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TASTE RESPONSES OF CHORDA TYMPANI PROPER NERVE IN THE WHITE-HANDED GIBBON (*HYLOBATES LAR*)

*G. Hellekant**, *G. DuBois*^, *T. Geissmann*+, *D. Glaser*+, and *H. Van der Wel**

* University of Wisconsin, Department of Veterinary Science, and Wisconsin Regional Primate Center Madison, WI 53706, USA.
^ The NutraSweet Company, IL 60056-1300, USA.
+ Anthropological Institute, University Zürich-Irchel, Switzerland.

ABSTRACT

Humans, chimpanzees and all Old World monkeys tested to date are able to taste the sweet compounds acesulfame-K, aspartame, D-tryptophan, sucrose, xylitol, monellin and thaumatin. In humans and chimpanzees, but not in rhesus macaques, gymnemic acid suppresses or abolishes the chorda tympani proper nerve response and the sweet taste of these compounds. This study examines the relationship between gymnemic acid and sweet taste responses in one species of the lesser apes, the white-handed gibbon (*Hylobates lar*). Recordings were made from the chorda tympani proper nerve during stimulation with these sweet compounds and some non-sweet taste stimuli before and after application of gymnemic acid to the tongue. Response amplitude and a number of other parameters were measured. Responses to the taste stimuli presented were similar to those found in other primate species. The effects of gymnemic acid on gibbons are intermediate between those of the greater apes and those of non-hominoid primates. As a tentative interpretation, we propose that sensitivity for gymnemic acid is a derived characteristic shared by gibbons and other hominoids, but not by other primates.

Key words: gibbon, taste, chorda tympani, sweet, gymnemic acid, thaumatin, monellin, *Hylobates lar*, primate evolution.

INTRODUCTION

The gibbons or lesser apes (Hylobatidae) are almost wholly arboreal and are distributed throughout the mainland and islands of Southeast Asia (e.g. Chivers 1977, Groves 1972, Marshall & Sugardjito 1986). Their diet is largely frugivorous, consisting of about 30-70% fruit, about 10-60% leaves, and occasionally flowers, birds' eggs, young birds, and insects (e.g. Chivers 1984, Leighton 1987).

It has become widely accepted that the gibbons diverged from the common stem leading to the great apes and humans after the Old World monkeys (Cercopithecoidea) had diverged. This view is supported by results from comparative studies using a wide array of morphological (Biegert 1973, Huber 1931, Sawalischin 1911, Schultz 1930, 1933, 1973, Wislocki 1929, 1932) and molecular data (Cronin *et al.* 1984, Darga *et al.* 1973, 1984, Dene *et al.* 1976, Felsenstein 1987, Goodman *et al.* 1983, Sarich 1968, Sarich & Cronin 1976, Sarich & Wilson 1967, Sibley & Ahlquist 1984, 1987, Turleau *et al.* 1983).

Our earlier have demonstrated that there are differences in the sense of taste among primates. The sense of taste approaches that of humans as one moves from prosimians through platyrrhine and to catarrhine primates (Brouwer *et al.* 1973, 1983, Glaser & Hellekant 1977, Glaser *et al.* 1978, 1984, Hellekant *et al.* 1974, Hellekant 1975, Hellekant *et al.*, 1976, Hellekant 1976, 1977, Hellekant *et al.* 1981, 1985).

In this context, gymnemic acid is of special interest in this context, since there is a clear cut difference between its effects in humans and great apes on the one hand, and in non-hominoid primates on the other. Gymnemic acid reduces or abolishes the sweet taste response to several compounds in humans and chimpanzees, *Pan troglodytes* (Diamant *et al.* 1965, Oakley 1985, Hellekant *et al.*, 1985), but not in several Old and New World monkeys, including long-tailed macaques *(Macaca fascicularis),* rhesus macaques *(M. mulatta),* green monkeys *(Cercopithecus aethiops),* and squirrel monkeys *(Saimiri* sp.) (Diamant *et al.* 1972, Hellekant 1977, Hellekant *et al.* 1974, Hellekant & van der Wel, 1989, Snell 1965). The question arises; Is the effect of gymnemic acid a characteristic which coincides with the phylogenetic dichotomy between the cercopithecoids and hominoids, or rather between gibbons and other hominoids?

In this study, we attempt to answer the following questions: what are the effects of gymnemic acid in gibbons? If it has an effect, what and how strong are these effects? Finally, how similar are the responses to sweeteners in gibbons compared with those found in some other primates?

METHODS

Animals

Four male gibbons weighing 4.8-6.6 kg were used in this study. The exact ages of the animals were not known, but, on the basis of body weight, they were assumed to be subadult or adult. The animals were housed at the Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP), New York Medical Center, Tuxedo, N.Y.

All gibbon species and even hybrids can readily be identified by their vocalizations (e.g. Geissmann 1984, 1988, Marshall & Sugardjito 1986). Several gibbon species have repeatedly been misidentified and interbred in captivity. In order to determine whether our group included

individuals from different species of gibbons or interspecific hybrids, one of us (T.G.) visited the gibbons and recorded their vocalizations (in August 1988). Based on characteristics of fur coloration, at least three of the four study animals could be identified as white-handed gibbons, *H. lar* (#G-53, #G-57, and #G-59); they all represented the buff brown color form of this highly variable species (see e.g. Marshall & Sugardjito 1986). Although the subspecies could not be identified with certainty, all three animals most closely resembled *H. lar entelloides*. Two of the three animals (#G-53 and #G-59) vocalized during this investigation. These vocalizations were identical to those of pure *H. lar*, with no traces of hybridization. The fourth animal (#G-55) was not available for examination, but was pale phase, too. Judging by the caretakers' description at LEMSIP, it is probable that the fourth animal (#G-55) belonged to the same species.

All four animals had lived for some time at the former gibbon colony of the 4niversity of California, Davis (see e.g. Kawakami & Kollias 1984, Kollias & Kawakami 1981). Two of the four animals had been born there (#G-57 and #G-59), but had different parents (Dr. Kawakami, pers. comm.). It is highly unlikely that any two of our gibbons are related, because each gibbon or its parents, respectively, had arrived at Davis from a different place. There is one possible exception: Gibbon #G-55 and the mother of gibbon #G-57 both came to Davis from the San Francisco Medical Center (Dr. Kawakami, pers. comm.).

Surgery

The animals were anesthetized with an i.m. injection of ketamine, 50 mg/animal, and atropine, 0.5 mg/animal. They were then intubated and maintained on i.v. nembutal, with 5% dextrose and lactated Ringer's solution. The right chorda tympani proper nerve was exposed through an incision along the mandibular angle between the rostral lobes of the parotid gland and the mandibular bone. First, the tissue attached to the mandibular angle was sectioned. Blunt dissection was then used to follow the caudo-medial side of the pterygoid muscle down to its origin at the pterygoid plate of the skull and to the chorda tympani proper nerve.

In three gibbons the nerve was located at a small caudal lobe of the medial pterygoid muscle and in the other animal in the major lobe of the muscle. The nerve forms one bundle and is surrounded by small veins. After the experiment, the wound was closed with vicryl and nylon. The nylon sutures were removed after 7 to 10 days.

