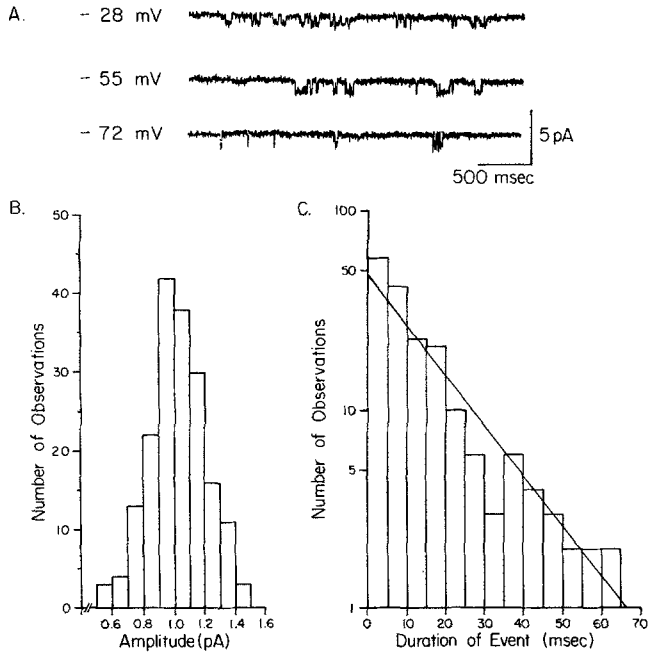

Figure 2. *A* Extracellular patch clamp records obtained from a giant cell in a 3-week-old culture of dissociated rat pancreatic islet. The recording was made in the cell attached configuration. The membrane patch was depolarized by applying the negative command voltages (V_c) indicated to the left of each trace. The depolarizations evoked outwardly directed single channel currents (downward deflections). *B* Histogram of the amplitudes of single channel currents recorded in this patch at $V_c = -55$ mV. Current amplitudes are unimodally distributed with an average value of 1.0 ± 0.83 pA (183 events). *C* Histogram of the duration of 185 single channel currents recorded in this patch at $V_c = -55$ mV. Note logarithmic scale of y-axis. The straight line was fitted to the data using least squares regression (correlation coefficient −0.960) and indicates a mean channel duration of 17.3 msec.

It is at present unclear whether these giant cells result from the fusion of normal islet cells, or represent the amitotic growth of single precursor cells.

Glucose induced release of insulin from adult β-cells in vivo is known to be accompanied by slow wave depolarization and action potential activity in the β-cell membrane[10]. The ionic basis of these events and their relation to insulin release have previously been studied using intracellular recording techniques[11,12]. Studies of this kind may be greatly facilitated if conducted using the giant insulin-containing cells reported above, since stable penetration with low resistance microelectrodes should be possible. Further, the present results show that these giant cells are also amenable to study using the extracellular patch clamp technique. This method allows identification of the types of ionic channel present in islet cell membranes. In addition, the modulation of these channels by secretagogues and by inhibitors of hormone release can also be investigated.

1 Amsterdam, A., and Jamieson, J.D., Proc. natl Acad. Sci. USA *69* (1977) 3028.
2 Maruyama, Y., and Peterson, O.H., Nature *299* (1982) 159.
3 Hellerstrom, C., Lewis, N.C., Borg, H., Johnston, R., and Freinkel, N., Diabetes *28* (1979) 769.
4 Neher, E., in: Techniques in Cellular Physiology, vol.I, part II, p.1. Ed. P.F. Baker. Elsevier, Amsterdam 1982.
5 Horn, R., and Patlak, J., Proc. natl Acad. Sci. USA *77* (1980) 6930.
6 Pearse, A.G.E., and Polak, J.M., Histochem. J. *7* (1975) 179.
7 Orci, L., Like, A.A., Amherdt, M., Blondel, B., Kanazawa, Y., Marliss, E.B., Lambert, A.E., Wollheim, C.G., and Renold, A.E., J. Ultrastruct. Res. *43* (1973) 270.
8 Marty, A., and Neher, E., J. Physiol. *326* (1982) 36P.
9 Colquhoun, D., and Hawkes, A.G., Proc. R. Soc. Lond. *B 211* (1981) 205.
10 Biegelman, P.M., Ribalet, B., and Atwater, I., J. Physiol. (Paris) *73* (1977) 201.
11 Sakamoto, Y., Biomedical Res. *1* suppl. (1980) 135.
12 Wollheim, C.B., and Sharp, G.W.G., Physiol. Rev. *61* (1981) 914.

