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EFFERENT CONTROL OF STIMULUS ACCESS TO THE HAMSTER VOMERONASAL ORGAN

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SUMMARY

Previous workers had identified the vomeronasal organ, on anatomical evidence, as an accessory olfactory organ, present in most terrestrial vertebrates. Lesion experiments had demonstrated its importance in sexual behaviour in the hamster. However, the sequestered position of the vomeronasal receptor epithelium within the organ raised questions concerning the access of olfactory stimuli. Using electrophysiological and pharmacological methods we have now demonstrated the following.

1. A pumping mechanism exists, powered by vasomotor movements, which can suck stimulus substances into the vomeronasal organ.

2. A mechanism also exists for the active expulsion of the contents of the vomeronasal organ.

3. These mechanisms are activated by fibres running in the nasopalatine nerve.

4. The suction mechanism is controlled by sympathetic, probably adrenergic, fibres from the superior cervical sympathetic ganglion. Control of the expulsion mechanism has not been definitely established but does not appear to be sympathetic.

5. The vomeronasal organ can be adequately stimulated by activation of the pumping mechanism in the presence of odour.

6. Odour responses of single units in the accessory olfactory bulb have been recorded for the first time.

7. The time course of neuronal response in the accessory olfactory bulb is more rapid than predicted by many authors. The response is sufficiently fast that the vomeronasal system cannot be ruled out as a possible sensory pathway in many odour related behaviours.

Possible modes of action of the pumping mechanism in awake animals are discussed.

INTRODUCTION

The vomeronasal organ is an accessory olfactory structure which is well developed in amphibians, reptiles and most mammalian species. The vomeronasal organ, also called Jacobson's organ, has generally been assumed to be chemoreceptive, even by the earliest investigators. This is because its neuro-epithelium and central connexions have a very similar organization to those of the main olfactory system (Negus, 1958). The sensory epithelium is freely exposed to the nasal airflow in some amphibians and reptiles. In other species, notably in snakes and some lizards, it is sequestered in a

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separate cavity which opens into the mouth. In these animals, anatomical, behavioural and electrophysiological evidence suggests that the organs function as chemoreceptors for substances delivered to them by the tongue (Burghardt & Pruitt, 1975; Kubie & Halpern, 1976; M. Meredith & G. M. Burghardt, 1978). In mammals the epithelium is also sequestered within a separate cavity, which may open into the nasal chamber or into the nasopalatine canal which connects nose and mouth (McCotter, 1912; Negus, 1958; Estes, 1972).

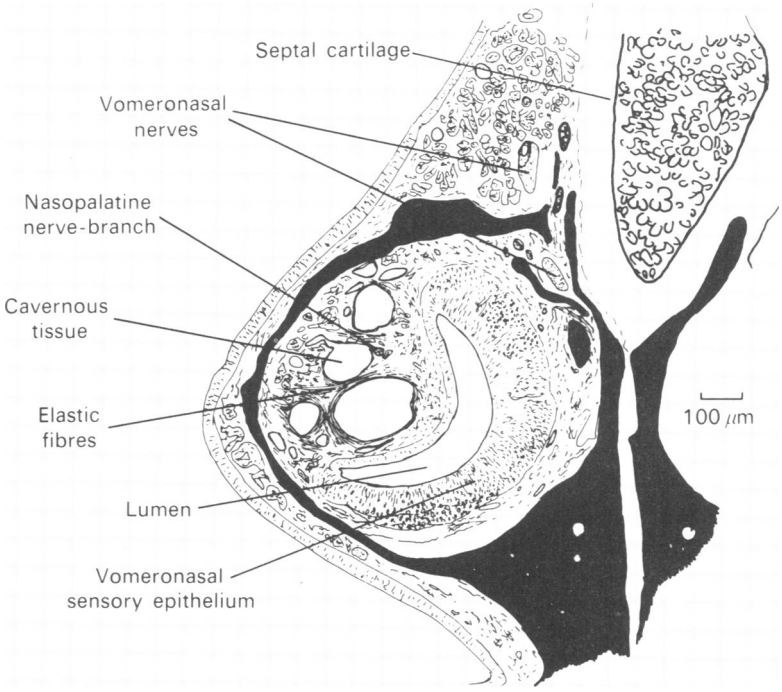

Fig. 1. Camera lucida drawing of a transverse section through the vomeronasal organ and the ventral part of the septum of one side. Bone is shown in solid black. The angle between the top of the vomeronasal capsule and the septal cartilage is filled with the acini and blood vessels of the vomeronasal gland.

The means by which chemical stimuli gain access to the sequestered epithelium in mammals has long posed theoretical questions. The paired organs consist of elongated tubes lying one at each side of the base of the nasal septum. They open only at the anterior end via narrow ducts and contain epithelium anatomically similar to that of the main olfactory organ (except that the receptor cells lack cilia; Kauer, 1968). The vomeronasal organ of each side, together with a longitudinal band of cavernous vascular tissue, is enclosed within a bony capsule (Fig. 1). In those mammals where the vomeronasal ducts open into the nasopalatine canal, it is conceivable that stimulus substances could be forced into the organ by the tongue (for another suggestion, see Estes, 1972). Where vomeronasal ducts open into the nasal cavity as in rodents, however, the question of stimulus access is especially puzzling. Diffusion through the narrow entrance duct (less than 50 μm wide and longer than 100 μm in the hamster) would necessarily be slow. This consideration has led to suggestions that the organ is

only sensitive to long-term changes in the odour environment (Negus, 1958) or that it is concerned with sensing the chemical composition of its own internal secretions (Kölliker, 1877). Several authors have suggested that there might be an auxiliary mechanism for transporting external stimulus molecules to the receptors within the lumen of the organ. Herzfield (1888) suggested that pressure changes in the vascular tissue surrounding the organ might compress the lumen. Hamlin (1929) investigated this hypothesis by modulating systemic blood pressure in the rabbit. He observed liquid and air emerging from the vomeronasal duct at high systemic blood pressures and fluid re-entering the duct as blood pressure fell.

