

DOPAMINE ELICITS FEEDING MOTOR PROGRAM IN *LIMAX MAXIMUS*¹

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Received December 20, 1982; Revised March 21, 1983; Accepted March 21, 1983

Abstract

A neural system within the cerebral and buccal ganglia of the terrestrial mollusc *Limax maximus* responds to lip chemostimulation by emitting a feeding motor program (FMP) *in vivo* and *in vitro*. We have analyzed chemically the cerebral and buccal ganglia of *Limax* for neurotransmitters involved in controlling expression of FMP. Dopamine was found in clusters of cells and in the neuropil of the cerebral ganglia at a concentration of 62 pmol/ganglion; a large proportion of such dopamine-containing cells projected to the lips. The buccal ganglia contained several small dopaminergic cells and large amounts of dopamine in the neuropil; the measured concentration was 10 pmol/ganglion. Exogenous dopamine applied to the cerebral and buccal ganglia *in vitro* between 10^{-7} M and 3×10^{-6} M excited an autoactive salivary duct motor neuron (FB) and inhibited an autoactive secretomotor neuron (BSN). Concentrations of dopamine between 3×10^{-6} M and 3×10^{-5} M triggered FMP output, with an increased probability of triggering at higher concentrations of dopamine. ADTN and SK&F38393 were potent agonists in this system, whereas ergonovine was the only potent antagonist found; none of the neuroleptics tested was effective. Thus, the *Limax* system shows agonist responses similar to the vertebrate D1 receptors, but its antagonist-binding properties appear to have requirements quite different from vertebrate receptors.

The effects of exogenous serotonin differed from dopamine's effects; serotonin excited BSN and several buccal motor neurons, could not elicit synchronized motor program cycling, and was not efficiently blocked by ergonovine.

These data suggest that dopamine is a good candidate as an endogenous triggering and sustaining transmitter for the *Limax* feeding motor program.

The neuronal system underlying feeding behavior in the terrestrial mollusc *Limax maximus* exhibits several forms of plasticity, including proprioceptive modulation (Reingold and Gelperin, 1980), inhibition by satiety (Gelperin, 1983), and associative conditioning (Sahley et al., 1981, 1983). Of particular interest, the radically dissected, isolated nervous system retains these properties for several days *in vitro* (Chang and Gelperin, 1980; Culligan and Gelperin, 1983; A. Gelperin and N. Culligan, submitted for publication). To approach a cellular basis of plasticity in *Limax*, we are delineating the groups of

neurons in the cerebral ganglia which receive sensory input and those which produce feeding-control decisions which then cause the buccal motor ganglia to initiate or modulate the feeding response. A central part of this analysis is to examine the neurotransmitters which may be used to modulate feeding behavior and the interactions of these neurotransmitters with target neurons. The monoamines serotonin and dopamine are among the major neuroactive substances in molluscs, and serotonin has already been shown in several molluscs to have a powerful modulatory effect on both the neuronal feeding network and the musculature involved in feeding (Gillette and Davis, 1977; Granzow and Kater, 1977; McCrohan and Benjamin, 1980; Gelperin, 1981; Kupferman and Weiss, 1981).

Earlier studies have detected catecholamines and serotonin in the *Limax* nervous system, including the cerebral and buccal ganglia, which are the primary sensory and motor ganglia controlling feeding behavior (Osborne and Cottrell, 1971; Wieland and Gelperin, 1982). In this paper we present evidence suggesting a major role for

¹ This work was supported by National Science Foundation Grant BNS 8005822 and National Institutes of Health Grant NSMN 15698 to A. G., and National Institutes of Health Training Grants 5T32 MH15799 at Princeton University and NS-07165 at the Marine Biological Laboratory, Woods Hole, Massachusetts to S. W. We wish to thank Dr. Frank Margolis of the Roche Institute for the use of his equipment for monoamine quantitations.

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dopamine in controlling the feeding motor system of *Limax*.

Materials and Methods

Specimens of *L. maximus* were maintained in laboratory culture on modified Purina Rat Chow with a light-dark cycle of 14:10 with lights on at 20:00 hr, at a temperature of 17°C. Animals used for both biochemistry and physiology were from 5 to 10 gm in weight. Individuals were cooled at 0°C for 30 min before dissection, which was carried out in *Limax* saline at 0 to 4°C. Saline composition was: 55.4 mM Na⁺, 4.2 mM K⁺, 7.0 mM Ca⁺², 4.6 mM Mg⁺², 80.1 mM Cl⁻, 0.2 mM H₂PO₄⁻, 2.5 mM HCO₃⁻, 5 mM glucose, pH 7.6. For feeding-response physiology, the isolated lip-cerebral ganglia-buccal ganglia preparation was used, as in Chang and Gelperin (1980); for measurement of monoamine contents and synthesis, only the cerebral and buccal ganglia were retained.

Determination of dopamine and serotonin. Oxidizable monoamines were determined by modification of the methods described by Mefford (1981). Isolated ganglia were homogenized in glass with 0.3 ml of 0.1 N HClO₄ in water, or in acetone containing 15% v/v 1 N formic acid, containing 100 pmol of deoxyepinephrine as an internal standard. The samples were centrifuged at 5000 rpm for 5 min, and the supernatants were stored at -20°C until assayed; recoveries averaged 80%. Samples were chromatographed on a reverse-phase C18 column (Waters) with the following solvent system: 5% methanol, 30 mg/liter of sodium 1-octanesulfonate, 0.1 mM EDTA, 0.8 M phosphate, pH 2.8; flow rate was 1.5 ml/min. Oxidation of eluted samples at +0.72 V was monitored with a Bioanalytical Systems electrochemical detector.

Metabolism of [³H]tyrosine. The production of radioactive metabolites from [³H]tyrosine was tested using the methods of Hildebrand et al (1971). Isolated ganglia were incubated at 12 to 15°C for up to 25 hr in the presence of 10 μCi/ml of [³H]tyrosine (specific activity 30 Ci/mmol) in *Limax* saline. They were then rinsed three times in nonradioactive *Limax* saline and homogenized in 0.2 ml of acetone containing 15% vol/vol of 1 N formic acid. Samples were spun at 5000 rpm for 5 min in a microcentrifuge; the supernatants were dried, redissolved in 10 μl of electrophoresis buffer (0.47 M formic acid, 1.4 M acetic acid, pH 1.9), and spotted on Whatman 3MM paper. The samples were subjected to 5000 V for approximately 1.5 hr in a Savant electrophoresis tank and dried, and the markers were visualized with diazotized sulfanilic acid spray. The paper was cut into 1-cm strips, acidified with 0.3 ml of 0.01 N HCl, and counted in 5 ml of Aqua-Sol (New England Nuclear), or Budget-Solve (Research Products International).

Histofluorescence. Isolated cerebral and buccal ganglia from *Limax* were frozen in powdered dry ice, sectioned 20 μm thick at -30°C in a cryostat, and processed with the glyoxylic acid method of de la Torre (1980). Fluorescence was visualized with epi-illumination from a 200-watt mercury-vapor lamp through a Zeiss BG 12 filter and viewed through a 470-nm cut-off filter.

Physiology. The isolated lip-cerebral ganglia-buccal

ganglia preparation was pinned out in a modification of the method of Gelperin et al. (1978). Three chambers were molded in a single piece of Sylgard: two flanking chambers, one for each half-lip, and the central chamber for the cerebral and buccal ganglia (Fig. 1); each chamber volume was 0.2 ml. Three lip nerves from each half-lip passed through Vaseline-sealed slits to the cerebral ganglia. Three or four polyvinyl chloride suction electrodes monitored the neural output of the buccal ganglia. Flow through the chambers was provided by three separate channels of a peristaltic pump (Rainin Rabbit) at 0.5 to 1.0 ml/min. Temperature was maintained at 15°C ± 0.5°C by Peltier refrigeration.

