tions and generally enhance our knowledge about the degree to which nonhuman species and young children understand their own problem-solving behavior.

DAVID PREMACK
Department of Psychology, University of Pennsylvania, Philadelphia 19174

GUY WOODRUFF
University of Pennsylvania Primate Facility, Honey Brook 19344

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Taste Responses in Sheep Medulla: Changes During Development

Abstract. *Response characteristics of taste neurons in the sheep solitary tract and nuclei alter during development. Solitary tract cells in younger fetuses respond tc stimulation of the tongue with fewer salts and acids than do cells in older fetuses, lambs, and adults. Further, responses to specific salts and acids develop in a particular sequence, not randomly. These changes may relate to maturation of taste receptor sites.*

Taste buds appear on the mammalian tongue early in development, at about one-fifth of gestation in the human fetus (*1*) and one-third of gestation in fetal sheep (*2*); studies on sheep have shown that fetal taste buds respond to chemical stimuli (*2*). In earlier experiments to determine whether neurophysiological taste response characteristics alter as the taste system matures, we recorded summed chorda tympani nerve responses to chemical stimulation of the tongue in 100- to 147-day (term) sheep fetuses (*2*). Fetal receptors were comparable to those of newborns and adults in that they responded to a variety of stimuli, including a wide range of NH_4Cl concentrations. More recently, recording from the central nervous system has enabled us to study the taste system in younger fetuses and to obtain more single- and few-unit neural responses (*3*). We now report changes in taste responses recorded from the solitary tract and nuclei (ST) in sheep fetuses from 84 days of gestation to term, in lambs, and in adults.

To record taste responses in fetal sheep, a pregnant ewe of known gestational age is anesthetized with sodium pentobarbital, and the fetus is delivered (*2*). The fetus remains attached to the maternal circulation throughout the experiment and can be maintained with stable internal temperature and heart rate for 4 to 16 hours (heart rate usually becomes erratic after 4 to 6 hours in fetuses between the ages of 80 and 95 days of gestation).

The fetal head is flexed at a right angle to the body and secured in specially designed head bars; sponges under the neck provide additional support. The cerebellum is removed by aspiration to reveal the floor of the fourth ventricle, which is then covered with mineral oil at 38°C. Coordinates for the position of the ST are determined from previously prepared sections of fetal brains at different gestational ages. Commercially made tungsten microelectrodes, with impedances ranging from 3 to 15 megohms and an exposed tip of < 1 μm are used to record neural activity. With the obex as a reference point, the electrode is positioned and advanced into the brain through the use of a three-coordinate microelectrode holder. Neural activity is amplified by a differential a-c preamplifier and monitored with an oscilloscope and audioamplifier. The neural activity is recorded on one channel of a magnetic tape recorder; voice cues are recorded on a second channel to provide information on experimental procedure.

As the microelectrode is driven through the medulla, units are located by applying frequent tactile and chemical stimuli (0.5*M* NH_4Cl or KCl) to the anterior tongue (approximately 30 percent of total tongue length). When a responsive unit is found, the tongue is stimulated with 20 ml each of 0.5*M* NH_4Cl, 0.5*M* KCl, 0.5*M* LiCl, 0.5*M* NaCl, 0.1*M* citric acid, and 0.01*N* HCl. Solutions are applied from a syringe, remain on the tongue for at least 20 seconds, and are then rinsed from the tongue with distilled water for 30 to 60 seconds. To monitor the reproducibility of the response, 0.5*M* NH_4Cl is used as every third or fourth stimulus. All chemicals are dissolved in distilled water and maintained at room temperature during experiments.

For comparative purposes, recordings from chemosensitive units are also made in the ST of lambs and adult sheep. Procedures for exposing the ST and recording from neurons are similar to those used in fetuses.

Twenty-three single- or few-unit recordings (*4*) were made in the ST while stimulating the tongues of 17 fetuses between the ages of 84 and 126 days of gestation. Ten single- or few-unit recordings were made in six lambs 42 to 82 days old, and nine recordings were made in five adult sheep. Electrolytic lesions were made after recording chemosensitive responses from 16 fetal and two lamb units; sites were located in the rostral region of the ST, extending from the level of incoming glossopharyngeal (ninth) nerve fibers to the level of incoming intermedius (seventh) nerve fibers.

Histograms of neural frequency before, during, and after chemical stimulation of the tongue were obtained by converting neural activity to standard pulses and counting the pulses with a rate meter. These histograms were analyzed to determine whether or not a response occurred during stimulation of the tongue with each of the six chemical stimuli. A response was defined as an increase in mean impulse frequency greater than the mean plus 2 standard deviations of the spontaneous discharge frequency for that unit (*5*).