In this paper we report experiments in the hamster which document the existence of a mechanism for drawing materials through the vomeronasal duct into the lumen of the vomeronasal organ. This mechanism appears to operate by local changes in vascular resistance which alter the degree of engorgement of the vomeronasal cavernous tissue rather than by large changes in systemic blood pressure. The changes in vascular resistance are under the control of fibres in the nasopalatine nerve which can be activated by electrical stimulation. In addition we show evidence for a dual innervation of the vomeronasal organ which can independently produce vasoconstriction, with a resulting inflow of fluid at the duct, and vasodilatation with a resulting outflow of fluid at the duct. The former is controlled by sympathetic, probably adrenergic, fibres from the superior sympathetic ganglion, whereas the latter is probably controlled by parasympathetic fibres. Finally we show that single units in the accessory olfactory bulb of anaesthetized animals can be influenced by odours when odour delivery to the vomeronasal pore is coupled with activation of the nasopalatine nerve. The time course of neuronal response in the accessory olfactory bulb to odours delivered in this way is faster than would be predicted in the absence of a pumping mechanism.

In the hamster, input from the vomeronasal organ has been directly implicated in the control of male sexual behaviour by the work of Powers & Winans (1975) and Winans & Powers (1977). These authors found serious deficits in the mounting behaviour of males whose vomeronasal nerves had been cut but whose main olfactory system was intact. Evidence has also been accumulating that vomeronasal organ/accessory olfactory bulb projections reach central nervous system regions known to be important in social and sexual behaviour (for review see Scalia & Winans, 1976; for projections in the hamster, see Davies, Macrides, Youngs, Schneider & Rosene, 1978).

METHODS

An ideal monitor of the vomeronasal pumping mechanism would incorporate direct measurements of both volume and velocity of fluid flow in the vomeronasal duct. Although this fluid movement is quite dramatic when viewed with the microscope (40 ×), its volume is small and its flow lacks sufficient power to allow any direct measurement without interfering with the flow itself. Therefore an indirect measurement was devised. This relies on detecting the movements of the vomeronasal organ side wall which accompany the pumping action. A small window (about $\frac{3}{4}$ mm across) cut in the bony capsule of the vomeronasal organ has no observable effect on the fluid flow but does allow a restricted region of the side wall to contract and dilate in a way which can be measured. When the nasopalatine nerve is stimulated electrically, the side wall of the organ which overlies the cavernous tissue is seen to retract into the capsule. The time course and duration of these movements are directly related to the time course of the fluid movement

observed simultaneously at the vomeronasal organ pore; that is, inflow at the pore is accompanied by retraction of the side wall and outflow by expansion of the side wall.

The side wall movement can be amplified with a lightweight expanded polystyrene lever and detected by arranging that the lever half-shadow a photo-transistor (Fig. 2). When the lever moves, the light reaching the phototransistor and the electrical output of the attached circuit increase or decrease accordingly. The system used in these experiments was sufficiently sensitive to record reliably a movement of about 5 μm.

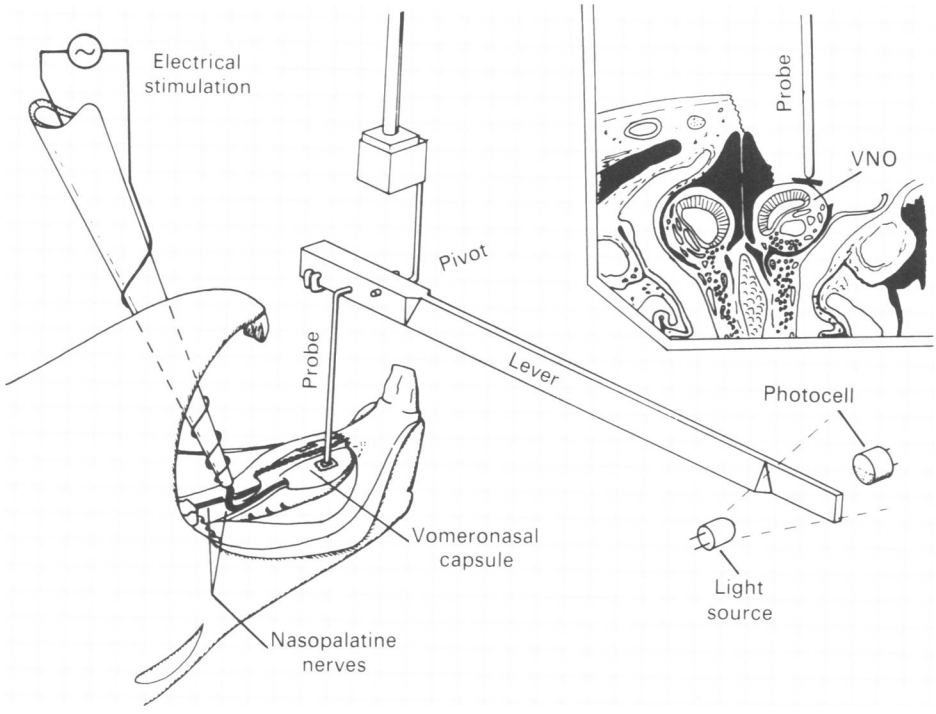

Fig. 2. Method of recording vomeronasal organ operation. The nasopalatine nerve is stimulated with a suction electrode. Movements are transmitted to the lever by a probe which rests on the vomeronasal organ side wall. The inset shows the ventral portion of the upper jaw in transverse section (dorsal down). The probe rests on a base-plate, cut from the bone removed in exposing the vomeronasal organ side wall.

Male hamsters obtained from Charles River-Lakeview (strain Lak-LVG (SYR)) and weighing between 95 and 150 g were initially anaesthetized with sodium pentobarbitone 80 mg/kg (I.P.), tracheotomized, and thereafter maintained on 1–2 % halothane. In those animals where adrenaline or atropine was to be used, anaesthesia was maintained with repeated injections of sodium pentobarbitone. Animals were fixed upside down in a modified rat stereotaxic frame (Fig. 2). The lower jaw was held open and the palatal skin dissected away from the hard palate. The bone of the palate was removed on the side to be stimulated, from the level of the first molar to a point halfway between the incisor and the anterior end of the palatal foramen. The vomeronasal capsule was exposed by easing back the overlying epithelium and a window was cut in the bone. In those cases where direct observation of fluid flow at the pore was desired, the palatal bone was removed up to the base of the incisor. The epithelium was then cut and reflected ventromedially to expose the pore. Observations of fluid flow at the pore are more conveniently made, however, with the animal prone and the incisor and the lateral nasal wall removed (see below).

