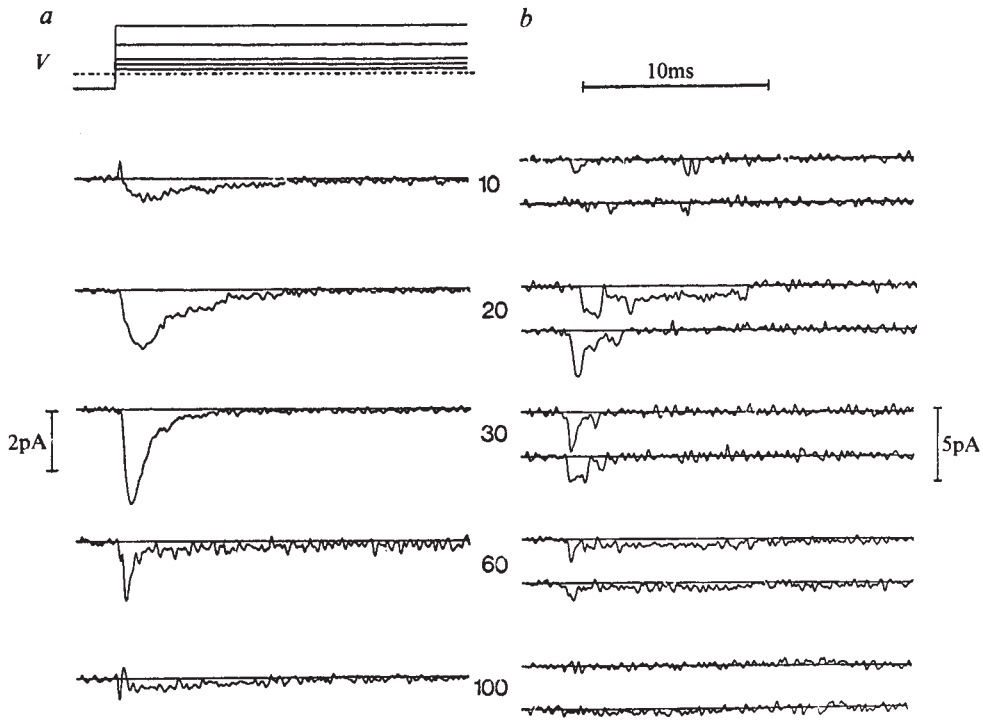

Fig. 3 Patch currents obtained with depolarizations to the various potentials V shown. *a*, Means calculated from 24–104 current records; *b*, representative individual records at each potential. Overlapping currents from 2–3 channels can be recognized at $V = 20$ and 30 mV. Note the decrease in current size near the reversal potential (approximately $V = 100$ mV). Leakage and capacitance currents were subtracted with averages from symmetrical hyperpolarizations. Recording system risetime was 220 μs. Cell 7.1, 3 days after colchicine treatment. Temperature, 22 °C.

Estimates of the cell resting potentials allow us to express values of V in terms of absolute membrane potential. Impalements with microelectrodes showed maximum resting potentials in the range −60 to −70 mV. h_∞ curves from averaged patch currents showed $h_\infty = 0.5$ in the range $V = 0$ to −15 mV whereas measurements with the microelectrode voltage clamp showed corresponding absolute potentials of −80 to −95 mV. We conclude therefore that the resting potentials were in the range −60 to −80 mV. This places the apparent reversal potential $V_{Na} \simeq 100$ mV in Fig. 3 in the absolute membrane potential range of +20 to +40 mV.

The size of the single-channel currents corresponds to a single-channel conductance $\gamma = 18$ pS. This value is at first sight surprisingly large, as γ estimates for frog nerve Na^+ channels, based on current fluctuation analysis, are in the range 6–8 pS (refs 1, 2). These values become twice as large, however, when they are corrected to the temperature and higher sodium concentration used in our experiments, assuming[14] $Q_{10} = 1.3$ and a linear concentration dependence of conductance.

