amounts of DNA were found by the Keck method (8) in any of these eluates. The DNA appears in the tubes beyond No. 79, mixed with the remainder of the RNA (that is, 27 percent) not accounted for in the eluents up to tube No. 79. The value of A_{490} of the DNA-indole "chromosol" produced by Keck's method parallels in percentage the value of A_{260} of respective eluates in tubes 70 to 112. This is shown by the shaded portions on this chromatogram (Fig. 1*B*). The distinct break in the nature of the peaks eluted after tube 79 is reflected in the change in the ratio A_{280}/A_{260} from 0.46 to 0.545 ± 0.01. The latter value is compatible with that of solutions of the DNA sample used (0.54).

From the data shown in Fig. 1*B*, the amount of RNA separated free of DNA was calculated to be 73 percent of the total amount of RNA present in the initial mixture. These experiments were performed in duplicate and have been repeated with 1.0 mg of RNA in the place of 2 mg, with essentially the same results. In contrast to these results on calcium phosphate columns, it has been reported (9) that RNA was not resolved from a mixture of RNA and DNA on columns of ECTEOLA-cellulose.

It is to be noted from Fig. 1 that RNA (2.0 mg) desorbed from the column much earlier (that is, at lower eluent concentrations) in the presence of DNA than it did in the absence of DNA (2 mg). One reasonable explanation of this finding implies that, as the full capacity of the column is approached (but not necessarily attained) by the load of 4 mg of mixed nucleic acids, the RNA is preferentially displaced by the DNA. This same displacement phenomenon also has been observed in the chromatography of proteins and of polynucleotides in the presence of DNA (10).

In experiments briefy reported elsewhere (5), bovine plasma albumin was cleanly separated from calf thymus DNA when 20 mg of the former and 2.0 mg of the latter were chromatographed as a mixture on columns of this absorbent (11).

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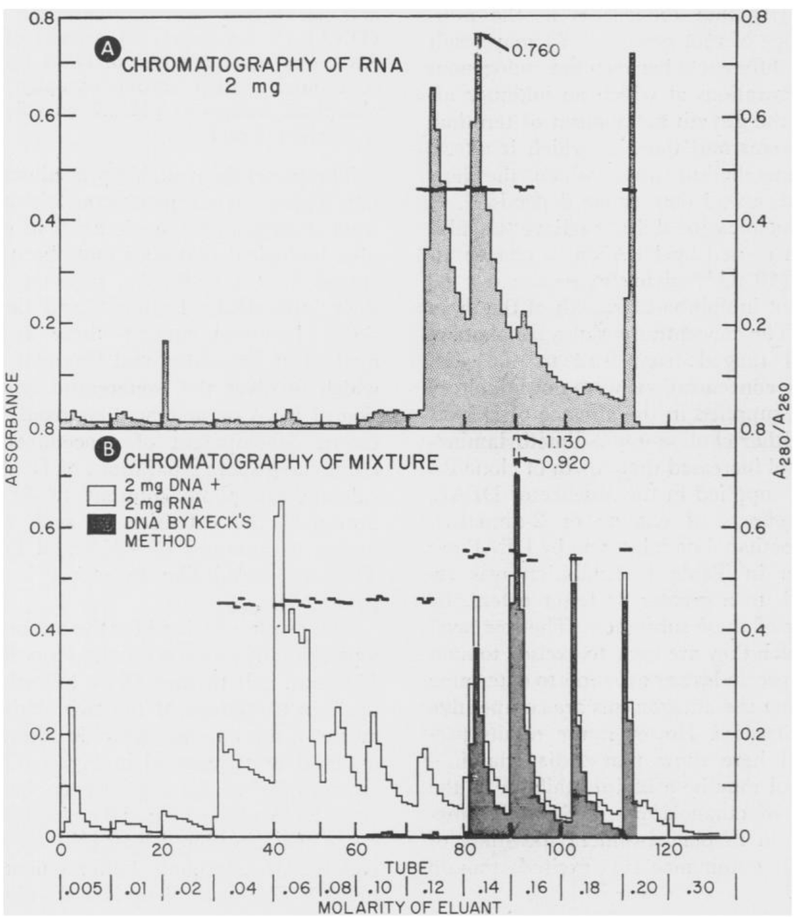