The nasopalatine nerve was exposed by slitting the ventral epithelium of the nasopharynx and then peeling off the septal epithelium overlying the nerve, which runs along the free edge of the nasal septum dorsal to the nasopharyngeal lumen. The nerve could be stimulated with a

suction electrode (tip i.d. 120 μm) either intact or after cutting. Electrical stimuli consisted of a train of from 10 to 90 biphasic pulses, each pulse 0·05–1·0 msec in duration with interpulse intervals ranging from 5 to 30 msec and constant current settings ranging from 20 to 500 μA DC. The most common stimulus parameters were 10 biphasic pulses of 1 msec duration with a 30 msec interpulse interval and a current of 100 μA. This usually produced a near maximal response of the vomeronasal organ. With monophasic pulses the effective current could sometimes be reduced below 5 μA and still produce a reliable response. When using nasopalatine nerve stimulation to deliver odour substances to the vomeronasal organ (see below), a 2 min recovery time was generally allowed between stimulus trains. When not using odours, a 1 min recovery time was used.

In order to study the role of the sympathetic nervous system in the control of vomeronasal organ function the sympathetic supply was either stimulated or eliminated. Stimulation was accomplished by raising the superior cervical sympathetic ganglion onto hook electrodes. The sympathetic supply to the vomeronasal organ was eliminated by removing the entire superior sympathetic ganglion 6–18 days before the acute experiment.

Further study of the autonomic supply involved local injections of pharmacologically active agents via a cannula (0·63 mm o.d. silastic tubing) in the common carotid artery. The cannula fitted loosely into the lumen of the vessel, allowing some arterial blood flow. When the tubing is inserted into the vessel through a hole made with a 25 gauge hypodermic needle, the muscular-elastic coat of the vessel contracts around it and prevents leakage.

Extracellular micro-electrode recordings of the activity of neurones in the accessory olfactory bulb were made with the animal prone and with the surface of the skull between bregma and the naso-frontal suture horizontal. This position increased the chance of encountering second-order neurones of the accessory olfactory bulb by providing for an optimum electrode path through it. The skin over the dorsal and lateral aspects of the skull from the zygomatic arch forward was reflected laterally and the nasal bones, the incisor and portions of the premaxilla and maxilla were removed. The bony supports of the nasal and maxillary turbinates (which remain after removing the external bones) and of endoturbinates II′ and II″ (Adams & McFarland, 1972) were carefully withdrawn from within their epithelial sheaths with minimum damage and loss of blood. The nasal epithelium was then slit dorsally and reflected laterally to expose the entire dorsoventral extent of the nasal septum from vestibule to nasopharynx. The epithelium overlying the nasopalatine nerve was peeled forward, the nerve freed from the substrate and a suction electrode positioned on it. The septal epithelium was carefully eased away from the dorsal edge of the septum and a second suction electrode lowered between septum and epithelium until it rested on one of the bundles of vomeronasal nerves located deep to the lamina propria. Slight suction was used to hold the intact nerve against the tip of the electrode without undue distortion of its normal position in the tissue. The bone overlying the accessory olfactory bulb was thinned with a dental drill until details of the cerebral vasculature could be observed (the intra-osseous sinuses were plugged with a mixture of bone chips and bone wax). The thinned bone was then removed over the accessory olfactory bulb just anterior to the lateral sinus, the dura was cut and glass micro-electrodes of 5–15 MΩ impedance filled with 3 M-NaCl were used to record extracellular neural activity. Second order accessory olfactory bulb units were identified using electrical stimulation of the intact vomeronasal nerve and the response of these units was then recorded during odour stimulation of the vomeronasal organ. Odours from a flow dilution olfactometer were presented at the vomeronasal pore and delivered to the vomeronasal organ through activation of the vomeronasal pump by nasopalatine nerve stimulation. A continuous flow of odourized air was passed over the vomeronasal pore during each odour test. Control records were taken in the absence of nasopalatine nerve stimulation. A second type of control record involved activation of the vomeronasal pump while pure air flowed over the vomeronasal pore at the same flow rate used for odour stimulation.

RESULTS

When the nasopalatine nerve was stimulated with a short train of pulses, fluid moved through the vomeronasal duct and the side wall of the organ contracted with the typical time course shown in Fig. 3*A*. There was an initial slight contraction and rapid dilatation. This was followed by a prolonged contraction reaching a peak

3–4 sec after the stimulus, then a slow relaxation back to baseline level. (The latency to the beginning of the response typically was 200 msec for a measured conduction distance of approximately 4 mm.) The size of the initial contraction and rapid dilatation were dependent on the base-line level, which fluctuated slightly over time and could be influenced by various experimental operations (see below). In general the time course of the movements had the appearance of a rapid dilatation superimposed on a slower shorter latency contraction. The amplitude of the main contraction varied with the strength of stimulation, i.e. number of pulses, pulse duration and current level. However, its time course was relatively constant with changes in these