Apparatus

The overall nerve impulse activity was recorded with a PAR 113 amplifier, monitored over a loudspeaker and an oscilloscope, and fed into a recorder (Gould ES 1000). The nerve impulses were also integrated using an absolute value circuit integrator with a time constant of 100 msec. The type of stimulus used and the stimulus duration were recorded on the same recorder as a binary coded signal. (An example of this will be shown in Fig. 2). In addition, an IBM PC-AT with a DAS-Keithley interface was used for storing each response.

The surface of the tongue was stimulated with a portable system. It delivers 12 solutions at given intervals and over a predetermined time under conditions of constant flow and temperature. The interval between each stimulation was 35 sec. Each stimulation lasted for 8 sec.

Test substances and procedure

The following sweet solutions were used for stimulation; 3.5 mM acesulfame-K, 3.4 mM aspartame, 14.5 mM D-tryptophan, 0.3 M sucrose, 0.75 M xylitol, 0.02% monellin, and 0.02% thaumatin. For comparison, taste responses to the following non-sweet stimuli were recorded: 0.04 M ascorbic acid, 0.04 M citric acid, 0.1 M NaCl, 0.002 M quinine hydrochloride. The aim was to repeat the sequence of stimulation at least three times before and after application of gymnemic acid. All but one compund were dissolved in artificial saliva (Hellekant *et al.* 1985). The exception was quinine hydrochloride which, for solubility reasons, was dissolved in distilled water. Artificial saliva was used to rinse the tongue between stimulations. The same preparation of gymnemic acid was used as described earlier (Hellekant *et al.* 1985). It consisted of 80% gymnemic acid A_1, 15% A_2, 5% A_3 and 5% A_4. Fresh solutions of gymnemic acid (10 mg in two ml 0.01 M NaH_2CO_3) were applied to the tongue for 5 min.

Data analysis

Fig. 1 illustrates the parameters measured on the summated recording. They are defined as follows: *Max*, the maximum amplitude of the response; *Area*, the surface area under the response; *Delay*, the time between the onset of the stimulus flow and the response (the moment for onset of response is defined as the first point 10% larger than the baseline); *Slope* (dy/dt), a measure of the change in magnitude of nerve activity with time during stimulation; *Rise*, time between onset of the current that opens the valve and maximum response amplitude; *Tonic*, the amplitude of the tonic activity during stimulation (determined 4.5 sec after onset of stimulation as a running average over the next 500 msec); *Resume*, time from the closing of the valve to the point of return of nerve activity to prestimulation level (i.e. the baseline activity). *N* (not illustrated) is defined as the total number of stimulations with a given taste stimulus.

The data for maximum amplitude were analyzed with a program for analysis of variance and covariance with repeated measures, 3-way ANOVA (Copyright, Regents of University of California).

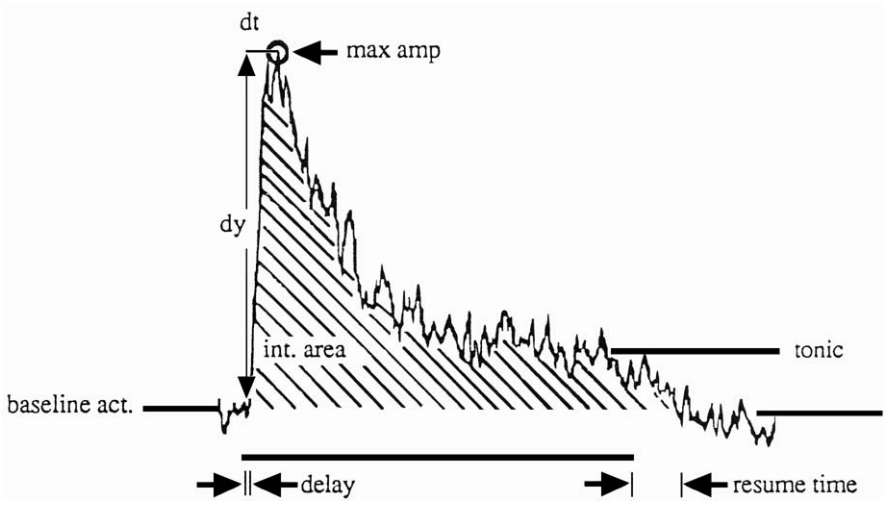

Fig. 1. Parameters measured on a summated recording and presented in Table I

RESULTS

Summated recordings to taste stimulation

Fig. 2 shows a recording before (top trace) and after (bottom trace) application of gymnemic acid. During the recording citric acid, acesulfame, ascorbic acid, aspartame, NaCl, sucrose, monellin and quinine hydrochloride were applied to the tongue as illustrated by the signals underneath the nerve traces. All compounds gave a taste response as shown by an increased impulse activity in the nerve. Of particular interest is the relatively large response to monellin and its cross-adaptory effects on the response to sucrose. This indicates that monellin elicits a response in some of the same fibers as sucrose. Similar cross-adaptory effects were observed between sucrose and thaumatin.

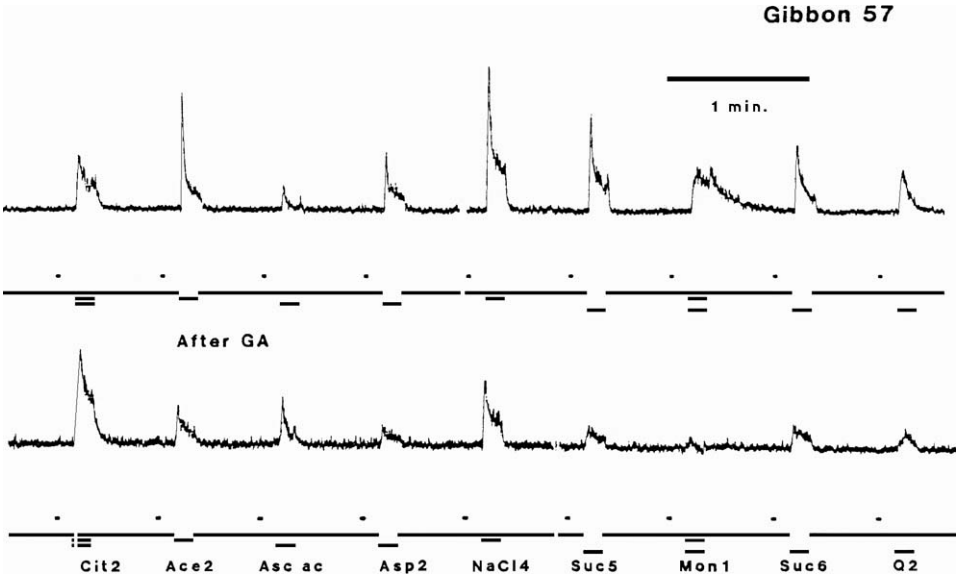

Fig. 2. Summated chorda tympani nerve recordings before and after 2 ml gymnemic acid solution, 5mg/ml for 5 min, on the tongue of gibbon #G-57. The nerve activity was recorded while the flow over the tongue was switched between artificial saliva and the stimuli indicated by the bottom trace. The stimuli are from the left: citric acid, acesulfame-K, ascorbic acid, aspartame, NaCl, sucrose, monellin and quinine hydrochloride.

