Neural Circuits for Taste

Excitation, Inhibition, and Synaptic Plasticity in the Rostral Gustatory Zone of the Nucleus of the Solitary Tract[a]

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ABSTRACT: The rostral nucleus of the solitary tract (rNST) plays a key role in modulating, organizing and distributing the sensory information arriving at the central nervous system from gustatory receptors. However, except for some anatomical studies of rNST synapses, the neural circuits responsible for this first stage in synaptic processing of taste information are largely unknown. Over the past few years we have used an *in vitro* brain slice preparation of the rNST to study synaptic processing, and it has become apparent that the rNST is a very complex neural relay.

Synaptic potentials recorded in rNST neurons resulting from stimulation of afferent taste fibers are a composite of excitatory and inhibitory post synaptic potentials. Pure excitatory postsynaptic potentials (EPSP) can be isolated by using γ-aminobutyric acid type A ($GABA_A$) receptor blockers to eliminate the inhibitory postsynaptic potentials (IPSP). Application of glutamate ionotropic receptor blockers effectively eliminates all postsynaptic activity, indicating that glutamate is the transmitter at the first central synapse in the taste pathway. Stimulation of the afferent taste fibers originating from the anterior (chorda tympani) and posterior (glossopharyngeal) tongue results in a postsynaptic potential that is a complex sum of the two individual potentials. Thus, rNST neurons receive convergent synaptic input from the anterior and posterior tongue.

The IPSP component of the synaptic potentials in rNST results from stimulation of interneurons. If these IPSPs are initiated by tetanic stimulation they undergo both short-term and long-term changes. Short-term changes result in the development of biphasic depolarizing IPSPs, and long-term changes result in potentiation of the IPSPs that can last over an hr in some neurons. This remarkable synaptic plasticity may be involved in the mechanism of learned taste behaviors.

Synaptic transmission in rNST consists of excitation combined with inhibition. The inhibition does not simply depress excitation but probably serves many roles such as shaping and limiting excitation, coordinating the timing of synaptic events and participating in synaptic plasticity. Knowledge of these synaptic mechanisms is essential to understanding how the rNST processes taste information.

INTRODUCTION

The rostral nucleus of the solitary tract (rNST) is responsible for receiving input from afferent nerve fibers innervating taste buds in the oral cavity and distributing this

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information to more central brain areas as well as to nuclei in the brainstem involved in oral-facial reflexes. Although the rNST was described at the turn of the century[15] and afferent terminations of the gustatory cranial nerves VII and IX to rNST have been documented using degeneration and central tracing techniques,[1,6,18] details of neural circuits within the nucleus remained largely neglected.

Recent anatomical studies have provided some information on the morphology of neurons in rNST, and there have been a few studies on synaptic connections.[21,22] However, the bulk of the electrophysiological studies are extracellular recordings usually in response to stimulation of the anterior tongue with taste stimuli. Rarely are tactile and thermal stimuli used despite the fact that rNST neurons often respond to more than just chemosensory stimuli.[13,14] Thus, most of the conclusions on what the rNST actually does are based on a very limited data set.

In other sensory systems investigators often have considerable information on the circuits, synaptology and properties of the second order neurons in the pathway. For example, investigators of the olfactory system have known the details of circuits within the olfactory bulb for some years[16] and have studied the basic biophysical properties of the principal and intrinsic neurons responsible for processing olfactory information. Until recently similar information was not available for the rNST, and yet conclusions were made of how this nucleus processes sensory information.

There are several possible reasons why this fundamental information on the rNST is lacking. Most of the effort in recent years by investigators of the taste system has been concentrated on peripheral receptor mechanisms, and it often seems that these investigators attribute gustatory perception to the receptors themselves. In addition, despite numerous extracellular investigations of the rNST in various species, intracellular recordings are nonexistent. Attempts have been made to make intracellular recordings but, because of the small size of the neurons and other technical difficulties, these attempts have failed. A further problem relates to the structure of the rNST. Instead of being a layered structure like the olfactory bulb it has no particular orientation, making it difficult to record from particular populations of neurons.