The extracellular patch clamp as used in this work promises to be a powerful electrophysiological technique for tissue-cultured cells. The ability to clamp and change the potential in small patches of membrane now allows voltage-clamp studies to be performed on cells that are too small or have unsuitable geometries for microelectrode work.

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Localization of [^{3}H]-2-deoxyglucose in single molluscan neurones

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The glucose analogue 2-deoxyglucose (2-DG) can be used quantitatively to measure metabolic activity[1] and is widely used qualitatively for mapping functional activity in the brain[2–4]. The resolution (meaning the full width at half maximum of the grain density distribution around a line source) of the technique using [^{14}C]-2-DG and X-ray film is limited to about 100 μm[5]. Attempts have been made to improve the resolution using [^{3}H]-2-DG (ref. 6) and cellular resolution has been achieved in the goldfish retina[7] and with cultured mouse neurones[8]. An anatomical technique for mapping the metabolic activity of individual neurones would be useful for studying invertebrate central nervous systems, which are relatively simple and stereotyped compared to vertebrate brains. The [^{3}H]-2-DG technique was applied to an invertebrate in a study of the *Drosophila* visual system[9], though without cellular resolution. We present here modifications of the [^{3}H]-2-DG technique to demonstrate localization of 2-DG in single neurones of *Limax maximus*, a gastropod mollusc, with a resolution of less than 1 μm.

Glucose and 2-DG compete both for transport into and out of a cell and for phosphorylation by hexokinase inside the cell. Once 2-DG is phosphorylated to 2-deoxyglucose-6-phosphate (2-DG-6-P), it is not metabolized further and accumulates in the cell. In the conventional [^{14}C]-2-DG technique[1], the tissue is frozen, sectioned on a cryostat, picked up on a warm coverslip

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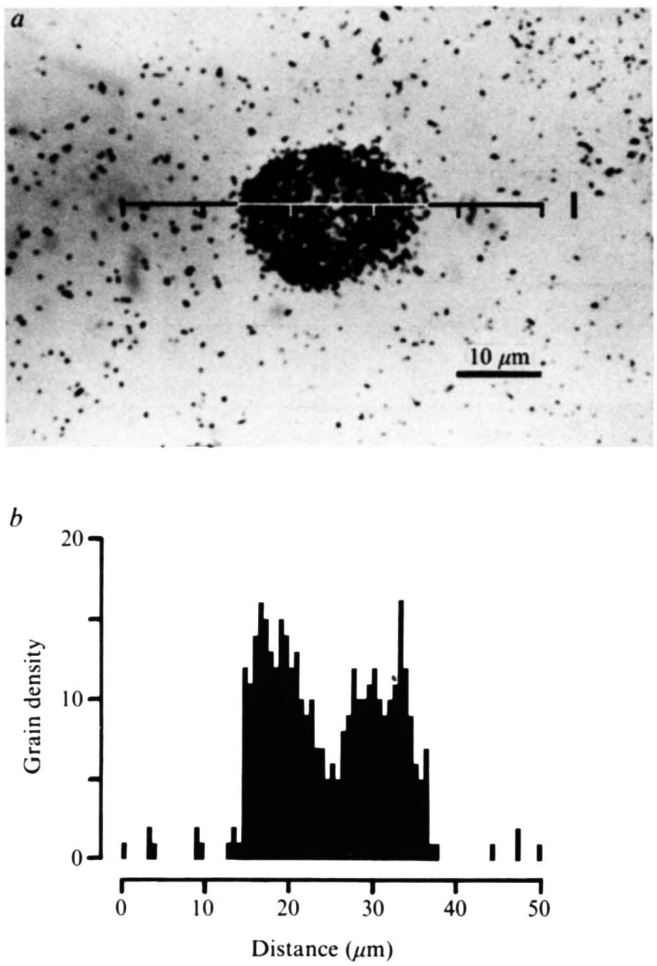

