

Blessing, Chapter 7: Eating and Metabolism, (part 1)

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The matter of daily bread, Pavlov noted in his Nobel address, dominates the life of all animals, linking them with surrounding nature. Particular internal states direct the attention toward appropriate environmental stimuli. Odors and tastes, colors and shapes, movement patterns and sounds, all signal the appropriateness or otherwise of particular foodstuffs, triggering capture and ingestive behaviors, eliciting salivary and gastric secretions, altering gastrointestinal motility and blood flow, and mobilizing the secretion of various hormones. After eating an appropriate amount, the animal usually rests, perhaps grooming itself or going to sleep. Toxins, mistakenly ingested, may initiate events that prevent digestion, delay absorption, induce vomiting, and alter behavior so that similarly tasting substances are subsequently avoided. Diet is influenced by nutritional deficiencies, by seasonal and circadian factors, by pregnancy and lactation, and by past experience with particular foodstuffs. Social and psychological factors unrelated to nutritional requirements influence the intake of daily bread.

Internal physiological states initiating eating were seen as deficits of key bodily fuels, such as glucose. However, the explanation of eating as correction of metabolic deficits has proven unsatisfactory. Receptors detecting a low blood glucose level may trigger eating, but the appropriate analogy is with oxygen chemoreceptors, which play little part in quiet respiration, being activated only in unusual circumstances. Normally, the pattern of eating is such that the plasma glucose level remains reasonably stable. Immediately after a meal, nutrients absorbed from the gastrointestinal tract provide the main source of fuel. Later, fuel is normally rapidly available from stores in liver, muscle, and adipose tissue (Woods and Strubbe, 1994; Smith, 1996).

Ingestive behavior may also be studied by focusing on why eating stops rather than on why it starts. An obvious hypothesis is that the presence of food in the stomach or the upper intestine generates a neurohumoral satiety signal that inhibits further eating until the signal dissipates. Paintal (1954), an early exponent of this idea, recorded from vagal afferents and demonstrated fibers activated by gastric stretch receptors. In recent years, as is described in this chapter, the importance of gastrointestinal vagal afferents has been even more appreciated. Since both vagal afferents and taste afferents terminate in the nucleus tractus solitarius, the medulla oblongata is obviously a strategic gateway to the central neural pathways regulating eating.

Pavlov (1910) divided the esophagus of a dog and sutured both ends to the edges of the skin wound, thereby creating a double fistula. Food could be placed into the stomach via the distal opening of the fistula, or the dog could be "pseudo" fed via normal ingestion, with exit of food through the proximal opening. Gastric secretions were collected from the stomach following either procedure. Pavlov summarized some of his findings in his Nobel address. Introduction of bread directly into the stomach "so as to prevent the dog from noticing it" failed to stimulate gastric secretion, because the bread lacked the substances that stimulate the gastric glands. But ingestion of bread or other food through the mouth so that "from the dog's point of view it had really been fed" elicited copious

gastric secretions, even though no food reached the stomach. This gastric secretion was not a reflex result of food stimulating the mouth and throat since chemical irritation of these regions did not cause gastric secretion, and secretion induced by food in the mouth was not prevented by sectioning the taste nerves.

Pavlov noted and confirmed the earlier observations of Bidder and Schmidt, determining that the mere sight of food causes gastric secretion. This response was related to canine personality. "There is not a dog in which skillful teasing with food does not evoke a more or less considerable secretion of juice in the empty and hitherto inactive stomach. At the mere sight of food nervous and excitable animals secrete several hundred cubic centimeters of gastric juice, while the sedate and quiet animal secretes only a few cubic centimeters" (Pavlov, 1955:141). This "psychic" or "cephalic phase" control of gastrointestinal activity has been studied in humans with chronic gastric fistulae (Beaumont, 1833; Carlson, 1916; Wolf and Wolff, 1943).

Cephalic phase CNS control is recognized for other visceral events, including, for example, salivation, adrenal medullary secretion of catecholamines, and pancreatic secretion of insulin. Cephalic phase control is contrasted with reflexly mediated central control. With cephalic phase control, the initiating event is complex and "cerebral," perhaps a genetically inbuilt response as exemplified by the manner in which most mammals reject carrion as a food source, perhaps a conditioned reflex as when we salivate at the sound of the bell. With reflex control, the initiating event is a signal that reaches the CNS by an identified visceral afferent pathway (e.g., a vagovagal reflex; see later in this chapter). The distinction between cephalic and reflex CNS control is artificial of course, to some extent reflecting the different frameworks involved in working with either conscious or anesthetized animals. Nevertheless, it is useful for describing neural regulation of various functions.

Pavlov's subsequent work on conditioned reflexes was important partially because he chose relatively natural environmental events as unconditioned (the food) and conditioned (the bell) stimuli, pairing them with the type of unconditioned response (e.g., salivation) that occurs naturally at the interface between internal visceral events and external behaviors. When psychologists tried to extend Pavlov's paradigm to operant (instrumental) conditioning of visceral events, with reward or punishment contingent on a visceral event such as alteration of heart rate, progress was much less spectacular. It is probably no accident that appreciation of another fundamentally important form of learning, conditioned taste aversions, also amplified an aspect of ingestive behavior that is part of the animal's natural response repertoire. Conditioned taste aversion, to be discussed in this chapter, is a form of one-trial learning in which animals (including humans) subsequently avoid a particular food when its ingestion is followed by nausea and vomiting. The robust nature of this form of learning emphasizes the close interrelationship between visceral events and the individual's interaction with the environment, reminding us that the distinction between cephalic and reflex control is indeed artificial. Since Pavlov, the contribution of cephalic phase responses to the regulation of gastrointestinal function via vagal efferent pathways has been well recognized. The involvement of visual and olfactory stimuli, and the influence of past experience, emphasized the role of the forebrain so that studies of the central control of metabolic regulation, ingestive behavior, and gastrointestinal function

were directed toward this region. In contrast, for many years there was little emphasis placed on the importance of visceral afferents in the regulation of gastrointestinal function. This presumably reflected a general neglect of visceral afferents (see Chapter 1), but no doubt the widespread use of vagotomy as therapy for peptic ulcer disease led to an under-estimation of the importance, for optimal function, of the interaction between local gastrointestinal events and the contribution of the CNS to their regulation. Digestion and absorption can occur in the absence of vagal innervation of the stomach and upper intestine (Carlson, 1916; McSwiney, 1931; Alvarez, 1948; Furness and Costa, 1987; Goyal and Hirano, 1996). However, apart from its obvious contribution to control of gastric motility and acid secretion (see later in this chapter) it is now understood that vagovagal reflex regulation is important for the efficient occurrence of processes as apparently simple as intestinal water absorption and pancreatic exocrine secretion (Zhang et al., 1992; Chu et al., 1993; Rogers et al., 1995).

Carlson recognized that because vagal afferents terminate in the nucleus tractus solitarius, this region must be important in the regulation of gastrointestinal function. Involvement of the medulla oblongata in the control of various gastrointestinal processes is now more widely appreciated, and the interconnections between the lower brainstem and various forebrain regions are better understood (see Chapter 3). The lower brainstem and the spinal cord contain all the central motoneurons regulating ingestive behavior, adrenal secretion, and gastrointestinal, hepatic, and pancreatic functions.

Although rats with midbrain transections do not seek out food, they will ingest suitable material applied near the mouth, accepting or rejecting foods according to taste (Grill and Norgren, 1978b). They can be kept alive by careful tube feeding, and in such animals acute food deprivation increases food intake (Grill and Kaplan, 1990, 1992). Ingestion responses to hypoglycemia also occur (Flynn and Grill, 1983). Anencephalic infants and adults with loss of forebrain function also eat after food deprivation (Nielsen and Sedgwick, 1949; Celesia, 1993). Obviously even in lower mammals the forebrain is necessary for full regulation of food intake so that, for example, the decerebrate rat fails to increase meal size when the number of feeding opportunities is reduced (Kaplan et al., 1993; Seeley et al., 1994). Thus it is now appreciated that neuronal circuitry in the lower brainstem is necessary for all aspects of ingestive, digestive, metabolic, and eliminative functions. An intact brain is required for the full coordination of these activities. Nevertheless, it is surprising how often lower brainstem circuits are sufficient for functions traditionally assigned to forebrain centers.

