As expected, PCPA treatment lowered the concentrations of 5-HT to about 10 percent of that in control animals, whereas concentrations of NE fell to 71 percent of that in normal animals (Table 2). After administration of pargyline to the animals treated with PCPA the 5-HT and NE concentrations rose to 20 and 100 percent of that in normal animals, respectively.

Brain serotonin inhibits sexual behavior in male rats. The increase in sexual excitement elicited by inhibition of monoamine oxidase suggests that the relative balance of serotoninergic and noradrenergic tone in brain may control sexual behavior in male animals. The demonstration that PCPA elicits sexual excitation in pinealectomized rats rules out the possibility that action of PCPA is mediated by inhibition of pineal indole hormones derived from 5-HT. The above considerations and the finding that sexual excitation is blocked by 5-hydroxytryptophan suggest that the changes in sexual behavior produced by PCPA alone or by PCPA with pargyline are the consequence of the depletion of 5-HT in the brain and of the secondary unbalance between 5-HT and catecholamine activity in brain. *p*-Chlorophenylalanine increases the number of copulations and ejaculations in male animals exposed to receptive females.

After we completed these studies, an abstract appeared which refers to the sexual stimulating effect of PCPA in male rats (*13*). The sexual stimulation produced by PCPA alone and in combination with pargyline is not restricted to male rats. Rabbits injected with PCPA and pargyline also displayed a compulsive sexual behavior that lasted up to 3 days.

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Avoidance Learning: Long-Lasting Deficits after Temporal Lobe Seizure

Abstract. *Microinjections of carbachol (carbamylcholine chloride) into the amygdaloid complex of rats produced behavioral and electrophysiological seizures which subsided within 24 hours. A persisting functional change caused a deficit in avoidance learning 1 to 3 weeks after the seizure. A cholinergic system is implicated by the fact that cholinergic blockade (scopolamine) of the amygdala during training reversed the effects of the seizures induced by carbachol.*

Microinjections of chemicals which initiate, facilitate, or inhibit synaptic transmission have been used extensively to produce temporary changes in the activity of discrete brain areas (*1*). In general, the induced changes are reversible, and the organism appears electrophysiologically and behaviorally normal within a few hours after the drug application. A notable exception has been reported after chemical stimulation of the amygdala with cholinergic substances such as acetylcholine or carbamylcholine chloride (carbachol). In the cat, highly abnormal electroencephalographic patterns have been seen in the affected region as long as 5 months after a single injection. This is accompanied by pronounced "personality changes" (extreme viciousness and aggressiveness) which make it essentially impossible to study in detail other behavioral reactions to the injection (*2*).

Long-lasting behavioral and electrophysiological changes have been seen after electrical stimulation of the amygdaloid complex (*3*), and a permanent reduction in amygdaloid seizure threshold has been produced by repeated electrical stimulation (*4*). The apparent lability of amygdaloid neurons is particularly intriguing in view of the evidence which suggests that this portion of the temporal lobe may play an important role in the acquisition of new responses (*5*). We therefore investigated the possibility that a carbachol-induced change in amygdaloid function might specifically affect an animal's ability to learn a novel conditioned response. We elected to perform these experiments on the albino rat in the expectation that behavioral testing might be possible in this species even after the expected changes in emotional reactivity.

Double-walled cannulas were stereotaxically implanted bilaterally into the ventral aspects of the amygdaloid complex of male albino rats of the Holtzman Sprague-Dawley strain. The surgical procedure and construction of the implant have been described (*6*). For some of the animals, the cannulas were insulated with a vinyl enamel except for the cross section of their tip, and an additional silver wire electrode was implanted into a third brain structure. A silver ball, embedded in the skull, served as the reference electrode.

