ACTIVITY OF NEURONS IN THE REGION OF THE SUBSTANTIA NIGRA DURING FEEDING IN THE MONKEY

F. MORA*, G. J. MOGENSON** and E. T. ROLLS***

University of Oxford, Department of Experimental Psychology, South Parks Road, Oxford OX1 3UD (Great Britain)

(Accepted January 7th, 1977)

SUMMARY

The activity of single neurons in the region of the substantia nigra of the monkey was recorded during feeding to investigate their function in ingestive behavior. It was observed that some neurons in the substantia nigra and the adjacent tegmentum altered their activity during feeding, in relation to mouth movements. The activity of these neurons was related to mouth movements in that the responses of the units were similar when the monkeys drank fluids with different tastes as long as the same movements were made, in that equally good responses could be obtained when the monkey moved his mouth to non-food objects, and in that in some units opposite responses were obtained when ipsilateral as compared with contralateral mouth movements were made. It was also shown that the responses of these units associated with mouth movements were similar when the monkeys were hungry and when they were satiated. These findings suggest that the activity of some neurons in the region of the substantia nigra is related to the execution of movements which may be involved in feeding, and that the activity of these neurons is not related to the initiation of feeding. Self-stimulation through the recording microelectrodes could be obtained just dorsal to the substantia nigra, but the neural basis of this self-stimulation is not known.

INTRODUCTION

Following the observation of Ungerstedt that aphagia and adipsia are produced by damage to the nigrostriatal dopamine system, there has been great interest in the

* F. Mora was a British Council Fellow.
** G. J. Mogenson was a Visiting Fellow of Wolfson College, Oxford. Present address: University of Western Ontario, Department of Physiology, London, Ont. N6A 5C1, Canada.
*** To whom correspondence should be addressed.
role of the nigrostriatal system in feeding\textsuperscript{4,5,10}. Because the similarities between the syndrome produced by lateral hypothalamic damage and the effects of nigrostriatal damage include aphagia, adipsia and motor dysfunction, it is likely that damage to the nigrostriatal system contributes to the lateral hypothalamic syndrome\textsuperscript{7}. To investigate the role of the nigrostriatal system in ingestive behavior, we decided to make recordings during ingestive behavior from single cells in the region of the substantia nigra, which contains in its pars compacta the dopamine-containing cells which project in the nigrostriatal pathway to the caudate and putamen. The microelectrode recordings were made from neurons in the substantia nigra and the other adjacent subthalamic structures in unanesthetized rhesus monkeys, and the activity of these neurons was studied during the behavior associated with the presentation and ingestion of different foods and fluids. The recordings made from neurons in subthalamic structures (such as the subthalamic nucleus, the tegmentum dorsal to the substantia nigra, and the posterior part of the zona incerta) as the microelectrode was lowered towards the substantia nigra were also of interest in view of their connections with extrapyramidal motor structures\textsuperscript{8,12} and because such recordings would provide information on activity of lateral hypothalamic neurons recorded in the same test situation\textsuperscript{2,11,14,15}.

Although there have been many investigations of the activity of neurons in subthalamic structures in the anesthetized animal to investigate the connections of these structures\textsuperscript{12}, the study reported here is the first we know which describes the activity of these neurons in relation to behavior. Because subthalamic structures have connections with the extrapyramidal motor system, and because damage to the nigrostriatal system is associated with the symptoms which include the akinesia of Parkinson's disease\textsuperscript{6}, the activity of the neurons was studied during different types of motor behavior in the alert monkey as well as during ingestive behavior.

METHODS

Recording method

The activity of single neurons was recorded with glass-insulated tungsten microelectrodes in 3 male rhesus monkeys (\textit{Macaca mulatta}) weighing 2.0–3.5 kg, using methods described elsewhere\textsuperscript{15}. When recordings were being made from a single neuron the oscilloscope was set to trigger on each action potential, so that the waveform could be monitored continuously. Spikes corresponding to each action potential, the firing rate of the unit, and event markers were recorded simultaneously on an ultraviolet polygraph and also analyzed on-line with a PDP-11 computer which computed the mean and S.E.M. of the firing rate in control and experimental periods.