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Estimation of serotonin and its action on oviducts and uteri of some oviparous and viviparous insects

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Summary. Serotonin was not found in the oviducts of *Blabera gigantea, Clitumnus extradentatus* nor in the uterus of *Glossina,* but it is present in the uterus of *Blabera.* It is also found in the central nervous system of all three insects. In vitro experiments confirm these data by showing that serotonin increases the contractions of the uterus of *Blabera,* but has no effect on the uterus of *Glossina.*

Key words. Central nervous system; insects; oviduct; serotonin; uterus.

In insects, serotonin (5-hydroxytryptamine) has been found in the central nervous system (CNS) (see Evans[1]) and the heart and gut[2–4]. It has been shown to stimulate several visceral organs: the semi-isolated heart[5,6], hindgut preparations[2,7,8] from cockroaches and locusts, and the oviduct from the horsefly *Tabanus sulcifrons* and *proximis*[9].

The purpose of this preliminary study was to investigate the presence of serotonin in the uteri of two viviparous insects, the tsetse fly *Glossina fuscipes* and the cockroach *Blabera gigantea* and to record the effects of this compound on isolated uteri. To establish a comparison, serotonin was also measured in the oviducts of *Blabera* and *Clitumnus extradentatus* (an oviparous insect) and in the central nervous systems of the 3 insects studied.

Methods. The content of serotonin in the tissues was estimated by the method of Reinhard et al.[10] using HPLC with electrochemical detection. The tissues were homogenized in 100 µl 0.1 M $HClO_4$ containing 0.2 mM ascorbic acid. The homogenates were centrifuged for 15 min at 30,000 × g; 20 or 50 µl of the supernatant was then injected into the HPLC. Experiments

Serotonin content of uterus, oviduct and CNS of *Clitumnus, Blabera* and *Glossina* adult females

Species	Uterus				Oviduct			Central nervous system			
	Exp. N	Organ N	Serotonin (pmol)* per organ	mg d.w. (f.w.)	Exp. N	Organ N	Serotonin (pmol)* per organ	Exp. N	Organ N	Serotonin (pmol)* per Organ	mg d.w. (f.w.)
Clitumnus extradentatus					3	10	0	1	2	22,2	49.3 (21.3)
Glossina fuscipes	3	11	0		–	–	–	3	10	0,6	4 (2.7)
Blabera gigantea	3	4	4.3	0.67 (0.14)	3	8	0	1	1	17	11.2 (2.4)

*Mean value; exp., experiment; d.w., dry weight; f.w., fresh weight. Values indicated in brackets cannot be precise since small pieces of fat body and small amounts of saline cannot be discarded exactly in the same way in each case.

were performed using a model 6000 solvent delivery system with a Model U6K sample injector (Waters Assoc.) and a thin-layer electrochemical detector with a glassy-carbon working electrode (Bioanalytical Systems, West Lafayette). The column was uBondapak C-18 (Waters Assoc.) and the mobile phase consisted of degassed 0.1 sodium acetate/acetic acid buffer, pH 4.7, containing 6% methanol and was delivered at a flow rate of 2.0 ml/min.

The contractions of the isolated oviducts and uteri, separated from the nervous system, were recorded using an electrical transducer[11]. The organs were fixed in small cuvettes where they were bathed in the appropriate saline solution to which neurotransmitters were added; each experiment was preceded by a change of the bathing fluid, followed by a 10-min recovery period.

Results. Estimation of serotonin in oviducts, uteri and CNS. The oviducts of *Clitumnus* and *Blabera* do not contain serotonin. As far as the uteri are concerned, that of the tsetse fly, which is very small, does not reveal the presence of this substance whereas in *Blabera* one uterus contains 4.3 pmol of serotonin, a fairly high content when compared to that of the entire CNS (17 pmol). In the stick insect and in *Glossina,* serotonin is also present in the CNS; 22.2 pmol in the former and 0.6 in the latter.

If the amount of serotonin present in the CNS is expressed in pmol per mg fresh or dry weight, it appears that the concentration varies; very high in *Clitumnus,* it is lower in *Blabera* and lower still in *Glossina* (table).

