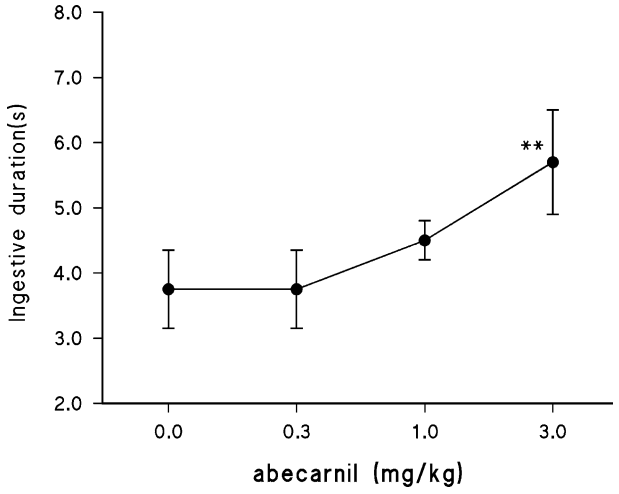

Fig. 3. Effect of abecarnil (0.3–3.0 mg/kg, i.p.) on positive ingestive responses measured in terms of cumulative duration (s), following the first episode of sucrose drinking. The results are shown as mean±S.E.M. ($N=16$).

3.2. Taste reactivity measures

Abecarnil exerted a significant effect to increase the cumulative duration of positive ingestive TR responses, in the period that followed the first bout of sucrose-licking [$F(3,45)=4.34$, $p<0.01$]. As Fig. 3 shows, abecarnil produced a significant effect at the 3.0 mg/kg dose [$p<0.01$]. In contrast to this abecarnil effect, there was no change in the durations of either neutral behavioural categories [$F(3,45)=2.45$, N.S.], or aversive components [$F(3,45)=0.3$, N.S.] following the abecarnil treatments (Table 2).

4. Discussion

The results demonstrate that the β-carboline BZR agonist, abecarnil, significantly increased the rats' licking responses for a palatable 3.0% sucrose solution. This increase in ingestive response was due to a substantial increase in the number of licks generated within the first continuous bout of drinking. It did not depend on any increase in the local rate of licking; indeed, abecarnil concurrently reduced the licking rate. These data are consistent with previous reports that abecarnil enhances palatable food and sucrose consumption (Cooper and Greenwood, 1992; Chen et al., 1996), and, more generally, fit the model that certain BZR agonists promote hyperphagia (Cooper, 1980; Filizola et al., 2000). However, the most informative feature of the present data is that the enhancing effect of abecarnil was essentially present at the very onset of ingestive behaviour. Thus, significant dose-related effects of abecarnil were in evidence within the first continuous bout of licking for sucrose.

Microstructural studies of licking responses, using computer-based automated recording of licks, have shown that the nonselective BZR full agonist, midazolam, exerts early-onset effects to increase the frequency of licks measures, and to extend the mean duration of licking bouts (Higgs and Cooper, 1997, 1998). Although a different experimental approach was used in the present study, the results are fully consistent with the previous data. BZR agonists exert an immediate impact on episodes of ingestive behaviour, and this effect most probably culminates in hyperphagia and increased meal size. There is compelling evidence from licking microstructural studies that the initial licking frequency and the mean duration of licking bouts provide a reliable index of taste palatability (Davis, 1998; Davis and Levine, 1977; Davis and Perez, 1993; Davis and Smith, 1992). Hence, the present evidence indicates that abecarnil acts immediately at the onset of ingestion, because it directly enhances palatability. By the same token, the effect of abecarnil reported here cannot be attributed to any effect on later postabsorptional factors determining the process of satiation.

Rats typically lick for water or solutions in a stereotyped manner, at a rate of about 7 licks/s within continuous bursts of drinking (Corbit and Luschei, 1969). Benzodiazepines act to reduce the local rate of licking, but this effect is pharmacologically dissociable from the hyperphagic or hyperdipsic effects of these drugs (Higgs and Cooper, 1997, 2000). The present results confirm that abecarnil decreased the local rate of licking to about 6.6 licks/s, which is a relatively small effect but nevertheless highly reliable. Since the i.p. administration of abecarnil produces reduced muscle-relaxant effects (Turski and Stephens, 1993), we are inclined to rule out a myorelaxant effect being principally responsible for the observed reduction in the local rate of licking. Importantly, stereotyped licking may fall under the control of a central pattern generator in the lower brainstem (Travers et al., 1997). Future research should address the possibility that pharmacological treatments, including benzodiazepines, may affect the outputs of the licking pattern generator.

The taste reactivity data obtained in the present study lend further weight to the view that abecarnil acts selectively, in a behavioural sense, to enhance positive ingestive responses, without affecting neutral or aversive responses. This result is consistent with other data which demonstrates that BZR full agonists, such as chlordiazepoxide, diazepam and midazolam, enhance the positive

Table 2
Duration of neutral and aversive categories following the administration of abecarnil (0.3–3.0 mg/kg, i.p.)