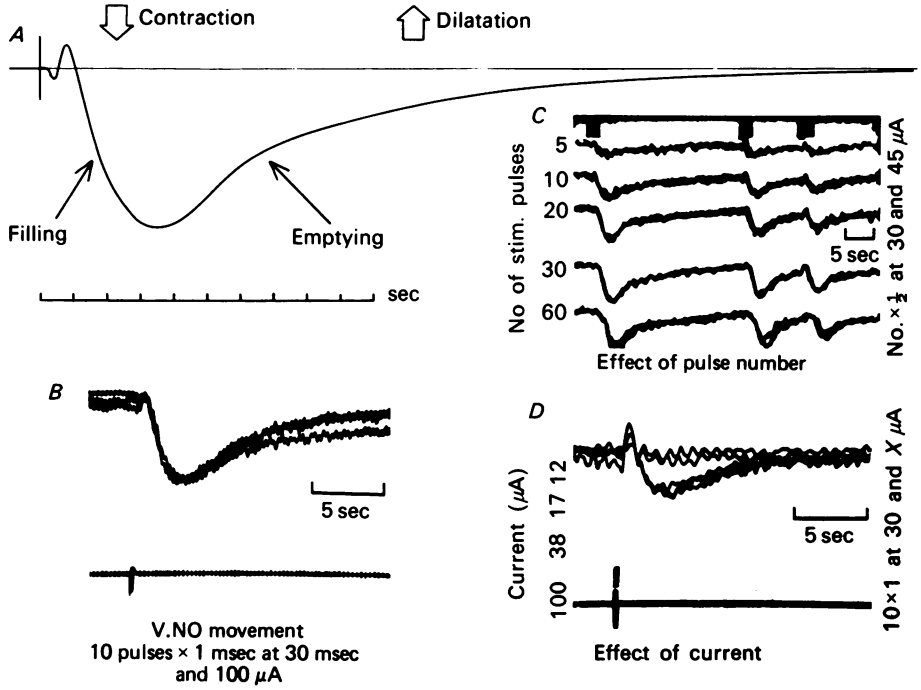

Fig. 3. Time course of vomeronasal organ movements. *A*, typical time course; the trace moves in a downward direction as the vomeronasal organ side wall contracts into the capsule and fluid moves into the vomeronasal organ pore. Preceding the major contraction is the upward trace movement and peak of the early rapid dilatation. Time base shown below: 1 sec divisions. *B*, three traces photographed from the oscilloscope screen showing vomeronasal organ movement. The time of the stimulus train is shown in the lower set of traces. Stimulus parameters are given below: 10 pulses, each 1 msec duration, at 30 msec intervals and 100 μA current. In later Figures the stimulus parameters are abbreviated; e.g. 10 × 1 at 30 and 100 μA. *C*, response to three stimulus trains (timing shown in the top trace) at two inter-train intervals. Five sets of traces showing the constant time course of response with varying numbers of pulses in each train (two traces superimposed in each set). The longest trains (top trace) overlap the beginning of movement and appear to suppress the early dilatation (bottom trace). *D*, four sets of traces (2 at 100 μA) showing constant time course with varying current. Increased current produced increased amplitude up to about 50 μA. The lowest two traces (almost superimposed) were produced by 100 μA current; the three successively smaller responses by 38, 17 and 12 μA respectively. Oscillations in the trace were partly due to respiration movements and partly due to resonance in the recording system.

parameters (Fig. 3*C*, *D*) and from animal to animal (compare Fig. 3*B*, *C*, *D*, which were recorded in different animals). Under the conditions of these experiments (i.e. deep halothane anaesthesia) the response to nasopalatine stimulation was the same with the nerve intact as it was with the nerve cut central to the point of stimulation.

Electrical stimulation of the sympathetic system and sympathectomy were used to investigate the contribution of that system to the nasopalatine nerve fibres controlling vomeronasal organ movement. Stimulation of the superior cervical sympathetic ganglion (Fig. 4*A*, *B*) produced contraction of the vomeronasal organ with inflow at

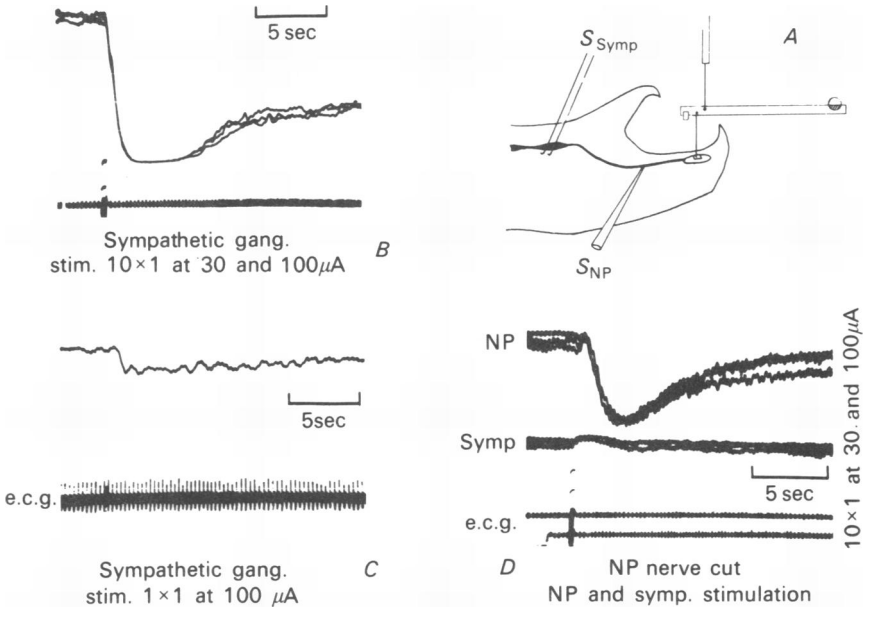

Fig. 4. Sympathetic ganglion stimulation. *A*, diagram of the experiment showing the recording lever (right) and the sites of nasopalatine nerve stimulation (S_{NP}: nerve intact in this case) and sympathetic ganglion stimulation (S_{Symp}). Similar diagrams are used in Figs. 5–8. *B*, Vomeronasal organ response to sympathetic stimulation. Three sets of traces superimposed. The amplitude of the movement caused saturation of the recording system (upper traces). The acceleration of heart rate caused the three superimposed e.c.g. traces which happened to be synchronized at the start of each trace to become desynchronized (lower traces); e.c.g. = electrocardiogram. *C*, response to a single stimulus pulse. Single trace. *D*, after cutting the nasopalatine nerve central to the stimulation site, nasopalatine stimulation gave a normal response. Sympathetic stimulation gave a slow dilatation movement. The lower e.c.g. and stimulus-mark trace shows a single sweep with ganglion stimulation.

the pore, although the time course was somewhat different from that following nasopalatine nerve stimulation. There was no early rapid dilatation and the relaxation following contraction was somewhat slower (Fig. 4*B*, *C*). In many animals, sympathetic stimulation was effective at a lower stimulus strength than the nasopalatine stimulation but it is unclear whether this was a reflexion of the relative efficiency of stimulation at the two sites or was due to the stimulation of pre- versus post-ganglionic fibres.