Drugs were applied for 2 min under constant flowing conditions; the inflow line to the ganglia passed through a temperature equilibration loop to eliminate temperature steps during drug additions. Trials were separated by at least 30 min; lip stimulation-induced feeding motor program (FMP) responses (Gelperin et al., 1978) were periodically tested between drug trials to monitor the stability of the preparation. The order of testing of drug concentrations did not affect response properties.

Chemicals. Drug stock solutions were made fresh at 1 mM or 10 mM concentrations in 1 mg/ml of ascorbic acid. Dilutions were made in *Limax* saline within 2 min of use; pH measurements of the saline showed no change due to drug additions over the range used. Dopamine-HCl, serotonin creatinine sulfate, chlorpromazine, ergo-

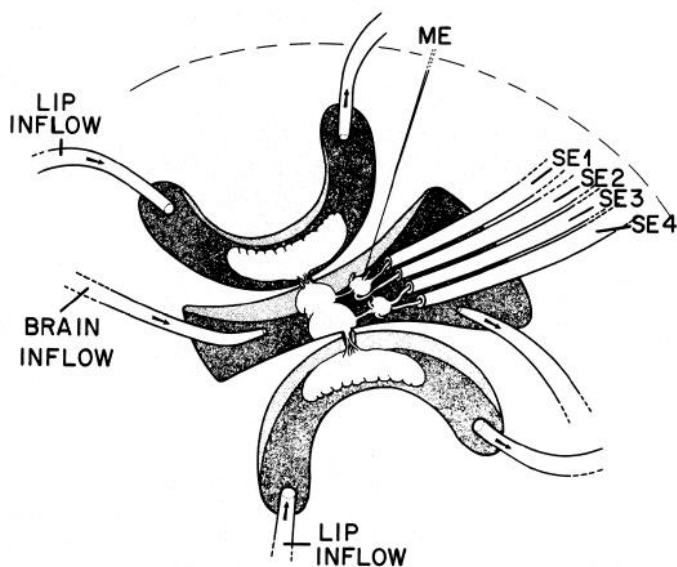


Figure 1. Schematic diagram of isolated nervous system in *Limax maximus*. Cerebral ganglia, buccal ganglia, and the lip chemosensory regions were left attached to one another via nerves and were dissected free of all other body structures. The two lip halves were placed in separate chambers in a multi-chamber unit molded in Sylgard, which allowed independent perfusion with food chemostimuli; the slits between the lip chambers and the ganglia were sealed with Vaseline. The ganglia themselves could be efficiently superfused with drugs, transmitters, or transmitter precursors. Extracellular suction electrodes (SE1, SE2, SE3, SE4) were attached to several motor nerves which exit from the buccal ganglia, and intracellular microelectrodes (ME) could be placed in either cerebral or buccal neurons.

novine, ergonovine maleate, and glyoxylic acid were purchased from Sigma Chemical Co. (St. Louis, MO). 2-Amino-6, 7-dihydroxy-1, 2, 3, 4-tetrahydronaphthalene (ADTN), (+)-butaclamol, and apomorphine were purchased from Research Biochemicals Inc. (Wayland, MA). The following drugs were gifts: fluphenazine, Schering Corp. (Bloomfield, NJ); haloperidol, McNeil Pharmaceutical (Spring House, PA); lysuride, Schering AG (Berlin); LY141865, Lilly Research Laboratories (Indianapolis, IN); phentolamine, Ciba Pharmaceutical Corp. (Summit, NJ); propranolol, Ayerst Laboratories (New York, NY); RU24213 and RU24926, Roussel Uclaf (Romainville, France); spiperone, Janssen R & D, Inc. (New Brunswick, NJ); (-)-sulpiride, Ravizza, S.P.A., (Milan, Italy); SK&F82526 and SK&F38393, Smith, Kline and French Laboratories (Philadelphia, PA); and bromocriptine, Sandoz Pharmaceutical (East Hanover, NJ).

Results

Presence of dopamine and serotonin in cerebral and buccal ganglia. To quantitate the catecholamine and serotonin content of ganglia controlling feeding behavior in *Limax*, we acid-extracted the cerebral and buccal ganglia and chromatographed the extracts using high performance liquid chromatography with electrochemical detection (HPLC-EC). This method will differentiate and detect the various catecholamines and serotonin, as these compounds are easily oxidized (Mefford, 1981). Figure 2 illustrates that ganglion extracts of *Limax* yielded two easily separated oxidizable components which co-chromatographed with dopamine and serotonin. No norepinephrine, epinephrine, or other metabolites of dopamine were detected. The ganglia contained large amounts of the two monoamines: 352 ± 166 (SD, $n = 4$) pmol of serotonin and 124 ± 33 (SD, $n = 4$) pmol of dopamine were found in the pair of cerebral ganglia (average wet weight, 4.1 mg). The paired buccal ganglia (average wet weight, 1.1 mg) contained 29.5 ± 17.3 (SD, $n = 4$) pmol of serotonin and 19.4 ± 2.4 (SD, $n = 4$) pmol of dopamine. Four other determinations without internal standards corroborated these values. From the standard deviations there appeared to be a much larger interanimal variance in serotonin values than in dopamine values. This was probably not due to variance in the recovery of serotonin, because cerebral and buccal ganglion serotonin values varied in parallel in samples from the same animal; a low buccal ganglion serotonin value predicted a low cerebral ganglion serotonin value.

As an independent confirmation of the presence of a dopaminergic system, we tested the ability of the ganglia to synthesize products from [^3H]tyrosine (Fig. 3). Both the cerebral and buccal ganglia produced a ^3H component which co-electrophoresed with authentic dopamine. Incubation times from 1 to 24 hr were used; longer incubation times produced larger quantities of labeled dopamine. Little evidence for the synthesis of either norepinephrine or octopamine was seen at any time point; however, the presence of either transmitter at a very low level is not ruled out.

Cells and neuropil exhibiting glyoxylic acid-induced fluorescence were found in both the buccal and cerebral

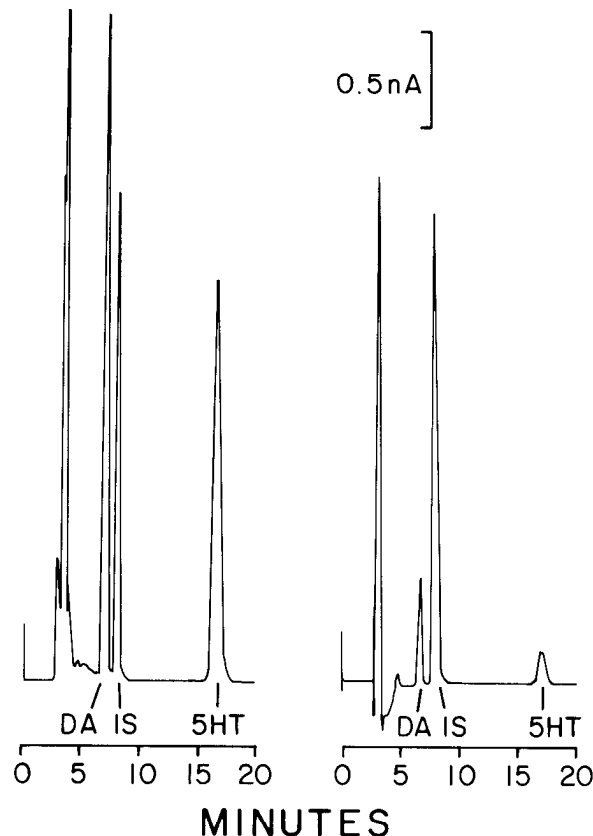


Figure 2. Quantitation of monoamines in *Limax* CNS extracts by HPLC-EC. Samples were extracted and chromatographed as described under "Materials and Methods." *Left*, 50 μl of a 300- μl extract from one pair of cerebral ganglia. *Right*, 50 μl of a 300- μl extract from one pair of buccal ganglia. *DA*, dopamine; *IS*, internal standard deoxyepinephrine; *5HT*, serotonin.

