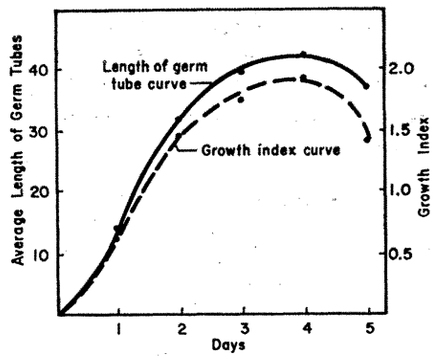

Fig. 1. Growth curves from one set of slides on which the conidia were germinated in water. The upper curve was obtained by actual measurement of the lengths of germ tubes (in micrometer units) and the lower curve by the growth index method.

nidial suspension (0.2 ml) was added to 2.5 ml of water in a screw-top tube, shaken thoroughly, warmed 20 seconds with gentle shaking in the bath, and poured into the vial which contained the agar. The agar and suspension were mixed for 20 seconds. The slides were then immediately dipped 3 cm into the suspension and hung with the dipped end downward to gel. As soon as dipping was completed the agar was scraped from the lower surface and the slides were labeled and slipped into sterile tubes containing 0.2 ml of guttation fluid. A loose roll of sterile moist filter paper was placed in the upper part of the tube above the slide, the tube was closed with a metal cap and the set of tubes was placed in an oven at 28°C. The slides were handled rapidly to prevent drying of the agar film.

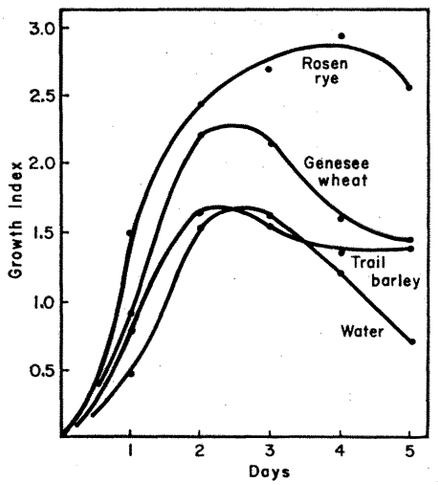

Fig. 2. Growth of *Claviceps purpurea* germ tubes in the presence of guttation fluids. Differences between treatments are: rye and wheat at 5 percent level, wheat and barley at 1 percent, barley and control at 1 percent. Susceptibility of hosts: rye, 39 percent; wheat, 11 percent; barley, none.

Growth was determined by examining 10 fields on each slide. The fields were chosen 1 cm from the lower end of the slide and at least 2 mm away from the edges. A square whose side equaled about one-third the diameter of the field was placed in the field as an ocular insert. The number of germ tubes which crossed the perimeter of the square and the number of conidia within the square were counted. Each crossing was counted regardless of the location of the conidium in the field, even though a single germ tube might be counted more than once because of position, curved growth, or branching. The growth index was computed by dividing the total number of crosses in all the fields by the total number of conidia inside the squares.

Figure 1 compares this method of determining growth with the actual measurement of the length of germ tubes in the same number of fields on the same set of slides. The curves are very similar. Since the growth index method required less than one-tenth the time, it was used in the comparative growth studies.

The guttation fluids caused a pronounced difference in growth of the germ tubes of the parasite (Fig. 2). The fluid from Rosen rye, the more susceptible host, produced the most growth; the fluid from Genesee wheat, a less susceptible host, produced less growth; and the fluid from Trail barley, the insusceptible host, produced about the same amount of growth as the water controls. Other experiments on different collections of guttation fluids and different preparations of conidia confirmed these results.

If similar results should be obtained for many other host-parasite combinations, the kind of experiment described here would be useful for testing available hypotheses (*5*) and for extending the general understanding of the host-parasite relationship. After further analyses have been completed and synthetic guttation fluids have been prepared, we may have approximated the parasite's "definitive nutrition"—that is, the complex nutritive environment which the parasite encounters once it penetrates into diffusion contact with host cells (*6, 7*).