Electrical stimulation of a number of brain sites was used during the recordings to assist in the identification of neurons in the substantia nigra and to provide information on the connections and function of the recorded neurons. Stimulation electrodes were permanently implanted in the head of the caudate nucleus 20 mm anterior to the earbars, 3 mm lateral to the midline, and 21 mm above the earbars, in an attempt to antidromically activate neurons in the substantia nigra, and were also implanted in the orbitofrontal cortex (25A, 8.0, 20 up), nucleus accumbens (18.5A, 2.5, 15 up),
lateral hypothalamus (14A, 2.0, 13 up), amygdala (15A, 9.0, 7 up), tegmentum close to the substantia nigra (6A, 4, 10 up), and locus coeruleus (2P, 2.5, 6 up), using the atlas of Snider and Lee\textsuperscript{16}. The stimulation currents used at these sites with 0.5 msec negative pulses were the lowest which produced self-stimulation, or an observable behavioral reaction such as a movement if no self-stimulation was obtained.

When it was found, as in earlier studies in the anesthetized animal\textsuperscript{8,9} that antidromic activation of neurons in the substantia nigra from the caudate nucleus was difficult to obtain, a pharmacological technique was used to assist the identification of neurons in the substantia nigra. The technique used was the systemic administration of a dopamine receptor agonist, which has been found to decrease the spontaneous firing rates of neurons in the substantia nigra of the anesthetized rat\textsuperscript{1}. However, in the unanesthetized monkey, although apomorphine (0.15–0.3 mg/kg, i.p.) was found to decrease the activity of several neurons in the region of the substantia nigra, the effect could not be dissociated from the sedation which occurred 5–10 min after the injection.

**Analysis of the behavior**

During the recording sessions the monkey sat in a primate chair which provided head restraint by pins attached to the implant. The monkey was fed by the experimenter during an experiment, and at the start of the experiment was approximately 6–12 h food deprived. When food such as a piece of orange, or blackcurrant juice from a syringe, was given to the monkey the following sequence occurred. First the experimenter showed the monkey the food, introducing it rapidly (from behind a screen) into the visual field of the monkey at a distance of 1 m, and then moving it gradually over 5 sec towards the monkey’s mouth. Second, when the food was at a distance of approximately 10 cm from the monkey and if it was a preferred food such as a grape, the monkey reached for the food. Third, as a plate prevented the monkey’s hand from reaching his mouth, the experimenter continued to guide the food towards the monkey’s mouth, which the monkey protruded towards the food when the food was approximately 1 cm away. Fourth, the food was placed in the monkey’s mouth so that the monkey opened his mouth and then touch and taste occurred. Fifth, the monkey swallowed the food. In the first instance, the experimenter determined whether the unit changed its rate in relation to any of these components of the sequence of ingestive behavior.

If a unit responded in relation to one of the components of the sequence of ingestive behavior described above, then the nature of the response was analyzed further. If the unit responded when the monkey reached for food or protruded his mouth towards food, then the activity of the unit was measured when the monkey made the same response towards a non-food object, when the monkey made the same response with the other hand or other side of the mouth, and when the monkey was touched by the experimenter. The response of the units was also measured when the monkey drank fluids with different tastes, to determine whether the response of the units was related to taste, or to movement occurring during ingestion. In this way it was often possible to determine whether the activity of a unit which altered during ingestive behavior was related to the sight or taste of food, or to a movement made to obtain non-food as well as food objects.
To obtain further evidence on whether the activity of the neurons was related to the control of feeding, or to movements made in the course of feeding, in some experiments the monkey was fed to satiety, i.e., until he refused more 20% glucose solution, to determine whether this affected the activity, or the responsiveness, of the units. Electrical stimulation was also sometimes applied through the microelectrode to determine whether feeding or movements were elicited, and to determine whether self-stimulation occurred when the microelectrode was in the vicinity of the analyzed neurons.

Localization of recording sites

The sites where the neurons described in this paper were recorded were located using X-radiographs made on every track and histological localization of lesions made through the recording microelectrodes, as described elsewhere. 5.

RESULTS

Recordings of the activity of 104 neurons in or immediately above the substantia nigra made in 3 rhesus monkeys are described here.

Activation of neurons in the region of the substantia nigra by electrical stimulation of different brain sites

Many neurons in the region of the substantia nigra were transsynaptically activated from the caudate nucleus with latencies of 8–10 msec, and antidromic activation was not observed. The number of occurrences of activation was 78, and the median latency was 10 msec (see e.g., Fig. 1). The transsynaptic (orthodromic) nature of the activation was evident from the variability of the latency, and by the absence of

Fig. 1. A: an example of the transsynaptic activation of a neuron recorded in the region of the substantia nigra produced by electrical stimulation (S) of the head of the caudate nucleus. B: an example of the transsynaptic activation of a unit in the region of the substantia nigra produced by electrical stimulation (S) of a number of different brain sites.
collision. A number of neurons in the region showed a second burst of action potentials with 20–40 msec latency (see Fig. 1). It is possible that the powerful relatively short-latency transsynaptic activation caused the neurons to fire before they could be antidromically activated by relatively slowly conducting nigrostriatal fibers.