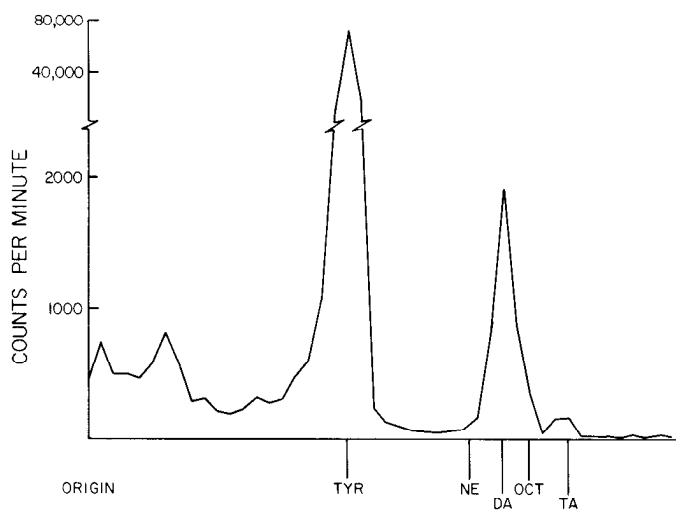


Figure 3. Synthesis of monoamines from [^3H]tyrosine by cerebral ganglia of *Limax*. Cerebral ganglia from a specimen of *L. maximus* were incubated in 10 $\mu\text{Ci/ml}$ of [^3H]tyrosine (3×10^{-7} M) in *Limax* saline for 24 hr at 11°C. The ganglia were then rinsed three times with saline, extracted, and subjected to high voltage paper electrophoresis as described under "Materials and Methods." *TYR*, tyrosine; *NE*, norepinephrine; *DA*, dopamine; *OCT*, octopamine; *TA*, tyramine.

ganglia (Fig. 4). This fluorescence was both heat and glyoxylic acid dependent; virtually all of the intensely fluorescent cells were relatively small, occurred in clusters, and exhibited the green-yellow fluorescence indicative of catecholamines. In Figure 4A, three clusters are apparent in a 20- μm section of the cerebral ganglion (*arrows*). The lowest cluster gave rise to fluorescent fibers which projected out the external lip nerve, and the middle cluster projected out the internal lip nerve; the connections of the upper cluster are not known at this time. The serotonin-containing metacerebral giant cell (Fig. 4A, *arrowhead*) is also visible in this section; the yellow fluorescence of the glyoxylic adduct of serotonin was much weaker than that of dopamine. This may be due to the weaker fluorescent yield of serotonin with glyoxylic acid (Moore, 1981) or to a low level of serotonin in the soma relative to the neurites. However, the yellow serotonin fluorescence was clearly differentiated from the dull red background fluorescence. Figure 4B shows cross-sections through 4 of the approximately 10 small green-yellow fluorescent cells in the buccal ganglion. Thus, in addition to the known distribution of serotonin, dopamine is also integrally associated in both the sensory

and motor ganglia with cells or structures underlying feeding behavior.

Dopamine target cells. Inasmuch as significant amounts of dopamine are present in the sensory and motor ganglia controlling feeding, dopamine might play an important role in the mediation of feeding behavior. We are seeking to identify major effects of dopamine and to identify both dopaminergic and target cells, if possible. As a first step, we examined the effects of exogenous dopamine on the feeding network and compared the dopamine-induced activity to the feeding motor program induced by lip chemostimulation. We also compared both of these effects to those of exogenously applied serotonin.

In an unstimulated preparation, most units projecting out the buccal roots are quiescent, except two burst-firing cells in the salivary nerve. Exogenous dopamine in the concentration range of 10^{-7} M to 3×10^{-6} M produced overt effects on these two motor neurons (Fig. 5). The autoactive fast salivary burster cell (FB), which controls salivary duct contractions (Prior and Gelperin, 1977; Beltz and Gelperin, 1980a, b), was excited. In Figure 5, the interburst interval of the right FB declined from 13 sec to 7 sec during dopamine exposure; the left FB