The diversity of ingestive behaviors emphasizes the complexity of their neural control. Intake may involve chewing, sucking, licking, lapping, and then swallowing. The muscles responsible for these actions are also involved in coughing, gagging, spitting, vomiting, vocalization, laughing, and crying. Since the relevant motor nuclei are not directly connected with each other, the generation of particular activities must depend on highly patterned, temporally organized inputs from premotor neurons.

Grillner and colleagues (1995) have used oscillatory analysis to examine the patterned discharge of anterior horn neurons required for activities as diverse as walking and swimming. Application of similar principles to brainstem somatic motor functions is a Herculean labor. The proximity of vital respiratory and cardiovascular control centers makes it difficult to study isolated *in vivo* preparations,

as can be done in investigations of the spinal cord. Indeed, these brainstem control centers, especially respiratory neurons, obviously have major inter-relationships with premotor neurons involved in ingestive and emetic behaviors (e.g., Miller and Nonaka, 1990a; Fukuda and Koga, 1992). Unfortunately, clinical studies of chewing and swallowing have not contributed greatly to our understanding of the brainstem control of these movements. This is in marked contrast to, for example, our understanding of brainstem control of eye movements, where the controlled structures are clearly bilateral and abnormalities are relatively easy to correlate with pathology.

Taste, a Sensory System Modified by the Needs of the Individual

The well being of all animals, including the unicellular amoeba, depends on their ability to incorporate nutrient substances and to avoid poisons. Certain species of fish have taste buds abundantly distributed on the skin around the mouth as well as within the mouth, emphasizing the role of gustation as transitional between exteroceptive and interoceptive afferent systems (Herrick, 1944, 1948). In mammals, the link between the two systems is further emphasized by the important role of gastric vagal afferents that function as an upper intestinal "taste" system, signalling to the brain the presence of poisons in the stomach and eliciting the sensation of nausea, as discussed later in this chapter. The nucleus tractus solitarius (nTS) is the central termination site of both true taste afferents and gastric vagal afferents. The functional similarity between the two systems is further emphasized when we remind ourselves of the importance of olfaction in the taste process. When the olfactory contribution is removed, as occurs when we have an upper respiratory viral infection (a cold), we no longer experience the sensation of taste.

Primary afferents selectively responsive to substances with a particular taste (salt, sweet, etc.) originate in different gustatory areas and terminate in the rostral nTS (Scott and Giza, 1990; McPheeters et al., 1990; Travers and Norgren, 1991). Gustatory afferents reach the rostral nTS and the spinal nucleus of the trigeminal nerve via the chorda tympani, greater superficial petrosal nerves (VII), lingual-tonsillar nerves (IX), and the superior laryngeal nerves (X), as discussed in Chapter 3 and illustrated in Figure 3.7. For an understanding of the central pathways involved in gustation, the reviews by Norgren (1983, 1985, 1995) are most helpful.

The link between gustation and behavior is emphasized by the manner in which dietary selection is responsive to nutritional and metabolic status. Sodium-deficient rats choose salty drinking water (Harris et al., 1933; Richter, 1942; Rodgers and Rozin, 1966; Cabanac, 1971; Denton, 1982). When dietary alternatives are socially constrained, nutritional deficiencies are more likely to occur, sometimes dramatically, as illustrated by the boy with salt-wasting adrenal hyperplasia who died when his normal self-selected sodium-rich diet was replaced by a hospital ward diet (Richter, 1942). Dietary correction may also result from less specific dietary selective mechanisms. Thiamine-deficient rats choose novel foods, thereby increasing the chance of ingesting thiamine. As discussed below, these behavioral changes may be related to changes in basic sensory physiology, rather than to cortically mediated changes in behavioral responses to relatively invariant sensory inputs. Processing of particular taste stimuli is remarkably contingent on the past experience and current metabolic status of the animal. As Richter envisaged, in deficiency states there may be alteration of specific sensory

thresholds. This contrasts with the situation in sensory systems such as vision and hearing, where physical properties of the stimulus more reliably predict the physiological responses of the receptors and neurons in the initial CNS pathways.

Electrophysiological studies of taste afferents have focused on the actions of water-soluble agents. Sensory mechanisms enabling animals to select and avidly consume fats and oils are more difficult to investigate. Single fiber chorda tympani studies of sodium responsiveness indicate that receptor sensitivity is modified by sodium dietary status (Contreras, 1977). Seemingly paradoxically, the number of impulses induced by applying a salt solution to the tongue is reduced by dietary sodium deprivation. More salt is therefore required to maintain normal activity in sodium-deficient animals (Contreras, 1977; Scott and Mark, 1986). Conversely, high dietary sodium enhances chorda tympani responses (Priehs et al., 1991). Sodium deficiency or excess thus affects taste responsiveness by an action on peripheral taste receptor mechanisms. How changes in gustatory sensory physiology could contribute to behavioral change in deficient animals has been analyzed by recordings of the discharge of neurons in the nTS, as shown in Figure 7.1.

Figure 7.1 Effect of dietary sodium deficiency on nTS neuronal discharge to salt, acid, sugar, and quinine stimuli in rats. **A**, Multi-dimensional neuron space obtained in sodium-replete rats, demonstrating clusters of neurons discharging in response to specific stimuli. **B**, In sodium-deficient rats the clusters are less distinct, with neurons that respond to sodium also responding to sugar. **C**, Histogram showing mean net discharge rates across cells with salt and sugar profiles in sodium-replete and sodium-deficient rats. Sodium deficiency reduces the discharge of salt-profile neurons to salt stimuli and increases the discharge to salt of neurons that also respond to sugar. (Modified from Jacobs et al., 1988.)

Neurons coded for sodium exhibit reduced discharge to equivalent sodium stimuli when the animal is sodium deficient (Jacobs et al., 1988). At the same time neurons normally coded for sugars increase their discharge to sodium. Given the hedonic properties of sugars (Cabanac, 1971), this alteration in the coding channel might explain the avidity for sodium in salt-deprived animals. In a similar manner, Cabanac showed in humans that sucrose-water gradually loses its appeal if the solution is swallowed. The same stimulus can thus be judged pleasant or unpleasant depending on the subject's internal state. The discharge of sweet-coded nTS neurons is modified by changes in blood glucose levels (Giza and Scott, 1983, 1987) in a manner supporting Cabanac's ideas, although satiety does not appear to alter the responses of these taste neurons in the monkey (Yaxley et al., 1985).

In the reviews cited above, Norgren emphasizes the status of gustation as both a somatic and a visceral sense, noting that the region of the nTS innervated by gustatory afferents overlaps with the region where abdominal vagal afferents terminate. Thus certain nTS neurons could have direct inputs from both gustatory and abdominal vagal fibers. Recordings from nTS neurons do reveal dual inputs from chorda tympani and cervical vagal afferents (Bereiter et al., 1981b), and gastric distension modifies the response of nTS neurons to various taste stimuli (Glenn and Erickson, 1976). However, possible interactions between vagal and taste afferents have not been extensively investigated.

Taste neurons in the nTS project to a pontine taste processing area, first described by Norgren and colleagues (1973, 1975) and now appreciated as part of the parabrachial complex (Fig. 7.2; see also Chapter 3).

Figure 7.2 Location of taste responsive neurons in the nTS and in the parabrachial nucleus determined by extracellular recordings. (Modified from McPheeters et al., 1990, and Halsell and Frank, 1991.) Abbreviations listed on pages xiii-xiv.