Several parameters have been measured on the summated recordings of the gibbons (see definitions above). The response amplitudes for each solution have been calculated using the response to NaCl as the standard. The values listed in Table I are compiled from all four animals. Table I shows several features of interest: The maximum amplitudes and surface areas of responses are highly correlated (r=0.88; monellin and thaumatin were not included in this comparison). The delay between onset of a stimulation and the nerve response was significantly longer for most sweeteners than for the salt and the acids. The response to NaCl stimulation has a

steeper slope and peaks earlier than any other stimulus. The difference between the time it takes to reach *Max* with monellin or thaumatin and that of the other stimuli is also striking.

Table I. Parameters measured on summated chorda tympani nerve recordings in four white-handed gibbons (*Hylobates lar*), following administration of test substances. The values are means (above) and standard errors of mean (below).

Solution	*N*	*Max*	*Area*	*Delay*	*Slope*	*Rise*	*Tonic*	*Resume*
Acesulfame-K	11	71.63	2189.37	330.55	28.25	820.91	28.55	2300.18
		13.08	398.63	117.26	4.07	183.32	11.04	497.40
Aspartame	14	47.18	1547.75	416.14	21.06	793.71	39.36	2203.71
		5.86	266.50	138.79	7.22	193.32	11.12	1034.94
D-tryptophan	11	66.48	2445.18	470.18	47.89	636.55	46.45	2181.82
		6.86	285.86	80.74	17.25	117.05	8.50	442.16
Sucrose	22	80.80	3062.92	482.18	55.27	570.27	101.41	2951.18
		11.34	423.91	188.36	19.38	221.26	9.52	588.02
Xylitol	11	71.98	2696.00	377.46	38.68	680.73	67.64	3834.55
		7.10	245.29	242.40	11.30	275.46	7.32	478.57
Monellin	5	68.98	3346.61	664.80	10.54	2107.20	574.00	4002.00
		15.86	756.76	270.00	1.51	99.00	34.50	
Thaumatin	6	56.86	1809.23	728.00	2.80	5365.00	116.50	4002.00
		13.02	332.21	578.42	0.23	1026.97	33.69	
Ascorbic acid	10	39.29	1910.41	537.60	20.69	785.40	25.80	1404.60
		4.56	64.67	94.57	8.28	303.34	3.55	679.73
Citric acid	16	60.40	3217.93	348.00	35.51	740.25	63.56	3722.50
		6.43	371.18	110.31	9.61	15.65	12.14	586.59
NaCl	27	100.00	5540.67	331.56	57.61	593.56	150.19	3430.44
		9.88	886.12	71.70	18.74	171.06	28.11	526.17
QHCl	9	41.15	1584.25	534.67	4.67	2241.33	34.11	3440.00
		4.11	174.09	236.27	1.36	587.07	3.49	1095.49

In Fig. 3, a comparison between the mean maximum responses obtained in white-handed gibbons (*Hylobates lar*), rhesus mcaques (*Macaca mulatta*) and chimpanzees (*Pan troglodytes*) is presented. The data for rhesus macaques have been extracted from previous unpublished studies, while those for chimpanzee are derived from Hellekant *et al*. (1985). In each species the responses are expressed in per cent of the response to NaCl in that particular species. Approximately the same stimulus concentrations were used in all species, except for quinine hydrochloride (2 mM in gibbons and 1 mM in chimpanzees and rhesus macaques). The diagram shows that the response magnitude to each substance tested is quite similar in the three species.

It is evident that the response magnitude to each substance tested is quite similar in the three species.

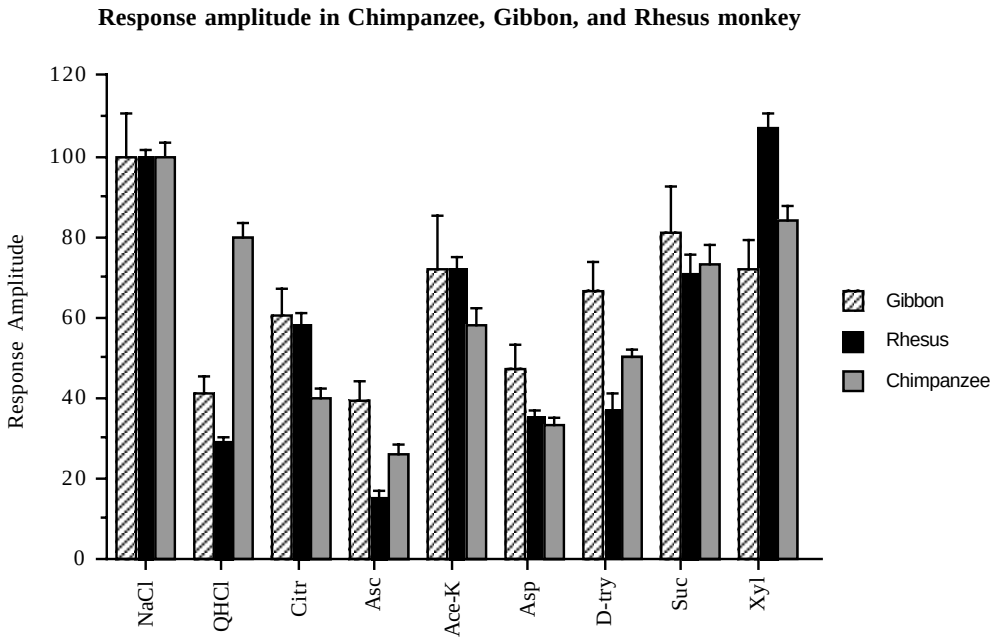

Fig. 3. Comparison among the maximum responses of chorda tympani nerve to test solutions in 4 white-handed gibbons (*Hylobates lar*), 3 rhesus macaques (*Macaca mulatta*), and 3 chimpanzees (*Pan troglodytes*). In each species the responses are expressed in per cent of that to NaCl. The values are means plus standard errors. Abbreviations for the stimuli, from left to right: NaCl, QHCl = quinine hydrochloride, Citr = citric acid, Asc = ascorbic acid, Ace-K = acesulfame-K, Asp = aspartame, D-try = D-tryptophan, Suc = sucrose, Xyl = xylitol.

Effects of gymnemic acid

Between the top and the bottom recording of the gibbon in Fig. 2, 10 mg gymnemic acid solution in two ml 0.01M NaH_2CO_3, was applied for 5 min to the tongue. A comparison between the recordings shows that the gymnemic acid application suppressed the responses to the sweet stimuli, as well as to NaCl and quinine, but enhanced the responses to the acids. The effect of gymnemic acid on monellin is of particular interest; the response of monellin was strongly suppressed and the cross-adaptation effect on sucrose by monellin, very evident in the

upper trace, disappears. A similar disappearance of cross-adaptory effects between sucrose and thaumatin after gymnemic acid was also observed.