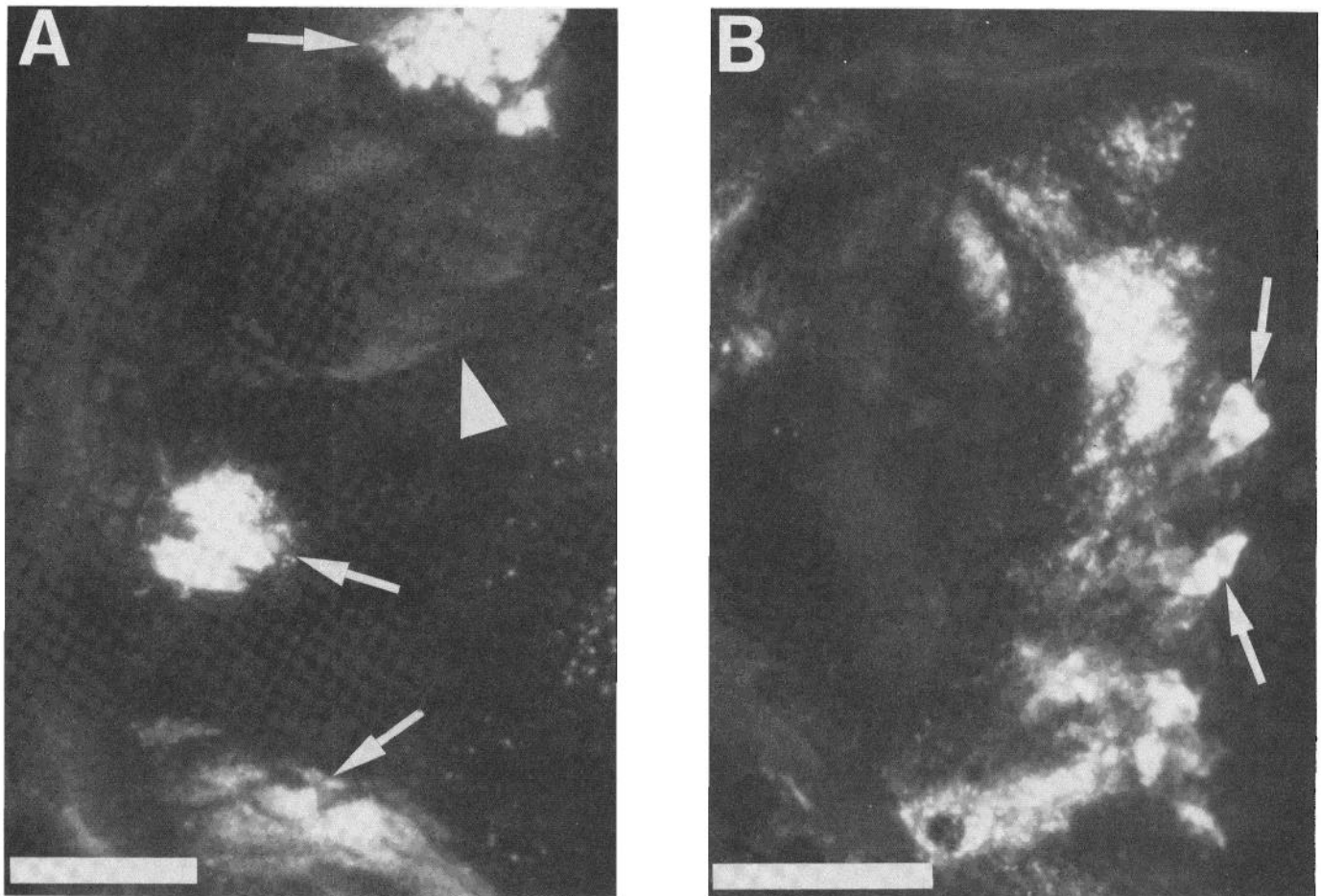


Figure 4. Induced fluorescence of catecholamine and serotonin-containing cells in *Limax*. A is a section through a cerebral ganglion; the other cerebral ganglion is outside the frame to the left of the area shown, and the buccal ganglia are outside the frame, below the area shown. The metacerebral giant cell is the large, dimly fluorescent cell (*arrowhead*); bright green-yellow fluorescent clusters of cells (*arrows*) are shown above and below the metacerebral cell. B is a section through a buccal ganglion illustrating several fluorescent cells (*arrows*) and fluorescent neuropil. Scale bars are 100 μ long.

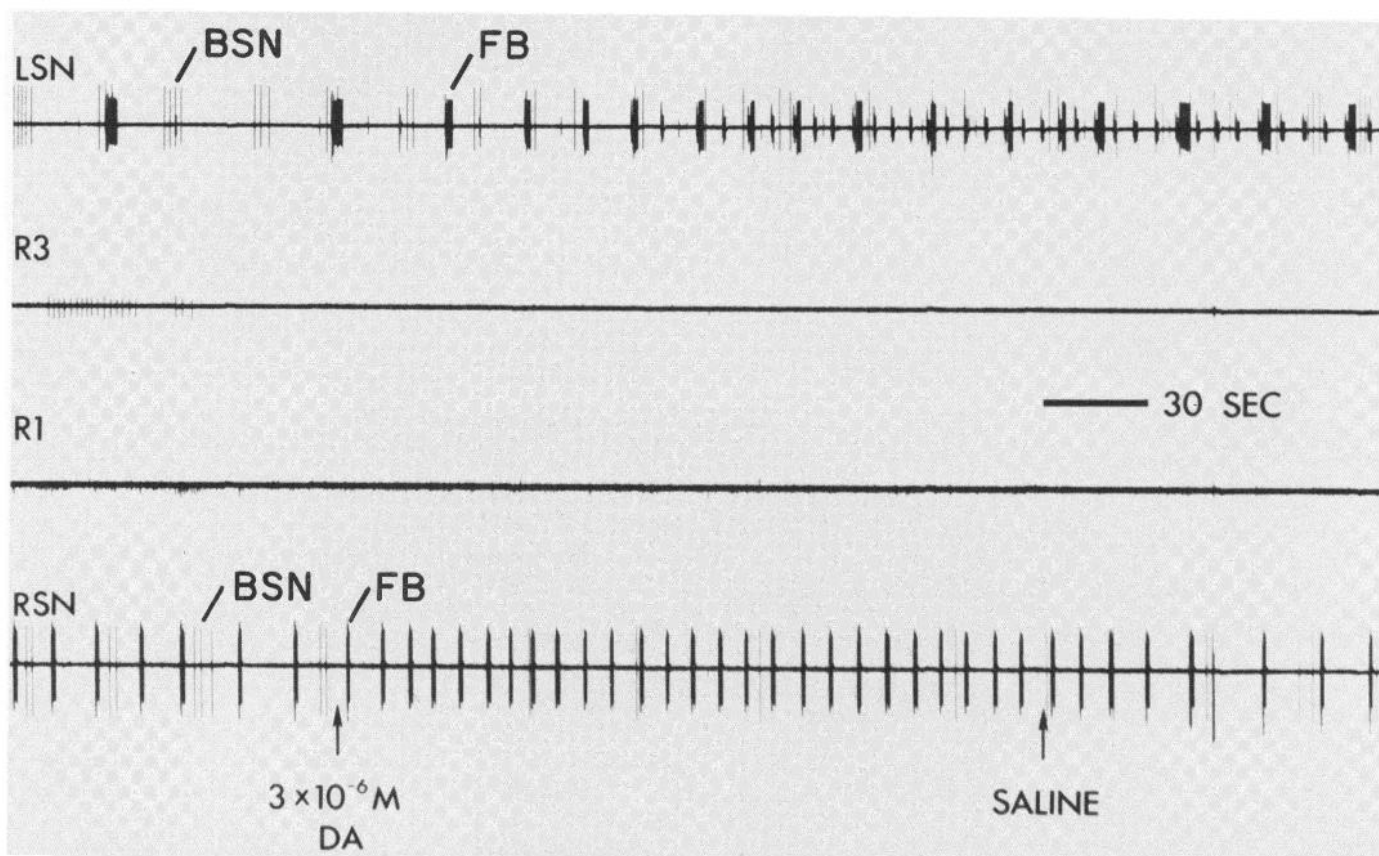


Figure 5. Selective excitation by dopamine of the buccal feeding network in *Limax*. Dopamine-containing saline was substituted for standard saline in a flow-through chamber containing a cerebral ganglion-buccal ganglion system. Extracellular recordings from the left salivary nerve (LSN) and right salivary nerve (RSN) show an increased number of bursts per unit time of the fast salivary burster neuron (FB). Each burst contains several unresolved spikes: 30 to 33 spikes/burst in LFB, 9 to 10 spikes/burst in RFB before dopamine; 22 to 25 spikes/burst in LFB, 7 to 9 spikes/burst in RFB during dopamine perfusion. The single, resolved spikes in LSN and RSN are from the slow salivary bursters (BSN); both the left and right BSN show inhibition of bursting during dopamine perfusion. Extracellular recordings from other buccal motor nerve roots show that no monitored neural activity exhibits excitation. R3, right buccal root 3; R1, right buccal root 1. Flow rate was 500 μ l/min.

interval declined from one burst every 65 sec to one burst every 20 sec in the presence of dopamine. In data pooled from six preparations, the burst frequency of FB increased in a concentration-dependent manner during dopamine exposure up to 10^{-5} M dopamine (Fig. 6). Although the excitatory effect of 10^{-7} M dopamine on FB was significantly above base line, we have not yet tried lower concentrations to determine a threshold. Above 3×10^{-6} M, dopamine sometimes triggered the feeding motor program (see below), which set the FB burst frequency to the cycle rate of the motor program; thus the FB burst rate at 5×10^{-5} M (where feeding was always triggered) in Figure 6 is lower than at 10^{-5} M.