With increasing age, ST units responded to a broader range of stimuli (Fig. 1). For example, almost half the units in fetuses responded to only three of the six stimuli. Only about one-tenth of fetal units responded to all six chemicals, whereas in lambs and adults, almost six-tenths responded to six. However, the transition to a broad responsive range was gradual, and any partition of units according to age groups is somewhat arbitrary.

A broad responsive range of chemosensitive neurons is characteristic of the adult taste system in other species. Doetsch and Erickson studied responses of rat chorda tympani fibers and ST cells to 14 stimuli, including ten salts and two acids (*6*). They reported that both first- and second-order taste neurons responded to "most" of the stimuli used.

Figure 2 illustrates responses from two or three "narrowly" responsive, 105-day fetal sheep units, compared with those from a "broadly" responsive, 122-day

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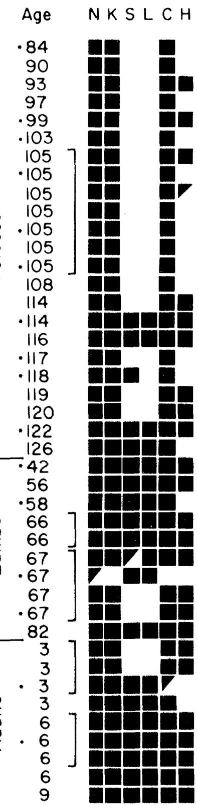

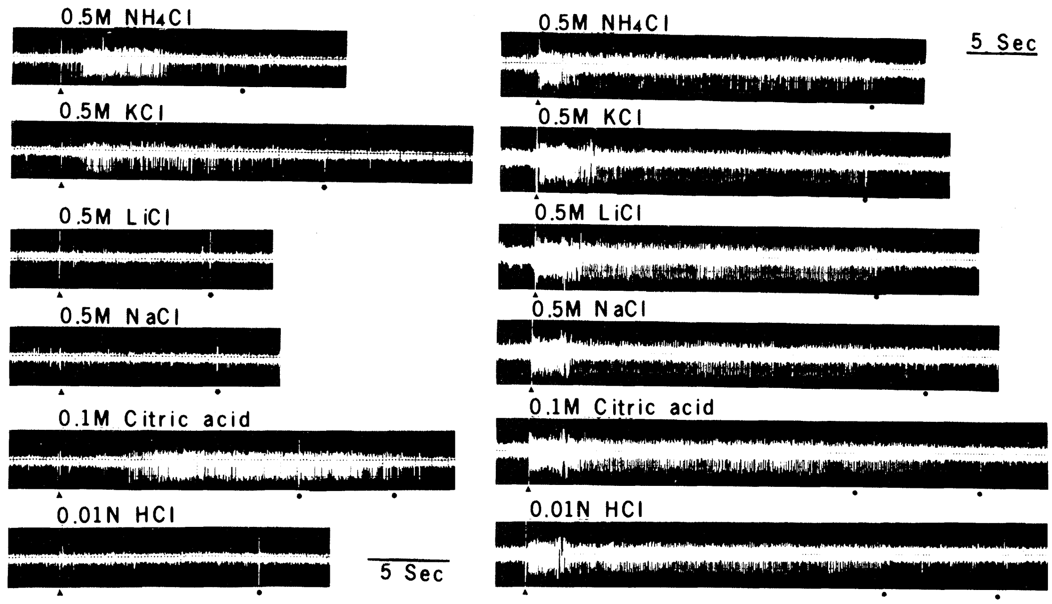

Fig. 1 (left). Responses of ST units in fetuses, lambs, and adults to 0.5*M* NH_4Cl (*N*), 0.5*M* KCl (*K*), 0.5*M* NaCl (*S*), 0.5*M* LiCl (*L*), 0.1*M* citric acid (*C*), and 0.01*N* HCl (*H*). Fetal and lamb ages are given in days of gestation or days after birth; adult ages are given in years. Responses to each chemical are indicated by a closed square (■); no response is indicated by a blank. If a unit was inconsistent (sometimes responded, sometimes did not), a half-square is noted (◩). Dots (·) at the far left signify single-unit recordings. Bracketed ages indicate that those units were recorded from one animal. Fig. 2 (right). Responses recorded from ST neurons in a 105-day fetus (left) and 122-day fetus (right) during chemical stimulation of the tongue. In each trace an arrow denotes the time of stimulus application and a dot denotes distilled water rinses.

fetal unit. Younger ST units show a long response latency and an adaptation of the neural discharge to the prestimulus frequency before NH_4Cl is rinsed from the tongue; in contrast, the older unit responds with a short-latency, slowly adapting discharge.