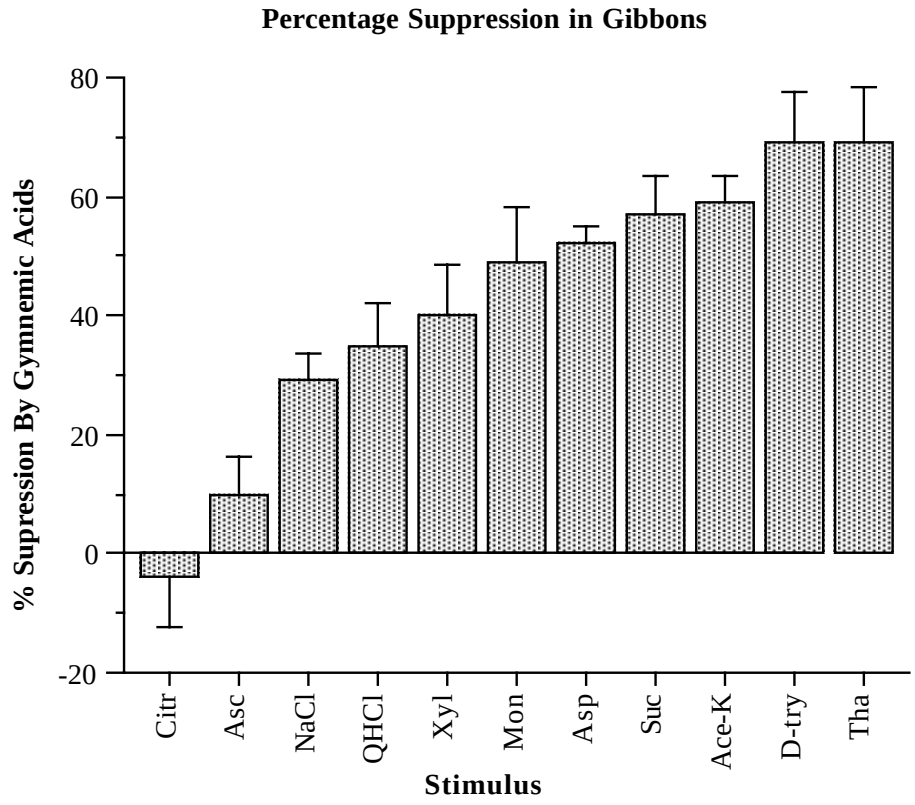

Fig. 4. Effects of gymnemic acid on the peak response in all four study animals. For each stimulus the amplitudes of the last three responses before and the first one after gymnemic acid were measured. The changes are expressed as per cent suppression. The vertical lines represent the standard errors. The stimuli are sorted by increasing suppression after gymnemic application. Abbreviations for the stimuli, from left to right: Citr = citric acid, Asc = ascorbic acid, NaCl, QHCl = quinine hydrochloride, Xyl = xylitol, Mon = monellin, Asp = aspartame, Suc = sucrose, Ace-K = acesulfame-K, D-try = D-tryptophan, Tha = thaumatin.

Fig. 4 presents a graph of the average effects of gymnemic acid on the response amplitudes. It is based on data from all four animals. For each stimulus, the amplitudes of the last three responses before application of gymnemic acid were used. To minimize the influence of possible declining effectiveness of gymnemic acid, only the first response of each compound after gymnemic acid was used. The suppression was expressed by first calculating the response after gymnemic acid application in per cent of the response before application and then deducting this value from 100. The responses to the sweeteners were most suppressed, varying from 70 to 40%. This suppression was statistically significant ($p<0.0001$). The graph also shows that, on the average, the responses to quinine hydrochloride and NaCl were suppressed, while there was a slight enhancement of the response to citric acid. The enhancement of the response to citric acid is not, due to strong individual differences, statistically significant.

Individual variations of the gymnemic effects

The effects of gymnemic acid varied among the gibbons. Table II attempts to summarize this. The contrasting effects on the acids and (to some extent) on NaCl are striking. Thus, there was no or little change of the acid responses in #G-59 and #G-57, while there was a strong suppression in #G-53, and an almost 100% enhancement was recorded in #G-55. These changes were consistent within the individuals. However, in all gibbons the responses to all sweeteners were suppressed, although the extent of suppression varied.

Table II. Individual variations in the effects of gymnemic acid on taste stimuli [1]

Animal (LEMSIP-No.)	Acids	NaCl	Sweeteners	QHCl
Gibbon #G-53	--	--	---	-
Gibbon #G-55	+++	--	--	-
Gibbon #G-57	0	0	--	--
Gibbon #G-59	-	0	-	0

[1] In the table, the effect of gymnemic acid is indicated by a scale ranging from --- to +++, with 0 indicating no effect, +++ a strong (about double size) enhancement, and --- a strong (about half size) suppression.

The duration of the gymnemic acid effect

The study of the duration of the effect had to be limited due to considerations for the animals' well-being. However, in all animals the effect of gymnemic acid could still be observed one hour after the application. We recorded for a longer time in gibbon #G-55 than in any other gibbon, The effect was still evident, although diminished, 2 hours after the application.

The effects of miraculin

Miraculin, which in all simian primates studied enhances the response to acids, was applied on the tongue of gibbon #G-55 more than 2 hours after the gymnemic acid application. The bottom record in Fig. 5, after the miraculin application, shows an enhancement of the responses to the acids, but virtually no effect on the response amplitude to the other stimuli. The amplitude of the acid responses after miraculin application increased about 1.6 times. Application of 5 mg gymnemic acid 35 min after the miraculin (not shown) decreased or abolished the responses to all sweeteners once again. It is difficult to state exactly how this second application of gymnemic acid affected the acid responses, because the relationship between the responses to NaCl (the standard) and the acids did not change, but the actual recording showed no decrease of the acid responses.

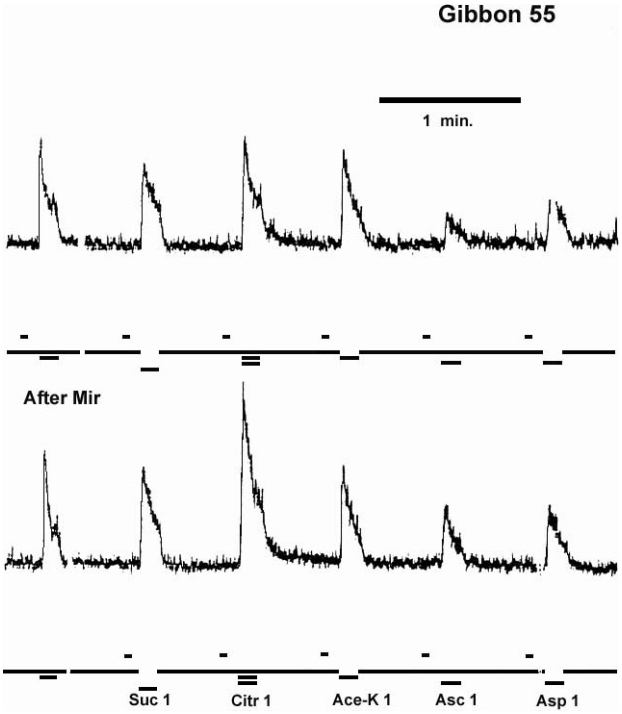

Fig. 5. Summated nerve recording of gibbon #G-55 before and after miraculin, 9 mg in 3 ml for 3 min, more than 2 hours after the gymnemic acid application. The record shows an enhancement of the responses to the acids with virtually no effect on the response amplitude of the other stimuli.