Fig. 1 Measurement of silver grain density over a single cell body in a buccal ganglion. *a*, Light-microscopic autoradiograph from a preparation incubated in [^{3}H]-2-DG for 45 min and freeze-substituted with acetone at −70 °C. During the incubation, a neurone in the buccal ganglion was stimulated with an intracellular electrode to elicit action potentials at the rate of 2 s^{-1}. Several cells in the buccal ganglion were labelled. Scale bar, 10 μm. *b*, A reticle with 100 lines was superposed over the cell at high magnification and the silver grains were counted by focussing through the emulsion of the autoradiograph. The number of silver grains between reticle lines (in a rectangle (0.63 × 4.6 μm) as shown to the right of the cell) is plotted as a function of distance along the line drawn through the cell body in *a*. The drop in density of silver grains over the centre of the cell occurs over the nucleus. The density of silver grains over the cytoplasm is 4.1 μm^{-2}, which is 40 times the average background density over the tissue surrounding the cell and 290 times the background density in the emulsion.

and dried on a hot plate, during which the 2-DG and 2-DG-6-P can freely diffuse and interfere with the localization of the label within cells. One way to prevent diffusion is to replace the water in the cell with a solvent in which 2-DG and 2-DG-6-P are insoluble and to exclude water from the tissue in all subsequent steps. We have localized [^{3}H]-2-DG using two methods: in the first, the tissue was frozen and freeze-substituted[10] with acetone at −70 °C, and in the second, the tissue was dehydrated in dry acetone at 4 °C.

The buccal ganglion of *L. maximus* innervates the animal's feeding musculature and contains several large identifiable neurones as well as several hundred smaller cells[11,12]. The stereotyped structure of the ganglion, its small size (about 1 mm in diameter) and its accessibility to electrophysiological techniques make it suitable for studying the uptake of 2-DG by single neurones. In each experiment, a pair of buccal ganglia was dissected and placed in saline modified by lowering Ca^{2+} to 0.07 times its normal concentration and raising Mg^{2+} by five times to reduce transmission at chemical synapses. (The composition of the solution was 56 mM Na^+, 4.2 mM K^+, 0.5 mM Ca^{2+}, 23 mM Mg^{2+}, 104 mM Cl^-, 0.2 mM $H_2PO_4^-$, 2.5 mM HCO_3^- and 5 mM dextrose.) Approximately 100 μCi ml^{-1} of 2-[1,2-^{3}H]-deoxy-D-glucose (New England Nuclear NET-549, specific activity 40 Ci mM^{-1}) was added to the bath and the ganglia were incubated for 1 h or less, depending on the experiment. Afterwards, the ganglia were rinsed in five washes of fresh saline over 30 min. For freeze-substitution, the ganglia were then rapidly frozen in Freon 22 cooled to −160 °C with liquid nitrogen, placed in a vial containing 20 ml dry acetone and 1 g of Al_2O_3