The presence of taste-responsive neurons in the lateral division of the parabrachial nucleus is of particular interest, since neurons in this region respond to general visceral inputs and some neurons in this lateral division respond to both vagal and gustatory afferents (Hermann and Rogers, 1985). From the parabrachial nucleus, third-order gustatory neurons project to the thalamus (Norgren and Leonard, 1973; Yamamoto et al., 1980; Block and Schwartzbaum, 1983) and thence to agranular insular cortex, subjacent to the primary somatosensory representation of the tongue (Norgren, 1985). The parabrachial nucleus also directly interconnects with hypothalamic and forebrain nuclei, including the central nucleus of the amygdala and insular, infralimbic, and lateral frontal cortices. There is a general similarity in the response patterns of neurons in the taste nerves, in the nTS, in the parabrachial nucleus, and in the amygdala (Norgren and Pfaffman, 1975; Schwartzbaum and Morse, 1978; Schwartzbaum and DiLorenzo, 1982; DiLorenzo and Schwartzbaum, 1982; Schwartzbaum, 1983; McPheeters et al., 1990; Halsell and Frank, 1991).

In the monkey the rostral portion of the nTS also projects directly to the ventral posteromedial thalamic nucleus so that direct transmission of gustatory information from nTS to thalamus may occur (Beckstead et al., 1980). Unfortunately, we do not have additional connectivity studies in primates. Information is also sorely required concerning interconnections between olfactory and gustatory pathways. Paradoxically, although MacLean's "limbic-visceral" forebrain was originally defined because of its extensive olfactory connections (see Chapter 1), we still have little idea of the physiology of olfactory-gustatory interactions or of the neuroanatomical connections between the olfactory forebrain and the (truly) visceral nTS.

Salivation

Since Pavlov's time there has been an emphasis on the occurrence of salivation following "cephalic phase" stimuli including the sight or smell of food and the anticipation of eating. Little emphasis has been placed on the neural pathways connecting salivation (the unconditioned response) with taste (the unconditioned stimulus). Salivary secretion occurs in anesthetized decerebrate rats following application of various stimuli to the oral region, indicating that the basic circuitry for the unconditioned salivary response is contained in the brainstem (Kawamura and Yamamoto, 1978; Matsuo et al., 1989). Salivation in response to particular gustatory stimuli, especially acid tastes, is such a basic unconditioned reflex that one might expect its lower brainstem pathway to be well understood. The location of the relevant preganglionic parasympathetic motoneurons in the lower pons is reasonably well established, and their content of nitric oxide synthase serves as a useful

marker (see Chapter 3, including Fig. 3.11A). Their location in the rabbit is shown in Figure 7.3, adapted from Matsuo and colleagues (1980). The termination of primary taste afferents in the rostral nTS is well documented (see earlier in this chapter and Chapter 3). However, the connection between the rostral nTS (where the relevant primary afferents terminate) and the parasympathetic motoneurons has not been studied by modern neuroanatomical procedures.

Figure 7.3 Location of parasympathetic preganglionic neurons projecting to submandibular salivary gland in rabbit. (Modified from Matsuo et al., 1980.) Abbreviations listed on pages xiii-xiv.

The close physiological relationship between salivation and gustatory stimuli suggests the likelihood of a direct projection from neurons in the rostral gustatory region of the nTS to parasympathetic preganglionic salivary motoneurons. By analogy with the central pathway for esopho-esophageal reflexes (see later in this chapter), there may be a "one central neuron" reflex pathway for taste-evoked salivation. Transneuronal viral tracing studies of inputs to salivatory preganglionic neurons (Jansen et al., 1992) demonstrate labeled neurons in the rostral nTS where gustatory primary afferents terminate, but the projection is not very dramatic. The full distribution of virus-positive neurons after injection of pseudorabies virus into the submandibular gland is shown in Figure 3.11B in Chapter 3.

There have been surprisingly few functional studies directed toward localizing the preganglionic salivary neurons. Electrical stimulation studies have demonstrated that copious salivary flow can be elicited by electrical stimulation in the medulla, in regions containing the appropriate parasympathetic preganglionic neurons (Chatfield, 1941; Magoun and Beaton, 1942; Wang, 1943; Matsuo et al., 1980), but the problems of fibers of passage make it difficult to be sure that the responses reflect activation of the preganglionic cell bodies. There is a need for further mapping studies using focal chemical excitation of neuronal function and for functional studies of the central pathway subserving the unconditioned taste-salivation reflex. Matsuo and colleagues (1989) obtained evidence that the nTS is essential for operation of taste-salivation reflexes, while various portions of the sensory trigeminal nuclei are involved in secretion of saliva in response to mechanical and thermal stimuli. Preganglionic neurons innervating the submandibular gland of the rabbit have been localized by extracellular recording procedures, with appropriate antidromic activation (Matsuo et al., 1982). The conduction velocity of the preganglionic axons ranges from 2 to 11 m/s. The neurons have also been identified by antidromic spike responses to tympanic nerve stimulation, and their responses to stimulation of the ipsilateral chorda tympani, glossopharyngeal, and vagus nerves have been investigated in cats. Many of these neurons can be excited by stimulation of more than one of the nerves (Murakami et al., 1989; Ishizuka and Murakami, 1992).

Swallowing and Esophageal Motility

Swallowing requires coordinated contractions of facial, jaw, tongue, palatal, pharyngeal, and esophageal musculature. The highly patterned response may be initiated voluntarily, or it may occur reflexly in response to gustatory and tactile stimulation of various lip, mouth, pharyngeal, and

laryngeal areas or by electrical stimulation of the superior laryngeal nerve (Doty, 1968; Jean, 1990; Miller, 1993). Once initiated, the coordinated response can proceed without further sensory input, indicating the existence of central programs for its execution.

The esophageal phase of swallowing transports the bolus from the pharynx to the stomach. Most experimental studies of neural regulation of esophageal action have been conducted in rats, dogs, and sheep, species in which esophageal musculature is entirely striated. In cats and humans, the lower third of the esophageal musculature is smooth. There is a debate as to the degree to which esophageal peristalsis is initiated by neural activity during swallowing (primary peristalsis) and the degree to which it is secondary to distention, either by the bolus or by alterations in air pressure (secondary peristalsis). The esophagus has sympathetic and vagal efferent innervation and spinal and vagal afferent innervation. In species with smooth musculature in the lower segment, there is an enteric neural contribution to esophageal contraction. Valuable reviews of neural control of esophageal activity are available (Cunningham and Sawchenko, 1990; Hendrix, 1993; Bieger, 1993a). The subject will be further examined at the end of the present section.

Since cranial nerve motoneurons are not directly interconnected, there must be premotor neurons whose activity coordinates the motoneurons so as to produce the required patterns of muscular contraction. Relatively normal reflex swallowing responses can be elicited in decerebrate animals and in anencephalic humans, indicating that the basic programs must be encoded in brainstem neural circuitry. As described in Chapter 3, there is good neuroanatomical evidence that premotor neurons for V, VII, and XII cranial nuclei are located lateral to the hypoglossal nuclei, extending laterally toward the dorsomedial portion of spinal nucleus of the trigeminal nerve (see Fig. 3.8 in Chapter 3). These premotor neurons could mediate some of the swallowing responses elicited by stimulation in the region ventrolateral to the nTS (see below). Except for a projection to the nucleus ambiguus from the central subnucleus of the nTS (see below), there are no direct projections from the nTS to cranial nerve motoneurons. Anatomical connections between the nTS and the relevant premotor neurons are poorly documented.

Doty and colleagues (1967) defined a medullary swallowing center by combining electrical stimulation of the superior laryngeal nerve with medullary lesions. The responsible region was localized to the medial reticular formation, extending rostrocaudally between the rostral pole of the inferior olive and the caudal pole of the facial nucleus. However, this center has not been confirmed by intramedullary stimulation studies. More rostral electrical stimulation in the ventrolateral pontine reticular formation of the rabbit (dorsolateral to the superior olive) induces swallowing and salivation (Sumi, 1972). However, these responses could reflect activation of afferent intramedullary fibers of the trigeminal nerve or nearby sensory nuclei of the trigeminal nerve, and the idea that there are swallowing centers in medial medullary or ventrolateral pontine regions is no longer accepted.