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Cholinergic Tracing of a Central Neural Circuit Underlying the Thirst Drive

Abstract. Cholinergic stimulation of any of a number of interrelated limbic and diencephalic structures in the rat elicits a rapid and marked increase in water intake. We postulate that a generalized Papez circuit mediates the thirst drive, that the circuit is specifically and functionally sensitive to cholinergic action, and that other primary drives depend on closely parallel neural circuits partitioned both structurally and biochemically.

Recent work with chemical stimulation of the brain (*1*) has correlated application of cholinergic drugs to the perifornical region with drinking behavior, and application of adrenergic drugs to the perifornical and far lateral hypothalamic areas with eating. The data suggest that the thirst drive is partly regulated by cholinergic action, and the hunger drive, by adrenergic action.

In early investigations in this area we were concerned with the possible central action of insulin or glucagon, or both, on brain systems related to hunger and satiety. No evidence for such action was uncovered, and early work with adrenergic drugs proved inconclusive.

A series of experiments with cholinergic drugs, however, are providing evidence that the perifornical region is only one of many brain areas in which localized application of minute quantities of cholinergic agents is followed by a marked increase in drinking.

The basic experimental procedures are as follows. Adult albino or hooded rats are prepared for experimentation by stereotaxic implantation of hypodermic guide shafts. The animals are then returned to special cages that contain feeding and drinking receptacles designed to permit continual and accurate measurement of food and water

intake. The water dispenser consists of the graduated section of a 50-cm³ buret and a detachable Plexiglas drinking well. The well is set in a plastic block to prevent spilling, and direct readings from the buret can be made during the test. On testing days, a 30-gauge cannula containing pure crystals or a chemical in solution is set in an implant connector and lowered to the target area. The technique permits introduction of 1 to 3 μg of crystalline substance or as little as 1/10,000 cm³ of solution per injection. Response to a series of placebos and experimental drugs is tested and retested at each brain site, each test being separated from the next by at least 24 hours. Food and water are continuously available to the animals, and the data recorded include measures of food and water intake for the pretest and the test hour, as well as daily 24-hour readings.

Thus far, 94 male adult rats have been permanently cannulated in a wide sampling of brain areas. Each rat has been given an extended series of brain injection tests which include at least four separate tests with carbachol. Table 1 gives the major positive brain areas thus far located in replicate testing, as well as the average amounts of water consumed, during the hour following cholinergic stimulation, by animals whose response was positive. Control measures obtained during pretest hours, and after injection of placebos and noncholinergic drugs, have been consistently and significantly below these levels. The mean hourly drinking response during pretest hours in animals whose response to cholinergic stimulation was positive was 1.4 cm³, and the mean drinking response in the hour following injection of placebos or noncholinergic test chemicals was 1.2 cm³. Brain areas which, to date, have yielded negative results (mean water intake of less than 4 cm³; maximum intake of less than 8 cm³) are as follows: posterior hippocampus, lateral hippocampus, midbrain tegmentum, amygdaloid areas, lateral thalamic areas, ventromedial area of the hypothalamus, paraventricular nucleus of the thalamus, frontal cortex, subiculum, entorhinal areas, piriform cortex, and most or all of the caudate nucleus (results were positive in one animal).

In the animals whose response was positive, drinking began 3 to 12 minutes after insertion of the carbachol-plugged cannula into the site and continued intermittently for 20 to 40 minutes in most cases. The longest periods of drinking response were recorded for animals with hippocampal placements. The precise time distribution of the drinking response after injection was not recorded in early stages of the study; additional tests are being made, with other animals, to determine whether onset and duration of drinking vary consistently with placement.

Table 1. Brain areas for which results were positive (that is, injected carbachol induced drinking).