The slow-bursting "bilateral salivary neuron" (BSN) of the buccal ganglion bifurcates and innervates both the ipsilateral and contralateral salivary glands through the salivary nerves (Copeland and Gelperin, 1983). In contrast to FB, BSN showed inhibition by exogenous dopamine (Figure 5). This result was seen in at least 12 experiments in which BSN spikes were resolvable in the salivary nerves, although the data are not yet sufficient to generate a concentration response curve. The left salivary nerve trace in Figure 5 shows that the BSN fired a burst of three to five spikes every 19 to 24 sec in normal

saline. By the last 60 sec of dopamine exposure, five isolated spikes with no clear burst pattern were recorded, indicating clear inhibition and depression of burst firing. Likewise, the record of the right salivary nerve also shows inhibition of the other BSN. From pre-exposure bursts of two to three spikes every 24 to 26 sec, the neuron-firing pattern declined to a single spike during the final 60 sec of dopamine exposure. In other cases, burst firing itself was not abolished, but the interburst intervals were still significantly lengthened (e.g., Fig. 8).

The effects of exogenous dopamine on these two neurons exhibited rapid onset and little desensitization with time. Excitation of the fast burster usually reached maximum in approximately 30 sec and was maintained as long as 20 min with continuous dopamine perfusion. Ascorbic acid alone at 5×10^{-5} M, twice its maximal concentration in any superfusion solution, had no effect after 40 min exposure.

Intracellular monitoring of FB (Fig. 7) during dopamine exposure in normal saline showed far less interburst hyperpolarization than in the absence of dopamine. Individual bursts tended to be more irregular and have fewer spikes during dopamine excitation. In Figure 7, many of the bursts contained only two or three spikes,

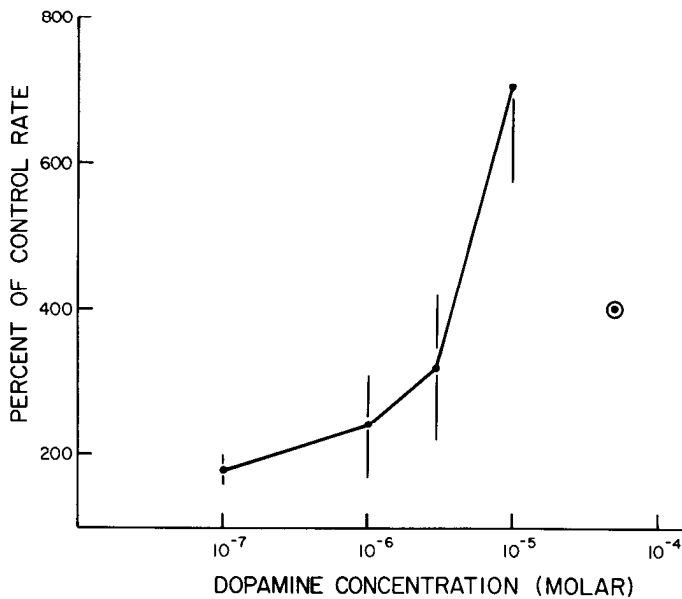


Figure 6. Effect of exogenous dopamine on fast burster neuron activity. Exogenous dopamine was added to isolated nervous systems as in Figure 5. The resulting burst frequency was defined as the number of bursts during the period between 60 and 120 sec after the beginning of the constant infusion of dopamine through the chamber. The control value for each run was the burst rate of the last 60 sec before dopamine infusion; average burst frequency in the absence of dopamine was 4.5 bursts/min. The average burst frequency dropped at dopamine concentrations above 10^{-5} M because the feeding motor program was reliably triggered (see Table I). Synchronization of the network during expression of the feeding motor program resulted in lower burst frequencies at 5×10^{-5} M.

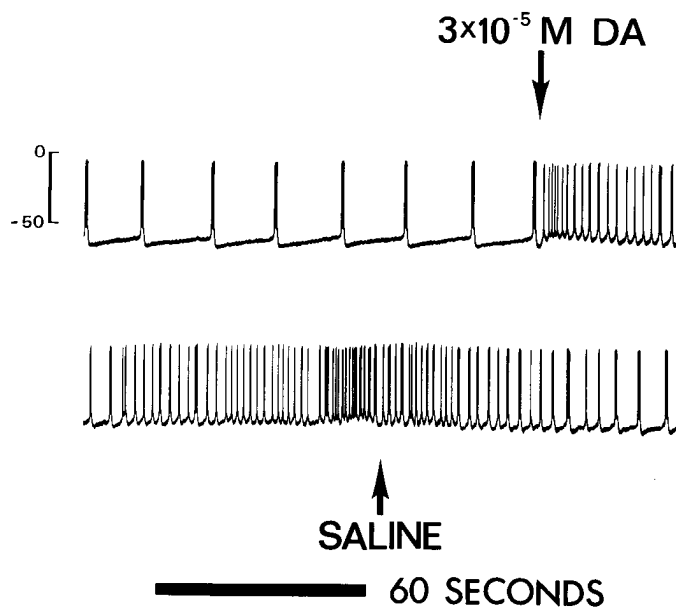


Figure 7. FB response during exposure to dopamine. Continuous intracellular record of FB activity before, during, and after exposure to 3×10^{-5} M dopamine in normal *Limax* saline. FB showed depolarization of the interburst interval and a highly increased rate of bursting during exposure to dopamine. Reversal of excitation was visible starting 30 to 60 sec after normal saline alone began flowing through the chamber.

compared to 11 to 12 spikes per burst before dopamine exposure. However, dopamine increased the average spike frequency of FB from 48 to 140 spikes/min.

Both the excitatory and inhibitory effects persisted in low Ca^{2+} -high Mg^{2+} saline (0.5 mM Ca^{2+} , 18 mM Mg^{2+}) which blocked chemical synapses (Prior and Gelperin, 1977; Prior and Grega, 1982), suggesting that the dopamine effects were directly on FB and BSN or on cells tightly electrically coupled to them (seven trials in three preparations). After 15 min in low calcium saline the autoactive cells had stabilized at new rates; the illustrated test of dopamine sensitivity in low calcium saline (Fig. 8) was run after 45 min. In the extracellular trace (Fig. 8, top), the slow bursts of both the ipsilateral BSN (larger spikes) and the contralateral BSN (smaller spikes) were visible (the contralateral BSN spikes are not detected in all salivary nerves due to their smaller size). Superfused dopamine at 3×10^{-5} M clearly decreased the interburst interval of the FB cell (Fig. 8, bottom) and strongly delayed the bursts of the BSNs (Fig. 8, top). The FB response to dopamine was not as extreme as in normal saline, perhaps due to the changed bursting properties which accompanied exposure to the modified saline.

Dopamine elicits expression of the feeding motor program in vitro. Tests were conducted with the isolated lip-cerebral ganglia-buccal ganglia preparation in order to directly compare the lip-stimulated cyclic, synchronized bursting pattern of the buccal motor units, which has been identified as the feeding motor program (FMP, Gelperin et al., 1978), with dopamine-stimulated outputs. Concentrations of dopamine above 3×10^{-6} M triggered FMP in the isolated preparation (Fig. 9). The dose dependency of the probability of triggering FMP (an all-or-none event for each 2-min trial) was measured in six preparations by scoring for each of several trials whether a given concentration of dopamine elicited FMP. This probability increased smoothly over the concentration range tested (Table I). In all cases, units monitored in the motor roots showed similar phase relations and firing frequencies in dopamine-excited and lip-stimulated feeding. In three cases, dopamine applied to buccal ganglia isolated from the cerebral ganglia also elicited rhythmic motor output similar to FMP, indicating that major targets of dopamine were present in the buccal network itself; however, in the absence of the cerebral connective, direct comparison with lip-stimulated feeding was not possible in these preparations.