Responses to the four salt stimuli in fetal, newborn, and adult sheep apparently develop in a particular sequence; responses to NH_4Cl and KCl appeared first, and only in fetuses older than 114 days of gestation did ST units respond during stimulation of the tongue with NaCl or LiCl. This change in response characteristics might result from biased sampling of discrete populations of neurons (*7*), although this seems unlikely. In fetuses younger than 108 days of gestation, we never recorded from units that responded to NaCl and LiCl (Fig. 1); however, in older animals we recorded from both units that were and those that were not responsive to NaCl and LiCl. Therefore, either units that respond to NaCl and LiCl are not present in younger fetuses, or else they are present but too small or too few for neural recording. The consistency of our findings speaks against the latter possibility.

As well as a change in responsiveness to salts, there was a differential response to acids during development (Fig. 1). All ages respond to citric acid, yet more units tended to respond to HCl as development progressed.

Although, to our knowledge, there are no other reports of changes in taste response characteristics during development, in a study of regenerating gerbil taste buds and chorda tympani nerves NH_4Cl was sometimes a more effective stimulus than NaCl during the earliest period of regeneration (*8*). The effect was not consistent, however; some nerves displayed little responsiveness to NH_4Cl. Rather than one type of response profile in the earliest-regenerating fibers, various chemicals yielded diverse response profiles, and the investigators concluded that "young receptor cells have normal responses" (*8*, p. 645). These results contrast with the more consistent responses in younger fetuses we report, but it is difficult to draw comparisons between the processes of regeneration (over a period of several days in gerbil) and development (several weeks in sheep).

The changes in ST taste responses throughout development in the sheep probably reflect maturation of the peripheral taste system. From studies of the adult rat (*6*), it is evident that second-order ST taste neurons resemble chorda tympani fibers in their broad range of sensitivity to 14 chemical stimuli; that is, the range of sensitivity to stimuli remains relatively unchanged from the first- to the second-order level. Thus, the range of responsiveness of our sheep ST units is probably similar to peripheral responsiveness and does not reflect extensive neural processing.

The altered range of responsiveness of sheep ST neurons could reasonably relate to the development of taste receptor sites. Since there are thought to be different receptor sites for the various molecules representative of one taste quality (*9*), it is possible that with structural maturation of taste cells, Na and Li sites are added. Alternatively, changes in the microenvironment around receptor sites could alter the binding capacity of sites for Na, Li, or HCl (*9*). Or the sensitivity of receptors to certain chemicals may change; for example, higher concentrations of NaCl, LiCl, and HCl than those used in this study may have elicited responses more frequently in younger fetuses. Studies of response-concentration functions could clarify this possibility.

Our data on fetal taste responses demonstrate not only that changes occur in neurophysiological function but also that functional taste responses can be recorded before structural development of taste buds is complete. Over the period from 84 days of gestation to term in the fetal sheep, changes occur in taste bud structure as observed with light microscopy (*2*). The apices of the taste bud cells are directly exposed to the oral cavity at 84 days; then, an oval taste pit is gradually formed at the apex of the taste cells, as surrounding epithelial cells grow over the outer margins of the taste bud. With increasing cornification of the tongue, a narrow channel forms between the taste pit and the oral environment. The number of taste buds per fungiform papilla also increases from one at approximately 84 days, to two at approximately 100 days of gestation, to three or more in lambs and adults. It is difficult to correlate these light microscopic anatomical changes with the electrophysiological data without the added insight that could be provided from ultrastructural studies, including observations on taste bud cell membranes and synapses.

From our studies of responses from ST chemosensitive units in fetal, newborn, and adult sheep we conclude that before structural development of taste buds is complete, taste responses can be recorded in the central nervous system; functional changes in range of responsiveness to salts and acids accompany morphological changes in taste buds. Cells in younger fetuses usually respond to lingual stimulation with NH_4Cl, KCl, and citric acid only; more units respond to HCl as development progresses, and sensitivity to NaCl and LiCl first appears in older fetuses. Therefore, taste responses seem to develop in a particular sequence, not randomly. These changes in the range of responsiveness may relate to maturation of taste receptor sites.