	Abecarnil (mg/kg)			
	0	0.3	1.0	3.0
Neutral responses	13.1±1.1	14.5±1.1	12.6±1.0	11.1±1.0
Aversive responses	5.2±0.7	4.2±0.8	5.1±0.7	5.2±0.9

Results are shown as mean±S.E.M. ($N=16$).
There was no significant effect of abecarnil on either of these measures.

hedonic evaluation (palatability) of tastants, without affecting elicited aversive reactions (Berridge, 1988; Berridge and Peciña, 1995; Berridge and Treit, 1986; Gray and Cooper, 1995). The general argument that BZR agonists specifically enhance palatability does not rest on data from a single test paradigm, but crucially depends upon several converging lines of evidence (Cooper, 1989a,b, 2004). Thus, the data for abecarnil can be summarised as follows: (i) abecarnil enhances the consumption of highly palatable foods and fluids in non-deprived animals; (ii) it increases both sweet-taste and salt-taste preferences in two-choice tests; (iii) it increases initial levels of licking at the onset of sucrose ingestion (this study); (iv) it selectively enhances positive ingestive responses in a taste reactivity paradigm (this study). Taken together, the data are strongly indicative that an abecarnil can act selectively to enhance taste palatability or hedonic evaluation. This effect should generalise, and we would predict, for example, that abecarnil would enhance salt-drinking responses (Cooper and Higgs, in press), and ethanol palatability and consumption (Söderpalm and Hansen, 1998).

Recent important work with genetically modified "knock-in" mice has indicated that the sedative side effect of BZR agonists is specifically mediated by the α_1-subunit present in the majority of the brain's $GABA_A$ receptors (McKernan et al., 2000; Möhler et al., 2002; Rudolph et al., 1999). There is strong evidence that the BZR-mediated hyperphagic effect is quite separate from induced sedation: thus, BZR partial agonists devoid of sedative side effects in rats promote a full hyperphagic response (Yerbury and Cooper, 1989). Moreover, direct microinjection of the full agonist midazolam into the pontine parabrachial nucleus elicits a robust hyperphagic response in the absence of any accompanying sedation (Higgs and Cooper, 1996b). We may tentatively conclude from these data that the $GABA_A$ receptor α_1-subunit is unlikely to play a role in the effects of BZR agonists (full, partial or selective) on hyperphagia or taste palatability. In contrast, zolpidem, which is highly potent in inducing sedation, an action exclusively mediated by α_1-$GABA_A$ receptors (Crestani et al., 2000), did not exhibit a hyperphagic effect in a palatable food consumption test (Yerbury and Cooper, 1989). Hence, other $GABA_A$ receptor subtypes must be considered in relation to food consumption and taste palatability. The tetrahydropyrazoloquinoline, CGS 17867A, exhibits anxiolytic activity, with reduced side effects (Bennett et al., 1987). New work indicates that CGS 17867A has significant efficacy at α_2- and α_3-$GABA_A$ receptor subtypes, with much reduced efficacy at the α_1-$GABA_A$ receptor subtype (Mitchinson et al., 2004). This is entirely consistent with the view that anxiolytic actions of BZR agonists might be mediated by α_2- and/or α_3-$GABA_A$ receptor subtypes (Atack, 2003; Basile et al., 2004; Möhler et al., 2002). Important for the present discussion, however, is the finding that CGS 17867A proved effective in promoting overconsumption of palatable food in non-deprived rats (Yerbury and Cooper, 1989). This strengthens the view that the α_2- and/or α_3-$GABA_A$ receptor subtypes, but not the α_1-subtype, is involved in the controls of food intake and taste palatability. If this were to be confirmed experimentally, one might predict that 2,5-dihydropyrazolo [4,3-*c*] pyridine-3-ones (Mitchinson et al., 2004), and the novel pyridoindole derivative SL651498 (Griebel et al., 2001) would prove positive in tests of hyperphagia and taste palatability. These drugs show selectivity for α_2- and α_3-$GABA_A$ receptor subtypes. Newer developments may provide useful pharmacological tools with which to investigate the potential roles of $GABA_A$ receptor α_2-/α_3-subtypes in tests of ingestive behaviour (Carling et al., 2004).

Abecarnil is somewhat difficult to characterize in terms of its affinity and efficacy at $GABA_A$ receptors α-subtypes (Atack, 2003; Basile et al., 2004). Evidence suggests that it is effective as an agonist at α_1-, α_2-, α_3- and α_5-subtypes, but may be effective as a full agonist at α_1- and α_3-subunit-containing receptors, and partial agonist at α_2- and α_5-subtypes (Knoflach et al., 1993; Pribilla et al., 1993; Smith et al., 2001). Nonetheless, its pharmacological profile indicates that it is effective as an anxiolytic in animal models, and has reduced side effects (Jung et al., 2000; Rex et al., 1996; Stephens et al., 1990). In the present study, its effects on licking responses and on taste reactivity measures are fully in accord with those of agonists such as midazolam. Within the brain, there is a highly heterogeneous distribution of the different $GABA_A$ receptor subunits (Pirker et al., 2000). Localization of target structure(s) within the brain may be a highly relevant factor in distinguishing between anxiolytic and hyperphagic effects of BZR agonists. Whereas the parabrachial nucleus is a principal candidate structure for the effects of BZR agonists on food consumption and taste palatability (Higgs and Cooper, 1996b; Söderpalm and Berridge, 2000), the medial prefrontal cortex and limbic structures in the forebrain appear to be critically involved in anxiolytic effects (Menard and Treit, 1999; Shah and Treit, 2004). Exploiting region-specific $GABA_A$ receptor subtypes may provide a means to dissociate hyperphagic from anxiolytic effects.

In summary, our results indicate that the β-carboline abecarnil not only produces hyperphagia and enhances taste preferences, but also enhances initial licking responses for sucrose, and selectively enhances positive ingestive responses in a taste reactivity test. These results are consistent with an effect of abecarnil to enhance taste palatability and to produce food overconsumption. In view of the global obesity epidemic, and the increasing evidence that obese individuals choose high-palatability, energy-dense foods, leading to high energy intakes (Blundell and Finlayson, 2004; Kral et al., 2004; Prentice and Jebb, 2003), the value of investigations into the pharmacological, molecular and neural bases of food/taste palatability cannot be underestimated.

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