To overcome these difficulties we pioneered a brain slice preparation of the rNST to provide information on the biophysical properties of the neurons and to study synaptic connections and synaptic processes.[2,3,7,8] Brain slice preparations have been used to great advantage to study other brain areas, most notably the hippocampus. The preparation is very stable, and the investigator has control over both the intracellular and extracellular environment of the neurons, as well as being able to elicit synaptic activity by electrically stimulating tracts and nerve roots. By adding an intracellular marker, such as biocytin, to the recording electrode filling solution, the neurons can be later identified providing structure-function correlations. Use of this preparation has permitted us to explore details of the circuits within the rNST and has revealed that the rNST is a much more complex nucleus than we could possibly have imagined when we embarked on these studies in 1987. In this review we plan to describe what we have discovered regarding the excitatory and inhibitory synaptic activity of the nucleus, in addition to recent findings that indicate that inhibitory synapses in rNST are remarkably plastic.

EXCITATION AND INHIBITION IN RNTS

Afferent input to the rNST consists of the chorda tympani (VII) and glossopharyngeal (IX) nerves. These nerves enter the brainstem and form the solitary tract that sends collateral branches to synapse with second order neurons of the rNST. Electrical stimulation of the solitary tract evokes, after a short latency, complex postsynaptic

potentials (PSP) in rNST neurons. These potentials can be characterized and separated into depolarizing and hyperpolarizing PSPs based on the predominant polarity of the potential. Application of bicuculline, which effectively blocks the action of the inhibitory neurotransmitter gamma-amino butyric acid (GABA) at the $GABA_A$ receptor, eliminates the inhibitory, hyperpolarizing component of the PSPs leaving only a short latency (about 5 ms) depolarizing excitatory component (FIG. 1A). Addition of blockers that affect the excitatory neurotransmitter receptor for glutamate eliminates initiation of all the PSPs, indicating that the excitatory PSPs (EPSP) at the primary synapse in the central taste pathway are mediated by glutamate.[5,20] This has recently been confirmed *in vivo* using extracellular recordings and iontophoresis of glutamate receptor blockers.[12]

By increasing the stimulus strength, after glutamate receptor block, it is possible to initiate pure inhibitory postsynaptic potentials (IPSP), presumably by direct activation of inhibitory interneurons.[5] Because the hyperpolarizing inhibitory component of the afferent PSPs occurs after a longer latency (~ 9 ms) than the depolarizing excitatory component, and because pure IPSPs can only be initiated after glutamate block, it follows that more than one synapse is required to produce IPSPs in rNST neurons. Thus, it can be concluded that excitation in rNST is primarily mediated by glutamate and inhibition by GABA.

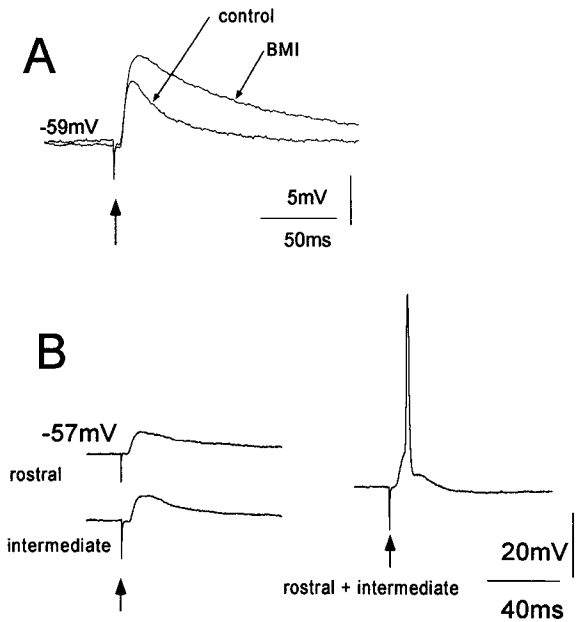

FIGURE 1. **(A)** Synaptic potential recorded from an rNST neuron in response to stimulation of the solitary tract *(arrow)* in control saline and after application of bicuculline (BMI). **(B)** Synaptic potentials recorded from an rNST neuron after stimulation of the rostral and intermediate locations of the solitary tract *(arrow)*. On the *left* the two locations were stimulated separately and on the *right* the two locations were stimulated simultaneously. Note that on the *right* the potentials sum sufficiently to depolarize the neuron and initiate an action potential.

