

## Involvement of the Mesolimbic System in Palatability-induced Ingestion

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### Introduction

The pleasantness of taste powerfully influences the preference for food and fluids. Particularly palatable tastes can lead to considerable overconsumption. Because palatable foods and fluids are potentially nutritive, animals are highly motivated to ingest them. The brain regions along the taste pathway and its anatomical interfacing with the brain reward system are thought to be involved in palatability-dependent consumption (Kelley *et al.*, 2002). However, the role of the mesolimbic system in the behavioral expression induced by taste pleasantness is still unclear. Our previous studies (Shimura *et al.*, 2002) demonstrated that lesions of the ventral tegmental area (VTA), the origin of the mesolimbic dopamine system, selectively affected the consumption of highly palatable solutions in rats. To obtain further information concerning the mechanisms of the reward system for palatability-driven ingestion, we recorded single neuron activities from the mesolimbic system during the licking of water and taste solutions in freely behaving rats.

### Methods

Male Wistar rats weighing ~300 g were anesthetized with Nembutal (50 mg/kg body wt) and fixed in stereotaxic apparatus. Three to four sets of bipolar chronic electrodes were inserted into the brain for the monitoring of neuronal activities with conventional electrophysiological methods. The electrode was made from two 80  $\mu$ m stainless steel wires insulated with polyurethane except at the tip. The wires were twisted together so that the tips were separated by 0.3 mm. All the leads were soldered to a miniature socket, which was then fixed securely to the skull with stainless steel screws and dental acrylics. The rats were given food and water ad libitum and allowed to recover for at least 1 week.

The apparatus used in this experiment was described in detail elsewhere (Yasoshima *et al.*, 1995). In the experimental chamber, the rats were previously trained to lick distilled water from each spout of the bottles through a small hole on the chamber's wall. In the recording session, the recording cables from the electrodes were attached to a slip-ring above the chamber. To eliminate the movement artifact, we amplified neuron activity with a miniature operational amplifier, which had field-effect transistor input in a voltage follower configuration, attached to the mating plug of the recording cable, the output of which was fed to the main amplifier. The single or multiple unitary activity from the neuron was monitored on an oscilloscope and recorded on magnetic tape.

Before the recording session, the rats were deprived of water for 16 h. In the recording session, the rats were presented with distilled water and taste stimuli consisting of 0.1, 0.5 and 1.0 M NaCl, 0.1 M sucrose, 5 mM saccharin, 0.1 mM quinine hydrochloride and 0.01 M HCl at each trial in pseudorandom order. A 2.5 kHz cue tone was presented for 2.5 s and then a shutter between the drinking spouts and the small hole in the chamber's wall were opened. Rats were allowed 5 s to access the spout until the shutter closed.

Neuronal activity was counted using a computer-aided data-acquisition and analysis system (CED 1401, Spike2; Cambridge Electronic Design). At the end of the last experimental session, rats were deeply anesthetized with Nembutal and marking lesions in the recording sites were made at the tip of one electrode by passing anodal current (20  $\mu$ A for 20s). Then the rats were perfused through the heart with phosphate-buffered saline and 10% formalin. The brains were removed and immersed in a 30% buffered sucrose solution for 1 week. Coronal sections were made through the electrode tracks and their locations were determined histologically.

### Results and discussion

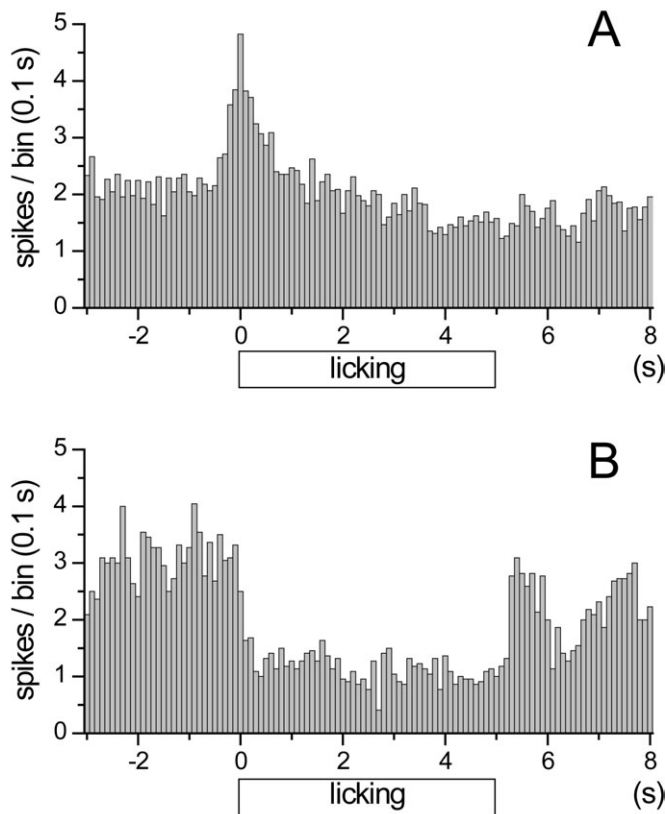
#### Neuron activities in the ventral tegmental area

In the VTA, 24 (38%) of the 64 recorded neurons showed increased firing just before the start of licking. A representative neuron is shown in Figure 1A. The firing of this neuron began to increase just before the start of licking. The firing reached maximal intensity at the start of licking, then gradually decreased to baselines. The firing rates of the other 40 neurons were unchanged before licking. Kosobud *et al.* (1994) have also observed similar increases in firing in four of 13 recorded neurons in the VTA of rats pressing a lever for a sucrose solution. The increased firing rate just before the start of licking may represent a highly motivational state of animals to obtain a fluid reward.