Fig. 1. *A*, Chromatography of tobacco mosaic virus RNA (2.0 mg). *B*, Chromatography of a mixture of tobacco mosaic virus RNA (2.0 mg) with calf thymus DNA (2.0 mg) on columns of modified calcium phosphate (1 by 7.5 cm) at 6° C. The solvent (4 ml volume) in which the nucleic acids were applied to the columns was 0.005*M* sodium phosphate buffer at *p*H 6.7; rate of elution, 4.8 ml/hr; tube volume 4.8 ml; discontinuous gradient elution. Eluents and phosphate buffers (*p*H 6.7) at molarities indicated. The value of A_{260} for each eluate is plotted according to the scale on the left. Values of bars, (A_{280}/A_{260}) are plotted according to the scale on the right. In *B*, the darkly shaded areas represent respective concentrations of DNA expressed in terms of A_{490} of the DNA-indole "chromosol" plotted according to the scale at the left. For concentrations of DNA (in terms of A_{260}) in the range between 0.40 to 1.15, the ratio A_{490} of the DNA-indole "chromosol" to A_{260} of a DNA solution was 1.12 ± 0.02 for any particular concentration of DNA. Recovery of DNA: 82 percent of that placed on column.

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29 September 1958

Centrifugal Arousal in the Olfactory Bulb

Abstract. The electrical activity of the olfactory bulb was recorded in awake, unrestrained cats with electrodes permanently implanted. It was found that any kind of sensory stimulation producing alertness or arousal brought about the appearance of bursts of rhythmic activity, the magnitude of which was related to the degree of alertness of the cat.

Some centrifugal fibers terminating around second-order sensory neurons, such as those of the retina and of the olfactory bulb, have been known for a long time, since Cajal's anatomical descriptions (1). However, it has only recently been shown that stimulation of these fibers may modify the electrical activity of those neurons. Electrical stimulation of the reticular formation of the brain stem, involved in wakefulness and arousal, inhibited sensory transmission at second-order neurons of the spinal cord (*2*), of the gracilis nucleus (*3*), and of the spinal fifth sensory nucleus (*4*). Photic impulses from the ganglion cells of the retina were either potentiated or inhibited (*3, 5*). As an apparent exception, the olfactory bulb was not shown to be controlled by centrifugal influences from the arousal system of the brain stem. Recruiting waves were recorded in the olfactory bulb during low-frequency stimulation of intralaminar thalamic nuclei (*6*), and suppression of olfactorily evoked activity was found during high-frequency electrical stimulation of basal rhinencephalic structures (*7*).

Pioneer exploration of the functional significance of those centrifugal influences coming from Magoun's "activating" system in the brain stem led to the discovery of sensory suppression of auditory signals at the cochlear nucleus during attention to other stimuli (*8*) and during habituation elicited by monotonous repetition of the same acoustic stimulus (*9*). Photic retinal signals recorded at the optic tract are also diminished when attention is distracted by other environmental stimuli (*10, 11*), whereas the same signals are facilitated when attention is focused upon that particular photic stimulus (*11*). So far, the retina is the only place where secondary sensory neurons have been found to be facilitated during activation of the brain-stem arousal system.

In an attempt to explore the functional significance of the centrifugal fibers to the olfactory bulb in the cat, multipolar electrodes were permanently implanted in this structure (*12*). Bipolar electrodes were also implanted in the region of the anterior commissure, and in the mesencephalic reticular formation. The electrical activity from the unanesthetized, unrestrained cat was recorded with an ink-writer oscillograph or with a cathode-ray oscilloscope. In this paper, centrifugal augmentation of the electrical activity of the olfactory bulb is reported.