The use of either GABA or glutamate receptor blockers to reveal 'pure' EPSPs and IPSPs not only demonstrates that the PSPs initiated in rNST neurons by solitary tract stimulation are complex mixtures of excitatory and inhibitory potentials, but reveals how the inhibitory component effectively alters the time course of the EPSPs. The IPSP component significantly shortens the PSP duration (50 ms before IPSP elimination, 113 ms after IPSP elimination) and therefore alters rNST synaptic excitability (FIG. 1A).[5]

The chorda tympani (VII) and glossopharyngeal (IX) nerves innervate the anterior two thirds and posterior third of the tongue, respectively. Thus, input from the oral cavity during mastication of food would travel over both of these nerves in a complex synchronous and asynchronous pattern. The projection patterns of the VII and IXth input to the rNTS overlap[6] and, therefore, converge on second order rNST neurons. To simulate the influence of convergent input from the chorda tympani and glossopharyngeal nerves on the postsynaptic responses of rNST neurons, we electrically stimulated the rostral solitary tract, at the site of termination of the chorda tympani nerve, and the intermediate extent of the solitary tract, at the site of termination of the glossopharyngeal nerve.

When synaptic responses were initiated by stimulation of the projection areas of *both* the chorda tympani and glossopharyngeal nerves, all rNST neurons tested responded to stimulation of both sites on the solitary tract. The resulting synaptic potential was a sum of the two individual synaptic potentials. When stimulation of the rostral and intermediate sites both elicited depolarizing potentials, the potential resulting from stimulation of both sites was the arithmetical sum of the two individual PSPs and often depolarized the neuron sufficiently to elicit an action potential (FIG. 1B). The EPSPs summed even if stimulation of the rostral and intermediate sites was separated by up to 100 ms. Inhibitory PSPs evoked by simultaneous stimulation of the rostral and intermediate solitary tract also summed. The summation was not linear and saturated at a mean membrane potential level of −66 mV. When the PSP at one stimulation site was excitatory, but inhibitory at the other site, the PSP wave form resulting from dual stimulation was a complex mixture of the two individual potentials. The inhibitory potential was capable of blocking action potentials resulting from the excitatory PSP.

These results indicate that synaptic responses in rNST are complex mixtures of excitatory and inhibitory potentials. The synaptic potentials result from excitatory afferent input mediated by glutamate, and the inhibitory component is mediated primarily by activation of GABAergic interneurons. Stimulation of the rNST afferent input derived from the chorda tympani and glossopharyngeal nerves reveals complex convergent input. The complexity of these synaptic interactions indicates that considerable processing of gustatory information occurs at the first central synapse in the taste pathway.

SYNAPTIC PLASTICITY IN RNST

Although GABA has been demonstrated in the rNST using immunocytochemical techniques, the role of these GABAergic neurons is not clear,[11] because most authors report only excitatory responses in the rNST using extracellular recording. Utilizing the slice preparation we have demonstrated that the majority of neurons in the rNST respond to application of GABA in a concentration dependent manner.[19] This indicates that inhibition plays a major role in synaptic processing in the rNST.

We have made extensive investigations of inhibitory mechanisms in the rNST and instead of using single shock stimuli to investigate synaptic potentials, we have used trains of stimuli to mimic the frequency of afferent input to the rNST evoked by gustatory stimulation. During gustatory stimulation of the tongue in rats and hamsters, afferent taste fibers are apparently capable of responding with impulse frequencies up to 60 Hz.[4,9] Thus, trains of stimuli or tetanic stimulation represents the type of input a rNST neuron would encounter *in vivo.*