Jean, Kessler, and colleagues, in extensive studies of central swallowing circuitry in sheep and rats, have suggested that the nTS and the immediately adjacent reticular formation are the only brain regions from which swallowing can be elicited by electrical or chemical stimulation in experimental animals (Jean, 1984a,b, 1990; Kessler and Jean, 1985; Kessler et al., 1990). Stimulation of the superior laryngeal nerve excites neurons in the nTS region in the sheep and the rat (Jean, 1984a;

Kessler and Jean, 1985). Longer latency responses can be recorded from neurons in the medial reticular formation, just lateral to the hypoglossal nucleus, as well as from neurons dorsal to the nucleus ambiguus in the ventrolateral medulla (see below for more recent studies on these neurons). Given the corresponding medullary distribution of premotor neurons innervating cranial motorneurons likely to be involved in swallowing, it may be that the physiological studies of Jean and colleagues have involved activation of these premotor cells (but see next paragraph for studies in the rat).

Detailed studies have demonstrated that microinjection of excitatory amino acid agonists into the rat nTS induces swallowing activity similar to that elicited by electrical stimulation of the superior laryngeal nerve (Kessler et al., 1990; Kessler and Jean, 1991). Positive responses were obtained from a very limited portion of the nTS, in the interstitial nucleus, and immediately ventral and medial to the tractus solitarius in the region of termination of superior laryngeal afferents (see Chapter 3), as shown in Figure 7.4.

Figure 7.4 **A**, Regions of the rat nTS in which microinjection of L-glutamate induces swallowing. **B**, Similarity between swallowing elicited by injection of L-glutamate into the nTS and by electrical stimulation of the afferent superior laryngeal nerve. (Modified from Kessler et al., 1990.)
Abbreviations listed on pages xiii-xiv.

In contrast with the ease with which swallowing can be activated by chemical stimulation of the nTS itself, similar microinjections into the region ventrolateral to the nTS fail to elicit swallowing (Bieger, 1991), even though this is the region containing many of the relevant premotor neurons.

Blockade of endogenous GABA-ergic, presumably inhibitory, inputs to nTS cells also elicits swallowing (Wang and Bieger, 1991). Evidently the output of certain nTS neurons results in the coordinated activation of cranial nerve motor neurons involved in swallowing. The pattern generator for swallowing could therefore be intrinsic to the nTS. Bieger (1993b) has provided a brief review of the role of excitatory and inhibitory amino acids in medullary circuitry mediating swallowing responses.

Ezure et al. (1993) made extracellular recordings just dorsomedial to the rostral nucleus ambiguus in cats, selecting for further analysis neurons that could be reliably orthodromically activated by electrical stimulation of the superior laryngeal nerve. These neurons could not be antidromically activated from the spinal cord, but most of them could be activated from the hypoglossal nucleus and from the more caudal nucleus ambiguus. Most displayed no spontaneous discharge. Fictive swallowing was elicited by continuous stimulation of the superior laryngeal nerve. Most neurons displayed a burst of impulses during swallowing; but the activity of a few was inhibited during swallowing. Characteristics of these swallowing interneurons are shown in Figure 7.5.

Figure 7.5 **A**, Distribution of ventrolateral medullary interneurons (open circles) activated orthodromically by stimulation of the superior laryngeal nerve (SLN) and active during the fictive swallowing that SLN stimulation induces. **B**, Discharge of one neuron orthodromically activated by

electrical stimulation of the SLN. C, Relationship between discharge and hypoglossal nerve activity. (Modified from Ezure et al. 1993.) Abbreviations listed on pages xiii-xiv.

Injection of excitatory amino acid antagonists into the region of the nucleus ambiguus prevents the occurrence of swallowing-related activity induced by stimulation of the superior laryngeal nerve (Kessler, 1993), but the location of the relevant parent neuronal cell bodies is not yet established. Swallowing causes temporary cessation of breathing, so we might expect a close integration of the control of the two activities. There may be respiratory modulation of motor axons innervating the upper esophageal sphincter (Miller, 1990). An intracellular recording and dye-filling study of rat nucleus ambiguus motoneurons innervating the middle and lower regions of the esophagus has revealed no evidence of respiratory modulation of the resting membrane potential (Kruszewska et al., 1994). It may be that the interaction is principally in the other direction, with swallowing command neurons inhibiting the activity of respiratory neurons.

The study relating swallowing to neurons in the nTS and the dorsomedial medulla (Kessler et al., 1990) focused on the region of the nTS where superior laryngeal nerve afferents terminate, a reasonable strategy given that stimulation of this nerve initiates swallowing. In the physiological situation other afferents from the oral cavity and esophagus are also relevant to swallowing. Afferents from these regions terminate more rostrally in the nTS (Altschuler et al., 1989). Esophageal afferents terminate in the central subnucleus of the nTS, probably directly innervating second-order neurons that themselves project directly from the central nucleus of the nTS to esophageal premotor neurons in the nucleus ambiguus (Altschuler et al., 1989; Cunningham and Sawchenko, 1989).

As discussed in Chapter 3 and illustrated in Figure 7.6, this key neuroanatomical evidence indicates that the simplest central circuitry linking esophageal afferents and esophageal efferents may require only one medullary bridging neuron, a second-order sensory neuron that also functions as an interneuron and a premotor neuron. A similar neuroanatomical arrangement is present in the rabbit (Gai et al., 1995). The central linking neurons have been shown to contain somatostatin and markers for nitric oxide synthesis (Cunningham and Sawchenko, 1989; Herbert et al., 1990; Gai et al., 1995). In cats, microinjections of L-glutamate into the general region of the nucleus centralis (not yet neuroanatomically defined in the cat) and adjacent dorsal motor nucleus of the vagus (dmnX), just rostral to the obex, increases lower esophageal sphincter tone, pyloric motility, and intragastric pressure (Rossiter et al., 1990). Similar injections in the more caudal dmnX relaxes the lower esophageal sphincter and reduces intragastric pressure.

Figure 7.6 A, Diagram summarizing a single interneuron CNS pathway mediating esophagoesophageal reflexes. Neuron ①, the primary sensory neuron in the nodose ganglion, projects into the brain and synapses on neuron ②, a nitric oxide-synthesizing neuron in the central nucleus of the nTS. This neuron projects directly to a nucleus ambiguus motoneuron (neuron ③), which innervates the esophageal striated muscle. **B**, Neuroanatomical study in rabbit showing anterograde transport of *Phaseolus vulgaris* leucoagglutinin (PHA-L) from the central nucleus of the

nucleus tractus solitarius to the nucleus ambiguus. (Modified from Yu et al., 1996a.) Abbreviations listed on pages xiii-xiv.

CNS Control of Gastrointestinal Function

Forebrain control of gastric motility and secretion

The stomach acts as an adaptable storage reservoir, as a mixer and a propellor of the gastric contents, and as a source of acidic digestive juices. An active stomach undergoes regional contraction and relaxation so that overall intragastric pressure, as assessed with a single balloon, may not provide a valid measure of gastric activity. This is important because many traditional studies of central nervous control of gastric motility have used the single in-dwelling balloon procedure. When problems with measurement are compounded with those besetting electrical stimulation and ablation of brain regions, it is not surprising that early studies of the central control of gastric motility failed to provide much of permanent significance (Eliasson, 1953; Hesser and Perret, 1960; Perret and Hesser, 1960; Thomas and Baldwin, 1968).

More recent studies have demonstrated that electrical stimulation of the rostral granular insular cortex inhibits gastric motility. Excitatory responses are located more caudally (Yasui et al., 1991a). Similarly, electrical stimulation of the medial frontal cortex in the rat decreases gastric motility, an effect mediated via the vagus nerves (Hurley-Gius and Neafsey, 1986).