Sympathetic stimulation would be expected to cause cardiovascular changes elsewhere in addition to the effects produced in the vomeronasal organ. The e.c.g. records in Fig. 4 (lower traces), however, indicate that the vomeronasal organ movement preceded the sympathetic acceleration of the heart, by at least 1 sec (Fig. 4*B*) and that the vomeronasal organ response was possible in the absence of any obvious tachycardia (Fig. 4*C*). The contraction was unchanged when the sympathetic trunk was cut proximal (i.e. posterior) to the stimulated ganglion but it disappeared, to be replaced by a small slow dilatation, when the nasopalatine nerve was cut. This dilatation, produced by sympathetic stimulation with the nasopalatine nerve cut,

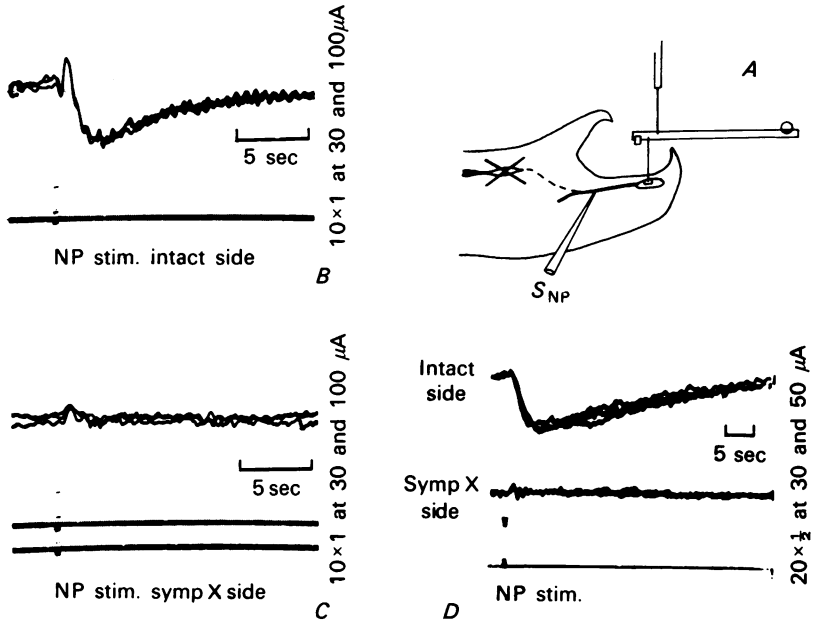

Fig. 5. Unilateral superior cervical sympathectomy. *A*, diagram of the experiment. *B* and *C*, vomeronasal organ response on the intact and sympathectomized (SympX) sides, with stimulation of the nasopalatine nerves on the side ipsilateral to the recording. Three traces in each case. Animal tested 6 days after SympX. *D*, another animal showing the same effect. Animal tested 10 days after SympX.

had a totally different time course from the early dilatation, produced by nasopalatine nerve stimulation. Stimulation of the peripherally connected end of the cut nasopalatine nerve under these conditions still produced a normal response (Fig. 4*D*).

The nasopalatine nerve survives the degeneration of sympathetic fibres following sympathectomy since it contains other nerve processes. After unilateral cervical sympathectomy (Fig. 5*A*), electrical stimulation of the nasopalatine nerve on the intact side produced a normal response (Fig. 5*B*), but stimulation of the nasopalatine nerve on the sympathectomized side produced only a small dilatation (Fig. 5*C*). This small dilatation had a similar time course to the early rapid dilatation of the normal response. It was coincident in time with the rapid dilatation phase seen on the intact side when the nerves were stimulated at anatomically equivalent points. Degeneration times up to at least 18 days (6 days shown in Fig. 5*B*, *C*; 10 days shown in Fig. 5*D*) produced equivalent results.

In order to investigate the chemical sensitivity of the target organs responsible for the vomeronasal organ movement, various pharmacological agents were injected into the common carotid artery (Fig. 6*A*). Fig. 6*C* shows the contraction produced by 10 μg adrenaline HCl (0·1 ml.), which was flushed in with 0·05 ml. physiological saline. The time course of the contraction was considerably longer than with nasopalatine or sympathetic stimulation and it was impossible to say whether an early dilatation occurred because of the artifactual dilatation produced by the injection itself. Nasopalatine nerve stimulation produced normal responses before (Fig. 6*B*) and

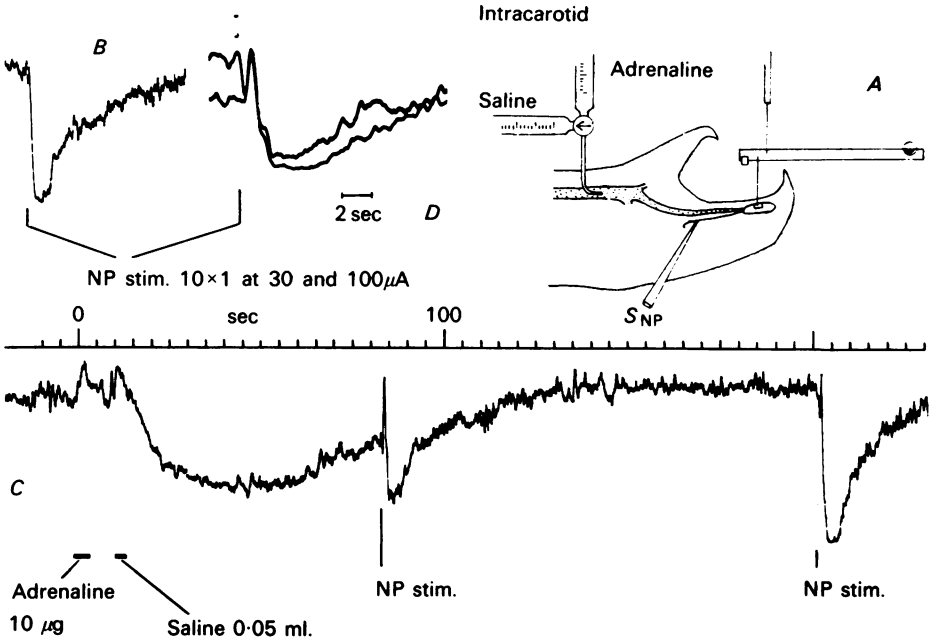

Fig. 6. Adrenaline injection. *A*, adrenaline in the intracarotid cannula can be flushed into the artery with saline from a second syringe. *B* and *C*, pen recorder traces showing the response to nasopalatine stimulation before (*B*), during and after the adrenaline driven contraction (*C*). Time base shown above *C*: 5 sec divisions. *D*, superimposed traces of response to nasopalatine stimulation before and during the adrenaline contraction (expanded time base).

after adrenaline injection (Fig. 6*C*) but the early dilatation appeared to be enhanced if elicited during the adrenaline-driven contraction (Fig. 6*C*). This was apparently because early dilatation tended to return the vomeronasal side wall to a constant position, independently of the state of contraction existing at the time of stimulation. This is more clearly seen in Fig. 6*D* where the oscilloscope was triggered by the stimulator synchronization signal. Here, nasopalatine nerve stimulation before and during an adrenaline contraction produced traces which exactly coincided at the peak of the early dilatation. When the nasopalatine nerve was stimulated repetitively, such that responses occurred during contractions elicited by previous stimuli, the early dilatations were also enhanced (Fig. 7*A*). Thus the side wall position at the peak of the dilatation was independent of the level of contraction at the time of stimulation

in this animal. In other animals the peak dilatation level with repetitive stimuli was relatively constant but of a lower level than that seen for the first response (Fig. 7*C*).