Whole cell recordings were made from second order rNST neurons after glutamate receptor blockade, and the solitary nucleus was stimulated at frequencies of afferent taste fibers (5–50 Hz). We found that in most neurons tetanic stimulation induced membrane hyperpolarization and increased conductance that could be blocked by bicuculline, indicating involvement of $GABA_A$ receptors. When compared to single shock stimuli, tetanic stimulation altered the characteristics of the evoked IPSPs. Tetanic stimulation at frequencies of 10–30 Hz resulted in sustained hyperpolarization due to summation of the individual IPSP amplitudes. In most neurons tetanic stimulation prolonged the decay time of the IPSP. Depending on the frequency, duration and magnitude of the stimulation, the decay time of the IPSP was lengthened several hundred orders of magnitude compared to single shock stimuli. Thus, tetanic stimulation can potentiate the IPSPs thereby increasing the time of inhibition.

In some neurons tetanic stimulation elicited a biphasic response with an initial hyperpolarization, which then became depolarizing and elicited action potentials. The depolarizing amplitude and the number of action potentials was dependent on the frequency, duration and magnitude of the stimulus. This type of biphasic GABA response has been described in the hippocampus.[10,17] We examined the influence of extracellular K^+ concentration on the IPSPs recorded from rNST neurons and concluded that tetanic stimulation results in an elevation of extracellular K^+ concentration and accumulation of intracellular Cl^-. This redistribution of Cl^- and K^+ produces a decay of the IPSP amplitude and as a consequence results in a biphasic or depolarizing IPSP.

Thus, GABA receptor activation, which is normally inhibitory, can become excitatory at these high stimulation frequencies. This short-term change in synaptic activity induced by afferent frequencies normally resulting from taste stimulation can alter the transmission of taste information in rNST and illustrates the importance of inhibitory activity in the gustatory relay nucleus.

In addition to short-term changes in the IPSPs evoked in rNST neurons, tetanic stimulation also produces remarkable long-term potentiation of the IPSP amplitude. After a series of control, single shock, evoked IPSPs, a 50-Hz, 2-sec duration tetanic stimulus was applied to the solitary tract, and then single test shock IPSPs were elicited at the same stimulus strength every 30 sec. In all neurons tested using this paradigm, the tetanic stimulation resulted in a marked potentiation of the evoked IPSP amplitude (mean amplitude is 275% of control immediately after the tetanic stimulation) and also resulted in an increase in the amplitude and frequency of the spontaneous IPSP activity (FIG. 2A). In the majority of neurons the potentiation lasted 5–20 min before returning to control levels, but in a few neurons the potentiation was sustained for over 1hr (FIG. 2B). After the potentiation had declined to control levels it could be induced again after a second tetanic stimulation (FIG. 2B).

Thus, tetanic stimulation can induce both short and long lasting changes in rNST inhibitory synapses. In the hippocampus changes in the effectiveness of synapses is related to learning mechanisms, and it is thus possible that the long-term potentiation of inhibition in rNST may be the cellular mechanism of learned taste behaviors.