Studies of central control of gastric acid secretion have benefited from a more easily measurable physiological endpoint, and forebrain regions in which electrical stimulation affects gastric secretion have been identified (Brooks, 1992). Electrical stimulation of the amygdala alters gastric acid secretion in various ways, depending on the subnucleus and the species. In monkeys there is a decrease in gastric acid secretion (Smith and McHugh, 1967). In intact conscious rats there is a vagally mediated modest increase in the volume of gastric acid secretion, without a change in plasma gastrin (Kim et al., 1990). The central nucleus is the portion of the amygdala directly connected with the dmX-nTS. Stimulation of this region usually increases gastric acid secretion (Hermann and Rogers, 1990), and lesions may attenuate centrally mediated gastric ulceration (Glavin et al., 1991). There is so far no strong evidence for transmitter candidates in the direct amygdalo-dmX pathway, but neuroanatomical studies suggest that somatostatin may be involved (Kawai et al., 1982; Higgins and Schwaber, 1983; Gray and Magnuson, 1987).

Little attention was paid to possible roles of the paraventricular nuclei of the hypothalamus in gastrointestinal control until modern neuroanatomical techniques established the brainstem and spinal projections of the nucleus. The paraventricular nucleus and the lateral hypothalamic area are the two major regions of the hypothalamus projecting directly to the dmX (see Chapter 3). Variable effects on gastric secretion and motility have been obtained following electrical stimulation of the nucleus (Smith and McHugh, 1967; Sakaguchi and Ohtake, 1985; Rogers and Hermann, 1986, 1987). Rogers and Hermann demonstrated that the increase in gastric acid secretion and the decrease in motility induced by electrical stimulation of the medial parvocellular division of the paraventricular nucleus could be suppressed by prior injection of an oxytocin antagonist into the dmX (see below). In an earlier study (Rogers and Hermann, 1985), gastric acid secretion was evoked by unilateral

electrical stimulation of the cervical vagus nerve. A lesion in the ipsilateral paraventricular nucleus reduced evoked gastric acid secretion by approximately 50%. This study was conducted on the assumption that unilateral stimulation of the vagus does not affect the contralateral paraventricular nucleus. However, as discussed in Chapter 3, there is substantial crossover of the primary afferents at the nTS level, and the ascending and the descending connections between the dmX-nTS and the paraventricular nucleus are bilateral. The effects of bilateral paraventricular nucleus lesions, therefore, may have been even more marked. Oxytocin neurons in the parvocellular paraventricular nucleus may therefore increase gastric secretion and decrease motility by an action in the dmX-nTS region of the medulla oblongata.

The oxytocin neurons in the paraventricular nucleus may be part of the central pathway mediating inhibition of gastric motility following peripheral administration of cholecystokinin (see below). Fos studies (Verbalis et al., 1991; Olson et al., 1992) have shown that peripheral cholecystokinin causes fos expression in parvocellular paraventricular oxytocin neurons, including some with identified projections to the dmX-nTS. However, administration of an oxytocin antagonist into the cerebrospinal fluid does not diminish the inhibition of gastric motility seen after systemic administration of cholecystokinin (Flanagan et al., 1992b).

Different investigators have injected neuropeptides into the hypothalamus, particularly the paraventricular nucleus, and obtained dose-response effects on gastrointestinal function. These studies do not specify whether the neuropeptide excites or inhibits paraventricular neurons. Controls are necessary to exclude secondary gastric effects mediated by adrenal hormones or by insulin released by the injections. Gastrin-17 increases gastric acid secretion by an atropine-sensitive vagal pathway in adrenal-ectomized rats (Ohtake and Sakaguchi, 1990). Bombesin suppresses gastric acid secretion, possibly indirectly by a sympatho-adrenal pathway (Taché and Yang, 1990). Injection of gastrin into the lateral hypothalamus stimulates secretion of gastric acid in rats (Tepperman and Evered, 1980). Injection of neuropeptide Y into the paraventricular nucleus reduces gastric acid secretion, possibly via a sympathetically mediated effect on vagal efferent terminals (Humphreys et al., 1992).

Vagal innervation of stomach, intestine and other abdominal organs

The stomach and other upper abdominal organs have an extensive afferent innervation that connects with the CNS via spinal and vagal afferents. Most investigators consider that pain arising from abdominal viscera depends on activity in spinal afferents. In this book, attention is focused on vagally-mediated control mechanisms. The anatomical relationships of the dmX-nTS and vagal afferent and efferent supply of the stomach are summarized in Figure 7.7.

Figure 7.7 A,B, Intracellular fill of an nTS neuron with discharge is increased by intestinal distention and a dmX neuron with discharge inhibited by intestinal distention. (Modified from Zhang et al., 1992.) C, Summary of the nTS-dmX neuroanatomical interrelationships after injection of cholera toxin-HRP into the stomach. Gastric vagal sensory axons terminate almost exclusively in the medial and gelatinous subnuclei of the nTS. Open circles represent vagal afferent boutons not

demonstrated to be in synaptic relationship with dmnX dendrites. Vago-vagal monosynaptic connections are indicated as closed circles. Some vagal afferent boutons form monosynaptic connections within the dmnX. (Modified from Rinaman and colleagues, 1989.) **D**, Diagram of connections between enteric nervous system in the rat fundic stomach and the nTS-dmnX. Vagal efferents innervate myenteric neurons, some of which contain nitric oxide synthase (NOS). Collaterals of vagal afferents also enter the myenteric plexus, possibly participating in local reflex activity. (Modified from Berthoud, 1995.) **E**, intracellularly filled dmnX neuron, initially identified by retrograde transport of tracer applied to the celiac branch of the nerve in the abdomen. The soma and dendrites ramified principally in the horizontal plane. (Modified from Fox and Powley, 1992.) Abbreviations listed on pages xiii-xiv.

The total number of nerve fibers in each midcervical vagus nerve of the human (light microscopic analysis, with supplementary electron microscopic examination) is about 100,000 of which about 20% are myelinated (Hoffman and Schnitzlein, 1961). The esophageal vagal plexus at the level of the diaphragm contains about 50,000 fibers (about 5% unmyelinated). The number of afferent versus efferent fibers could not be determined in this study. An electron microscopic study in the ferret determined that the cervical vagus contains about 28,000 fibers, of which 86% are afferent and 96% are unmyelinated; a similar pattern occurs in the abdominal vagus (Asala and Bower, 1986). Similar findings have been reported for rabbits and cats (Evans and Murray, 1954; Agostoni et al., 1957). Vagal efferents innervating the stomach and upper bowel project to enteric neurons in the myenteric plexus (see Fig. 7.7); the identity of these neurons is still under investigation (Berthoud and Powley, 1992, 1993; Kirchgeßner and Gershon, 1989; Neuhuber, 1987; Berthoud, 1995). For the liver and pancreas, organs without an enteric nervous system, the vagal input is to parasympathetic final motoneurons in the particular organs (Berthoud and Powley, 1991).

Distal processes of gastric vagal afferents are found in close contact with enteric neurons within the myenteric and submucosal plexi, within the muscle, and extending into the lamina propria of the mucosa, toward the epithelial cells (Mei, 1978; Sato and Koyano, 1987; Neuhuber, 1987; Berthoud et al., 1990b). The perikarya of the motor vagal fibers that innervate the stomach, the liver and pancreas, the upper intestine, and the cecum derive principally from the dmnX, with a smaller contribution to the cardia region from the nucleus ambiguus (see Chapter 3). As judged by single fiber vagal efferent recordings in the ferret, perikarya in the dmnX are usually spontaneously active in the anesthetized animal, discharging at up to 6 spikes/s (Grundy et al., 1981). Vagal efferent fibers projecting to the abdomen are thinly myelinated or unmyelinated axons, with conduction velocities of about 1 m/s (Zhang et al., 1995).