The apparent independence of the dilatation and contraction elicited by nasopalatine nerve stimulation was supported by the results of other experiments. Sympathetic ganglion stimulation did not produce dilatation even with repeated stimuli at high current levels (Fig. 7*C*, lower) or during an ongoing adrenaline driven contraction (Fig. 7*D*). Chronic sympathectomy on the other hand eliminated the

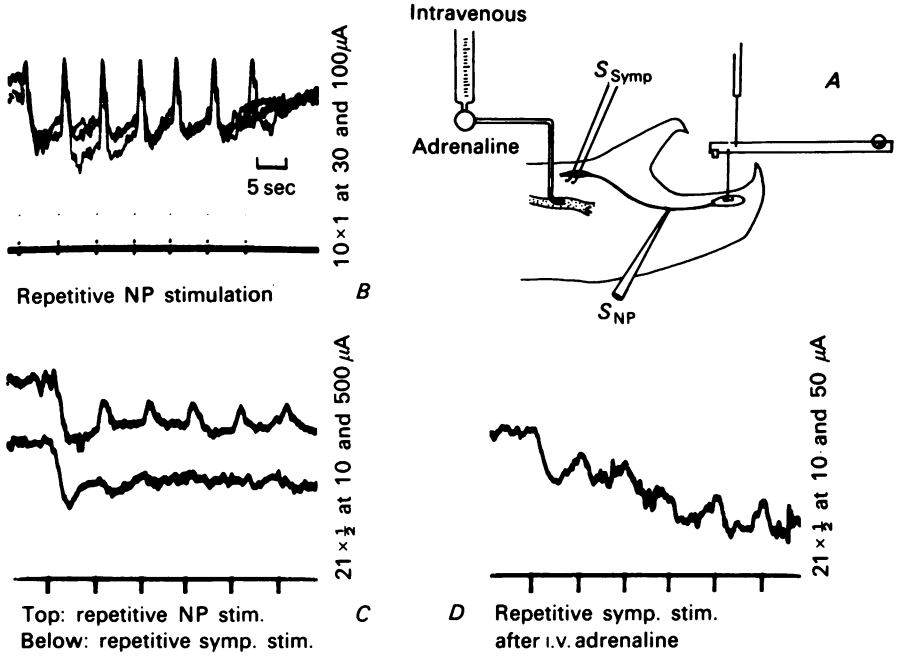

Fig. 7. Independence of contraction and dilatation. *A*, the adrenaline used for Fig. 7*D* was injected into the jugular vein. *B*, repeated nasopalatine stimulation: three traces. The stimulus train timing is shown below. *C*, repeated nasopalatine stimulation (top) and sympathetic ganglion stimulation (below) at high current. Same time base as *B*. Early rapid dilatation movements follow each stimulus train with nasopalatine stimulation. *D*, repetitive sympathetic stimulation after injecting 50 μl. adrenaline (1:1000) IV. Same time base as *B*. The upward movements of the trace preceding the second and subsequent stimulus marks are due to relaxation following the preceding stimulus.

contraction but not the dilatation produced by nasopalatine nerve stimulation (Fig. 5*C*). Furthermore, the current required to elicit a reliable dilatation was in some preparations more and in others less than for a reliable contraction. It should be pointed out that this apparent independence of the dilatation movements applies only to the early rapid dilatation. The slow dilatation which follows the major contraction appears to be a passive relaxation, rather than an active dilatation.

In an attempt to identify the source of the fibres driving active dilatation, atropine sulphate was injected intracarotidly and/or intraperitoneally. In two cases (one I.P., one intracarotid), animals were given repeated injections of atropine resulting in an accumulated dose of 1·2 mg (8–9 mg/kg). In all cases where atropine was injected,

changes in the form of the response were no greater than those produced by an equal volume injection of Ringer solution. There was no evidence for a decrease in the rapid dilatation movements following atropine injection whether or not the dilatation movements were emphasized by first injecting adrenaline or using repetitive stimulation.

Vomeronasal pump used to deliver stimuli. The inflow and outflow of fluids through the vomeronasal duct produced by nasopalatine nerve stimulation could be used to deliver stimulus substances to the receptor neurones within the vomeronasal organ while recording electrophysiological responses from the accessory olfactory bulb. Our experiments in this direction are not complete but are reported here to outline the functional significance of the suction mechanism.

