Halothane-induced sleeping time could be repeatedly determined in the same animals with no evidence of tolerance, although a marked diurnal variation was observed. We have made repeated halothane sleeping time determinations in rats pretreated both acutely and chronically with a number of CNS depressant drugs and have found that drug exposure is followed by a cross-tolerance to halothane. This tolerance is frequently followed by a post-tolerance hypersensitivity to halothane and brain halothane concentrations determined on awakening confirm that the tolerance and hypersensitivity are due to changes in the sensitivity of the CNS to the anaesthetic.

Thus it is concluded that determination of halothane-induced sleeping time appears to be a sensitive index of the excitability of the CNS and has the advantage that repeated measurements can be made over a relatively short period of time.

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Selective autoradiographic markers for GABA-releasing interneurones and nerve terminals

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In this light and electron microscopic autoradiographic study the cerebellum was used as a test model since only in this region of the brain has ultrastructure been fully correlated with function (Eccles, Ito & Szentagothai, 1967). In addition, neuropharmacological data is available to allow us to suggest that γ-aminobutyric acid (GABA) is the sole transmitter of the inhibitory interneurones of the cerebellum.

Either [^{3}H]-DABA (DL-2,4-diaminobutyric acid) or [^{3}H]-GABA dissolved in 2 μl of artificial CSF were microinjected under pressure into the centre of a single cerebellar folium of anaesthetized cats or rats at a depth of about 1 mm using a fine glass microelectrode (tip diameter <20 μm). Twenty minutes later the animals were killed by perfusion fixation with 5% glutaraldehyde. Subsequently 300 μm thick coronal slices of the lobule were cut on an Oxford 'vibratome' and prepared for electron microscopic autoradiography as described previously (Schon & Kelly, 1974).

Light and electron microscopic autoradiographs from the cerebellar folia following the microinjection of [^{3}H]-DABA (50 μCi) and [^{3}H]-GABA (25 μCi) provided similar results and were characterized by intensely and discretely labelled cell bodies in all layers of the cerebellum. At the electron microscopic level the labelled cells were identified as stellate cells in the molecular layer, as basket or Golgi cells in the Purkinje cell and upper granular layers. The Purkinje cells themselves, however, were virtually devoid of silver grains and their somas were silhouetted against a dense rim of radioactivity which became broader towards their rounded ends. Electron microscopic observations showed this dense rim to be composed of dense cluster of sliver grains located over unmyelinated axons and nerve terminals in synaptic contact with the Purkinje cell soma. These axons and terminals were tentatively identified to be basket cell axons or Purkinje cell recurrent collaterals.

Clusters of silver grains were also found in association with the cerebellar glomeruli; the silver grains were located exclusively over Golgi axon terminals in close contact with the granule cell dendrites. Neither the granule cell bodies or their dendrites nor the mossy fibre terminals were labelled.

In this study we have shown that light and electron microscopic autoradiographic techniques are now available to identify and map the distribution of the neurones in the mammalian CNS which use GABA as their transmitter. This technique is based on the hypothesis that such neurones possess a unique high affinity uptake site for the amino acids that they employ as their transmitter.

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Topographical study of the distribution of GABA in the human substantia nigra

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In 1971, Precht & Yoshida first proposed the hypothesis that striatonigral fibres release GABA as a transmitter. Since then, interest has been focussed on the high concentrations of GABA which occur in the substantia nigra of most mammals and man (Perry, Hansen & Kloster, 1973). Since GABA, glutamic acid decarboxylase and the high affinity uptake of GABA appear to be specifically located in the nerve terminals of certain inhibitory neurons, it has been proposed that GABA in the substantia nigra may well be exclusively localized in nerve terminals. The main cellular components of the human substantia nigra are the melanin-rich cells which send their axons to the striatum and are believed to be dopaminergic in function. It would be of interest, therefore, to know the morphological distribution of GABA in the substantia nigra and its relationship to the dopaminergic neurons. In addition it now appears that a knowledge of the distribution of GABA in the substantia nigra could be of clinical value since a decreased level of GABA in the substantia nigra appears to be a feature of Huntingdon's chorea.

The midbrain from a neurologically normal 28-year-old male was obtained at autopsy within four hours of death. Using the method of Miyata & Otsuka (1972), transverse sections (150 μm thickness) of the rostral, middle and caudal substantia nigra were placed in a cold box (-20°C) and cut under a binocular microscope into 500 x 500 μm square blocks with a razor blade. The map of GABA distribution in each level of the substantia nigra was obtained by superimposing photographs taken before and after cutting the sections into square blocks.

In the rostral substantia nigra, the GABA distribution was markedly uneven, and the highest concentrations (more than 11.0 mM) were found in the pars reticulata. In the middle and caudal substantia nigra the GABA distribution was again uneven and the highest GABA levels were equally divided between the pars reticulata and the pars compacta. More detailed analysis of the results also supported the view that in the substantia nigra the highest concentrations of GABA are due to the presence of striato-nigral nerve terminals as they synapse with the dendrites of the nigral dopaminergic neurons whose cell bodies are located in the pars compacta (Rinvik & Grofova, 1970). In the cat more than half the synapses in the pars reticulata undergo degeneration following lesions of the ipsilateral caudate nucleus (Grofova & Rinvik, 1970) and the same kind of lesion in the rat is accompanied with a reduction in the GABA concentration of the substantia nigra.

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