When the vagus is involved in both afferent and efferent limbs of an aspect of gastrointestinal control, the term *vagovagal* is used to describe the function. Cephalic phase vagal responses are initiated by more complexly triggered forebrain function. Strictly, a vagovagal reflex implies a monosynaptic connection between afferent and efferent vagal fibers, presupposing either that dendrites of vagal motoneurons extend into the nTS or that some vagal afferents terminate in the dmnX. As discussed in Chapter 3, there are monosynaptic vagovagal synapses. Demonstration of the functional role of this

circuitry is very difficult. In practice, the term *vagovagal* is used loosely to indicate that a particular response is initiated via vagal afferents, integrated via a CNS pathway (complex or simple), and executed via vagal efferents (Rogers et al., 1995). Transection experiments indicate that at least some vagovagal reflexes require only intramedullary connections (Nakazato and Ohga, 1971), perhaps utilizing local connections from the nTS to the dmX (Shapiro and Miselis, 1985b; Rogers and McCann, 1993).

Presumably the medulla and other lower brainstem regions also contain spinally projecting neurons that innervate sympathetic preganglionic cells controlling gastrointestinal function. However, little is known concerning these directly descending pathways. Their likely presence should be kept in mind because, by analogy with sympathetically mediated control of the cardiovascular system, relevant neurons are likely to be located in the ventrolateral medulla, not too far from the nucleus ambiguus. They are likely to be inadvertently stimulated in studies directed at the nucleus ambiguus.

Role of the dmX-nTS and of TRH-containing neurons in medullary raphe nuclei, in control of gastrointestinal function

Electrical stimulation of the efferent end of the cut vagus has both excitatory and inhibitory effects on gastric function. Both effects are blocked by hexamethonium, but many of the inhibitory effects are resistant to muscarinic receptor blockade (Andrews and Scratcherd, 1980). This suggests that the vagal preganglionic neurons all excite the relevant ganglionic neurons, with inhibition of gastric function being mediated by noncholinergic transmitters, probably including vasoactive intestinal polypeptide and nitric oxide (Grundy et al., 1993; Meulemans et al., 1993; Takahashi and Owyang, 1995; Berthoud, 1995).

Activation of the efferent vagus following intravenous 2-deoxyglucose causes secretion of both acid and pepsin, with a concomitant increase in gastric motility (see review by Hirschowitz, 1989). After complete bilateral vagotomy in humans there is decreased acid secretion and alteration in gastric motility with delay in gastric emptying (Alvarez, 1948). The importance of the vagus in insulin hypoglycemia-induced gastric acid secretion is indicated by the reliance placed on the absence of this form of acid secretion as a test for the completeness of the nerve transection when vagotomy was used to treat peptic ulcer (Stein and Meyer, 1948; Alvarez, 1948). In experimental animals, decerebration has variable effects on insulin-induced gastric acid secretion (Jogi et al., 1949), so whether forebrain function is essential for this process is not yet determined.

Since the dmX is the site of medullary preganglionic parasympathetic neurons regulating gastric motility and acid secretion, one would expect that these functions could readily be affected by alteration of neuronal function in this region. This issue has not been easy to study because the proximity of the nTS to the dmX means that experimental procedures often affect both nuclei. Chronic ablation directed at the dmX reduces insulin-induced gastric acid secretion in cats (Kerr and Preshaw, 1969). Electrical stimulation of the dmX-nTS causes both excitatory and inhibitory effects on gastric motility (Semba et al., 1969). Injection of L-glutamate into the nTS reduces tonic and phasic gastric activity (Spencer and Talman, 1986a,b). These effects are difficult to interpret in terms

of neuronal effects, given the proximity of primary afferent terminals in the nTS, the nTS interneurons, and the dmnX preganglionic cells.

Inhibitory effects on gastric motility are also seen after injection of L-glutamate into the dmnX; the effects are abolished by vagotomy and hexamethonium, but not by atropine (Raybould et al., 1989). Since L-glutamate presumably always excites the vagal neurons, this inhibitory action on the gut may reflect excitation of the noncholinergic nonadrenergic inhibitory final motoneurons in the gut wall. Injection of oxytocin into the dmnX-nTS excites vagal pregastric motoneurons, thereby inhibiting gastric motility; an oxytocin receptor antagonist increases motility and prevents the inhibition of gastric motility induced by electrical stimulation of the paraventricular nucleus (Charpak et al., 1984; Rogers and Hermann, 1987; McCann and Rogers, 1990). This suggests that vagal pregastric neurons are tonically excited by an oxytocin-utilizing pathway originating in the paraventricular nucleus, with a consequent inhibitory action on gastric motility.

Injection of thyrotropin-releasing hormone (TRH) or its analogues into the dmnX excites a subpopulation of neurons whose activity increases gastric motility and gastric acid secretion by the atropine-sensitive vagal pathway; TRH-containing inputs to the dmnX may arise from the paraventricular nucleus and the medullary raphe (Rogers and Hermann, 1986, 1987; Stephens et al., 1988; Hornby et al., 1989; McCann et al., 1989a,b; Raybould et al., 1989; Garrick et al., 1989; Travagli et al., 1992;). Both electrical and chemical activation of the raphe neurons increases gastric motility (Hornby et al., 1990; McCann et al., 1989a; Krowicki and Hornby, 1993a). TRH may increase the sensitivity of dmnX neurons to the excitatory action of 5-HT, a possible cotransmitter in medullary raphe neurons (McCann et al., 1988a). Blockade of TRH receptor activation by injection of TRH antibodies into the dmnX reduces the secretion of gastric acid normally observed after chemical activation of neurons in raphe pallidus (Yang et al., 1993). Injection of TRH into the nucleus ambiguus also stimulates gastric motility and acid secretion (Ishikawa et al., 1988; Garrick et al., 1989), but the pathway is not entirely clear since nucleus ambiguus preganglionic cells are not thought to project to the stomach. Injection of TRH into nucleus raphe obscurus also increases gastric motility, presumably by activating neurons that project to the dmnX; injection of substance P into raphe obscurus decreases gastric motility, possibly by activating a noncholinergic vagal pathway to the stomach (Krowicki and Hornby, 1993b). Injection of TRH into the cerebrospinal fluid also has effects on upper intestinal function, increasing net secretion of water, via vagal efferent pathways and a principally vasointestinal peptide-mediated postganglionic mechanism (Lenz and Silverman, 1995). Detailed electrophysiological and morphological studies of nTS and dmnX neurons whose discharge is affected by distension of the stomach or small intestine have been carried out in rats (Zhang et al., 1992, 1995; Renehan et al., 1995; Fogel et al., 1996). Distension of the stomach usually increases the activity of dmnX neurons, whereas distension of the small intestine and the colon usually reduces neuronal discharge. An example of the response of one such dmnX neuron, and the morphology of this cell, is shown in Figure 7.7. More complex response patterns were observed for nTS neurons, as might be expected given the difficulty of determining whether any recording is from a secondary sensory neuron or from an interneuron without primary afferent input. However, most of the nTS

neuronal responses were excitatory (Fig. 7.7), suggesting, for small intestinal and colonic distension, that the discharge of nTS neurons may inhibit dmX cells.

Neurally Mediated Changes in Blood Glucose Level

Sudden changes in the level of blood glucose often reflect the occurrence of neurally mediated changes in secretion of adrenaline, insulin, or glucagon, with consequent effects on stores of hepatic glycogen. As for many other parameters, control of the secretion of these hormones by the brain can be described in terms of cephalic and reflex phases, as long as we remember that the distinction is ultimately artificial (see the introduction to this chapter).

Reflexly evoked CNS initiation of adrenaline secretion occurs during periods of hemorrhage-induced hypotension and during experimental hypoglycemia (for review, see Yamaguchi, 1992) These stimuli have been used to probe the relevant CNS pathways. Secretion of adrenaline in response to hypoglycemia does not occur after transection of the upper spinal cord, either in experimental animals or in humans (Cannon et al., 1924; Crone, 1965; Cantu et al., 1968; Brodows et al., 1973; Mathias et al., 1979a). Secretion still occurs after midbrain transection in sheep (Crone, 1965), dogs (Cantu et al., 1968), and rats (DiRocco and Grill, 1979), indicating that neither the hypothalamus nor the rest of the forebrain is necessary for this response. Adrenal medullary secretion in response to hypoglycemia can therefore be mediated entirely via brainstem pathways to the relevant sympathetic preganglionic neurons in the spinal cord. The central pathway for hypotension-induced secretion of adrenal catecholamines, with subsequent elevation of plasma glucose, is discussed in Chapter 5. In contrast to adrenaline, glucagon is still secreted in response to insulin-induced hypoglycemia in humans with cervical cord transection (Palmer et al., 1976), although in intact individuals sympathetically mediated neural impulses may augment this process (Kurose et al., 1990). Electrical and chemical stimulation of the rostral ventrolateral medulla oblongata and the paraventricular nucleus of the hypothalamus increases plasma adrenaline (Ross et al., 1984b; McAllen, 1986c; Katafuchi et al., 1988; Martin et al., 1991) and, in the case of the paraventricular nucleus, plasma glucose (Paton, 1957). It is not known whether these responses are physiologically related to CNS pathways regulating plasma glucose in the natural state.