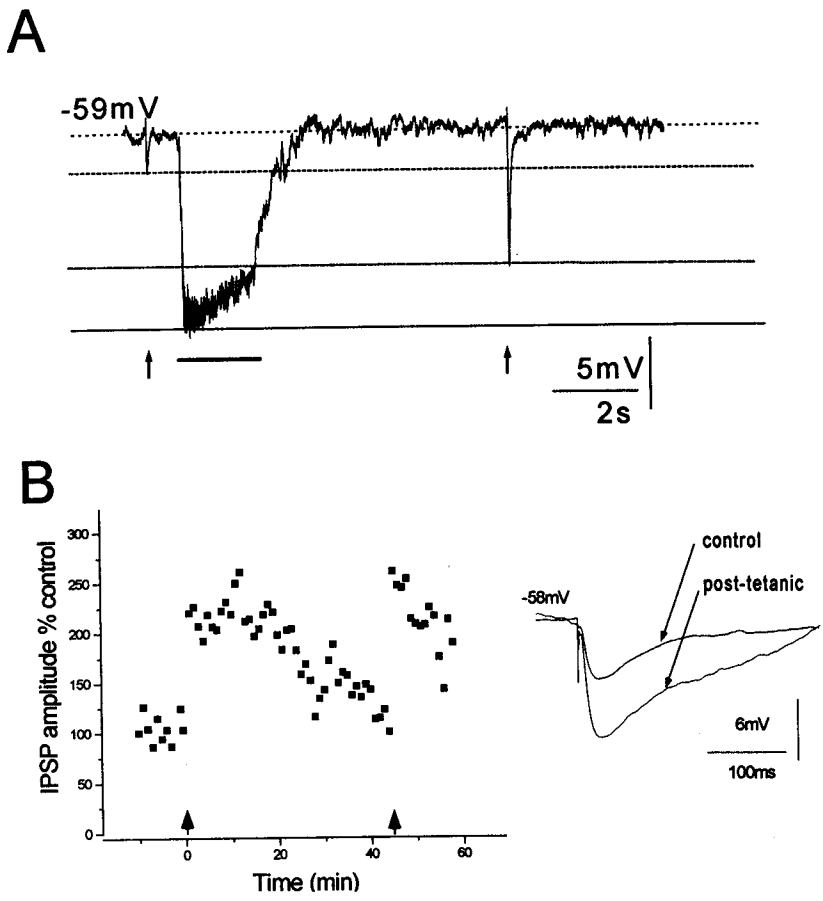

FIGURE 2. **(A)** IPSPs recorded from an rNST neuron after glutamate receptor blockade. A single shock-evoked IPSP *(arrow)* is followed by tetanic stimulation *(horizontal bar)* at 50 Hz for 2 sec. Note that following the tetanic the stimulation, the single shock-evoked IPSP *(second arrow)* is of a much greater amplitude. All the IPSPs were evoked by the same stimulus strength. **(B)** *Left.* Relationship of the amplitude of the single shock-evoked IPSP after tetanic stimulation *(arrow)*. The IPSP amplitude is potentiated following tetanic stimulation for 40 min before returning to control values. A second tetanic stimulation *(arrow)* results in a further potentiation of the IPSP. *Right.* Fast trace recording of a single shock IPSP evoked under control conditions and after tetanic stimulation (post-tetanic).

CONCLUSIONS

The results presented in this paper are beginning to reveal details of synaptic activity in the rNST. Synaptic potentials recorded from second order neurons in the rNST are complex mixtures of excitation and inhibition. These complex potentials are derived from excitatory afferent synapses and are mediated by glutamate. All neurons in the

rNST receive excitatory input from afferent fibers of the chorda tympani and glossopharyngeal nerves. Furthermore, second order neurons receive convergent input from both the chorda tympani and glossopharyngeal nerves. Inhibitory interneurons that receive excitatory input are responsible for generating the inhibitory component of the synaptic potentials and are mediated by $GABA_A$ receptors.

By using tetanic stimulation at frequencies and durations that mimic the afferent neural input recorded by investigators of peripheral gustatory nerve experiments, we have discovered that this stimulus paradigm leads to both short-term and long-term alterations in inhibitory synaptic activity, usually referred to as synaptic potentiation. Thus, inhibitory synaptic potentials are potentiated for up to one hr after tetanic stimulation. This kind of synaptic activity has been demonstrated in brain areas usually associated with learning and memory. It is possible therefore that some learning may take place at the first central relay in the taste pathway and may be involved in various kinds of learned taste behaviors.

It is apparent from these studies that the rNST is a complex neural structure. Investigators should be aware of this when formulating hypotheses on how the rNST processes information derived from taste buds and other sensory receptors in the oral cavity.