Dopamine agonist and antagonist effects. Exogenous ADTN, a rigid analogue of dopamine, was equipotent with dopamine over the range 10^{-7} M to 10^{-5} M (Table II). The compound SK&F38393, a dopamine D1 receptor agonist in vertebrates (Stoof and Keabian, 1981), and an analogue, SK&F82526, were also relatively potent in producing a dopaminergic effect in *Limax*. Lower concentrations (10^{-5} M) excited FB and inhibited BSN, whereas higher concentrations (above 10^{-4} M) triggered the feeding motor program. Apomorphine, bromocriptine, and the vertebrate D2 agonists lysuride (Keabian and Calne, 1979) and RU24213 and RU24926 (Euvrard et al., 1980) were ineffective on FB, BSN, and the *Limax* feeding system.

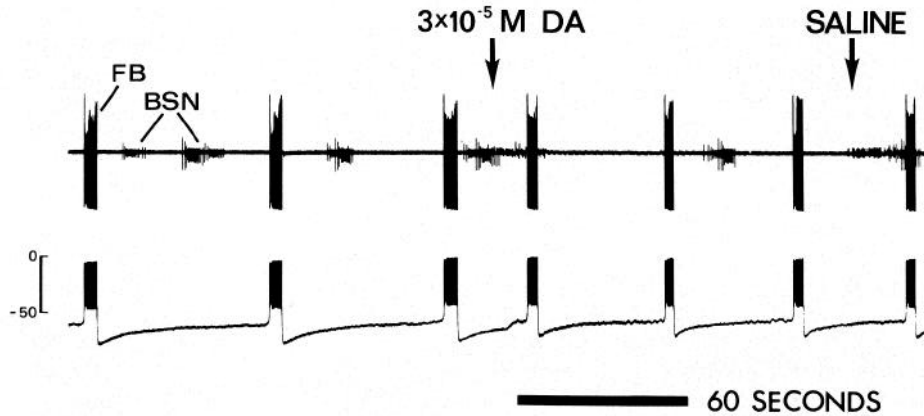


Figure 8. Effect of dopamine in low Ca^{2+} , high Mg^{2+} saline. An intracellular electrode monitored the right FB (lower trace) while an extracellular electrode monitored the activity of the right FB and both BSNs in the right salivary nerve (upper trace) of the same preparation shown in Figure 7. In normal saline, both BSNs had 8 to 10 spikes/burst with a 22- to 30-sec interburst interval. In modified saline (0.5 mM Ca^{2+} , 18 mM Mg^{2+}), the BSNs stabilized at 16 to 20 spikes/burst, with interburst intervals of 30 to 50 sec. FB stabilized at 80 to 85 spikes/burst after 15 min, with a 61- to 65-second interburst interval. FB showed hyperpolarization to -78 mV at the end of each burst. Dopamine perfusion reduced the FB interburst interval to 29 sec for the first, then 40 to 48 sec for the remaining intervals. Both BSNs showed delays of 86 sec after dopamine exposure before their next bursts.

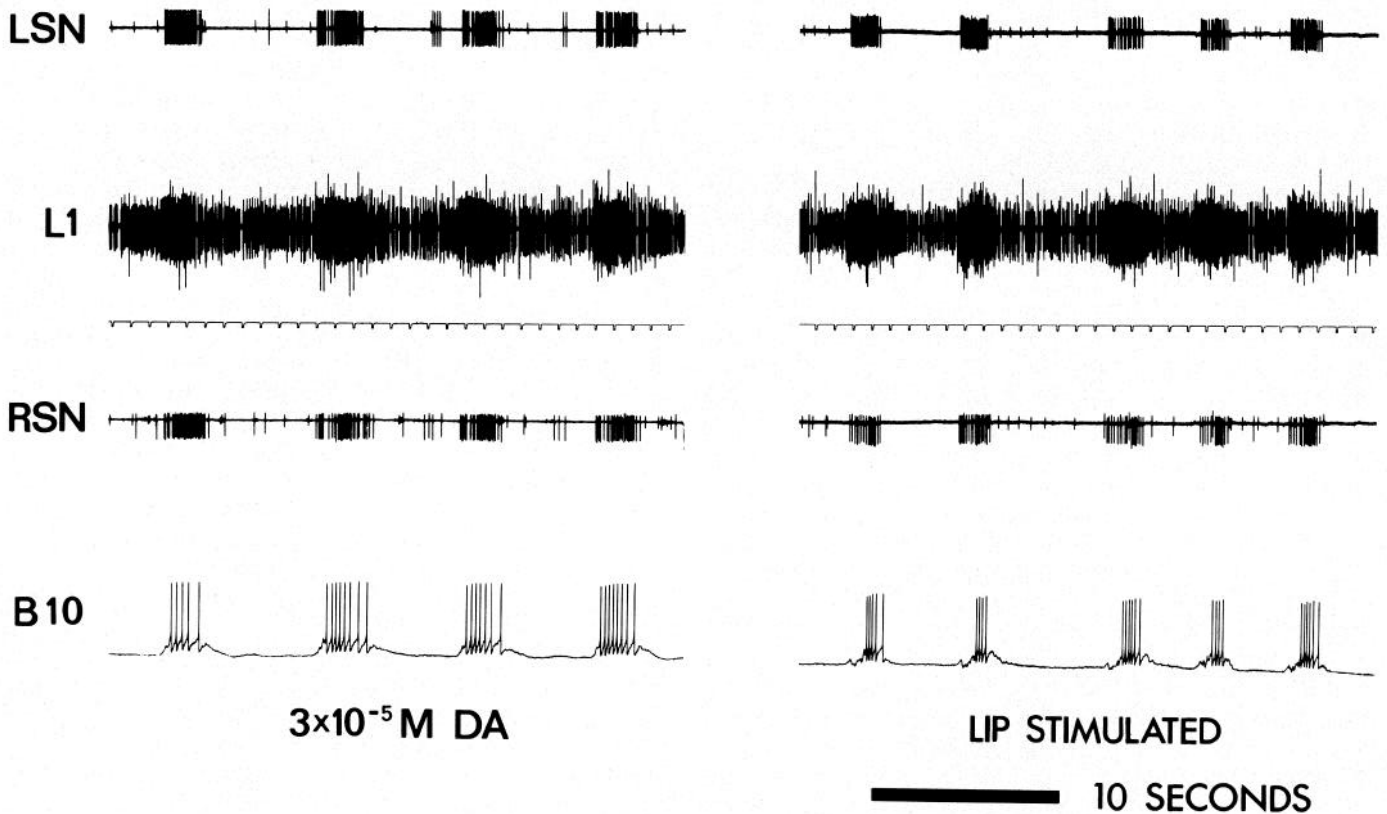


Figure 9. Comparison of cycles of the feeding motor program (FMP) induced by exogenous dopamine and lip-stimulated FMP in the same preparation. Left, Dopamine (3×10^{-5} M) applied to the nervous system triggered a synchronized oscillatory response in the buccal motor roots. An intracellular recording of an identified buccal neuron (B10) showed smooth recruitment into the phase-locked feeding in a similar pattern to that seen in chemostimulus-induced feeding. Right, Lip-stimulated feeding motor program in the same preparation. Both lips were stimulated by 20 sec of diluted apple juice (50% in saline). LSN, left salivary nerve; RSN, right salivary nerve; L1, left buccal root 1.