During the period of licking, the firing rates increased in 11 (17%), decreased in 29 (45%) and remained unchanged in 24 (38%) neurons. Figure 1B illustrates an example of a neuron showing decreased firing during the period of licking. Although Kosobud *et al.* (1994) found significant decreases in the firing rate coincident with sucrose consumption, changes in the firing pattern during licking in the present experiment did not depend on the taste presented at each trial. That is, there was no difference in the responsiveness to taste solutions and water. Because the rats were deprived of water for 16 h before the recording session, they drank not only palatable sweet solutions, but also otherwise aversive sour or bitter solutions. Thus, these VTA neurons may be involved in fluid reward regardless of taste. Another possibility is that these neurons may be related to motor coordination in the licking response to solutions.

#### Neuron activities in the nucleus accumbens shell

Although the total number of recorded neurons was small ( $n = 19$ ), we found similar firing patterns in neurons in the nucleus accumbens shell (NAcS) to those in VTA neurons. Three neurons (16%) showed a phasic increase in firing about a half second before the start of licking. The firing rates increased in six (32%), decreased in seven (37%) and remained unchanged in six (32%) neurons coincident with fluid consumption. However, further experiments are currently



**Figure 1** Examples of neuron activities in the VTA during the licking of water and taste solutions. **(A)** Increased firing at the start of licking. **(B)** Firing suppression during the period of licking. PSTHs are aligned at the start of the licking of fluids. Horizontal bars below each PSTH show the period of licking.

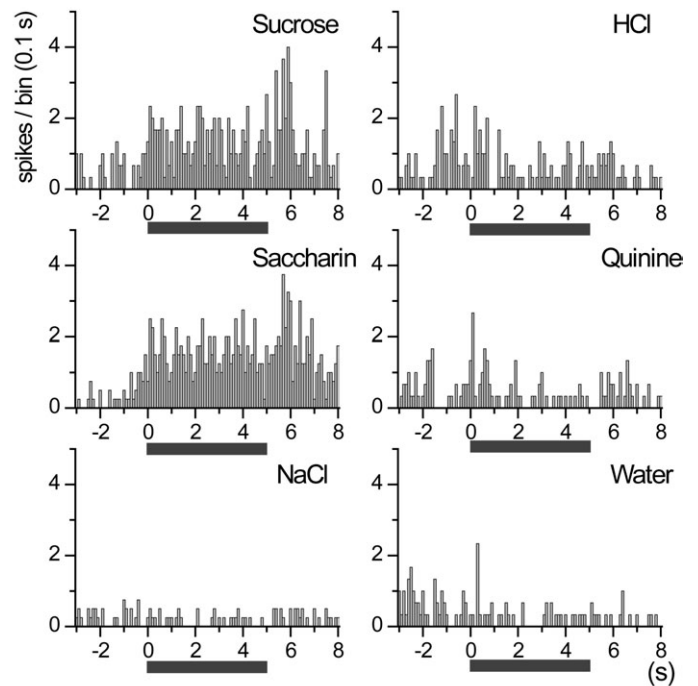
underway to evaluate the responsiveness to taste stimuli of NAcS neurons.

### Neuron activities in the amygdala

Because the amygdala is thought to be one of the limbic regions that influences the reward system, we recorded neuron activities from the central as well as the basolateral nuclei of the amygdala. A small number of units in the amygdala responded to given solutions: six (14%) of 42 neurons in the central nucleus and three (7%) of 46 neurons in the basolateral nucleus (BLA). In particular, three BLA neurons (7%) selectively responded to sucrose and saccharin, suggesting that they are related to the evaluation of hedonic palatability. A representative neuron activity pattern is shown in Figure 2.

### Conclusion

The electrophysiological and lesion results show that the VTA is necessary to motivate animals to enhance the consumption of normally preferred food and fluids. In addition, our recent lesion and pharmacological experiments suggest that the ventral pallidum (VP), one of GABAergic projections from the NAcS, is another candidate for the control of palatability-dependent consumption



**Figure 2** Differential responsiveness to taste stimuli and water in a basolateral amygdalar neuron. This neuron increased its firing rate in response to sucrose and saccharin but not other fluids. PSTHs are aligned at the start of the licking of fluids. Horizontal bars below each PSTH show the period of licking.

(unpublished data). However, the VTA and VP have no direct access to taste pathways. Thus, these structures appear to make use of information about the hedonic value of taste from the amygdala via the NAcS. The mesolimbic system seems to mediate palatability-dependent ingestive responses through its connection to the lateral hypothalamic area.

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