satellite cells (Adams & Brown, 1974). This raises the question whether release of GABA from such cells results from neural excitation. Several observations suggest that this may not be so. (a) Release following electrical stimulation was not prevented when transmission and/or conduction were blocked by (i) 0 mM [Ca^{2+}], (ii) 0 [Ca^{2+}] + 10-30 mM [Mg^{2+}], (iii) 0 mM [Ca^{2+}] + 1 mM [EDTA] and (iv) 3 mM procaine or 300 μM amethocaine. (b) Carbachol (550 μM), which depolarizes ganglionic neurones but not glial cells (Adams & Brown, 1974), did not accelerate [^{3}H]-GABA release. (c) When the effluent radioactivity from the trunks and soma was measured separately during pre-ganglionic trunk stimulation, an increased efflux rate from the pre-ganglionic trunk but *not* from the soma was detected. In contrast, accumulated [^{3}H]-choline *was* released from the soma following pre-ganglionic stimulation.

Thus, release of GABA from ganglionic glial cells by 'depolarizing' stimuli appears to result from a direct and local action on the glial cells *per se*

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The effects of depleting brain amines on the behavioural actions of γ-hydroxybutyric acid

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It has been well-established that γ-hydroxybutyric acid (GHB) produces a dose-dependent increase in the concentration in the brain of dopamine (Gessa, Vargiu, Crabai, Boero, Caboni & Camba, 1966; Roth & Suhr, 1970; Hutchins, Rayevsky & Sharman, 1972; Clifford, Taberner, Tunnicliff, Rick & Kerkut, 1973; Stock, Magnusson & Anden, 1973) and serotonin (Clifford *et al.*, 1973; Spano & Przegalinski, 1973). There is a close correlation between the duration of the sleep-like state induced by GHB and the increase in dopamine level (Gessa *et al.*, 1966; Clifford *et al.*, 1973), although no causal relationship has been

Table 1 Effects of 6-hydroxydopamine on brain amine levels and γ-hydroxybutyric acid (GHB) sleeping time

Dose schedule	*Whole brain amine levels (µg/g wet weight)*			*Sleeping time (min)*
	Dopamine	*Noradrenaline*	*Serotonin*	
Control (saline)	0.936 ± 0.104	0.569 ± 0.042	0.485 ± 0.063	–
GHB (4 mmoles/kg i.p.)	1.23 ± 0.14***	0.446 ± 0.073	0.688 ± 0.151**	58.9 ± 7.8
6-Hydroxydopamine (0.8 µmoles)	0.197 ± 0.130	0.074 ± 0.017	0.573 ± 0.392	–
GHB (4 mmoles/kg i.p.)	0.142 ± 0.054	0.123 ± 0.057	0.676 ± 0.094	24.3 ± 4.9*

* $P < 0.001$; ** $P < 0.05$; *** $P < 0.01$.

Results are the means ± s.d. of at least 5 determinations. Mice were injected intraventricularly with either 6-hydroxydopamine or vehicle in a volume of 5 µl 72 h prior to assay of brain amines or determination of the GHB sleeping time. Amine levels were determined in GHB-treated mice 40 min after the injection of GHB.

demonstrated. We have therefore selectively depleted brain dopamine and noradrenaline by the intraventricular injection of 6-hydroxydopamine in the mouse in order to determine whether or not the behavioural response to GHB can be modified. GHB, at a hypnotic dose, increased the whole brain dopamine concentration and pretreatment with 6-hydroxydopamine 72 h prior to the assay produced a significant decrease in dopamine and noradrenaline concentration (Table 1). GHB did not increase the dopamine concentration in 6-hydroxydopamine-treated animals and, at the same time, the sleeping time (defined as the duration of the loss of righting reflex) was significantly reduced. The GHB sleeping time was not significantly altered in mice pretreated with 4-methyl-α-ethyl-m-tyramine (H75/12), a depletor of brain serotonin (Carlsson, Corrodi, Fuxe & Hokfelt, 1969), at a divided dose of 0.93 mmoles/kg i.p. given at 2 and 4 h prior to the GHB. It is therefore possible that the behavioural effects of GHB are related to its action on dopamine synthesis.

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Effects of central nervous system depressants and stimulants on the acetylcholine concentration of leech ganglia *in vivo*

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Little is known about the effects of drugs on acetylcholine (ACh) metabolism in invertebrate nervous tissue. Since leech ganglia contain cholinoceptive cells (Kerkut & Walker, 1967) as well as ACh, cholinesterase (Cammelli, De Bellis & Nistri, 1974) and choline acetyltransferase (Perkins & Cottrell, 1972), the effects of some centrally acting drugs on the ACh concentration of this preparation were studied.

Leeches (*Hirudo medicinalis*) were placed in beakers containing 100 ml distilled water (controls) or a drug dissolved in distilled water, for 10, 20 or 40 min, after which the ventral nerve cord was rapidly removed. Two cords were pooled as a single sample for the extraction and bioassay of ACh (Cammelli, De Bellis & Nistri, 1974).

Table 1 ACh content of the leech ventral nerve cord

	ACh (ng/ganglion)	n	*Dose*	*Period of exposure (min)*
Controls	3.09 ± 0.29	11	–	–
Ethanol	2.91 ± 0.10	4	10%	10
Ethanol	3.07 ± 0.66	5	10%	20
Leptazol	1.62 ± 0.14*	5	10 mM	10
Eserine	4.80 ± 0.35*	4	0.2 mM	40

Mean ± s.e.m., n = number of experiments; * = $P < 0.01$ when compared with controls.