CHARLOTTE M. MISTRETTA
Department of Oral Biology, School of Dentistry, and *Research Area, School of Nursing,* and *Center for Human Growth and Development, University of Michigan, Ann Arbor 48109*

ROBERT M. BRADLEY
Department of Oral Biology, School of Dentistry, University of Michigan

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4. Recordings were characterized as single-unit when neural impulses of one consistent amplitude were obtained and as few-unit when impulses of different amplitudes were obtained.
5. The mean spontaneous discharge frequency for each unit was calculated by averaging the impulse frequency during the 5-second periods preceding each lingual stimulation. The frequency of spontaneous activity for all units ranged from 0.06 to 9.10 impulses per second. Mean impulse frequency during chemical stimulation was calculated from neural activity during seconds 3 through 7 after application of the stimulus to the tongue. The first 2 seconds of response were not included, to eliminate counting errors resulting from stimulus artifacts.
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Aphagia and Adipsia After Preferential Destruction of Nerve Cell Bodies in Hypothalamus

Abstract. *Microinjections of the excitatory neurotoxin kainic acid into the lateral hypothalamus of rats produced a period of aphagia and adipsia. Kainate-treated rats displayed transient motor effects during the first hours after the injection but did not show the persisting sensory-motor and arousal disturbances typically observed in animals with electrolytic lesions in this part of the hypothalamus. Histological examination revealed a significant reduction in the number of nerve cell bodies in the lateral hypothalamus. Silver-stained material indicated no evidence of damage to fiber systems passing through the affected region. Assays of dopamine in hypothalamus, striatum, and telencephalon did not indicate significant differences between experimental and control animals. These results are in agreement with recent reports of the anatomical and biochemical effects of intracerebral kainic acid injections and suggest that the observed effect on feeding behavior is related to the destruction of neurons in the lateral hypothalamus.*

Food and water intake (hunger and thirst) are thought to be regulated by excitatory and inhibitory mechanisms intrinsic to the hypothalamus. This idea is based on an extensive body of research demonstrating that ingestive behavior is abolished or exaggerated by certain hypothalamic lesions and that food or water intake can be elicited or inhibited by electrical stimulation or microinjections of putative neurotransmitters and related compounds into the diencephalon (*1*). The "hypothalamocentric" interpretation has been seriously questioned in recent years because (i) it is not clear that the effects of hypothalamic lesions or stimulation can be ascribed to a direct effect on soma and dendrites rather than fibers of passage, and (ii) the behavioral effects of hypothalamic lesions or stimulation are not specific to ingestive behavior (*2*).

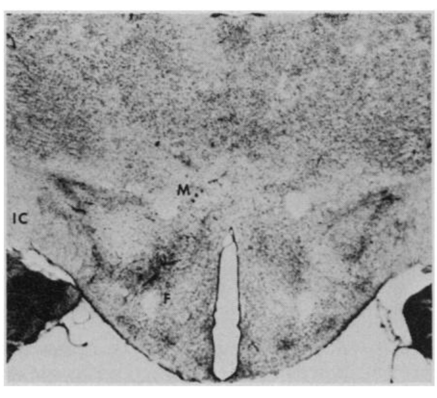

Fig. 1. Sections (50 μm) through the brain of a kainic acid-treated rat of group 7. The area damaged by the injections shows a loss of neurons and increased glia. This animal was aphagic and adipsic when killed 10 days after the injection. Cresyl violet stain; *IC*, internal capsule; *F*, column of the fornix; *M*, mammillothalamic tract.

The focus of the recent criticism has been on the lateral hypothalamus because this region is relatively cell-poor and is traversed by numerous diffuse fiber systems, several of which originate or terminate in areas of the brain (such as the globus pallidus, substantia nigra, and ventral tegmentum) where electrolytic or more selective chemical lesions produce effects on behavior that are similar to those seen after damage to the lateral hypothalamus (*3*).

In all cases, severe sensory-motor disturbances (lack of endogenous arousal, absent or impaired responsiveness to external stimuli, and akinesia) are prevalent throughout the period when the animals are aphagic and adipsic. Although it was suggested (*4*) that the sensory-motor impairments failed to correlate with the severity and persistence of the aphagia and adipsia syndrome, more recent investigators (*5*) have demonstrated parallel recovery functions and concluded that a lack of endogenous arousal or impaired arousal response to external or internal stimuli might be responsible for or contribute to the aphagia and adipsia syndrome.

We have investigated the role of some of the major fiber systems which course through the lateral hypothalamus by means of surgical knife cuts (*6*, *7*). The results of these experiments indicate that