causing the animal to step down from the shelf more quickly: as the stimulant effect wore off so the enhanced retention would become increasingly evident. Alternatively, the drug might produce a delayed effect upon motor activity, causing the subjects to become less active after a period of 7 days, thereby increasing the step-down latencies and producing an artifactual improvement in learned response retention. However both these possibilities were eliminated since it was observed that there were no indications of any effects on motor activity affecting step-down readiness amongst the non-shocked control subjects. Moreover, a subsidiary experiment on a further 10 subjects, 5 of which were given methylamphetamine hydrochloride (in the dose level previously noted) and the remainder given saline, confirmed that no elevated locomotory or rearing activity occurred 1, 3 or 5 days after injection. This does not, of course, rule out the possibility that some other characteristic of the drug obscures elevated step-down latencies on retest days 1, 3 and 5, though it is very difficult to see what the mechanism of this might be. This same subsidiary experiment also showed that no impairment of motor responses occurred 7 days after drug treatment such as would be necessary to explain the elevated latency sores observed in the previous experiment.

Other explanations, not related to changes in motor activity, may also be considered. It may be, for example, that methylamphetamine hydrochloride produces an effect which increases over time and which operated in such a way as to facilitate the expression of the learned response, i.e. to enhance memory retrieval. At a physiological level this might be due to the progressive development of a change in synaptic functioning, or to the build-up of some psychoactive metabolite of the drug. There is, however, no evidence to support either of these proposals.

A second possibility is that the drug affects the process of memory consolidation so as to enhance the establishment of a long-term trace and to make it less susceptible to disruption. However, experimental animal studies[5-7] on memory consolidation have indicated that the transformation of short-term into long-term traces is likely to be essentially completed within a matter of minutes, or at the most within 2 or 3 h, following the learning experience. This does not, of course, mean that other processes of more extended duration might not be involved in the fixation of long-term traces. These extended processes might be susceptible to modification by emergent effects of previous drug administration. In the case of the drug under consideration, there is little known about the form which such emergent effects might take: acute methylamphetamine hydrochloride administration produces catecholamine depletion in nerve terminals, particularly in the hypothalamus, amygdala and hippocampus[8], but the time course of this effect is uncertain, as are the functional implications for memory consolidation.

The phenomenon of a pharmacologically induced apparent improvement of memory occurring after a 7-day delay is very curious, if indeed it is a phenomenon which can be replicated under different experimental circumstances, and its further and closer examination may help to elucidate some of the consolidation processes which occur during memory establishment.

Résumé. L'hydrochlorure de méthylamphétamine, drogue qui était administrée aux rats après qu'ils aient subi une seule expérience d'entrainement, améliorerait la mémoire des animaux testés, mais cet effet se n'est produit que 7 jours après l'expérience.

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Binding of 5-Hydroxytryptamine and Noradrenaline by Rat Lung

Rat lungs in vitro have been shown to remove both 5-hydroxy-tryptamine[1] (5-HT) and noradrenaline[2] (NA) from the pulmonary circulation. Alabaster and Bakhle[1] reported that NA, in concentrations up to 10 times those of 5-HT, failed to influence removal of the latter by lung, suggesting that removal sites for the 2 amines may not be identical, or that their affinity is greater for 5-HT than for NA. In an effort to identify possible binding sites for both amines, radioactive 5-HT and NA were administered simultaneously to rats and the subcellular distribution of each amine determined subsequently in sucrose homogenates of lung.

Male rats (180 to 250 g) were anesthetized with Dial/urethane. Solutions of ^{14}C-5-HT and ^{3}H-NA (New England Nuclear Corporation, specific activities 15.3 mC/mM and 9.95 C/mM respectively) were prepared in 0.9% sodium chloride at concentrations such that 6 µg of 5-HT base and 0.1 µg NA base were infused (via a juglar cannula) per min. This mixture of amines was infused for 10 min, after which lungs were removed either immediately or, in some experiments, 30 min later. Lungs were rinsed in saline, blotted, weighed and homogenized in 0.25 M sucrose (total of 7–8 ml). The ^{14}C-5-HT and ^{3}H-NA in an aliquot of the homogenate was measured as described earlier[2,3]. The homogenate was centrifuged at 3000 $\times g$ for 10 min. In some experiments, an aliquot of the supernatant was layered over a linear gradient of sucrose (see below). The remainder of the supernatant was centrifuged at 105,000 $\times g$ for 30 min. The resulting sediment was resuspended in 2.0 ml of 0.25 M sucrose. 0.5 ml of the suspension was used for measurement of ^{14}C-5-HT and ^{3}H-NA. The remainder (1.5 ml) was layered over an 11.5 ml linear gradient of sucrose, ranging in concentration from 0.25 to 1.0 M and prepared with a Beckman Gradient former. Before making the gradient 2.0 ml of 1.5 M sucrose was added to each tube. Gradients were centrifuged at 132,000 $\times g$ for 1 h, after which 22 fractions, each of approximately 0.6 ml,