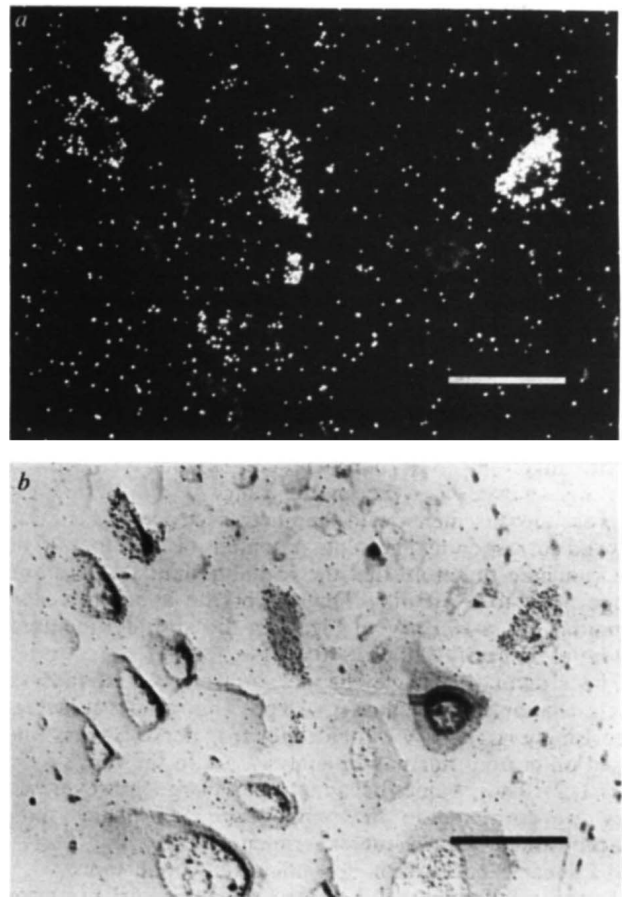

Fig. 2 Light-microscopic autoradiograph of several labelled cell bodies in a buccal ganglion. The ganglion was incubated in [^{3}H]-2-DG for 20 min and dehydrated in dry acetone at 4 °C. During the incubation a neurone was stimulated with an intracellular electrode to elicit action potentials at the rate of 2 s^{-1}. The sections were lightly stained with methylene blue. *a*, Five labelled cells (dark-field illumination). The cell bodies could be traced in serial sections through the ganglion. The nuclei of the two labelled cells on the far left and the cell on the far right were not labelled above background. The average density of silver grains over the labelled cells was 0.1 μm^{-2}–0.5 μm^{-2}, which was approximately 10–50 times the background density over surrounding tissue. *b*, The same section in *a*, photographed using Nomarski differential interference contrast optics. Scale bar, 40 μm.

(Brockman grade 1), and kept at −70 °C for 48 h. For acetone dehydration, the ganglia were placed in an identically prepared vial and kept at 4 °C overnight. No fixatives were used. (Fixation with glutaraldehyde[6,7] results in a loss of over 90% of the radioactivity from the tissue[7,8].) After either freeze-substitution or dehydration, the tissue was gradually warmed to room temperature and embedded in Araldite to which 1% by weight Dow-Corning silicone fluid[13] had been added. The embedded

tissue was then dry-sectioned at 2–4 μm on an ultramicrotome and placed on a slide coated with a thin layer of anhydrous glycerol. The glycerol was evaporated by setting the slide on a hot plate at 110 °C. The slides were then dipped in Kodak NTB-2 nuclear emulsion, dried, exposed at 4 °C for 2–4 weeks, developed in Kodak D-19 at 16 °C for 6 min, washed and stained with either 1% methylene blue or 1% toluidine blue.

Scintillation counting was used to determine the uptake of [^{3}H]-2-DG by ganglia during incubation and the loss of label during subsequent processing. The amount of radioactivity left in the acetone following either freeze-substitution or dehydration was less than 0.5% of the total radioactivity remaining in the ganglion. The loss of label during autoradiography was not quantitatively assessed, but it was probably low because spreading of label around the edges of the section or around discretely labelled structures within the section was never seen.

Autoradiographs of buccal ganglia incubated in [^{3}H]-2-DG revealed labelled individual neurones in all preparations. No cells were labelled in control ganglia incubated without [^{3}H]-2-DG, thus ruling out positive chemography. In seven heavily labelled cells from three preparations, the density of silver grains over cell bodies was between 0.1 μm^{-2} and 4.5 μm^{-2}, which was 10–50 times the mean background density over adjacent tissue. The glucose utilization is therefore 10–50 times greater within some cells than without. The localization of [^{3}H]-2-DG in a single heavily labelled buccal neurone is shown in Fig. 1*a*. The density of silver grains drops sharply at the boundary of the cell body, with the transition from maximum grain density to background occurring in less than 1 μm (Fig. 1*b*). The resolution of the technique appears to be limited by the size of a developed silver grain, which has a diameter of 0.5 μm. Whenever the stained cell membrane of a labelled cell is visible, the grain density drops abruptly at the cell membrane. In some labelled cells, such as those shown in Fig. 2, the grain density also drops abruptly at the nuclear membrane, with much lower labelling over the cell nucleus than the cytoplasm. In addition to labelled cell bodies, labelled structures could also be detected in the neuropil.