DISCUSSION

The described combination of stimulation and data acquisition techniques record several parameterss in the nerve responses. One of these wwas the maximum response amplitude. Traditionally, it is used as the major parameter for the characterization and comparison both between solutions and those in aminals. We will first compare the responses in gibbons with those in other non-human primates, and then with those in chimpanzees and rhesus macaques. Then we will outline the possible relationship between the other parameters in Table I, and the taste sensation. Finally some aspects of the effects of gymnemic acid will be discussed.

Acesulfame-K, aspartame, D-tryptophan, sucrose, xylitol, monellin, and thaumatin all elicit a response in the gibbons. These sweeteners have elicited a taste response in all representatives of the catarrhine group tested, to which the gibbons belong. In the platyrrhine and prosimian primates, aspartame and thaumatin do not elicit any response (Brouwer *et al.* 1973, 1983, Glaser 1986, Hellekant 1975, 1977, Hellekant *et al.* 1974, 1976, 1980, 1981, 1985).

There were no major taste response differences between gibbons, chimpanzees and rhesus macaques (Fig. 3). The general order in which the stimuli could be arranged was the same. Thus, among the sweeteners, aspartame gave the smallest response, and sucrose the largest, and the order of response amplitudes to the sweeteners was similar. The exception was xylitol. Among

the non-sweet stimuli, NaCl elicited the largest response. Further, the size of and relationship between the responses to the acids were similar. The large response to quinine hydrochloride in the chimpanzees may indicate a better developed sensitivity to bitter compounds in this species. However, caution must be shown when drawing conclusions on the intensity of a sensation from the size of the neural taste response. Observations in humans indicate that for different taste qualities, there is no correlation between the size of the nerve response and intensity of taste sensation. However, there is a good correspondence (Diamant *et al.* 1965, Zotterman 1971) between these parameters within on compound.

Among the parameters presented in Table I, the delay, slope and rise time are parameters which can be expected having an impact on the taste of a compound. There is a correlation between the delay and the onset of the taste sensation. The long delay between the tongue application of thaumatin (Brouwer *et al.* 1973) and the onset of its sweet taste demonstrate this. But the time to reach the peak of the response, the result of the delay and the rise time, should also affect the taste sensation. Here, the respponse to NaCl peaked first, followed by the non-protein sweeteners, the acids, then quinine and lastly, the sweet proteins. This corroborates with psychophysical findings with NaCl and non-protein sweeteners (Yamamoto & Kawamura 1981, Kelling & Halpern 1983) and behavioral data in rats (Halpern 1985). Rats recognize 0.5 M NaCl within 229 msec and respond to 0.5 M sucrose within 854 msec.

Further, as shown in Table I the response of sucrose and acesulfame-K peaked first, closely followed by xylitol and D-tryptophan and lastly aspartame, while the sweet proteins, especially thaumatin, lagged. No data on the sensory temporal profile of these four stimuli have, to our knowledge, been published. It is, however, known that the sweet taste of aspartame grows slower than that of sucrose and acesulfame-K. Further, the slow increase of sweetness of monellin and thaumatin is well known.

The time for decline (resume) of nerve activity post stimulation should account for the amount of lingering taste in a compound. The sweet proteins once again stand out from the other sweeteners, as is evident for anybody who has tasted them. The above suggests that parameters in the summated nerve response, which normally are not measured in electrophysiological recordings, can be measured and used to evaluate the taste of compounds. These parameters can be used as a first screening process of compounds.

The discussion on gymnemic acid addresses two questions: First, is the gibbon more similar to the chimpanzee than to Old World monkeys in its reactivity to gymnemic acid? Second, where and how does gymnemic acid exert its sweet suppressing effects; what are its mechanisms of action?

In humans, gymnemic acid suppresses or abolishes the neural responses to 0.5 M fructose, sucrose, 0.004 M saccharine and 0.03 M cyclamate. The response to 0.2 M NaCl, 0.02 M citric acid and 0.01 M quinine hydrochloride are unaffected (Oakley 1985). In chimpanzees, the gymnemic acid effects range from a complete abolishment of the response to 0.02% thaumatin, 3.5 mM aspartame and acesulfame-K, to a 75% suppression of 0.3 M sucrose and a 50% suppression of 0.76 M xylitol, to a slight increase on the response of 0.04 M ascorbic and citric acid (Hellekant *et al.* 1985). In the gibbons, gymnemic acid suppressed the response to the same sweeteners and concentrations as in chimpanzee, but to a lesser extent. Its strongest effects were observed on D-tryptophan and thaumatin, followed by sucrose and acesulfame-K. Xylitol was least suppressed, as in the chimpanzees. Gymnemic acid also increased slightly the response to citric acid, although there were large individual differences as mentioned.

In the Old and New World monkeys tested so far (*Macaca fascicularis, M. mulatta, Cercopithecus aethiops, Saimiri* sp.), gymnemic acid exerted no effect on sweeteners or non-sweet compounds (Diamant *et al.* 1972, Hellekant 1977, Hellekant *et al.* 1974, Hellekant & Van der Wel 1989, Snell 1965; see also Glaser, 1986, for evidence in other primate species). Thus, the reaction of white-handed gibbons to gymnemic acid is more similar to that of humans and great apes than that of monkeys. However, the effects on sweeteners are smaller, and some of the

effects observed on non-sweet compounds in non-hominoid mammals occur as will be mentioned later.

The discussion on the mechanisms of action of gymnemic acid will by necessity be hypothetical: an increasing number of single fiber, genetic and phylogenetic studies suggest the existence of multiple sweet receptors (cf Hellekant 1975, Faurion 1987). Here we assume that the sweet receptors to high potency sweeteners have a higher degree of specificity than those to low potency sweeteners. We assume the binding of stimulus initiates a sequence of intracellular events which is shared by several sweet receptors. The taste of a sweetener is determined by the identity of the nerve fiber and not by the uniqueness of the intracellular transduction (Brouwer *et al.* 1983).

The question then arises: by what mechanisms does gymnemic acid affect gustatory response to taste stimuli in the apes? It may be fruitful to bring up earlier thoughts on the mode of action of gymnemic acid. Apparently, gymnemic acid can act in two different ways (Kennedy & Halpern, 1980, DeSimone *et al.* 1980), although Riskey *et a1.* (1982) were unable to confirm the conclusions by DeSimone *et a1.* (1980). One is as a specific antagonist of sweetness. The second is as a saponin-like substance with general disruptive effects on cell membranes. Here the effects of gymnemic acid on the non-sweet compounds can possibly be explained by its saponin-like action. This is in analogy to some earlier results in non-primates: the dog *Canis familiaris* (Anderson *et al.* 1959, Hellekant 1976), and the hamsters *Cricetus* and *Mesocricetus auratus* (Bartoshuk cited in Pfaffmann 1970, Faull & Halpern 1971, Hagstrom 1957, Hellekant & Gopal 1976, Hellekant & Roberts 1983, Yackzan 1969). But its effects on sweeteners in apes and humans must be ascribed to a selective effect.