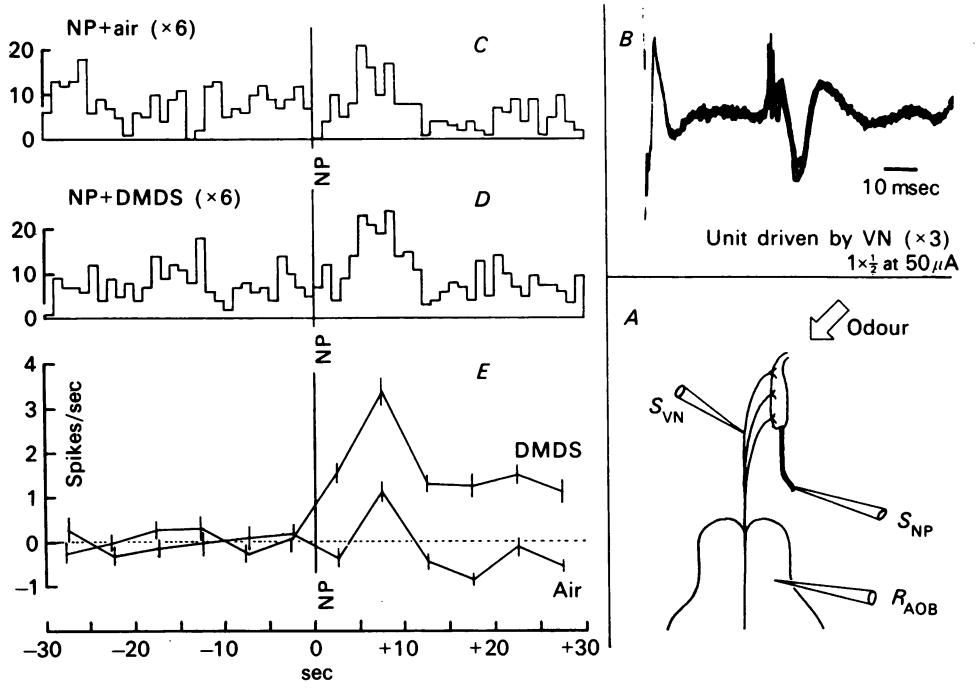

Fig. 8. *A*, odour or air is blown continuously over the vomeronasal pore. *B*, a unit recorded in the accessory olfactory bulb (R_{AOB}) can be driven by electrical stimulation of the vomeronasal nerve (S_{VN}). *C* and *D*, peristimulus-time histograms for six repetitions of the nasopalatine nerve stimulation (S_{NP}) during continuous pure air flow (*C*) or continuous flow of dimethyl disulphide (DMDS) at 10^{-2} of vapour saturation at 22 °C (*D*). 1 sec time bins. Nasopalatine nerve stimulation occurred at time 0, indicated by the vertical line labelled NP. *E*, data from *C* and *D* replotted onto the same axes. Firing rate expressed as the difference from the average rate during the prestimulus 30 sec, for each 5 sec period. Short vertical bars show 2 S.E. (centred on each of the points).

When odourized air was simply blown over the vomeronasal pore, no response was apparent in the accessory olfactory bulb. However when the nasopalatine nerve was stimulated during continuous odour delivery (Fig. 8*A*), odourized mucus and/or air was sucked into the lumen of the organ and a reliable response could be demonstrated in the activity of some single neurones in the accessory bulb. Fig. 8*B* shows a unit in the accessory olfactory bulb which was driven by electrical stimulation of the

intact vomeronasal nerve (Fig. 8*A*; S_{VN}). The relatively constant latency and the position of the spike on the field potential establish this as an accessory bulb unit and suggest that it was driven monosynaptically by vomeronasal input (unit of second order). The activity of this cell before and after nasopalatine nerve stimulation (S_{NP}) is shown in Fig. 8 during continuous delivery of pure air (Fig. 8*C* and *E*) or of odourized air (Fig. 8*D* and *E*) to the vomeronasal pore. There was a significant increase in firing rate following nasopalatine nerve stimulation in both conditions ($P < 0{\cdot}01$; Kolmogorov Smirnov test, Siegel, 1956) but in the superimposed plot of Fig. 8*E*, the increase when odourized air was used is clearly greater than that when pure air was used ($P < 0{\cdot}025$; Wilcoxon test, Siegel, 1956). The odour used in this case was dimethyl disulphide (DMDS), a component of hamster vaginal discharge which is highly attractive to male hamsters (Singer, Agosta, O'Connell, Pfaffmann, Bowen & Field, 1976) but not to females (R. J. O'Connell, unpublished observation).

Of eight units recorded in two animals, five gave statistically significant responses to one or more odours delivered in this way. A further thirty-two units in fourteen additional animals gave apparent responses but these were not evaluated statistically. Changes from spontaneous activity when they occurred were generally found within the first 5 sec following nasopalatine nerve stimulation. The responses could involve either increased or decreased spike frequency and could be obtained with biologically significant odours (e.g. DMDS) as well as with arbitrarily selected odours (e.g. amyl acetate).

DISCUSSION

This study has demonstrated that there is an active mechanism for the introduction of stimulus materials into the lumen of the hamster vomeronasal organ and that it is under the control of sympathetic, probably adrenergic fibres connected to the superior sympathetic ganglion. The action of this pump in the hamster does not appear to depend on the modulation of systemic blood pressure as had been suggested by Hamlin (1929) for the rabbit.

Since the measurements of the action of the pumping mechanism have been necessarily indirect, the details of the volume and flow rate of the fluid movement itself have not been established. As far as can be judged by simultaneous observation, however, the separate phases of the side wall movement and of the fluid flow correspond exactly in time; that is, there is fluid inflow during the initial contraction and during the major contraction; outflow during the early rapid dilatation and during the final relaxation. The output of the measuring device cannot be considered as a graph of fluid flow versus time, but the constancy of time course of the side wall movement does suggest a similar constancy in the fluid movements. The mechanism responsible for the pumping movements can be inferred from our observations, from the anatomy of the organ (Fig. 1) and from the previous evidence that the sympathetic system controls vasoconstriction in the general nasal mucosa (Blier, 1930; Tucker, 1963; Eccles & Wilson, 1973; Wilson & Yates, 1976). Sympathetic control of vasoconstriction in the nasal mucosa is supported by our observation that stimulation of the sympathetic ganglion or of the nasopalatine nerve causes visible constriction of blood vessels in the nasal mucosa and vomeronasal side wall.

Constriction of arteries supplying the cavernous tissue of the vomeronasal side wall

should cause a local drop in blood pressure, allowing the elastic fibres spanning the cavernous tissue (Hamlin, 1929; see Fig. 1) to pull the lateral wall of the organ away from the medial wall. This of course would increase the volume of the organ and cause suction at the pore. Active dilatation on the other hand would compress the lumen and cause outflow at the pore. Such a mechanism depends on the presence of a rigid capsule (Vomer bone) surrounding the cavernous tissue.