Activation of neurons in the region of the substantia nigra was produced by stimulation of a number of other brain sites tested, namely the orbitofrontal cortex (number of occurrences, n = 29, median latency = 15 msec), the nucleus accumbens septi (n = 19, median latency = 50 msec), the lateral hypothalamus (n = 12, median latency = 45 msec), the amygdala (n = 36, median latency = 7 msec) and the midbrain tegmentum (n = 29, median latency = 10 msec) (see e.g., Fig. 4). Activation of these neurons did not appear to be closely related to whether the stimulation produced reward, in that self-stimulation did not occur through some of these stimulation electrodes in some of the animals. For example, there was no self-stimulation from the hypothalamic electrodes, which were 1–2 mm too far posterior, and no self-stimulation from the electrode in the midbrain tegmentum, which was 2 mm posterior to the substantia nigra. It was also noted that there was almost no activation of units in the region of the substantia nigra from good self-stimulation sites near the locus coeruleus.

Activity of units in the region of the substantia nigra during feeding

Of the 104 neurons in the region of the substantia nigra from which recordings were made, 41 showed responses which were related to the feeding behavior of the monkeys. Clear responses often occurred when the monkey opened his mouth and drank fruit juice from a syringe. These responses depended on touch to the mouth or movements made by the monkey, in that they could be obtained just as well, and could often be maximized, by moving the mouth of the monkey with an empty syringe or other non-food object. The maximal response of these units was usually obtained when a syringe was held against the side of the mouth and the monkey moved his mouth laterally to accommodate it, or when the monkey protruded his lips to make contact with the syringe as it approached.

The relationship of the activity of these neurons to feeding was further investigated as follows. First, similar neuronal responses were obtained when solutions with different tastes were ingested, as illustrated in Fig. 2A. Second, the neuronal response obtained depended on the mouth movements made rather than on the solution consumed, so that different effects on the firing rate of the neuron were sometimes obtained depending on whether the monkey drank from a syringe placed in the side of his mouth ipsilateral or contralateral to the recording electrode (see e.g. Fig. 2B). Third, the neuronal responses were not directly related to the motivational state of the animal, in that they occurred whenever the monkeys made a particular movement, irrespective of whether the animal was hungry or satiated. For example, the unit shown in Fig. 2C increased its firing rate to 60–70 spikes/sec from the baseline of 10–20 spikes/sec when a syringe containing 20% glucose was placed in the mouth, and the response did not diminish after drinking 75 ml. The monkey started to become satiated on trial 15, as indicated by failure to swallow the solution and by attempts to avoid having the glucose-containing syringe placed in his mouth.
Fig. 2. A: the firing rate of a neuron in the region of the substantia nigra while the monkey drank different fluids. Similar responses (shown by the mean and the S.E. of the firing rate above the baseline spontaneous rate of 22 spikes/sec) were obtained to 5% glucose, water, 0.9% saline, 0.3% citric acid and blackcurrant juice. B: firing rate of a neuron in the region of the substantia nigra while the monkey drank from a syringe placed in the side of the mouth ipsilateral to or contralateral to the recording microelectrode. The control period with no drinking is shown before and after the drinking to indicate the spontaneous baseline firing rate of the neuron. C: Effect of feeding the monkey to satiety on the responses of a neuron recorded in the region of the substantia nigra. The neuron responded (with an increase of firing rate to 50–80 spikes/sec) when the syringe was placed in the monkey's mouth (the mean and S.E.M. are shown). On each trial the monkey was fed 5 ml of the 25% glucose solution, until by trial 15 satiety, measured by attempted rejection of the syringe by hand and failure to swallow the glucose, started to develop. The unit continued to respond after this when the glucose was placed in the monkey's mouth.

Of the 36 neurons which responded in relation to mouth movements, 26 responded predominantly by increasing their firing rates, and 10 predominantly by decreasing their firing rates, although opposite effects on firing rate were sometimes obtained by movements of opposite sides of the mouth (see e.g., Fig. 2B). The spontaneous firing rates of these neurons were between 0 and 30 spikes/sec. Five more neurons in this region fired both in relation to mouth movements and in relation to arm movements such as reaching. Examples of the sites of units which responded in relation to mouth movements are shown in Fig. 3. Some of the units were in the substantia nigra, and others were within 2–3 mm dorsal to the substantia nigra, in the adjacent tegmentum and in the region of the subthalamic nucleus. The units which were activated by electrical stimulation as described above, but which did not respond in relation
to mouth movements, were found throughout this same region, particularly just above the substantia nigra. The recordings were from cell bodies in this region and not fibers of passage as shown by the size and waveform of the action potentials, and by the injury discharges which were sometimes observed.