When the cat was awake but relaxed, a uniform low-voltage and fast-frequency activity was observed in the olfactory bulb, simultaneously with large, slow waves in the septal area. But when the cat was alerted by any environmental stimulus, simultaneously with a "desynchronization" of the tracing in the septal area, typical burst discharges of rhythmic (34 to 48 per second) activity appeared in the olfactory bulb. The magnitude and duration of the "arousal discharges" had a direct relationship to the degree of alertness or excitement of the animal. Often, the intermittency of these discharges varied between 20 and 25 per minute—that is, within the range of the respiratory rate. However, no constant relationship was observed between any of the respiratory phases and the initiation of the "arousal discharges."

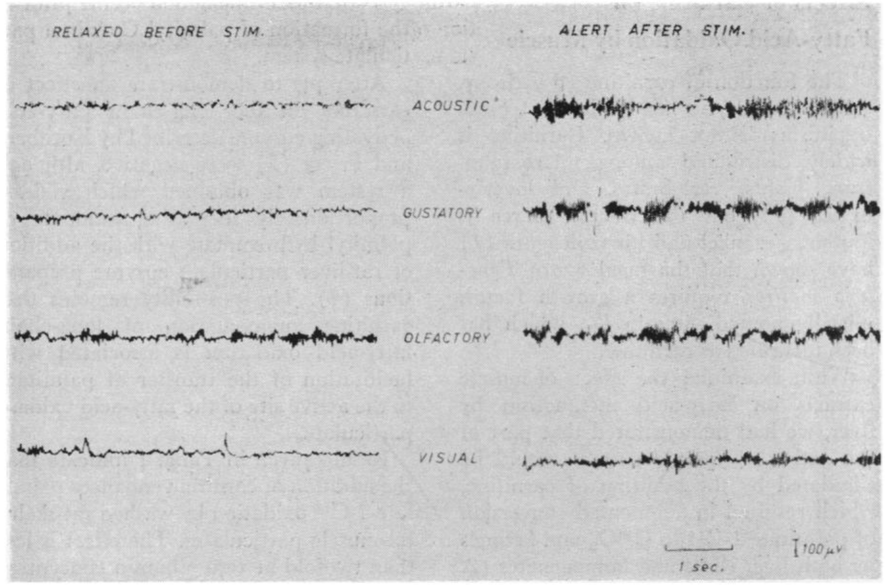

Fig. 1. Electrical activity of the olfactory bulb, showing the effects produced by stimuli of various sensory modalities which elicited behavioral alertness.

It must be emphasized that the observed increase of activity in the olfactory bulb was elicited not only by odors but also by visual, acoustic, somatic, or gustatory stimulation. Figure 1 shows the effects produced in the cat's olfactory bulb by stimulation of various sense modalities. The fact that stimuli of multiple sensory modalities were capable of yielding the same activating effect in the olfactory bulb suggested that the responsible centrifugal influence proceeds from a region of sensory convergence related to arousal. Indeed, electrical stimulation of the mesencephalic reticular formation (50 cy/sec during 2 to 3 sec) brought about, simultaneously with the alerting behavior of the cat, the appearance of "arousal discharges" in the olfactory bulb and "desynchronization" of the electrical activity in the septal area. On the other hand, central anesthesia or a lesion in the mesencephalic reticular formation which rendered the cat unconscious eliminated and prevented the appearance of "arousal discharges" through any kind of sensory stimulation.

From the above-mentioned experiments it is evident that, during arousal and alertness, activation is produced in some intrinsic elements of the olfactory bulb by centrifugal influences from the mesencephalic tegmentum of the brain stem.

Centrifugal activation of the olfactory bulb during arousal may be a physiological mechanism for enhancing the sensitivity of the olfactory apparatus—so important for exploration of the environment in macrosmatic animals such as the cat. The observation that olfactory stimulation inhibits the "arousal discharges" in the olfactory bulb (*13*) supports this interpretation. Besides discharges from silent elements, cessation of background activity produced by the olfactory stimulus provides useful information to the brain.

The "arousal discharges" from the olfactory bulb may prove to be a useful index for objective recording of the degree of alertness in the cat.

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24 July 1958