The training apparatus consisted of a box, 92.5 by 92.5 by 61 cm, with a grid floor, white Plexiglas walls, and transparent Plexiglas top. The box was partitioned into four equal compartments. The middle of each partition contained an opening (10 by 10 cm) covered with a black door which opened in one direction only. The doors were locked by a solenoid until the beginning of a trial. The conditoned stimulus (CS) consisted of the illumination of a light bulb mounted in the center of the ceiling of each compartment. The unconditioned stimulus (USC) was a 300-μa grid shock supplied by a high-voltage, vacuum-tube-controlled constant-current source (*7*).

Immediately before training, the animals were shaped, by a series of successive approximations, to open the doors between compartments. The CS and UCS were not presented during these habituation trials. After an animal was habituated, all the doors were locked, and the animal was confined in one compartment. After 60 seconds the door to the next compartment was un-

locked, the CS was presented alone for 10 seconds, and then the shock was administered. The CS or CS-UCS combination was terminated, and a 60-second interval was initiated as soon as the animal opened the door to the next compartment. If no escape response occurred after 90 seconds of shock, the trial was terminated, and the interval started. If the animal did not leave the shock compartment after opening the door, in order to avoid or escape on the next trial, it had to go through two successive doors.

Seven animals received bilateral injections of 0.5 to 2.0 μg of crystalline carbachol into the amygdaloid complex. Within minutes after these injections all animals developed clonic convulsions accompanied by high-voltage epileptiform spike activity in the temporal lobe electroencephalogram. The motor disturbances subsided within a few hours, and the abnormal brain activity disappeared completely within 24 to 48 hours. All animals appeared behaviorally and electrophysiologically normal within 1 to 3 weeks after the injection. At this time avoidance training was begun. Records of electroencephalogram activity were obtained from one-half of the carbachol-treated animals immediately before and after each of the first seven daily training sessions. The performance of the carbachol-treated animals was compared with that of unoperated (UC, $N=6$) and cannulated controls (CC, $N=4$). Six additional animals received bilateral injections of 2 to 4 μg of scopolamine hydrobromide into the ventral amygdala immediately before each daily test session. All animals received 10 trials per day for 16 consecutive days; training was then terminated because all groups appeared to have reached asymptotic performance.

At the end of the experiment frozen sections of the brains (50 μm) of all cannulated animals were stained with Luxol blue and cresylecht violet (*8*). Microscopic examination of the material showed that the tips of the cannulas were located in the ventral amygdala in all animals. An attempted correlation between the electrophysiological, behavioral, and histological data failed to demonstrate evidence for discrete localization of effects within the amygdaloid complex.

Microscopic analysis of the material showed necrotic tissue within 0.2 to 0.5 mm of the tip of the cannula implants. The amount of discernible structural damage was entirely comparable for carbachol- and scopolamine-treated animals which showed opposite behavioral effects after treatment. Visual inspection of encephalographic recordings obtained from the injection sites before and after the training period did not reveal evidence of electrophysiological abnormalities at the time behavioral training was begun.

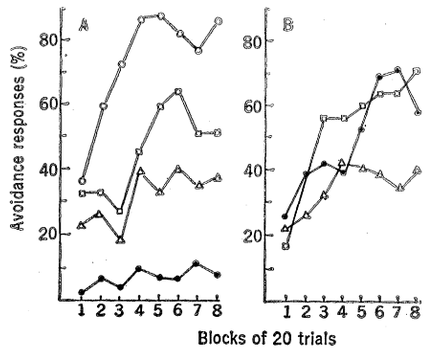

Fig. 1. Average percentage of avoidance responses for consecutive blocks of 20 trials. (A) Comparison of animals receiving a single injection of carbachol into the amygdala 1 to 3 weeks prior to training (solid circles) or daily scopolamine injections into the amygdala during training (open circles) with normal (triangles) and cannulated (squares) controls. (B) Comparison of animals receiving amygdaloid lesions (squares) or a single injection of carbachol into the amygdala followed by daily scopolamine injections into the amygdala during training (solid circles) with normal controls (triangles).