Natelson et al. (1973) electrically stimulated the perifornical region of the lateral hypothalamus in unanesthetized monkeys. When the stimulation was intense enough to cause behavioral arousal there was a marked increase in plasma glucose accompanied by a small rise in plasma insulin, less than would normally be secreted in response to the rise in glucose; both glucose and insulin responses were largely prevented by prior adrenalectomy. In adrenal-ectomized animals blood glucose actually decreased, perhaps as a result of insulin secretion mediated via vagal pathways activated during the hypothalamic stimulation. Similar findings, i.e., increases in plasma glucose with insulin levels well below those obtained with corresponding glucose increases following a meal, were obtained in response to noxious tail stimulation in unanesthetized monkeys. When 2-deoxy-D-glucose was used to simulate hypoglycemia in unanesthetized monkeys, plasma glucose also increased without corresponding increases in insulin secretion, an effect probably reflecting an inhibitory effect on insulin secretion by high levels of circulating adrenaline (Smith et al., 1973), as well as an inhibitory

effect on insulin secretion and an excitatory effect on glucagon secretion mediated by sympathetic nerves innervating the pancreas (Kurose et al., 1990). Since insulin is required for glucose entry into muscle, but not for entry into brain, such results suggest that plasma glucose elevations during the defence reaction, and during hypoglycemic stress, serve the brain rather than the muscles. Whether direct spinal projections from the paraventricular nucleus of the hypothalamus to the spinal cord mediate the activation of relevant spinal preganglionic neurons is not yet known.

The pancreatic islets are richly innervated (for reviews, see Woods and Porte, 1974; Yamaguchi, 1992), and neural, especially vagal, control of insulin secretion is well established. Britton (1925) provided evidence for pancreatic release of a hypoglycemia factor in response to electrical stimulation of the peripheral end of the cut vagus nerve; others have documented the release of insulin by the efferent vagus and established the expected sensitivity to hexamethonium and atropine (Bergman and Miller, 1973; Woods and Porte, 1974; Bloom and Edwards, 1981, 1985a; Berthoud et al., 1990a). Vagovagal reflexes, initiated when hepatic vagal afferents are stimulated by an increase in blood glucose, mediate both pancreatic secretion of insulin and an additional neurally mediated increase in glycogenesis in rats (Niiijima, 1985, 1986). The reflex secretion of insulin persists after midbrain transection (Niiijima, 1984). So far, however, there is little indication that such responses are physiologically important (Adkins-Marshall et al., 1992; Cardin et al., 1992).

The "cephalic" early phase of insulin secretion that occurs with the sight, smell, and taste of attractive food and during the first part of a meal depends on vagal efferent innervation of the pancreas (Louis-Sylvestre, 1976; Parra-Covarrubias et al., 1971; Berthoud, 1984; Powley and Berthoud, 1985). This form of insulin secretion is present in animals with an esophageal fistula and is therefore not dependent on gastrointestinal actions of food; it is absent in animals with islet cells denervated by transplantation or by vagotomy (Berthoud et al., 1980, 1981; Woods and Porte, 1974; Berthoud and Powley, 1990; Woods, 1972; Louis-Sylvestre, 1976). In most species, but not in the cat or the pig, it is also abolished by muscarinic receptor blocking agents (Berthoud, 1984). Insulin release after ingestion of a meal (late phase) depends principally on direct pancreatic effects of the meal-induced increase in plasma glucose. There is no major alteration in this phase of insulin secretion after vagotomy, after anticholinergics, or after sympathetic blocking agents (Woods and Porte, 1974; Bloom and Edwards, 1985b).

Lesions of the hypothalamic ventromedial nucleus cause pancreatic secretion of insulin (Hales and Kennedy, 1964; Bray et al., 1981; Powley and Laughton, 1981). The effect is abolished by vagotomy, but the pathway from the hypothalamus to the dmNX has not been determined. The relevant vagal preganglionic neurons are in the medial columns of the dmNX (Rinaman and Miselis, 1987; Berthoud et al., 1990a). Injection of oxytocin into the dmNX decreases secretion of insulin, an effect prevented by bilateral vagotomy (Siaud et al., 1991). Since injection of an antagonist of oxytocin into the dmNX actually increases secretion of insulin, it is possible that there is a tonic inhibitory oxytocin-mediated input to the dmNX, presumably originating in the paraventricular nucleus of the hypothalamus.

Electrical stimulation of the region of the ventrolateral medulla containing the nucleus ambiguus also causes secretion of insulin (Bereiter et al., 1981a). However, no nucleus ambiguus neurons project to

the pancreas in the rat (Rinaman and Miselis, 1987); earlier contrary reports probably reflected spread of retrograde anatomical tracers to structures outside the pancreas. Thus Luiten and colleagues (1987) reported a projection of nucleus ambiguus neurons to the pancreas, but they also noted retrogradely labeled motoneurons in the C4 region of the spinal cord, presumably phrenic motoneurons labeled after spread of tracer from pancreas to diaphragm. It is therefore unlikely that the secretion of insulin reflects a direct stimulation of cell bodies of vagal preganglionic neurons in the nucleus ambiguus. It is possible that the stimulation affects intramedullary axons of efferent fibers of neurons with cell bodies in the dorsal motor nucleus of the vagus.

Blood Glucose Level, Hunger, and Food Intake

Traditional theories emphasized that regulation of hunger and food intake was closely related to local factors, including gastric function, and to general factors, including blood glucose level. Carlson (1916), like Cannon and Washburn (1912), performed experiments to study preconditions for the occurrence of sensations related to hunger. The investigators swallowed balloons to measure their own gastric contractions under various conditions of food deprivation or excess. Dull, gnawing epigastric aches and hunger pangs were correlated with gastric contractions, and a localized source of the hunger sensation was emphasized by both Carlson and Cannon. They relegated to secondary importance the more general sensations of emptiness and associated fatigue, faintness, irritability, and headache (Grossman and Stein, 1948; Janowitz and Ivy, 1949), partially because these aspects disappeared during continued fasting. Carlson (1916) did summarize the alternative tradition, in which hunger is related to activity in a brain hunger center, stimulated directly by a fall in blood glucose level.

Subsequently, a great deal of research focused on the identification of a brain "hunger center" or "glucostat," presumably a collection of neurons selectively responsive either to plasma glucose itself or to some more complex index of the amount of intracellular fuel available for metabolism (Stricker et al., 1977). Thus, administration of insulin, or production of intracellular glucopenia by administration of 2-deoxyglucose, elicits reports of increased hunger in humans, as well as initiating eating behavior in humans and experimental animals (Mackay et al., 1940; Grossman and Stein, 1948; Smith and Epstein, 1969; Houpt, 1974; Thompson and Campbell, 1977; Ritter et al., 1981). However, in normal fasting humans, fluctuations in spontaneous hunger sensations bear little relationship to minor variations in fasting plasma glucose (Janowitz and Ivy, 1949). In this study it was found that intravenous administration of glucose has little effect on hunger sensations. However, intravenous injection of insulin did cause hunger sensations that occurred approximately 10 minutes after the lowest value recorded for plasma glucose (Fig. 7.8).