TABLE I
Induction of FMP by dopamine

Dopamine Concentration	Episodes of FMP/ No. of Trials	Percentage
10^{-6} M	0/7	0
3×10^{-6} M	1/6	17
10^{-5} M	5/15	33
5×10^{-5} M	9/9	100

The ergot alkaloid ergonovine was a potent dopamine antagonist (Table II). At a concentration of 10^{-6} M, ergonovine or ergonovine maleate prevented excitation of FB, inhibition of BSN, or induction of FMP by concentrations of dopamine up to 3×10^{-5} M (15 trials in seven preparations). Serotonin, which excites many neurons in the buccal ganglion (see final section), was more weakly blocked by ergonovine (eight trials in four preparations). The excitation of FB (measured as bursts per minute) produced by 10^{-5} M serotonin was reduced by approximately 50% in 5×10^{-6} M ergonovine, and excitation by 3×10^{-5} M serotonin was unaffected by 3×10^{-6} M ergonovine. These results indicate that dopamine was much more susceptible to blockade by ergonovine than serotonin and that ergonovine did not induce non-specific inexcitability, because serotonin could produce excitation in its presence.

The vertebrate dopamine receptor blocking drugs (+)-butaclamol, haloperidol, spiperone, and fluphenazine, were ineffective at 10^{-5} M in showing any inhibition of the effects of 3×10^{-6} M dopamine. In addition, phentolamine (an α -adrenergic blocker) and propranolol (a β -adrenergic blocker) were also ineffective at 10^{-5} M in inhibiting 3×10^{-6} M dopamine.

Ergonovine blocks expression of the feeding motor program in vitro. Ergonovine was a potent blocking agent of changes in neuronal activity induced by exogenous dopamine, including excitation of FB, inhibition of BSN, and induction of FMP. Therefore, we tested a possible role of dopamine in the expression of FMP in response to lip chemostimulation by food extracts *in vitro* by attempting to inhibit this response with ergonovine (five trials in three preparations). Application of 0.5 to 5×10^{-6} M ergonovine maleate or ergonovine inhibited lip stimulus-induced expression of FMP in each case. Figure 10A illustrates the normal response of an isolated preparation to 15 sec of lip stimulation with a saline extract of potato. The cerebral and buccal ganglia of this same preparation were then exposed to 5×10^{-6} M ergonovine maleate starting 90 sec before lip stimulation (Fig. 10B). The response to potato stimulation was then severely inhibited; two weak "bite" cycles were completed, and even these showed fewer units firing than the nontreated test (Fig. 10A). Lower concentrations of ergonovine produced partial inhibition of the feeding response: at 0.5×10^{-6} M of ergonovine maleate, the number of bite cycles in response to 15 sec of lip stimulation was 25% of the control response.

Both onset and recovery times with ergonovine treatment were slower than with other drugs. With dopamine already present in the bathing medium, added ergonovine took up to 60 sec to block dopamine stimulation. Likewise, complete removal of the blockade took 30 to 60 min

TABLE II
Feeding system response to dopamine drugs

Agonist	Relative Potency ^a	No. of Tests
Dopamine	+++	>60
ADTN	+++	8
SKF38393	++	10
SKF82526	++	3
LY141865	-	4
Apomorphine	-	2
Bromocriptine	-	3
Lysuride	-	3
Antagonist	Relative Potency ^a	No. of Tests
Ergonovine	+++	15
Spiperone	+	14
Sulpiride	-	4
(+)-Butaclamol	-	9
Haloperidol	-	2
Fluphenazine	-	3
Chlorpromazine	-	3
Phentolamine	-	7
Propranolol	-	2

^a Approximate potency: +++, most potent drug (see the text for numbers); ++, 10% as potent; +, 1% as potent; -, ineffective even at high concentration.

for low concentration (10^{-6} M) ergonovine treatments and up to 5 hr for 5×10^{-6} M ergonovine effects to be fully reversed.

Contrast of serotonin and dopamine effects. Since serotonin also exerts excitatory effects in the feeding system (Gelperin, 1981), this monoamine was applied to isolated preparations in the identical manner as dopamine (18 trials in five preparations). Under these conditions, the effects of serotonin could be distinguished from those of dopamine in four ways: (1) serotonin (10^{-7} M to 10^{-5} M) excited BSN as well as FB (Fig. 11), whereas dopamine inhibited BSN and excited FB (Fig. 5); (2) serotonin (10^{-7} M to 10^{-5} M) excited several motor units in other buccal roots (Fig. 11), whereas dopamine did not (Fig. 5) unless FMP was triggered; (3) serotonin at greater than 10^{-5} M elicited broad excitation of multiple buccal motor units, but these exhibited little or none of the synchronization found in the feeding motor program (Gelperin, 1981); (4) serotonin effects were more weakly blocked by ergonovine than dopamine effects (see "Dopamine agonist and antagonist effects").

Discussion

Dopamine and serotonin are major neuroactive chemicals in both invertebrates and vertebrates (Jacobs and Gelperin, 1981). Serotonin plays a role in exciting several invertebrate behaviors, for example, stimulating the flexed posture of the lobster (Livingston et al., 1980), swimming behavior in the leech (Willard, 1981), sensitizing the gill withdrawal reflex of *Aplysia* (Kandel and Schwartz, 1982; Siegelbaum et al., 1982), and intensifying the feeding behavior of several molluscs (Kupfermann and Weiss, 1981; Gelperin, 1981).

The behavioral roles of dopamine are not yet as extensively documented in invertebrates. However, it has been found to be a strong positive modulator of the pyloric