The number of avoidances in successive blocks of 20 trials (Fig. 1A) were analyzed statistically with a two-way analysis of variance for main effects (*9*) and the Newman-Keuls test for comparison of individual groups (*10*). A similar statistical analysis was made on response latencies.

The carbachol-treated animals showed little or no evidence of conditioning throughout the experiment and their performance was significantly poorer than that of either the normal ($P<.05$) or cannulated ($P<.01$) controls. An analysis of the animals' response latencies showed a similar, statistically reliable deficit in all instances.

Scopolamine injections, given immediately before each daily training session, produced a marked facilitatory effect. The overall avoidance performance of the scopolamine-treated animals was reliably better than that of the unoperated ($P<.01$) and cannulated ($P<.05$) controls and vastly superior ($P<.01$) to that of the carbachol-treated animals.

These results suggest that a single injection of carbachol into the amygdaloid complex produces a long-lasting facilitation of a cholinergically mediated pathway which inhibits the acquisition of conditioned avoidance responses. To further demonstrate the specificity of this effect a second experiment was performed.

Large bilateral lesions were made in the amygdaloid complexes of six animals by passing 2 ma for 40 seconds through a stainless steel electrode inserted at the same coordinates as the cannula placements of the preceding experiment. Four additional animals received a single bilateral injection of carbachol into the amygdaloid complex, 1 to 3 weeks before the beginning of training, and daily bilateral injections of scopolamine during training just before the first trial of each day. The performance of the experimental animals was compared to that of normal animals shown to be essentially similar to cannulated controls in the first experiment.

Microscopic analysis of the histological material from all experimental animals showed that all lesioned animals sustained damage to both corticomedial and basolateral nuclei of the amygdala. Some animals showed additional damage to the pyriform cortex, and two animals had slight damage to the ventral hippocampus. Animals with amygdaloid lesions made more conditioned avoidance responses than control animals (Fig. 1B). Daily scopolamine injections apparently reversed the inhibitory effects of preceding carbachol injections and facilitated avoidance acquisition above the level of controls. The scopolamine-injected animals in this experiment were superior to the carbachol-treated animals of the first experiment ($P<.01$). The differences between scopolamine-treated and control animals in this experiment were somewhat more variable and did not reach customary statistical significance ($P>.05$). However, since the performance of the animals in this experiment was clearly opposite in direction to that produced by carbachol injections, we feel justified in offering the following conclusions.

The injection of a cholinergic stimulant into the amygdaloid region of rats' brains produces long-lasting functional changes which are reflected in an inhibition of learning in a simple avoidance situation. This impairment seems to reflect an increase in sensitivity

rather than a functional lesion since electrolytic lesions, as well as microinjections of a cholinolytic inhibitor, improved learning. A cholinergic mechanism seems to be implicated since local injections of a cholinolytic agent reversed the effects of cholinergic stimulation.

The apparent increase in sensitivity of amygdaloid neurons following intense activation may be due to processes similar to posttetanic potentiation. The duration of the facilitatory effects in our experiments is of a different order of magnitude than that typically observed after tetanic stimulation. However, Goddard's (*11*) finding that repeated electrical stimulation of the amygdala produces similarly prolonged facilitatory effects on seizure thresholds suggests that the amygdaloid complex may be uniquely sensitive to such influences.

Although it is tempting to relate the apparent lability of cholinergic components of the amygdala to registration processes potentially useful in learning, our data do not require such an interpretation. An alternative explanation would stress changes in emotional sensitivity, but our animals did not demonstrate any evidence of excessive emotionality at the time of training. Goddard (*12*) has reported changes in some fear-motivated behaviors following microinjection of smaller doses of carbachol to the amygdaloid complex, but these injections did not alter the acquisition of active avoidance responses. The carbachol-induced inhibitory effect must be studied in appetitive learning situations before interpretations in terms of changes in emotional reactivity can be discounted.