Positive brain structures	Animals (N)	Water intake (cm³)*	
		Mean (4–8 tests/N)	Maximum
Dorsomedial hippocampus–area of dentate gyrus†	6	20.7	56
Septal regions†	6	14.0	31
Area of diagonal band†	3	17.5	40
Reuniens-mesial thalamic†	5	13.7	45
Perifornical	5	9.7	16
Preoptic regions	5	10.0	19
Lateral hypothalamic–medial forebrain bundle	6	8.5	17
Mammillary-interpeduncular regions	4	10.2	17
Anterior nuclei of thalamus (anterodorsal, anteroventral)	5	8.9	15
Cingulate cortex	3	12.5	22

* During the hour following cholinergic stimulation. † Water intake above 20 cm³ was frequently recorded.

All animals tested had a single brain implant and could be stimulated only in a single vertical plane. The cannula containing the crystalline chemical plug could be inserted to several successive depths but was never lowered beyond the point where a positive response was first obtained in the individual animal.

After a series of control and experimental tests the animals were sacrificed and the brains were sectioned and stained. The end of the track left by the implant guide shaft, or by the internal cannula if it was lower, was considered to be the stimulation site.

Several points of theoretical interest should be particularly noted. First, all of the positive areas for drinking thus far found lie within the positive reinforcement system traced by Olds and others in rats in self-stimulation experiments. Second, the data may become even more meaningful when related to a circuit postulated and outlined by Papez in 1937 (*2*). Papez suggested that a series of structures, principally including the hippocampus, the fornix, the mammillary regions of the hypothalamus, the mammillothalamic tract, the anterior nuclei of the thalamus, the cingulate gyrus, and again the hippocampus, composed an interconnected, functional circuit related to the expression of emotion in man and animals. Most of the brain areas found by us to be positive for the drinking response are either part of the original Papez circuit or closely integrated with it.

Our findings suggest that the controlling units involve the dentate gyrus and the H_1 pyramidal cell fields of the medial-dorsal hippocampus (*3*), which project principally to the mammillary regions by way of the dorsal fornix. Nauta has stated that fibers from the dorsal hippocampus of the rat also project to the septal regions, the nucleus of the diagonal band, the preoptic regions, and the anterior and midline nuclei of the thalamus. Other studies confirm his statement for many of these projections. Midline thalamic nuclei, in turn, project to the cingulate gyrus, and there is some evidence for an interconnection between lateral preoptic, lateral hypothalamic, and septal region, the septal region providing a rich two-way circuit with the dorsal hippocampus and presumably with the H_1 pyramidal fields by way of the dentate gyrus (*3*, *4*).

It is thus feasible to include all major positive areas within a medially oriented, generalized Papez circuit, although it is perhaps equally relevant at this stage to postulate a more complex interaction between medial forebrain bundle and fornix systems. The preoptic regions could be much more clearly implicated through involvement of the medial forebrain bundle, and we have preliminary but unreplicated evidence that the medial parolfactory area, a contributor to the medial forebrain bundle, may be a positive area. The overall data thus provide close correlation with findings reported in MacLean and Ploog's recent paper (*5*) concerned with the identification of primate brain areas in which electrical stimulation was correlated with penile erection, and in Robinson and Mishkin's report (*6*) relative to food and water ingestion after electrical stimulation of the primate brain. Many of the areas implicated in the three separate studies are the same, and the evidence appears strong that circuits mediating each of the primary drives will be found to follow generally parallel courses through the limbic system and diencephalon.

Specific chemical keys or stimulation techniques may be necessary to isolate and trace separate functional circuits, but such tools seem to be rapidly becoming available (*1*, *5–7*).

In this connection, our recent emphasis has been on testing animals under conditions which maximize the possibility of measuring changes related to any primary drive during chemical stimulation of the brain. One animal has been found that consistently responds to injection of carbachol by drinking, to injection of noradrenalin by eating, and to injection of a soluble steroid by building nests. All three chemicals have been applied to the same rhinencephalic locus at the junction of the area of the diagonal band of Broca and the medial preoptic region, and all three effects are specific with respect to the chemical or chemical family implicated.