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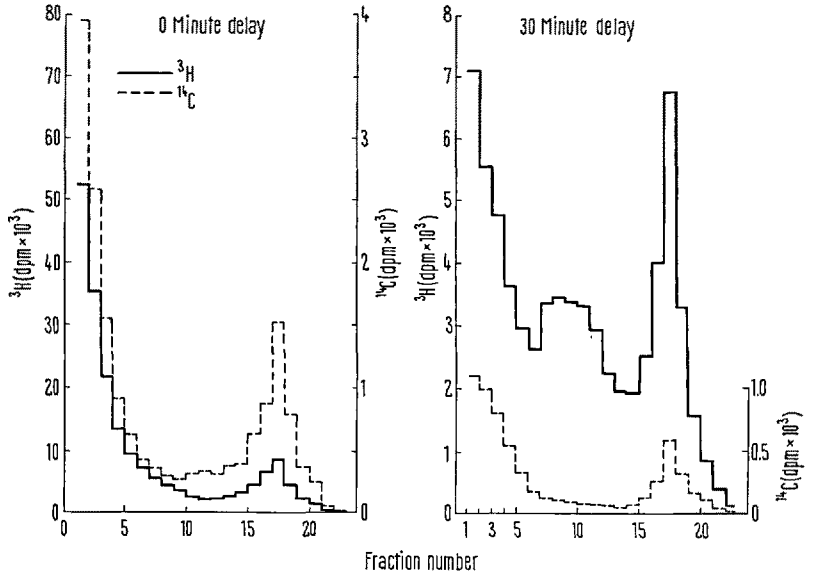
Unchanged ^{3}H-noradrenaline and ^{14}C-5-hydroxytryptamine in subcellular fractions of rat lung

Fraction	Time after end of infusion (min)	Noradrenaline[a]	5-hydroxytryptamine[a]
Whole homogenate	0	31.0 (29–42)	16.7 (12–20)
Whole Homogenate	30	41.3 (26–50)	34.3 (20–44)
High-speed sediment[b]	0	58.6 (54–72)	35.6 (28–47)
High-speed sediment	30	78.0 (67–94)	59.7 (48–70)

[a] Noradrenaline or 5-hydroxytryptamine presented as per cent of total ^{3}H or ^{14}C respectively. Each value is the mean of 3 or 4 separate experiments. The range is given in parenthesis. [b] Sediment isolated by centrifugation of 3000 × *g* supernatant at 105,000 × *g* for 30 min.



Distribution of tritium and ^{14}C carbon in the subcellular fraction of lung homogenate isolated between 3000 × *g* and 105,000 × *g*. Rats received i.v. infusions of ^{14}C-5-HT and ^{3}H-NE for 10 min: lungs were removed immediately (left panel) or 30 min (right panel) later. Note the 10-fold decrease in tritium scale between left and right panels.

were prepared[4] and collected in scintillation counting vials to which had been added 0.02 ml of 4.0 *M* perchloric acid. 10 ml of Brays phosphor[5] was added to each vial and both tritium and ^{14}C-carbon determined simultaneously by means of a Packard 3320 liquid scintillation spectrometer with external standardization feature.

Density gradient centrifugation of the 3000 × *g* supernatant of lung homogenates from animals killed immediately after a 10 min infusion resulted in a very similar profile for both ^{14}C and ^{3}H. A small peak of both isotopes was observed corresponding to a sucrose density of approximately 1.0 *M*. It was probable that the distribution of isotopes in the 3000 × *g* supernatant reflected largely that of metabolite since only 17% of total ^{14}C in the original homogenate was unchanged 5-HT while the corresponding figure for NA was 31% (Table). Although lung homogenates prepared from animals killed 30 min after the infusion had increased proportions of total radioactivity present as unchanged 5-HT and NA (34% and 41%, respectively, see Table) the profile of isotope distribution in the 3000 × *g* supernatant was unaltered. The distribution of ^{14}C and ^{3}H in resuspended 105,000 × *g* sediment is illustrated in the Figure. It can be seen that whether lungs were removed immediately after the infusion or 30 min later, the major peak of both isotopes occurs at fractions 16–18. Sucrose density at this level was approximately 1.0 *M*. The Table shows that when lungs were homogenized immediately after the infusion, unchanged 5-HT constituted 36% of total ^{14}C while 59% of total ^{3}H in this fraction was NA. When 30 min elapsed after the infusion, unchanged 5-HT accounted for 60% of total ^{14}C while 78% of ^{3}H was associated with NE. Although in the latter experiments, the bulk of NE was present in the heavy peak at 1.0 *M* sucrose, a smaller and lighter peak of ^{3}H was observed in 2 of 3 separate experiments at a density corresponding to 0.4–0.5 *M* sucrose. This peak was never seen for 5-HT, regardless of when lungs were removed after the infusion.

