definitely. The vapor pressure of perfluorodecalin is 14 torr and that of perfluoromethyldecalin is 5 torr.

Our results show that there are classes of PFC which form some kind of chemical bond with the liver substance. Such PFC contain atoms other than carbon and fluorine. All of them contain either a C-O-C or a C-N-C linkage. One possibility for such a coupling is that the unshared electron pair on the oxygen or nitrogen atom may passively form bonds with the substance of the liver. If the binding was to protein then it is surprising that it was not bound as much to, say, muscle. Ullrich's finding (*9*) that perfluorohexane is bound to, or at least interacts with, cytochrome P450 suggests that certain PFC may form complexes with certain iron-containing proteins. The straight-chain fluorocarbons studied so far, on the other hand, rapidly leave the liver of the intact animal.

Another possibility is that an active metabolic process is involved not only in the binding, but in the release from the liver. It may be, for example, that the perfluorocyclocarbons are actively excreted by the liver because they resemble steroid fragments.

This new family of compounds containing only carbon and fluorine and having cyclic structures may make possible PFC emulsions capable of being safely used in intact animals.

LELAND C. CLARK, JR.
FERNANDO BECATTINI
SAMUEL KAPLAN, VIRGINIA OBROCK
DAVID COHEN, CHARLES BECKER
Children's Hospital Research Foundation, Cincinnati, Ohio 45229

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1. The nomenclature for these compounds is not universally accepted. Perfluorochemical is used here to mean organic compounds that have been fluorinated until no hydrogen remains. Fluorocarbon refers to compounds having only fluorine and carbon in the molecule. PP5 is *cis*- and tris-perfluorodecalin and some impurities. PP9 is a mixture of isomers of perfluoromethyldecalin, some PP5, and some impurities. PP5 and PP9 are trade names of I.S.C. Chemicals, Ltd. P11D is a perfluorodiisopropoxybutane synthesized by the Allied Chemical Company. After this manuscript was read in proof we found that our perfluorodimethyldecalin (product 10964, PCR, Fla.) has the same infrared spectrum as the monomethyl compound.
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Impaired Learning and Decreased Cortical Norepinephrine after Bilateral Locus Coeruleus Lesions

Abstract. *Bilateral lesions of the nucleus locus coeruleus in rats deplete the cerebral cortex of norepinephrine and significantly diminish the rate of increase of running for food reward in a simple L-shaped runway. As assessed in this situation, learning was absent in those rats with the most complete ablations of the locus coeruleus, although these rats showed normal weight gain and normal motor and exploratory activity.*

Studies with the Falck-Hillarp histochemical technique (*1*) reveal the presence of a network of norepinephrine-containing nerve terminals in the mammalian cerebral cortex. Studies with lesions (*2*) and stimulation (*3*) show that these terminals are derived from cell bodies situated in the nucleus locus coeruleus in the floor of the fourth ventricle. Electrical self-stimulation can be obtained through electrode tips in close proximity to this nucleus (*4*), and this noradrenergic system may be one of two catecholamine-containing systems that will support this behavior (*5*, *6*). On the basis of theoretical considerations, it has been proposed (*5*, *6a*) that the norepinephrine-containing neurons arising from the cell bodies of the locus coeruleus function as a "reinforcement" system in the sense that this term is used in theories of learning.

Our experiments were designed to test the theory that the norepinephrine-containing neurons innervating the cerebral cortex form an essential component of the mechanisms of learning. Male hooded Lister rats (initial weight, 200 ± 10 g) were anesthetized with pentobarbitone, immobilized in a Kopf stereotaxic apparatus, and had burr holes drilled bilaterally in the skull over the cerebellum. In one group (BH) of six rats, no further procedures were carried out before the wound was resutured. In three further groups of rats, bilateral electrolytic lesions were made by passing a charge of 15 to 20 millicoulombs through the bare tip of a varnished steel electrode to an anal cathode. In one group (CB) of six rats, the electrode was located at symmetrically placed points in the cerebellar