It is not established if gymnemic acid's main action is outside or inside the cell membrane. If we assume that gymnemic acid blocks the access of the stimulus to the receptors, then this assumption would require that gymnemic acid affects all sweet receptors, because it suppresses all sweeteners. It is easier to assume that it interferes with a common intracellular mechanism shared by several receptors. It is also well known that the presence of a sweetener does not prevent the development of the gymnemic acid effects. On the other hand, if the transduction is the target for gymnemic acid, the effects of gymnemic acid should be the same on all sweeteners. Why would a low-intensity sweetener like sucrose give a residual response after gymnemic acid, when a most potent sweetener, like thaumatin, doesn't? This points to an effect of gymnemic acid on the membrane surface.

The effects on the sweet enhancement by miraculin may contribute to the solution as to where gymnemic acid exerts its effects. As mentioned above, the effects of gymnemic acid on the miraculin enhanced acid responses in the gibbons were similar in all simian species (humans, chimpanzees, and Old World monkeys) (Hellekant 1977, Hellekant *et a1.* 1985). As has been documented several times in simian species, miraculin gives acids a sweet taste (Bartoshuk *et al.* 1969, Brouwer *et al.* 1983). Neural recordings show that the effect is caused by an enhancement of nerve responses in sweet fibers to acids after miraculin (Hellekant *et al.* 1974, Hellekant *et al.* 1977, Brouwer *et a1.* 1983). After the application of miraculin, cross adaptation between sucrose and acid can be observed. The response to a mixture of sucrose and citric acid is less enhanced by miraculin than is citric acid alone (Hellekant *et al.* 1974). The last two observations suggest competition for the same receptors by sucrose and acid after miraculin. Gymnemic acid applied after miraculin abolishes the miraculin enhancement, but the enhancement returns after some time without further application of miraculin. It is possible that gymnemic acid blocks the miraculin effects by affecting transduction, but this would leave unexplained the complete absence of gymnemic acid effects on sweet compounds in the monkey. We think it is more likely that miraculin remains on the membrane and that the gymnemic acid through its binding prevents miraculin from stimulating (Kurihara *et al.* 1971). Finally, studies with labelled miraculin

(Farbman & Hellekant 1989), intravascularly applied miraculin, and intravascular stimulation with acids after application of miraculin on the tongue (Hellekant *et a1*. 1986), all indicate that miraculin remains on the oral side of the taste cell (Hellekant *et a1*. 1986). Thus everything in the effect of miraculin indicates that miraculin remains and acts on the apical cell plasma membrane. It all points to the idea that the target for gymnemic acid is on the cell membrane and not intracellularly. We assume that gymnemic acid blocks sweet responses by binding to a structure on the microvillie. This structure is not the sweet receptors, but it is so close that it affects the function of the receptors.

If we assume that this gymnemic sensitivity emerged with evolution in primates, then the following explanation for the results presented above appears plausible: Miraculin does not elicit a sweet taste and gymnemic acid exerts no effects on sweeteners in prosimian primates. Consequently prosimian primates lack gymnemic sensitive sweet receptors. In platyrrhine and catarrhine species, gymnemic acid blocks the effects of miraculin. The receptors stimulated by miraculin are also sensitive to gymnemic acid; the first gymnemic acid sensitive sweet receptor has emerged. In hominoidea species, gymnemic acid suppresses both the miraculin effects and the responses to sweeteners. Gymnemic sensitive receptors to all kind of sweeteners have evolved. However, the effects of gymnemic acid on sweeteners were smaller in gibbons than in chimpanzees. This may be interpreted as coexistence of sweet receptors with and without gymnemic acid sensitivity and a higher proportion of the "old" gymnemic acid unsensitive sweet receptors in the phylogenetically older gibbons than in the younger humans and chimpanzee.

Individual differences in the proportions of these two hypothetical sweet reseptor populations may explain the large individual variations among individuals in Table II. Chimpanzees always showed a residual response to sucrose after application of gymnemic acid; consequently, they have a mixture of both types of sweet receptors, but since the effects were larger, the proportion of the "young" receptors is larger. In humans, finally, the gymnemic sensitive type of sweet receptors dominates.

If this interpretation is correct, sensitivity for gamnemic acid in gibbons and other hominoids would suggest a common phylogenetic stem for these primates, and would appear to represent the first such physiological evidence to support this conclusion. It should, however, be stressed that all the above interpretations are tentative.

In summary, the **sweeteners** used in this study gave responses in white-handed gibbons which were similar to those recorded earlier in chimpanzees and rhesus macaques. However, the effects of **gymnemic acid** in the gibbons differ from its effects in chimpanzees and humans, as well as from those found in Old World monkeys. Thus, in gibbons, gymnemic acid exerts effects that fall between those of the great apes and the Cercopithecoidea tested so far. This finding indicates a larger similarity between gibbons and chimpanzees than between gibbons and Old World monkeys.

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REFERENCES

ANDERSON, B., LANDGREN, S., OLSSON, L. & ZOTTERMAN, Y. 1950. The sweet taste fibres of the dog. *Acta Physiol Scand* **21**, 105-119.

BARTOSHUK, L.M., DATEO, G.P., VANDERBELT, D.J., BUTTRICK, R.L. & LONG, L. Jr. 1969. Effects of *Gymnema sylvestre* and *Synsepalum dulcificum* on taste in man. In: C. Pfaffman (ed.) *Olfaction and taste*, vol. III, **1**, pp. 436-449. Rockefeller University Press, New York.

BIEGERT, J. 1973. Dermatoglyphics in gibbons and siamangs. In: D.M. Rumbaugh (ed.) *Gibbons and siamang*, vol. **2**, pp. 163-184. Karger, Basel.

BROUWER, J.N., GLASER, D., HÅRD AF SEGERSTAD, C., HELLEKANT, G., NINOMIYA, Y. & VAN DER WEL, H. 1983. The sweetness-inducing effect of miraculin; behavioural and neurophysiological experiments in the rhesus monkey *Macaca mulatta*. *J Physiol (Lond.)* **337**, 221-240.

BROUWER, J.N., HELLEKANT, G., KASAHARA, Y., VAN DER WEL, H. & ZOTTERMAN, Y. 1973. Electrophysiological study of the gustatory effects of the sweet proteins monellin and thaumatin in monkey, guinea pig and rat. *Acta Physiol Scand* **89**, 550-557.

CHIVERS, D.J. 1977. The lesser apes. In: Prince Rainer III and G.H. Bourne (eds.) *Primate conservation*, pp. 539-598. Academic Press, New York.

CHIVERS, D.J. 1984. Feeding and ranging in gibbons: A summary. In: H. Preuschoft, D.J. Chivers, W.Y. Brockelman and N. Creel (eds.) *The lesser apes. Evolutionary and behavioural biology*, pp. 267-281. Edinburgh University Press, Edinburgh.