REFERENCES

1. BECKSTEAD, R. M. & R. NORGREN. 1979. An autoradiographic examination of the central distribution of the trigeminal, facial, glossopharyngeal, and vagal nerves in the monkey. J. Comp. Neurol. **184:** 455–472.
2. BRADLEY, R. M. & R. D. SWEAZEY. 1989. Intrinsic characteristics of gustatory neurons in rat solitary nucleus [abstract]. Soc. Neurosci. Abstr. **15:** 930.
3. BRADLEY, R. M. & R. D. SWEAZEY. 1990. *In vitro* intracellular recordings from gustatory neurons in the rat solitary nucleus. Brain Res. **508:** 168–171.
4. FRANK, M. E., S. L. BIEBER & D. V. SMITH. 1988. The organization of taste sensibilities in hamster chorda tympani nerve fibers. J. Gen. Physiol. **91:** 861–896.
5. GRABAUSKAS, G. & R. M. BRADLEY. 1996. Synaptic interactions due to convergent input from gustatory afferent fibers in the rostral nucleus of the solitary tract. J. Neurophysiol. **76:** 2919–2927.
6. HAMILTON, R. B. & R. NORGREN. 1984. *Central projections of gustatory nerves in the rat.* J. Comp. Neurol. **222:** 560–577.
7. HARRISON, T. A. & R. M. BRADLEY. 1988. An *in vitro* brain slice preparation to study gustatory-salivatory nucleus interactions [abstract]. Chem. Senses **13:** 696.
8. HARRISON, T. A. & R. M. BRADLEY. 1988. Characteristics of parasympathetic secretomotor neurons studied *in vitro* [abstract]. Soc. Neurosci. Abstr. **14:** 1182.
9. HILL, D. L., C. M. MISTRETTA & R. M. BRADLEY. 1982. Developmental changes in taste response characteristics of rat single chorda tympani fibers. J. Neurosci. **2:** 782–790.
10. LAMBERT, N. A. & L. M. GROVER. 1995. The mechanism of biphasic GABA responses. Science **269:** 928–929.
11. LASITER, P. S. & D. L. KACHELE. 1988. Organization of GABA and GABA-transaminase containing neurons in the gustatory zone of the nucleus of the solitary tract. Brain Res. Bull. **21:** 623–636.
12. LI, C. S. & D. V. SMITH. 1997. Glutamate receptor antagonists block gustatory afferent input to the nucleus of the solitary tract. J. Neurophysiol. **77:** 1514–1525.
13. OGAWA, H., T. HAYAMA & Y. YAMASHITA. 1988. Thermal sensitivity of neurons in a rostral part of the rat solitary tract nucleus. Brain Res. **454:** 321–331.
14. OGAWA, H., T. IMOTO & T. HAYAMA. 1984. Responsiveness of solitario-parabrachial relay neurons to taste and mechanical stimulation applied to the oral cavity in rats. Exp. Brain Res. **54:** 349–358.
15. RAMÓN Y CAJAL, S. 1909. Histologie du Système Nerveux de l'Homme et des Vertébrés. Maloine. Paris.

16. SHEPHERD, G. M. & C. A. GREER. 1990. Olfactory Bulb. *In* The Synaptic Organization of the Brain. G. M. Shepherd, Ed. : 133–169. Oxford University Press. New York.
17. STALEY, K. J., B. L. SOLDO & W. R. PROCTOR. 1995. Ionic mechanisms of neuronal excitation by inhibitory $GABA_A$ receptors. Science **269:** 977–981.
18. TORVIK, A. 1956. Afferent connections to the sensory trigeminal nuclei, the nucleus of the solitary tract and adjacent structures—an experimental study in the rat. J. Comp. Neurol. **106:** 51–141.
19. WANG, L. & R. M. BRADLEY. 1993. Influence of GABA on neurons of the gustatory zone of the rat nucleus of the solitary tract. Brain Res. **616:** 144–153.
20. WANG, L. & R. M. BRADLEY. 1995. *In vitro* study of afferent synaptic transmission in the rostral gustatory zone of the rat nucleus of the solitary tract. Brain Res. **702:** 188–198.
21. WHITEHEAD, M. C. 1986. Anatomy of the gustatory system in the hamster: Synaptology of facial afferent terminals in the solitary nucleus. J. Comp. Neurol. **244:** 72–85.
22. WHITEHEAD, M. C. 1988. Neuronal architecture of the nucleus of the solitary tract in the hamster. J. Comp. Neurol. **276:** 547–572.