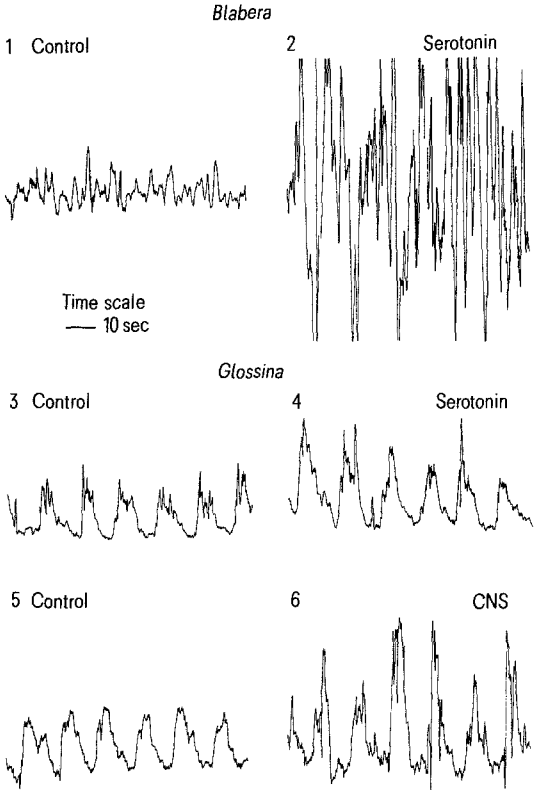
Stimulation of oviduct and uterine contractions in vitro. Serotonin appears to have no effect upon either *Blabera* or *Clitumnus* oviducts. Its effects upon the uteri, at the concentration of 10^{-4} and 10^{-6} M depend on the insect studied. In the tsetse fly, where the muscular sheath of the uterus is very thin, serotonin has no effect, whereas in *Blabera,* whose uterus possesses large muscles, serotonin causes a strong increase of the contractions (fig.).

Comparative assays were performed with other neurotransmitters (acetylcholine, GABA, glutamate), the neuropeptide proctolin and CNS extracts (fig.). No clear-cut effects were observed, except for CNS extracts which stimulate uterine contractions in both *Blabera* and *Glossina.* If the *Blabera* uterus is maintained in connection with the last abdominal ganglion, the contractions follow a distinct pattern, which is abolished by serotonin (10^{-6}, 10^{-4} M) as well as by Gaba (10^{-4} M). Glutamate (10^{-6}, 10^{-5} M), on the other hand, suppresses the contractions completely. All these effects, not observed in denervated organs, probably result from a central effect exerted by the transmitters.

Concluding remarks. Serotonin is present in the CNS of *Blabera, Clitumnus* and *Glossina.* In *Blabera,* the total amount of serotonin contained in the CNS is about 17 pmol. Comparatively, in *Periplaneta,* dopamine and octapamine content seems more abundant (48.8 and 57 pmol, respectively[12]). When serotonin content is calculated per fresh weight unit, it appears that *Clitumnus* contains much more of this neurotransmitter than either of the other species.

Serotonin has not been found either in the oviducts of *Clitumnus* and *Blabera* or in the uterus of *Glossina,* whereas it is present in the uterus of *Blabera.* These results are in accord with the in vitro effects of serotonin. The *Blabera* uterus responds to serotonin by strong contractions, whereas the *Glossina* uterus, as well as the *Clitumnus* and *Blabera* oviducts are not sensitive to this substance. The results with *Glossina* are not surprising since, in vivo, serotonin injections were not able to cause parturition[13].

The role of serotonin in the regulation of visceral muscle functionning is thus observed in some, but not all, species and it seems that this regulation may involve several neurotransmitters and neurohormones. Glutamate was shown to depress the contractions of the uterus of *Blabera* in the same way as it acts on the hyperneural muscle of *Periplaneta*[14]. Proctolin, on the contrary, reveals no effect in our experiments although it has been shown to increase both hindgut[15] and oviduct contractions[9] in some species. Other neurohormones, still chemically unknown, probably intervene since CNS extracts cause an increase of uterine contractions in *Blabera* and in *Glossina.* In the latter, parturition, which involves uterine contractions, was obtained by injections of CNS extracts[13, 16].



Uterine responses to saline, serotonin and CNS extracts in *Blabera* (1, 2) and *Glossina* (3–6). The controls are represented on the left side; the graphs on the right side represent the effects of serotonin (10^{-4} M) in *Blabera* (2) and *Glossina* (4). The effects of the central nervous system extract (CNS) in *Glossina* are seen in 6.

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1 Evans, Adv. Insect. Physiol. *15* (1980) 317.
2 Colhoun, E.H., Experientia *19* (1963) 9.
3 Cook, B.J., Raker, J.E., and Anderson, G.R., J. Insect Physiol. *15* (1969) 445.
4 Hiripi, L., and S.-Rózsa, K., J. Insect Physiol. *19* (1973) 1481.
5 Collins, C., and Miller, T., J. exp. Biol. *67* (1977) 1.
6 S.-Rózsa, K., and Miller, T., Adv. Physiol. Sci. *22* (1981) 557.
7 Brown, B.E., Gen. comp. Endocr. *5* (1965) 387.
8 Cook, B.J., and Holman, G.M., Comp. Biochem. Physiol. *61C* (1978) 291.
9 Cook, B.J., Int. J. Invert. Reprod. *3* (1981) 209.
10 Reinhard, J.F. Jr, Moskowitz, M.A., Sved, A.F., Fernstrom, J.D., Life Sci. *27* (1981) 905.
11 Véró, M., and Miller, T., Med. Biol. Eng. Computing *17* (1979) 662.
12 Dymond, G.R., and Evans, P.D., Insect Biochem. *9* (1979) 535.
13 Robert, A., Grillot, J.P., Guilleminot, J., and Raabe, M., J. Insect Physiol. *30* (1984) 671–684.
14 Miller, T., and James, J., J. Insect Physiol. *22* (1976) 981.
15 Brown, B.E., Life Sci. *17* (1975) 1241.
16 Robert, A., Grillot, J.P., and Raabe, M., C.r. Acad. Sci. Paris *293* (1981) 611.