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Why Is the Moon Gray?

If water ever was emitted from the lunar surface, as several scientists believe (*1*), there should have been a tendency for the surface to turn orange as a result of the oxidation of ferrous oxide in the rocks by photolytically liberated oxygen. The question therefore arises—"Why is the moon gray and colorless?" I suggest that the answer lies in the solar wind's bringing in atomic hydrogen to replace that lost by the photolytic decomposition of water vapor.

For a gaseous molecule leaving the lunar surface with a speed v (in excess of the escape velocity) the chance of escaping is $1 - v_E/v$ where v_E is the escape velocity (2.4 cm/sec). By averaging we obtain $0.28\ [\exp(-v_E/\bar{v})^2]/(v_E/\bar{v})$ where $\bar{v}$ is $(2kT/m)^{\frac{1}{2}}$, where T is the absolute temperature (400°K for the lunar day), k is the Boltzmann constant, and m is the mass of the molecule. Thus, for water, the escape probability per pass is 2×10^{-8}, and the escape time is substantial. Each pass takes about 240 seconds ($\bar{v}/g$) on the average; so with no time spent in the adsorbed state the escape time is about 300 years spent entirely in the lunar atmosphere. (Actually it will be twice as long because escape will not take place in the lunar night.) It will be longer if there is appreciable time spent in the adsorbed state between passes, as seems likely.

My essential point is that the escape time is so long that the solar ultraviolet light will dissociate the water molecule to give a hydrogen atom, which will escape, and a hydroxyl radical. The hydroxyl in turn will either oxidize the surface rocks or will be further photolyzed to give atomic hydrogen and oxygen. The oxygen will certainly oxidize the rocks on contact. Thus we may be able to use the color of the surface rocks on the moon as an indicator of the oxidizing or reducing nature of the lunar environment.

Other than the oxidizing influence of escaping water vapor we have the solar wind, which is reducing, and the color may give us a means of balancing these two influences—water causes atomic hydrogen to leave the moon, and the solar wind, being essentially pure hydrogen, counterbalances by bringing atomic hydrogen into the moon (*2*).

The solar wind consists, on the average, of about five protons per cubic centimeter traveling at about 300 km/sec (*3*). Thus about 10^8 hydrogen atoms each with an energy of 450 ev impinge each second on each square centimteter of the sunlit side, amounting in 4.5×10^9 years to the equivalent of ½ m of water uniformly spread over the lunar surface. As the hydrogen plasma consisting of equal numbers of protons and electrons impinges, electrons neutralize the approaching protons and 450-ev hydrogen atoms hit and stick on the lunar surface. Atomic hydrogen, especially when very energetic, is a most powerful reducing agent.

It seems likely that lunar water as well as that on earth evolved very early [some terrestrial life forms are 3 billion years old (*4*)]; therefore, according to our analysis, the moon may have started out as colored and it may have gradually been reduced to its present dull gray.

The argument that planetary and lunar water must have evolved in the early part of the history of the solar system is simply that the radioactivity heat source was much stronger in the beginning. If all of the heat were derived from radioactive potassium (half-life 1.25 billion years), the average age of the oceans would be about 3 billion years, on the assumption that the rate of water vapor emission is proportional to the rate of generation of heat. It seems likely that some such number is applicable even though U^{235} (half-life 0.7 billion years), U^{238} (half-life 4.5 billion years), and Th^{232} (half-life 14 billion years), as well as shorter-lived radioisotopes such as Pu^{244} (half-life 87 million years) were substantial additional heat sources.

Therefore, according to the Rubey (*5*) theory for the origin of the atmospheres and oceans, any lunar water liberated was probably released early in the lunar history. Subsequently it disappeared, probably oxidizing the rocks. Then, slowly, over the long eons, these may have been reduced by the solar wind. If the lunar rocks have been stirred in the last 3 billion years or so