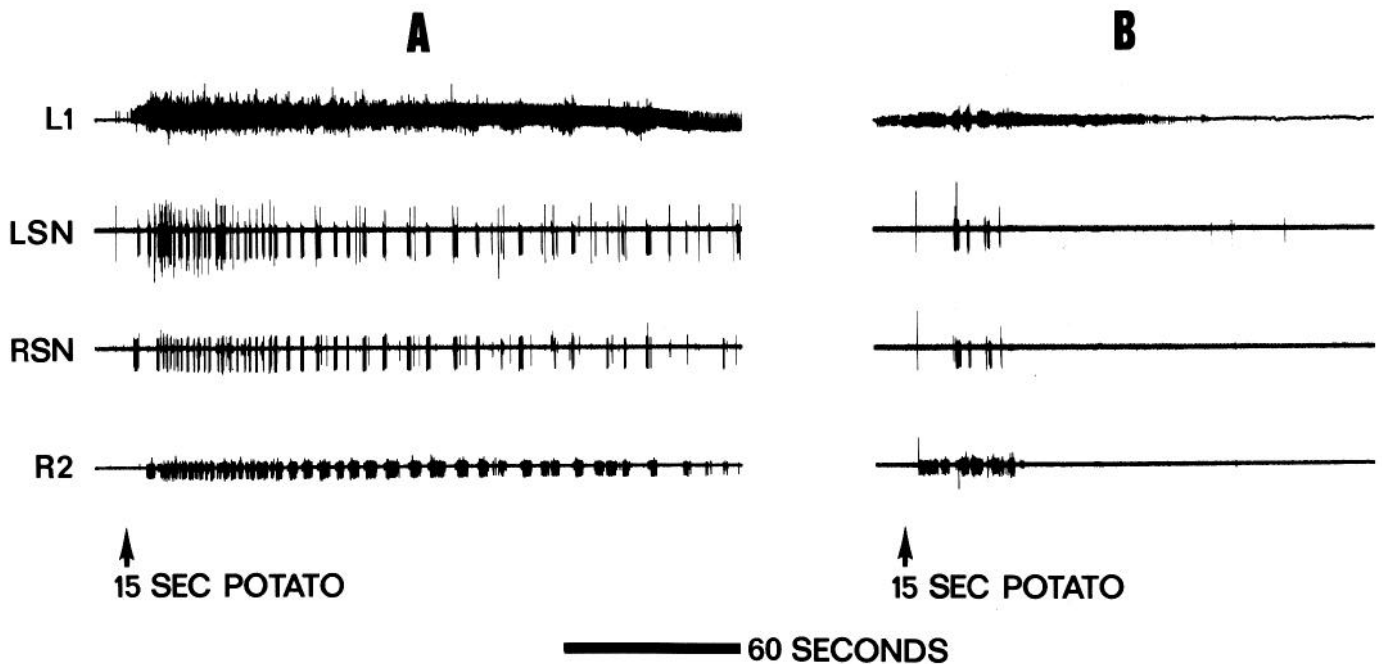


Figure 10. Blockade of feeding response by ergonovine. *A*, A standard lip-cerebral ganglion-buccal ganglion preparation was stimulated by the application of 15 sec of a saline extract of potato to the lips. The entire response of feeding motor program is illustrated. *B*, The cerebral and buccal ganglia of the same preparation as in *A* were exposed to 5×10^{-6} M ergonovine maleate in saline continuously, starting 90 sec before the arrival of a potato stimulus to the lips. The resulting response to the lip stimulus is illustrated. *L1*, left buccal root 1; *LSN*, left salivary nerve; *RSN*, right salivary nerve; *R2*, right buccal root 2.

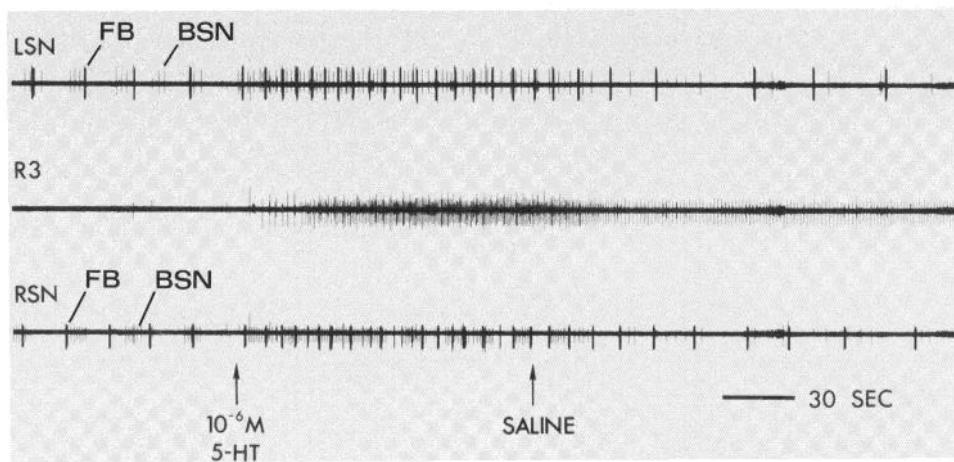


Figure 11. Serotonin excitation of the buccal feeding network in *Limax*. Serotonin-containing saline was substituted for standard saline in a flow-through chamber containing a cerebral ganglion-buccal ganglion system as in Figure 5. Extracellular recordings from buccal motor nerve roots show that all monitored neural roots exhibited excitation. *LSN*, left salivary nerve; *R3*, right buccal root 3; *RSN*, right salivary nerve; *FB*, fast burster neuron; *BSN*, bilateral salivary neuron; *5-HT*, serotonin.

rhythm in the lobster stomatogastric ganglion (Kushner and Maynard, 1977; Barker et al., 1979; Anderson and Barker, 1981). Our data, and those of Trimble and Barker (1982) in *Helisoma*, bring dopamine into consideration for a major modulatory or command role in molluscan feeding behavior. The effect of dopamine on the stomatogastric network and on the buccal network is excitatory and shows little desensitization, in contrast to the desensitization of excitatory and inhibitory effects seen in

single-neuron studies of dopamine effects in *Aplysia* (Ascher, 1972).

Two identified potential target cells for dopamine were found in the buccal ganglion. Extracellular monitoring indicated that the autoactive "bilateral" slow burster neuron (Copeland and Gelperin, 1983) and the salivary fast burster motor neuron (Prior and Gelperin, 1977; Beltz and Gelperin, 1980a, b) were oppositely affected by superfused dopamine: BSN was inhibited, and FB was

excited. The excitation of the autoactive bursting cell FB by dopamine appears to be a novel finding; however, the inhibitory effects of dopamine on BSN may have a parallel in the inhibition of *Aplysia* R15 bursting by dopamine. Dopaminergic inhibition of R15 has in turn been compared to "long duration synaptic inhibition" (Boisson and Gola, 1976; Wilson and Wachtel, 1978; Adams et al., 1980; Gospe and Wilson, 1980). In R15, it has been argued that at least part of the long duration synaptic inhibition as well as dopamine-induced inhibition was mediated by a decrease in slow sodium and/or calcium conductances, whereas a shorter term component of inhibition was mediated by a potassium conductance increase (Wilson and Wachtel, 1978; Adams et al., 1980). A study of the mechanisms of dopamine action on BSN and FB may provide models for the modulation of the rest of the feeding network in *L. maximus*.

There may also be a parallel between dopamine's inhibitory action on BSN and its effect on some hippocampal pyramidal neurons. Dopamine's action on BSN is to shorten bursts and lengthen interburst intervals (Fig. 5). Bernardo and Prince (1982) showed that dopamine augments the postspike hyperpolarization when applied to guinea pig hippocampal pyramidal neurons, probably by interaction with the adenylate cyclase-linked D1 receptor (Iversen, 1975; Seeman, 1980). Since some hippocampal pyramidal neurons normally fire rhythmic bursts of action potentials (Kandel and Spencer, 1961; Wong and Prince, 1978), it seems likely that dopamine application to such bursting hippocampal neurons would also shorten each burst and lengthen the interburst intervals, as it does to BSN.

The role of dopamine in modulating the feeding behavior of *Limax* is not limited to the buccal system; a large amount of dopamine is present in the cerebral ganglia. Fluorescent adducts of dopamine and serotonin reveal an extensive system of cerebral neurons which project to the lips (Osborne and Cottrell, 1971; Wieland and Gelperin, 1982) as well as other cells whose projections have not been mapped. The roles of these cells in feeding behavior have not yet been determined.

In vitro ligand-binding studies of vertebrate tissues have defined at least four forms of dopamine-binding sites (Seeman, 1980). However, only two major categories of vertebrate dopamine receptors have been defined on the basis of response physiology (Kebabian and Calne, 1979; Creese et al., 1983). One apparently stimulates adenylate cyclase (D1), whereas the other (D2) does not stimulate and may even inhibit it. Certain agonists and antagonists can affect D1 model systems and D2 model systems quite differently (Stoof and Kebabian, 1981). Our survey of agonists and antagonists suggests that the *Limax* feeding system responses to various dopamine agonists are similar to those of a vertebrate D1 receptor. However, these responses cannot be blocked by standard vertebrate dopamine antagonists. The only potent antagonist we have found thus far is ergonovine. Ergonovine, also known as ergometrine, has been noted as a dopaminergic antagonist in several molluscs: *Planorbis corneus* (Cottrell et al., 1974), *Helix aspersa* (Walker et al., 1968), and *Aplysia* (Ascher, 1972). One interpretation of these data is that the molluscan dopamine agonist-bind-

ing site is similar to the vertebrate D1 dopamine agonist-binding site but that the antagonist-binding sites of molluscs and vertebrates have dissimilar requirements. This description leaves open the question of whether the agonist- and antagonist-binding sites are truly separate but allosterically linked or are two conformations of the same site. It also suggests that if endogenous antagonists for dopamine receptors exist, they may be different molecules in vertebrates and molluscs.

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