Another aspect of the data worth discussing is the finding that some of the positive areas produce a significantly greater drinking response than others (see Table 1). The density of selectively sensitive neurons in an area, or the precision of delivery to a positive locus, may be involved, but other possibilities suggest themselves. All but one of the positive areas (reuniens) for which an attendant water intake of 20 cm^3 or more is frequently recorded are in the hippocampus or directly project to it. It is thus possible that sustained hippocampal afterdischarge is responsible for the prolonged and accentuated drinking that follows cholinergic stimulation of these areas. Electroencephalographic recording should reveal any correlations between separate types of hippocampal electrical activity and drinking. A second, but currently less likely, hypothesis is that the hippocampus normally functions as an inhibitory part of the drive system and that carbachol produces local seizures which temporarily disrupt hippocampal function and indirectly increase drive. Again, it should be possible to select between hypotheses by utilizing electroencephalographic techniques with animals prepared with multiple combinations of chemical and electrical implants.

Finally, it should be stated that the correlation between drinking and cholinergic stimulation of the brain is remarkably specific. It is true that we have found five cases in which injection of carbachol increased both eating and drinking, and one in which injection of either carbachol or strychnine consistently led to marked increases in food and water intake, but such cases or loci are quite rare. Animals injected with carbachol in the designated brain areas show a highly selective water-ingestion response and typically ignore stimuli allied to other primary drives for at least 20 to 30 minutes after drug injection. Such facts appear to weigh against the possibility that random firing or seizure activity in the limbic system underlies the response and that no true circuit is being traced. Indiscriminate neural firing would be expected to disrupt integrated response, or to influence a number of drives, rather than to selectively increase drinking.

In summary, the data of this study seem of particular interest because of the implication that a functional neural circuit can be traced through a selective sensitivity to a chemical agent, or to a particular range of concentration of that agent. Our own research (*7*) and that of others (*1*) has previously implicated only single or isolated loci, with little indication that entire circuits or their synaptic interconnections might be biochemically distinct. In addition, the new evidence, coupled with other recent data from studies of chemical and electrical stimulation of the brain, suggests the probability that relatively parallel neural circuits coursing through the limbic system and associated brain areas underlie the mediation of the primary drives (*8*).

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Quantitative Analysis of Blood Circulation through the Frog Heart

Abstract. Analysis of the oxygen content of blood in the frog heart and its major vessels has shown that in *Rana pipiens* the carotids receive primarily left atrial blood, which is highly oxygenated, whereas the pulmocutaneous vessels receive blood almost exclusively from the right atrium. Only the aortas receive blood subjected to considerable mixing.

In the anuran heart, blood from the left and right atria enters a single ventricle as two separate streams. This blood, after passing through a divided conus arteriosus, is distributed to three bilateral arterial trunks: the carotids, aortas, and pulmocutaneous vessels. Despite the absence of a ventricular septum, Brücke (*1*) postulated that the oxygenated left atrial stream and the less-oxygenated right atrial stream remain nearly separate when passing through the ventricle, that the more-oxygenated blood enters the carotids, mixed blood enters the aortas, and that the less-oxygenated blood enters the pulmocutaneous arteries. Vandervael (*2*) observed the passage of India ink through the ventricle and arterial trunks of *Rana temporaria* by transillumination and concluded that the blood leaving the heart had been thoroughly mixed in the ventricle. Simons (*3*), who also followed the passage of a dye injected into *R. temporaria,* and Sharma (*4*), on the basis of anatomical studies on *R. pipiens,* concluded that the blood entering the ventricle from the atria is selectively distributed to the arterial trunks. The degree to which this separation or mixing of oxygenated and less-oxygenated blood occurs in the ventricle and conus arteriosus is therefore controversial.

In the experiment reported here, oxygen occurring naturally in the blood was used to trace the distribution of the returning blood through the heart into the major arteries. Continuous breathing activity throughout the experiment was assured by only spinal pithing the frog, thus preventing injury to the nerve innervating the pharyngeal respiratory pump. A slit in the body wall was then made, extending about an inch caudad and craniad from the sternum, which itself was split lengthwise. If bleeding occurred, thrombin was applied to the cut surface and the amount of bleeding was thus kept to an estimated 0.05 ml or less. Samples of 0.15 to 0.3 ml of blood were withdrawn from the desired vessel with a No. 27 needle attached