Origin of fibres controlling vomeronasal organ movements. Our experiments using sympathetic stimulation, sympathectomy and adrenaline injection provide evidence for an adrenergic sympathetic control of the suction phase of the vomeronasal organ pumping action. Section of the sympathetic trunk posterior to the stimulated ganglion had no effect on the vomeronasal organ contraction whereas nasopalatine nerve section eliminated it. This suggests that the fibres concerned run directly from the ganglion to the nasopalatine nerve (presumably via the internal carotid, deep petrosal and vidian nerves) and that the systemic effects of sympathetic stimulation are not important in the vomeronasal organ movements observed here. The small slow dilatation movement produced by sympathetic stimulation after nasopalatine section would be expected from the shunting of blood through the denervated vomeronasal organ vessels, when vasoconstriction occurred in other nasal regions having an intact sympathetic supply.

The experiments on the active dilatation phase are more difficult to interpret. Blier (1930) had suggested a sympathetic vasodilator innervation of the nasal mucosa to explain the results of some of his experiments on nasal volume. The failure in the present experiments to demonstrate an early rapid dilatation with sympathetic stimulation, even under the most favourable circumstances (repetitive stimulation or following adrenaline injection) argues against a sympathetic vasodilator contribution to the rapid dilatation. On the other hand the obvious alternative control system, the parasympathetic supply, could not be clearly implicated using atropine. Atropine would be expected to block the cholinergic parasympathetic activity at the post-ganglionic axon terminals, preventing any active dilatation controlled by that system, but in our hands large doses of atropine did not have any reliable effect on the rapid dilatation. The intra-carotid route for injection was chosen to allow substances to be delivered as directly as possible to the target organ, reducing prior dilution in the blood stream and allowing smaller doses to be used. This in turn minimizes the effects on other systems. In the case of atropine, however, large doses were used and systemic effects would be expected, but these doses seemed to have little effect on systems which are known to be atropine sensitive in other animals. The heart rate, for example, was not noticeably increased as would be expected if vagal activity were blocked. Although some post-ganglionic parasympathetic junctions have been shown to be insensitive to atropine (Goodman & Gilman, 1975; Ambache & Zar, 1970), the lack of systemic effects of large doses of atropine by either of two routes of administration suggests a general rather than a specific insensitivity. It is possible that the hamster is one of those animals which possesses circulating atropine-esterase (Goodman & Gilman, 1975) and that this enzyme deactivates the blocking agent before it reaches the target tissue. We have not attempted so far to distinguish between these possibilities.

The vomeronasal organ contraction appears to be produced by the sympathetic

innervation which also controls vasoconstriction elsewhere in the nasal mucosa. The parasympathetic system controls vasodilatation in the general nasal mucosa and it seems reasonable that this system should be responsible for active dilatation in the vomeronasal organ. This has not been clearly demonstrated by the methods used here, although some evidence against the most likely alternative (sympathetic vasodilatation) has been produced.

Time course of vomeronasal organ movements. Although autonomic control of the vomeronasal organ is clearly available, it is not yet possible to say whether the mechanisms demonstrated here are operational in the behaving animal. The temporal relationship of the contraction and active dilatation shown here is presumably a consequence of the electrical stimulation of a multifunction nerve. This relationship is not necessarily maintained if the system is operated by the behaving animal (although, if it were, it would ensure that previous odours were to some extent cleared before each influx). The time course of the contraction, however, may be a property of the smooth muscle target tissue rather than a property of the nerve fibre activation. The constancy of time course with variations in stimulus parameters is consistent with this possibility. The time course seen in the present experiments, with an inflow lasting approximately 3 sec, is considerably faster than had been predicted by some authors (Mihailkovics, 1899; Negus, 1958). Unit activity in the accessory olfactory bulb can also show significant changes from spontaneous activity within the first 5 sec after nasopalatine stimulation. Vomeronasal participation in behaviours mediated by environmental chemicals cannot therefore be ruled out simply on the basis of short latency unless this were less than about 3 sec.

Possible strategies for odour sampling by the vomeronasal organ. If the vomeronasal organ pumping mechanism is indeed functional in the behaving animal, two reasonable alternatives come to mind for the circumstances under which it would be activated. It is unlikely that the autonomic innervation would be under voluntary control, but it could be activated either continuously or reflexly. Continuous sampling of the air flow at each inspiration could be brought about by a modulation of sympathetic and parasympathetic tone during the respiratory cycle, as seen in other parts of the autonomic system (Tucker, 1963).

The afferent limb of a reflex initiation of pumping might be activated by the arrival at the olfactory epithelium of an odour requiring further analysis by the vomeronasal receptors or by a more complex cluster of chemical and non-chemical stimuli.

Unit response in the accessory olfactory bulb. The preliminary single unit recordings reported here provide only limited data on the response properties of the accessory olfactory bulb. They do provide, however, the first evidence for odour sensitivity and the first odour responses in single units in the mammalian accessory olfactory bulb. There was no evidence for specific responses to chemicals which could be regarded as sex attractant pheromones although considerably more work will be required to provide an adequate description of potential coding mechanisms.

Odour sensitivity of the vomeronasal system is well established in turtles where the receptor epithelium is not sequestered in an enclosed organ (Tucker, 1971). Electro-olfactogram (e.o.g.) recordings have been obtained from the mammalian vomeronasal epithelium (Müller, 1971) but Adrian (1955) and Tucker (1963) were unable to detect neural responses to odour in the vomeronasal nerve of the rabbit when odours were

passed over the vomeronasal pore. In the present experiments a very slight change in spontaneous activity could sometimes be detected when odour first appeared in a continuous flow of air over the vomeronasal pore even if the pumping mechanism had not been activated. However, this could only be detected if spike trains were averaged over many repetitions and was considerably smaller even than the response to air when the pumping mechanism was activated (Fig. 8*C*, *E*). The responses of accessory olfactory bulb units during such nasopalatine nerve stimulation without concomitant presentation of odours may reflect some sensitivity of the receptors to the movements associated with the pumping mechanism, or it could have been due to residual odours dissolved in the mucus which was sucked in during these control stimulations.

Where no signal averaging methods were used, no detectable response to odours passed over the vomeronasal pore would be expected. Tucker, using a combination of aqueous odour delivery and pharyngeal occlusion in the rabbit, was able to show some instances of (multi-unit) vomeronasal nerve response to odour (Tucker, 1963, Fig. 18). It is possible that the pharyngeal occlusion triggered a sympathetic response which caused suction in the vomeronasal organ in that particular case. The fact that odour delivery and activation of a pumping mechanism have not been deliberately combined by other authors probably explains their failure to record reliable odour responses in the mammalian accessory-olfactory system.

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