**Effects of electrical stimulation in the region of these neurons on behavior**

When electrical stimulation was applied through the recording microelectrode while it was in the region of the neurons described here, when recording was finished, the following effects were obtained. First, mouth movements were elicited by trains of stimulation lasting 2–3 sec (0.5 msec negative pulses of 100–500 $\mu$A at 100 Hz). When the monkey was given the opportunity to work for 0.3 sec trains of this stimulation,
Fig. 4. The current required to obtain self-stimulation through the microelectrode is shown beside the units recorded in the microelectrode track. The graph at the right presents rates of self-stimulation for 2-min periods. The units were activated transsynaptically (S) with the latencies shown (in msec) from stimulation sites in the head of the caudate nucleus (Ca) or in the orbitofrontal (OF) cortex.

good self-stimulation was obtained. To investigate the focus of this self-stimulation, the microelectrode was raised and lowered to different positions so that tests for self-stimulation could be performed at different depths. Good self-stimulation was obtained when the microelectrode was just dorsal to the substantia nigra, but self-stimulation could only be obtained at higher currents or was not obtained at any current at other depths (see Fig. 4).

DISCUSSION

These findings show that some neurons in the region of the substantia nigra in the monkey are active during feeding. Activity in these neurons was associated with mouth movements, such as protrusion of the lips forwards or sideways. The neurons did not show consistent responses during other aspects of feeding, such as the initiation of feeding behavior as soon as food was shown to the animal, or chewing or swallowing. The activity of the units appeared to be related to the execution of the mouth movements described rather than the initiation of feeding, in that different responses were sometimes obtained depending on the side of the mouth from which the animal was fed (see e.g., Fig. 2B). Further evidence for this is that the units responded to different food and non-food objects or fluids similarly if the monkey made the same...
movements to them (see e.g., Fig. 2A), and that the units responded during feeding independently of the degree of hunger of the monkey (see Fig. 2C). Thus present evidence suggests that the activity of neurons in the region of the substantia nigra is related to the execution of mouth movements and not to the control of feeding behavior. If a deficit in feeding behavior occurs after destruction of these neurons, the present results suggest that this is probably a motor and not a motivational deficit. It is of interest that the feeding and drinking deficits which follow lesions of the substantia nigra and nigrostriatal pathway\textsuperscript{17,18} may occur because of a generalized behavioral deficit not related specifically to feeding and drinking\textsuperscript{10,17}.

It is of interest to compare the activity of neurons in the lateral hypothalamus and substantia innominata of the monkey\textsuperscript{2,13–15} with the activity of the neurons in the region of the substantia nigra recorded in the same test situation and described here. Some of the hypothalamic neurons altered their activity as soon as and while the hungry monkey looked at food but not at non-food objects. In contrast, the neurons in the region of the substantia nigra were only activated when the food was close to the monkey’s mouth (within approximately 5 cm) and clear movements towards the syringe were made, or in a smaller population of neurons when arm or mouth movements were made. This comparison suggests that the activity of neurons in the lateral hypothalamus and substantia innominata during feeding is different from the activity of neurons in at least some motor areas, and thus that the hypothalamic neurons may have some different function in feeding\textsuperscript{2,13–15}.

Self-stimulation through the microelectrode was obtained as the microelectrode was lowered and raised through the substantia nigra. The highest rate of responding for brain-stimulation reward was observed when the electrode was just dorsal to the level of the substantia nigra, pars compacta, and responding disappeared abruptly when the electrode was lowered (see Fig. 4). Although this finding is consistent with the hypothesis that dopamine-containing cells in the pars compacta are involved in brain-stimulation reward\textsuperscript{19}, we found that cells in this region have activity related to motor movements, and note that there are neural structures other than dopamine-containing cells in the region where the stimulation was rewarding.

In conclusion, the recordings made from neurons in the region of the substantia nigra of the unanesthetized monkey show that these neurons have activity which is related to the execution of identifiable movements. It will now be of interest to determine the function in the execution of movements of these neurons.

ACKNOWLEDGEMENT

This work was supported by the Medical Research Council.

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