CRONIN, J.E., SARICH, V.M. & RYDER, O. 1984. Molecular evolution and speciation in lesser apes. In: H. Preuschoft, D.J. Chivers, W.Y. Brockelman and N. Creel (eds.) *The lesser apes. Evolutionary and behavioural biology*, pp. 467-485. Edinburgh University Press, Edinburgh.

DARGA, L.L., GOODMAN, M. & WEISS, M.L. 1973. Molecular evidence on the cladistic relationships of the Hylobatidae. In: D.M. Rumbaugh (ed.) *Gibbon and siamang*, vol. **2**, pp. 149-162. Karger, Basel.

DARGA, L.L., BABA, M.L., WEISS, M.L. & GOODMAN, M. 1984. Molecular perspectives on the evolution of the lesser apes. In: H. Preuschoft, D.J. Chivers, W.Y. Brockelman and N. Creel (eds.) *The lesser apes. Evolutionary and behavioural biology*, pp. 448-466. Edinburgh University Press, Edinburgh.

DENE H.T., GOODMAN, M. & PRYCHODKO, W. 1976. Immunodiffusion evidence on the phylogeny of the primates. In: M. Goodman, R. E. Tashian and J. H. Tashian (eds.) *Molecular anthropology. Genes and proteins in the evolutionary ascent of the primates*, pp. 171-195. Plenum Press, New York.

DESIMONE, J.A., HECK, J.G. & BARTOSHUK, L.M. 1980. Surface active taste modifiers: a comparison of the physical and psychophysical properties of gymnemic acid and sodium lauryl sulfate. *Chem Senses* **5**, 317-330.

DIAMANT, H., HELLEKANT, G. & ZOTTERMAN, Y. 1972. The effect of miraculin on taste buds of man, monkey and rat. In: D. Schneider (ed.) *Olfaction and taste*, vol. **IV**, pp. 241-244. Wiss. Verlagsges., Stuttgart.

DIAMANT, H., OAKLEY, B., STROM, L., WELLS, C. & ZOTTERMAN, Y. 1965. A comparison of neural and psychophysical responses to taste stimuli in man. *Acta Physiol Scand* **64**, 67-74.

FARBMAN, A.I. & HELLEKANT, G., 1989. Evidence for a novel mechanism of binding and release of stimuli in the primate taste bud. *J. Neurosc.* **9**, 3522-3528.

FAULL, J.R. & HALPERN, B.P. 1971. Reduction of sucrose preference in the hamster by gymnemic acid. *Physiol Behav* **7**, 903-907.

FAURION, A. 1987. Physiology of sweet taste. *Prog. Sens. Res.* **8**, 130-186.

FELSENSTEIN, J. 1987. Estimation of hominoid phylogeny from a DNA hybridization data set. *J Mol Evol* **26**, 123-131.

GEISSMANN, T. 1984. Inheritance of song parameters in the gibbon song, analzsed in 2 hybrid gibbons *(Hylobates pileatus x H. lar)*. *Folia Primatol* **42**, 216-235.

GEISSMANN, T. 1987. Songs of hybrid gibbons *(Hylobates pileatus x H. lar)*. *Int J Primatol.* **8**, 540 (Abstract only).

GLASER, D. 1986. Geschmacksforschung bei Primaten. *Vierteljahresschrift Naturf. Ges. Zürich* **131**(2), 92-110.

GLASER, D. & HELLEKANT, G. 1977. Verhaltens- und elektrophysiologische Experimente über den Geschmackssinn bei *Saguinus midas tamarin* (Callitrichidae). *Folia Primatol* **28**, 43-51.

GLASER, D., HELLEKANT, G., BROUWER, J. N. & VAN DER WEL, H. 1978. The taste responses in primates to the proteins thaumatin and monellin and their phylogenetic implications. *Folia Primatologica* **29**, 56-63.

GLASER, D., HELLEKANT, G., BROUWER, J. N. & VAN DER WEL, H. 1984. Effects of gymnemic acid on sweet taste perception in primates. *Chem Senses* **8**, 367-374.

GOODMAN, M., BABA, M.L. & DARGA, L.L. 1983. The bearing of molecular data on the cladogenesis and times of divergence of hominoid lineages. In: R.L. Ciochon and R.S. Corruccini (eds.) *New interpretations of ape and human ancestry*, pp. 67-86. Plenum Press, New York.

GROVES, C.P. 1972. Systematics and phylogeny of gibbons. In: D.M. Rumbaugh (ed.) *Gibbon and siamang*, vol. **1**, pp. 1-89. Karger, Basel.

GROVES, C.P. 1984. A new look at the taxonomy and phylogeny of the gibbons. In: H. Preuschoft, D.J. Chivers, W.Y. Brockelman and N. Creel (eds.) *The lesser apes. Evolutionary and behavioural biology*, pp. 542-561. Edinburgh University Press, Edinburgh.

HAGSTROM, E.C. 1957. Nature of taste stimulation by sugar. Ph. D. Thesis, Brown University.

HALPERN, B.P. 1985. Time as a factor in gustation: Temporal patterns of taste stimulation and response. In: D.W. Pfaff (ed.) *Taste, olfaction, and the central nervous system*, pp. 181-209. The Rockefeller University Press, New York.

HELLEKANT, G. 1975. Different types of sweet receptors in mammals. In: D.A. Denton and J.P. Coghlan (eds.) *Olfaction and taste*, vol. **V**., pp. 15-21. Academic Press, New York.

HELLEKANT, G. 1976. On the gustatory effects of gymnemic acid and miraculin in dog, pig and rabbit. *Chem Senses Flavor* **2**, 85-95.

HELLEKANT, G. 1977. Effects of miraculin and gymnemic acid in the rhesus monkey *(Macaca mulatta)*. In: Y. Katsuki, M. Sato, S.F. Takagi and Y. Oomura (eds.) *Food intake and chemical senses*, pp. 201-210. University of Tokyo Press, Tokyo.

HELLEKANT, G., BROUWER, J. N., VAN DER WEL, H., NINOMIYA, Y., HÅRD AF SEGERSTAD, C. & GLASER, D. 1977. The effect of miraculin on the response of single taste fibers in the rhesus monkey. In: *Olfaction and taste*, vol. **VI**., p. 285 (Abstract only). IRL Press Ltd., London.

HELLEKANT, G., GLASER, D., BROUWER, J.N. & VAN DER WEL, H. 1976. Gustatory effects of miraculin, monellin and thaumatin in the *Saguinus midas tamarin* monkey studied with electrophysiological and behavioural techniques. *Acta Physiol Scand* **97**, 241-250.

HELLEKANT, G., GLASER, D., BROUWER, J.N. & VAN DER WEL, H. 1980. Study of behavioural and neurophysiological experiments on the sweet taste in five primates. In: van der Starre (ed.) *Olfaction and taste*, vol. **VII**, pp. 183-186. IRL Press, London.