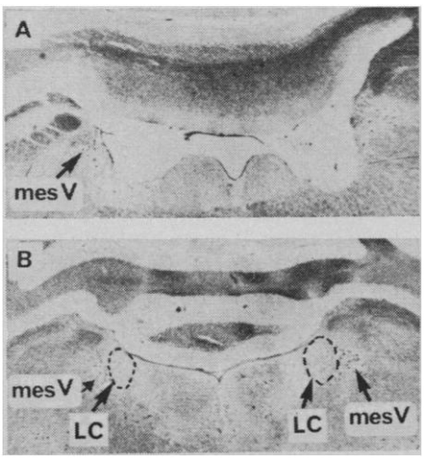

Fig. 1. Histological preparations of the region of the locus coeruleus in the floor of the fourth ventricle. (A) The locus coeruleus on each side has been ablated, although cells of the mesencephalic tract of the trigeminal nerve (*mesV*), situated laterally to the locus coeruleus, have been spared. This rat showed no increase in running speed in the course of behavioral testing. (B) The locus coeruleus (*LC*) is intact on both sides in a rat in group BH.

substance [coordinates according to the Fifkova and Marsala atlas (*7*): P1.8 (from lambda), L1.0, V4.0]; and in a second group (BS) of six rats, it was located at points 0.5 mm lateral to the midline in the reticular formation of the brainstem (coordinates P1.8, L0.5, V8.5). In the third group (LC) of 28 rats, the lesions were aimed at the region of the locus coeruleus [coordinates P1.8, L1.0, V6.4, determined as described by Crow *et al.* (*4*)].

Three weeks after the operation, the animals were starved to 90 percent of normal body weight. All animals were given five trials for a food reward on each of the next 16 days in an L-shaped runway, and were timed on the long initial arm by photocells 120 cm apart. Subsequently the animals were killed by decapitation under ether anesthesia. The brains were removed and the brainstem region, including the cerebellum, was prepared for histological study by the technique of Klüver and Barrera (*8*). After the cerebral cortices were dissected away, norepinephrine was assayed by a modified aluminium oxide–trihydroxyindole method (*9*) in each side of the cortex, and a mean value was calculated for each animal.

Examination of the histological material showed that ablation of the locus coeruleus had been incomplete in many of the animals in the LC group. The six animals with the most extensive lesions of the locus coeruleus were selected for comparison with the control groups. In three of these rats, the nucleus was completely ablated on both sides, with minimal damage to other brainstem or cerebellar structures (Fig. 1). In the remaining three rats, there was complete ablation of one locus coeruleus and extensive damage to the contralateral nucleus. In the CB group, lesions of similar size to those in the LC group were found in the cerebellar substance dorsal to the cerebellar nuclei; in the BS group, lesions were in the reticular formation of the brainstem in the region of the nucleus reticularis pontis caudalis.

The cortical norepinephrine content and behavioral performance of the six rats with the most extensive locus coeruleus lesions were compared with those of the three control groups. The cortical norepinephrine content (mean ± standard error of mean per gram of cortical tissue) was 102 ± 24 ng/g for the six rats with extensive locus coeruleus lesions; 290 ± 53 ng/g for group CB animals; 357 ± 62 ng/g for group BH animals; and 328 ± 55 ng/g for group BS animals. There was a significant reduction in cortical norepinephrine in the group with locus coeruleus lesions ($P < .001$ compared to the three control groups combined; $P < .005$ compared to group BS alone). When the latency measures for the four groups during the 16 days of runway training were subjected to analysis of variance, a significant groups effect was obtained ($F = 19.629$; d.f. = 3, 20; $P < .001$). The group with locus coeruleus lesions showed a considerably smaller daily decrease in running time than the other three groups (Fig. 2). Runway performance was most strikingly impaired for the three rats with the most complete ablations of the locus coeruleus (these animals also had the lowest cortical norepinephrine contents). In contrast, the control rats all showed a rapid decrease in running time within the first 5 days of testing. There were no differences between the groups in the rate of weight gain after the operation, nor in the amount of exploratory activity as measured in an open field apparatus at the completion of runway testing. Most of the 22 group LC rats with incomplete lesions showed decreases in latency comparable to those in the three control groups, although in a few animals the decreases were somewhat delayed. None was as impaired as the six rats with the most extensive ablations of the locus coeruleus. The mean cortical norepinephrine content in these 22 rats was 175 ± 14 ng/g.