These data suggest that most of the unchanged 5-HT and NE in lung is bound to subcellular organelles that are either identical for both amines or behave similarly in the sucrose gradient used. Also, it is clear that metabolites of both 5-HT and NA are more readily lost from lungs than the amines since the latter constitute larger proportions of the whole homogenate prepared 30 min after the infusion than immediately at its termination. Sedimentation and resuspension of particulate elements in the 3000 × *g* supernatant yields a preparation with considerably higher proportions of unchanged amine. Yet even when, in one ex-

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periment (see Table), NA and 5-HT in the high-speed sediment accounted for 94% and 70% of total ^{3}H and ^{14}C respectively, considerable amounts of both isotopes appeared in the first 4 or 5 fractions of the gradient. This observation suggests that binding of the amines is not sufficiently firm to prevent some loss during resuspension and centrifugation in a sucrose gradient.

Résumé. Après injection i.v. de 5-hydroxatryptamine-C^{14} et de noradrénaline-H^{3} à des rats, on a préparé et centrifugé des homogénates de leurs poumons dans un gradient linéaire de sucrose. Après sacrifice immédiat ces deux amines sont retrouvées dans une grande fraction de particules pulmonaires sédimentant dans 1 *M* de sucrose. Après 30 min les poumons des rat offrent un pic additionel de noradrénaline-H^{3} correspondant à une densité de sucrose de 0.4 à 0.5.

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Neuronal Types in Long-Term Culture of Avian Retina

Tissue culture of nervous system has provided an ideal model system for experimental studies in the various disciplines of the neurosciences. For understanding and interpretation of any experimental studies, precise definition of neuronal types in a given culture is most crucial, since no valid conclusion can be drawn without such information. Although there have been a few reports on culture of mammalian and avian retina, little is known about the neuronal types differentiated in culture[1–3]. The present communication is primarily concerned with the identification of neuronal types in silver impregnated chick retinal cultures.

The eyes of 10–14-day-old chick embryos were dissected out under sterile conditions and the globes were incised anterior to the equatorial plane. The retinae were detached gently and cut in small pieces for explantation. The explants were placed on rat tail collagen coated coverslips (11 by 22 mm rectangular), and then sealed in Maximow's slides[4] or in roller tubes[5]. The nutrient fluid consisted of equal parts of horse serum, medium 199, Hanks' balanced salt solution and supplementary glucose giving a concentration of 600 mg per 100 ml nutrient. The cultures were incubated at 36 °C. At various time intervals (12–34 days in vitro) cultures were taken out and fixed for silver impregnation by a modification of Bodian's protargol method[6,7].

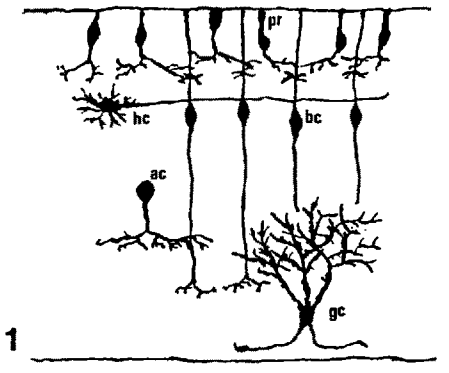

Fig. 1. Neuronal elements of 13–14-day-old chick embryo retina. Pr, photoreceptors (rods and cones); bc, bipolar cells; hc, horizontal cell; ac, amacrine cell; gc, ganglion cell (modified from Ramon y Cajal[8]).

The normal developmental stage of the retina from 13–14-day-old chick embryos is depicted in Figure 1, which is modified from the sketches of Ramon y Cajal[8]. The cell elements illustrated are photoreceptor cells (rods and cones), bipolar cells, ganglion cells, and two types of association cells, horizontal and amacrine cells. Neurons demonstrated in silver impregnated cultures were closely correlated morphologically to the neuronal types shown in Figure 1.

The development of chick retinal cultures in general was similar to the descriptions made by previous authors in cultures of new-born rat retina such as the rosette formation of photoreceptors[1,2]. Amongst the heavy population of photoreceptors and glial cells (including Müller cells), various neuronal types were observed. Although nerve cells in culture were irregularly oriented, 4 principal types of neurons were identified as such by characteristic features of their morphology and size. Ganglion cells were the largest among the neurons observed in retinal cultures (14–20 µm soma), with multipolar orientation of dendrites. They closely resembled typical neurons demonstrated in cultures of other regions of the chick central nervous tissue[9,10] (Figure 2). Bipolar cells were distinguished from other neurons by their smaller size (6–10 µm soma) and 2 long processes extending from the cell body (Figure

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