Figure 7.8 Relationship between change in blood glucose level and hunger sensation in a human subject after intravenous administration of insulin. (Modified from Janowitz and Ivy, 1949.)

It appeared that the function of plasma glucose in hunger was analogous to arterial oxygen content in respiratory control, only stimulating the relevant receptors in abnormal circumstances.

Glucose receptors that might initiate ingestion were thought to be central rather than peripheral because insulin hypoglycemia continued to cause diffuse hunger sensations in vagotomized patients, and in those with dorsolumbar sympathectomy or cervical cord transection (Grossman and Stein, 1948; Janowitz and Ivy, 1949), although in tetraplegic patients hypoglycemia does not produce the same anxiety and restlessness seen in neurologically intact patients (Mathias et al., 1979a). This suggests that direct effects of plasma glucose on glucoreceptors within the CNS must be important in inducing sensations of hunger. This is difficult to prove in humans. There are no clear-cut CNS diseases or injuries associated with selective increases or decreases in the sensations of hunger. Anorexia nervosa may be relevant, but this condition is exceedingly complex (McHugh et al., 1989). In experimental animals, stimulation of central glucoreceptors is usually measured by changes in eating or by changes in plasma glucose secondary to secretion of adrenal catecholamines. It is generally assumed that the same receptors are responsible for initiating eating and for activating the adrenal medulla. However, this may not always be the case. One study in rats demonstrated that intravenous infusion of fructose, a sugar that does not cross the blood-brain barrier, prevented increased feeding behavior during insulin-induced hypoglycemia, but it did not prevent secretion of adrenaline (Stricker et al., 1977). Infusion of carbohydrates or ketone bodies that could be oxidized by the brain prevented both responses.

The hypothalamus in the regulation of food intake and body weight

As discussed in Chapter 1, theoretical formulations of MacLean and others considered the hypothalamus as the headquarters of the autonomic nervous system. The clinical concept of hypothalamic obesity is well established (Bray and York, 1979). Thus early theories of central regulation of food intake and body weight concentrated on the hypothalamus (Brobeck, 1960). These studies will be discussed because they are typical of many early approaches to the study of CNS control of visceral function. The hypothalamus was considered in isolation from the lower brainstem so that little attention was paid to the actual neural pathways connecting the brain to the gastrointestinal tract.

The early work of La Barre (1930) is often cited as supporting the existence of glucose receptors in the forebrain, perhaps the hypothalamus. However, the conclusion was based on very complicated cross-perfusion experiments between three dogs! Simpler experiments have demonstrated that plasma glucose level is increased more by intracarotid infusion of 2-deoxy-D-glucose than by intravenous infusion (Sakata et al., 1963), suggesting the presence of glucose-sensitive neurons in the territory supplied by the internal carotid. Intracarotid infusion of glucose increases plasma noradrenaline (but not plasma adrenaline) to a much greater extent than do similar intravenous infusions, again indicating the presence of glucose receptors in brain regions supplied by the carotid arteries (Levin, 1991). Electrolytic lesions in the ventromedial nuclei of the hypothalamus cause hyperphagia and obesity (Hetherington and Ranson, 1939), while similar lesions in the lateral hypothalamic area inhibit feeding (Anand and Brobeck, 1951). Both lesions cause hyper-insulinemia, a direct vagally mediated function of the injury rather than a secondary effect of change in food intake (Hales and Kennedy, 1964; Bray et al., 1981; Powley and Laughton, 1981). Loss of glucostat neurons after the

ventromedial lesions, with consequent decreased inhibition of neurons in the lateral hypothalamic area, was proposed as a mechanism underlying the changes in eating behavior. In accord with this hypothesis, Mayer and Marshall (1956) administered gold thioglucose to rats and related the resulting hyperphagia and obesity to ventromedial hypothalamic damage. However, other regions, including medullary nuclei, were also damaged by the gold thioglucose (Powley and Prechtl, 1986).

The concept of the hypothalamic glucostat has been loosely defined. Theories have not always stipulated whether the glucose-sensitive neurons are supposed to increase or decrease their discharge rate in response to changes in plasma glucose. Extracellular recording studies suggested that neurons in the ventromedial nucleus were stimulated by application of glucose (Anand et al., 1964). Other electrophysiological studies suggested that the glucose-sensitive neurons were concentrated in the lateral hypothalamic area (Oomura et al., 1974). However, there is little correlation between the manner in which locally and systemically administered glucose affects the discharge rate of neurons in the lateral hypothalamus (Orsini et al., 1990). Increases in plasma glucose in response to a systemically administered glucoprivic stimulus were smaller after injections of local anesthetic (total of 18µl of 2% lignocaine) into the lateral hypothalamic area (Himsworth, 1970). Increase in adrenal nerve activity in response to intracerebroventricular injection of 2-deoxyglucose was markedly attenuated by electrical lesions of the lateral hypothalamic area, but not by lesions of the ventromedial nucleus (Katafuchi et al., 1985). These results could simply imply that hypothalamic neurons, or axonal pathways, modulate the activity of glucose-sensitive neurons located outside the hypothalamus. Lesions of the paraventricular nucleus do not impair the increase in food intake normally occurring in response to 2-deoxyglucose (Calingasan and Ritter, 1992). Direct intracerebroventricular injection of 2-deoxyglucose increases food intake, but this effect is not observed after direct injection into the ventromedial nucleus, the lateral hypothalamus, or other hypothalamic areas (Miselis and Epstein, 1975).

The available evidence thus provides no clear consensus on the roles of ventromedial and lateral hypothalamic regions, or other hypothalamic areas, in the regulation of glucose and insulin secretion or in the control of food intake and body weight. Nor have modern studies confirmed the neuroanatomical connections postulated as part of the functional theories. A projection from the ventromedial nucleus to the lateral hypothalamic area was a central assumption. Autoradiographic evidence (Saper et al., 1976b) did suggest such a pathway, but modern anterograde tracing studies have failed to substantiate either this projection or a projection from the lateral hypothalamic area to the ventromedial nucleus (Luiten et al., 1987; Larsen et al., 1994). The major descending projection of the ventromedial nucleus is to the periaqueductal grey (Luiten et al., 1987). It does not project to the spinal cord or to the paraventricular nucleus of the hypothalamus or the rostral ventrolateral medulla, regions that contain spinally projecting neurons. It does not project to the dorsal motor nucleus of the vagus or to the nTS. Intra-hypothalamic projections that have been substantiated are summarized in Figure 7.9.

The lateral hypothalamus does contain neurons with projections to the dorsal motor nucleus of the vagus and the nTS (see Chapter 3), but whether these cells are involved in the aphagia seen after lateral hypothalamic lesions is doubtful. The passage of medial forebrain bundle fibers through the

lateral hypothalamus is a most important factor in the interpretation of lesion and stimulation experiments. Lateral hypothalamic aphagia may, for example, result from damage to nigrostriatal dopamine pathways in the medial forebrain bundle (Ungerstedt, 1971b).

Modern studies on hypothalamic regulation of metabolism and eating behavior focus more on the paraventricular nucleus. Injecting noradrenaline, neuropeptide Y, or galanin into this region increases food intake; injection of antibody to neuropeptide Y has the opposite effect (Leibowitz, 1991; Menéndez et al., 1992; White, 1993; Shibasaki et al., 1993; Corwin et al., 1993; Hanson and Dallman, 1995). These findings are robust, providing a coherent framework for future studies. It is notable that noradrenaline, neuropeptide Y, and galanin are present in the terminals of lower brainstem catecholamine groups that project to the hypothalamus.

Figure 7.9 **A**, Anatomical relationships in hypothalamus according to theoretical views of Anand and Brobeck, based on hypothalamic lesion studies. Triangles, normal food intake; squares, hyperphagia; circles, no food intake. **B**, Intrahypothalamic anatomical relationships based on axonal tracing experiments by Luiten et al. (1987) and Larsen et al. (1994.) Abbreviations listed on pages xiii-xiv.

Blessing, chapter 7, Eating and Metabolism, (part 2) continued, p.350