These experiments show that small bilateral lesions in the mid-pontine region, which include the nucleus locus coeruleus on both sides and deplete the cortex of norepinephrine, can markedly impair or even abolish the reward-induced increase in running speed in an L-shaped runway. It is unlikely that this impairment can be attributed to a "motivational" deficit, since the food intake and rate of weight gain of rats with lesions were similar to those of the controls. It is also unlikely that the impairment can be explained in terms of a motor or performance deficit, since

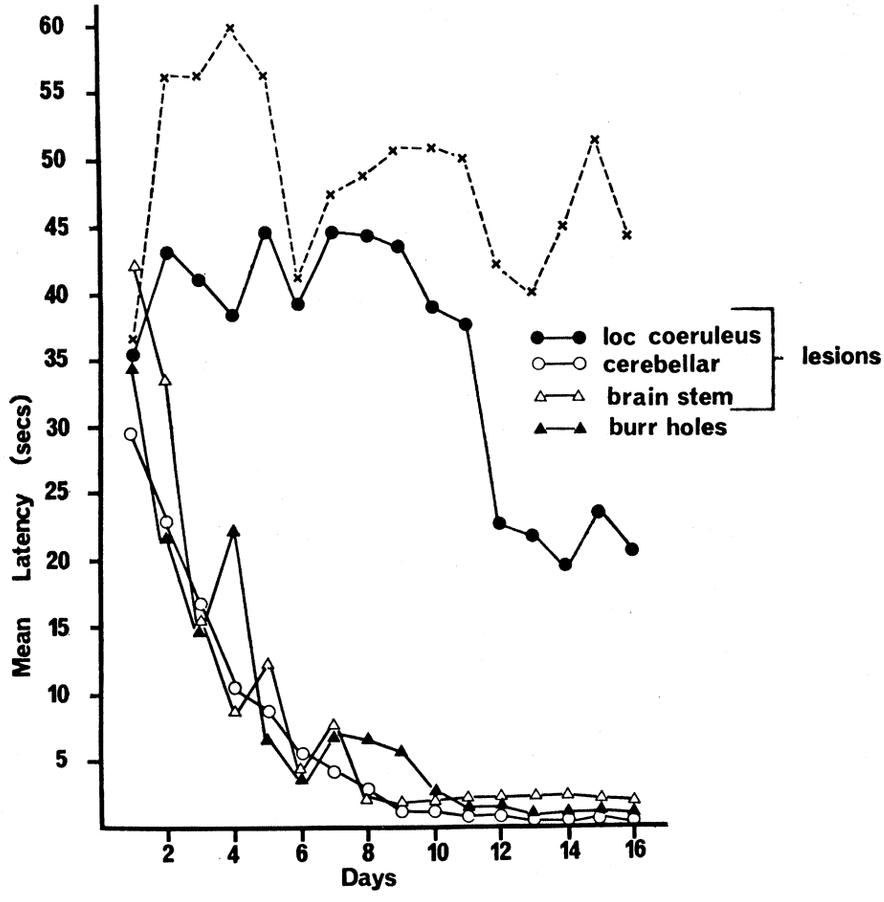

Fig. 2. The mean latencies (time taken to run 120 cm) in the initial arm of an L-shaped runway for the four groups of rats. The dotted line shows mean latencies for the three rats in group LC with the most complete bilateral ablations of the nucleus locus coeruleus; loc, locus.

the initial latency in the runway was not significantly different from that of controls, no motor defect was apparent, and the animals showed the same levels of exploratory activity as did the controls. In other experiments we have shown that these animals are not impaired in ability to discriminate sucrose solution from water. We therefore interpret our data as evidence of defective learning capacity, and our results are in obvious contrast with those of Lashley (*10*), who found that even large lesions of the cerebral cortex itself had relatively little effect on the animal's ability to learn simple tasks.