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Both thermal and nociceptive afferents influence the unit activity of the neurons in the corpus striatum[1]

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Summary. Both thermal and nociceptive stimulation in the periphery were shown to influence the neuronal activity recorded in the striatal area. Both the thermal and nociceptive sensitvity of the striatal neurons were closely related.
Key words. Rat, striatum; striatum, rat; nociceptive afferents; thermal afferents; single-unit activity.

The striatum (caudate and putamen) receives dopamine-containing fibers arising from cell bodies in the zona compacta of the substantia nigra[2]. In addition, the striatum receives glutamate fibers from the cortex, serotonergic fibers from the dorsal raphe, and cholinergic inputs from the thalamic region[2]. The striatum in turn projects to the pallidum and to the substantia nigra zona reticulata[3]. The striatum is usually thought to be involved in the central control of motor performance. Recently, evidence has accumulated to suggest that striatal neurons are also involved in the regulation of thermoregulation[4,5] and the nociceptive reflex[6,7]. The present study was an attempt to assess the effects of thermal stimulation of scrotal skin and noxious stimulation of the tail on single unit activity in the striatum of urethane-anesthetized rats, to test whether or not there exist thermally responsive and/or nociceptive responsive units in the striatum.

Adult male Sprague-Dawley rats weighing between 250 and 300 g were used. Each animal was anesthetized with urethane (1.25 g/kg, i.p.). Supplementary doses were occasionally needed during an experiment. The rectal temperature was maintained between 36.8 and 37.5 °C using a water-perfused pad under the animal. All the fur of the scrotum was removed with clippers. The animals were mounted stereotaxically with the heads fixed according to the König and Klippel coordinates system[8]. A piece of bone was removed from the right half of the skull and the underlying dura was removed. Recording of single unit discharges were made from the right half of the striatum at stereotaxic coordinates of A: 7.0–1.0; L: 2.0–3.6; and H: 2.0–0.0 mm[8]. Single-barrel micropipettes were filled with 4 M NaCl saturated with fast green dye and used for extracellular recording. The overall tip diameter of the micropipette was 2–5 μm. It generally had an impedance of 2–3 mΩ. After the micropipettes were lowered to the desired location in the striatum, a hydraulic microdrive was used to advance the micropipettes slowly. Single unit activity was processed using standard cathode follower and amplication circuitry for extracellular spike potential[9]. Impulses were counted at 1-sec intervals by WPI Scope Raster/Stepper Model 140 and displayed on a Grass polygraph recorder. Rectal temperature and scrotal temperatures were all displayed on the same polygraph record. The method used for thermal stimulation of the scrotum was similar to that described by Hellon and Misra[10]. Skin temperature was measured by a thermocouple connected to the surface of the thermode which was in contact with the skin. The noxious stimulation was produced by pinching the rat's tail with forceps.

A total of 77 single units in the striatal area were examined in 26 rats under urethane anesthesia. Each unit was subjected to change in scrotal temperature and to tail pinch (TP). Each unit was classified as either warm-responsive, cold-responsive or thermally unresponsive according to their thermal sensitivity. The thermal sensitivity or thermal coefficient was determined by dividing the maximum increase or decrease in discharge rate by the maximum change in scrotal temperature. Units that increased their firing rate with a rise of scrotal temperature and had a positive thermal coefficient greater than 0.8 impulses sec °C to changes in scrotal temperature were considered as warm-responsive. Units that increased their firing rate with a fall of scrotal temperature and had a negative thermal coefficient greater than 0.5 impulses $\cdot sec^{-1} \cdot °C^{-1}$ to changes in scrotal temperature were considered as cold-responsive[11]. Units that displayed no change in firing rate with changes in scrotal temperature were classified as thermally unresponsive. At the end of

Effects of tail pinch on striatal units classified as cold-responsive, warm-responsive, or thermally unresponsive

No. of units	Noxious stimuli produced by pinching the tail with forceps
24	Cold-responsive unit response
13	Facilitated
11	Inhibited
0	None
33	Warm-responsive unit response
22	Inhibited
8	None
3	Facilitated
20	Thermally unresponsive unit response
12	None
5	Inhibited
3	Facilitated