HELLEKANT, G., GLASER, D., BROUWER, J. & VAN DER WEL, H. 1981. Gustatory responses in three prosimian and two simian primate species *(Tupaia glis, Nycticebus coucang, Galago senegalensis, Callithrix jacchus* and *Saguinus midas niger)* to six sweeteners and miraculin and their phylogenetic implications. *Chem Senses* **6**, 165-173.

HELLEKANT, G. & GOPAL, V. 1976. On the effects of gymnemic acid in the hamster and rat. *Acta Physiol Scand* **98**, 136-142.

HELLEKANT, G., HAGSTROM, E.C., KASAHARA, Y. & ZOTTERMAN, Y. 1974. On the gustatory effects of miraculin acid in the monkey. *Chem Senses Flavor* **1**, 137-145.

HELLEKANT, G., HÅRD AF SEGERSTAD, C., ROBERTS, T., VAN DER WEL, H., BROUWER, J.N., GLASER, D., HAYNES, R.J. & EICHBERG, J.W. 1985. Effects of gymnemic acid on the chorda tympani proper nerve responses to sweet, sour, salty and bitter taste stimuli in chimpanzee. *Acta Physiol Scand* **l24**, 339-408.

HELLEKANT, G. & ROBERTS, T.W. 1983. Study of the effect of gymnemic acid on taste in hamster. *Chem Senses* **8**, 195-202.

HELLEKANT, G. & VAN DER WEL, H. 1989. Taste modifiers and sweet proteins. In: R.H. Cagan (ed.) *Neural mechanisms in taste*, pp. 85-96. CRC Press.

HELLEKANT, G., VAN DER WEL, H., BROWER, J.N., GLASER, D., HÅRD AF SEGERSTAD, C. & ROBERTS, T., 1986. Chorda tympani proper nerve responses to intra-arterial and surface stimulation of the tongue in rhesus monkey and rat. *Chem Senses* **11**, 557-566.

HUBER, E. 1931. *Evolution of facial musculature and facial expression*. Johns Hopkins Press, Baltimore, and Oxford University Press, London.

KAWAKAMI, T.G. & KOLLIAS, G.V. 1984. Breeding and rearing lar gibbons in captivity. In: H. Preuschoft, D.J. Chivers, W.Y. Brockelman and N. Creel (eds.) *The lesser apes. Evolutionary and behavioural biology*, pp. 44-50. Edinburgh University Press, Edinburgh.

KENNEDY, L.M. & HALPERN, B.P. 1980. Extraction, and characterization of a sweetness- modifying component from *Ziziphus jujuba*. *Chem Senses* **5**, 123-147.

KELLING, S.T. & HALPERN, B.P. 1983. Taste flashes: reaction times, intensity and quality. *Science* **219**, 412-414.

KOLLIAS, G.V. & KAWAKAMI, T.G. 1981. Factors contributing to the successful captive reproduction of white-handed gibbons *(Hylobates lar). Am Ass Zoo Vet Ann Proc* **1981**, 45-47.

LEIGHTON, D.R. 1987. Gibbons: Territoriality and monogamy. In: B.B. Smuts, D.L. Cheney, R.M. Seyfarth, R.W. Wrangham and T.T. Struhsaker (eds.) *Primate societies*, pp. 135-145. University of Chicago Press, Chicago and London.

MARSHALL, J.T. & SUGARDJITO, J. 1986. Gibbon systematics. In: D.R. Swindler and J. Erwin (eds.) *Comparative primate biology, vol. 1: Systematics, Evolution, and anatomy*, pp. 137-185. Alan R. Liss, New York.

OAKLEY, B. 1985. Taste responses of human chorda tympani nerve. *Chem Senses* **10**, 469-481.

PFAFFMANN, C. 1970. Physiological and behavioural processes of the sense of taste. In: Wholestenholme and Knights (eds.) *Taste and smell in vertebrates*, pp. 45-50. Churchill, London.

RISKEY, D. R., DESOR, J. A. & VELLUCCI, D. 1982. Effects of gymnemic concentration and time since exposure of intensity of simple tastes: A test of the biphasic model for the action of gymnemic acid. *Chem Senses* **7**, 143-151.

SARICH, V.M. 1968. The origin of the hominids: An immunological approach. In: S.L. Washburn and P.C. Jay (eds.) *Perspectives on human evolution*, pp. 94-121. Holt, Rinehart and Winston, New York.

SARICH, V.M. & CRONIN, J.E. 1976. Molecular systematics of the primates. In: M. Goodman, R. E. Tashian and J. H. Tashian (eds.) *Molecular anthropology. Genes and proteins in the evolutionary ascent of the primates*, pp. 141-170. Plenum Press, New York.

SARICH, V.M. & WILSON, A.C. 1967. Immunological time scale for hominid evolution. *Science* **158**, 1200-1203.

SAWALISCHIN, M. 1911. Der *Musculus flexor communis brevis digitorum pedis* in der Primatenreihe, mit spezieller Berücksichtigung der menschlichen Varietäten. *Morph. Jahrbuch* **42**, 557-663.

SCHULTZ, A.H. 1930. The skeleton of the trunk and limbs of higher primates. *Hum Biol* **2**, 303-438.

SCHULTZ, A.H. 1933. Observations on the growth, classification and evolutionary specialization of gibbons and siamangs. *Hum Biol* **5**, 212-255, and 385-428.

SCHULTZ, A.H. 1973. The skeleton of the Hylobatidae and other observations on their morphology. In: D.M. Rumbaugh (ed.) *Gibbon and siamang*, vol. **2**, pp. 1-54. Karger, Basel.

SIBLEY, C.G. & AHLQUIST, J.E. 1984. The phylogeny of hominoid primates, as indicated by DNA-DNA hybridization. *J Mol Evol* **20**, 2-15.

SIBLEY, C.G. & AHLQUIST, J.E. 1987. DNA hybridization evidence of hominoid phylogeny: Results from an expanded data set. *J Mol Evol* **26**, 99-121.

SNELL, T.C. 1965. *The response of the squirrel monkey chorda tympany on a variety of taste stimuli*. M.Sc. Thesis, Brown University.

TURLEAU, C., CRÉAU-GOLDBERG, N., COCHET, C. & DE GROUCHY, J. 1983. Gene mapping of the gibbon. Its position in primate evolution. *Hum Genet* **64**, 65-72.

WISLOCKI, G.B. 1929. On the placentation of primates, with a consideration of the phylogeny of the placenta. *Contr Embryol* **111**, 51-80, + 7 plates.

WISLOCKI, G.B. 1932. On the female reproductive tract of the gorilla, with a comparison of that of other primates. *Contr Embryol* **135**, 163-204, + 12 plates.

YACKZAN, K.S. 1969. Biological effects of *Gymnema sylvestre* fractions. II. Electrophysiology. Effect of gymnema acid on taste receptor response. *Alabama J Med Sci* **6**, 455-463.

YAMAMOTO, T. & KAWAMURA, Y. 1981. Gustatory reaction time in human adults. *Physiol Behav* **26**, 715-719.

ZOTTERMAN, Y. 1971. The recording of the neural response from human taste nerves. In: *Handbook of sensory physiology. Chemical senses 2, Taste*, pp. 102-115. Springer Verlag, Berlin, Heidelberg, New York.