Previous experiments suggest that coerulocortical norepinephrine-containing neurons are one of two catecholamine systems supporting electrical self-stimulation behavior (*5, 6*). The earlier data, taken together with the present results, suggest that this pathway may function as a "reinforcement" system. This concept would be consistent with hypotheses that the noradrenergic terminals in the cerebral cortex mediate the synaptic changes assumed to take place during learning (*11, 12*).

G. M. ANLEZARK
Physiology Department, Marischal College, University of Aberdeen, Aberdeen, Scotland AB9 1AS
T. J. CROW
Psychiatry Department, University Hospital of South Manchester, Manchester, England M20 8LR
A. P. GREENWAY
Psychology Department, King's College, University of Aberdeen, Aberdeen, Scotland AB9 2UB

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Memory Mechanisms in Instrumental Responding

John's main discovery (*1*) is that when the occurrence of an instrumental response (for example, lever pressing to obtain food or to avoid an electric shock) has been brought under the control of a positive discriminative conditioned stimulus (CS+), each presentation of the CS+ releases a neural "readout component," which is recordable as a set of uniform high-amplitude evoked potentials in diverse brain structures. Such a readout component—the particular evoked-potential waveform—is not produced by a negative discriminative conditioned stimulus (CS−); the waveform produced by a CS− is quite different. The further observation that the readout component is not produced by a generalization test stimulus (CS^G) on occasions when it fails to instigate the instrumental response, but is produced by the same CS^G on occasions when it instigates the instrumental response, indicates that the release of the readout component may be a necessary link in the sequence of neural events that results in response occurrence. The interpretation that John places on his findings is essentially that the effective stimuli (CS+ or CS^G) are successful in instigating the response because they activate a unique, specific memory that has become organized during training with CS+. He equates the activation of a memory with the release of the readout component. This interpretation is disputable on two points: Does the readout component represent memory? How is it produced?

It is unlikely that the readout component represents memory per se. A memory presumably is a neural correlational organization representing the correlation (or contingency) that an animal has observed to exist between two environmental events. Thus the memory CS+ : US^I represents the correlation that CS+ is followed (with or without an intervening response) by an unconditioned stimulus with incentive (or "reinforcing") properties (US^I), and the memory CS− : $\overline{US}^I$ represents the correlation that CS− is followed by the absence of the incentive stimulus ($\overline{US}^I$). It is known that behaviorally the presentation of a CS− is not equivalent to either the absence of CS+ or to the presentation of a familiar neutral test stimulus (CS^0)—one which is not correlated, positively or negatively, with any incentive stimulus and to which the animal has been habituated. In general, compared to the presentation of a CS^0, CS+ facilitates instrumental responding while CS− suppresses responding (*2*). Clearly, a memory is organized not only in the case of positive learning (CS+ : US^I), but also in the cases of negative learning (CS− : $\overline{US}^I$) and habituation learning (CS^0 : no important consequence). Thus, if a readout component represents a memory, it (the component) ought to be produced not only in the case of the response-facilitative CS+ but also in the cases of the response-suppressive CS− and the habituated CS^0.

John, however, uses the label "readout component" to describe only the waveform produced by CS+, not that produced by CS− (or CS^0). But if a waveform represents a memory per se, each training stimulus (CS+, CS−, or CS^0) should produce some characteristic readout component. Any difference between the waveform produced by CS+ and the waveform produced by CS− (or CS^0) could then be attributed to the contents of the two memories. But, alternatively, the difference in waveforms could also be attributed to some other process affecting response output. This could be a motivational process; response-facilitative in the case of CS+ which is linked to the presentation of the incentive stimulus (US^I), and response-suppressive in the case of CS− which is linked to the nonpresentation of the incentive stimulus ($\overline{US}^I$). Such an alternative interpretation could be tested by determining whether variations in motivational