The high resolution obtained with this technique is probably the result of removing water from the tissue. During freeze-substitution, the maximum concentration of water at the interface between ice and acetone is 4% at −70 °C (ref. 14). Thus, molecules insoluble in acetone diffuse very little from the site at which they were immobilized during freezing. For example, freeze-substitution has been used to localize Ca^{2+} in subcellular compartments[15]. More diffusion of 2-DG and 2-DG-6-P occurs within the cell with acetone dehydration at 4 °C than with freeze-substitution at −70 °C. However, the labelling of single cell bodies using acetone dehydration had the same resolution as with freeze-substitution. We can therefore conclude that both freeze-substitution and acetone dehydration are effective in localizing [^{3}H]-2-DG within single neurones.

The [^{3}H]-2-DG technique presented here can be used to measure the qualitative change in the distribution of label under different conditions. Preliminary experiments indicate that the labelling of single neurones demonstrated here is correlated with spontaneous and stimulated electrical activity in the ganglion[16]. The technique could be useful in studying networks of neurones underlying behaviour in the isolated central nervous system[17]. In particular, the participation of small neurones, which are difficult to study electrophysiologically, can be monitored as easily as large neurones. It may also be possible to measure the metabolic activity of axon terminals in the neuropil. As the resolution reported here is close to the limiting resolution of light microscopy, further improvement will require the use of electron microscopy.

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Repeated tricyclics induce a progressive dopamine autoreceptor subsensitivity independent of daily drug treatment

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Dopamine (DA) has largely been ignored in considering possible mechanisms underlying the therapeutic effects of tricyclic anti-depressants (TCA)[1–3]. Most previous work done has focused on the ability of some TCAs to block the *in vitro* re-uptake of DA[1]—an effect which unfortunately requires very high doses. Recently, however, Serra *et al.*[3] proposed that TCAs may exert their therapeutic effects by inducing a subsensitivity of presynaptic receptors located on the dendrites and soma of DA neurones (DA autoreceptors). This hypothesis was directly tested by examining the influence of TCAs on the demonstrated ability of the DA agonist, apomorphine, to depress selectively the spontaneous activity of single DA cells. We now report that repeated administration of both typical and atypical TCAs induces a progressive subsensitivity of DA autoreceptors, and that this gradual augmentation of DA autoreceptor subsensitivity depends on the passage of time rather than daily TCA administration. The latter finding suggests that daily drug administration may not be therapeutically necessary.

The 53 male albino rats (200–250 g; Zivic Miller) used in these studies were housed two per cage with free access to food and water and maintained on a 12 h light/dark cycle. Animals were handled and weighed daily for 2 weeks before experiments began. All experiments were done in double-blind conditions. After a period of drug treatment (specified below) the animals were anaesthetized with chloral hydrate [400 mg per kg, intraperitoneally (i.p.)], mounted in a stereotaxic apparatus and the spontaneous discharge rates of single DA neurones located within the zona compacta of the substantia nigra (SNC; co-ordinates: anterior 1,300–2,400 μm, lateral 1,300–2,400 μm [ref. 4]) were monitored as previously described[5,6]. Briefly, extracellular electrical activity was recorded through glass micropipettes filled with 2 M NaCl which was saturated with fast green dye (tip size 1–3 μm, *in vitro* d.c. impedance of 4–12 MΩ). Neuronal signals were fed into a high input–impedence differential amplifier (bandpass filters: 100 Hz and 3 kHz) whose output was led into a window discriminator, a signal integrator and digital frequency meter. The femoral vein was catheterized for the intravenous (i.v.) administration of all pharmacological agents. All drug doses are based on the weight of the salt. At the termination of each experiment the recording

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