percent aqueous solution of gelatin (*4*); control rats received the vehicle alone. Upon weaning, the animals were fed diets containing 0, 80, or 160 ppm of *N*-hydroxy-*N*-2-fluorenylacetamide (*5*). After 26 weeks the rats were killed and carefully autopsied; selected organs were weighed and fixed for histopathological examination.

Male and female rats treated once at birth with estrogen had atrophic gonads; the females also had smaller uteri. The livers of the control animals not treated with carcinogen and injected with hormone were lower in weight than is customary in Fischer rats (2.9 to 3 g per 100 grams of rat; Table 1). The incidence of hepatoma in male rats fed 160 ppm of carcinogen was similar in groups injected with estrogen or vehicle. At the lower dosage of carcinogen, however, the single neonatal dose of hormone resulted in more rats with heavier livers and with hepatoma than among the controls. In female rats the effect was accentuated, especially at the higher carcinogen dosage; the hormone-treated groups had higher liver weights and cancer incidence at the 160-ppm carcinogen dosage, while at the lower dosage the precancerous lesions were more advanced.

It appears, therefore, that neonatal injection of hormone exerts a powerful effect on both the direct sexual behavior of rats (*1*) and the physiological and pathological processes subject to hormonal control mechanisms—including the induction of liver cancer. Detailed analysis of the complex elements participating, such as function and differentiation of the components of the endocrine system, in relation to the hormonal requirements for liver carcinogenesis may contribute to both psychophysiology and knowledge of cancer.

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Inhibition of the Carotid Sinus Reflex by Stimulation of the Inferior Olive

Abstract. *A projection of nerve fibers from rostral brainstem areas, which produce pressor responses and tachycardia, terminates in the inferior olive. Electrical stimulation of the olive in the cat produces no cardiovascular response but inhibits the depressor component of the carotid sinus reflex.*

Other than as a source of afferent input to the cerebellum, the inferior olive has been considered a nearly "nonfunctional" structure, despite its size and interesting morphology. The afferent projections to the inferior olive from other central neural structures (*1*), as well as the systematic projection from the inferior olive to the cerebellum (*2*), and the olive's status as the font of the olivospinal tract are well known anatomically. These connections, however, account for only an ill-defined role in motor coordination.

Surprisingly, anatomical study of the autonomic pathways in the brainstem included a projection to the medial portion of the olive from various diencephalic and midbrain structures in rats (*3*), cats, and dogs (*4*). The common feature of these disparate structures is that electrical stimulation of them uniformly results in large sustained increases in arterial pressure and tachycardia. Although stimulation of the portion of the olive receiving these projections might consequently be expected to have cardiovascular sequelae, stimuli localized to this area produced little or no change in cardiovascular function.

The consistent anatomical projection from cardiovascularly responsive areas in the more rostral portions of the brainstem can be reconciled with the absence of such responses to stimulation of the inferior olive, by postulating that the olive provides an inhibitory input to a cardiovascular reflex. The carotid sinus reflex immediately comes to mind. It is known that large sustained pressor responses accompanied by tachycardia are elicited by exercise or emotion as well as by diencephalic stimulation. Why the powerful carotid sinus reflex does not curtail this pattern of responses has never been explained.

The hypothesis that the "silent" effect of inferior olive stimulation is inhibitory to the carotid sinus reflex was tested in the following manner.

Ten cats were anesthetized with α-chloralose in water (40 mg/kg body weight), administered intraperitoneally. Femoral arterial blood pressure was recorded on a polygraph. The carotid arteries were exposed bilaterally, and loops of heavy suture were placed around them although the arteries were not occluded. A tracheal cannula was inserted. The cats were placed in a stereotaxic instrument, and the medial portion of the cerebellum was exposed in preparation for the insertion of stimulating electrodes into the medulla. The loops of suture around the arteries were connected to the shaft of a synchronous clock motor so that application of current to the motor stretched the arteries until the tissue resistance stalled the motor, thereby maintaining a constant pull on the arteries. Stretching of the carotids in this fashion gave a consistently repeatable bradycardia and a decrease in blood pressure.

After several control sinus reflex responses were obtained in this manner a coaxial stimulating electrode was stereotaxically placed in the brainstem in the area ranging from posterior 9 to 13, and 0.5 to 1.5 mm off the midline. The dorsal-ventral dimension

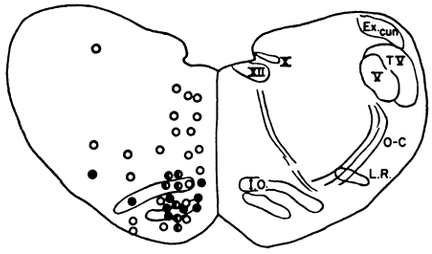

Fig. 1. Map of stimulation loci in the caudal portion of the inferior olive. Open circles are stimulation sites which exert no inhibitory effects on carotid sinus reflex. Half-filled circles are sites which showed an inhibition of only the cardiac component of the sinus reflex. Filled circles are sites which inhibited both bradycardia and depressor effects of the reflex. Dorsolateral portion of the medulla was not investigated systematically because the larger pressor responses elicited from that area make the resultant interaction difficult to interpret.

was systematically explored from DV − 6 to DV − 12 in steps of 0.25 mm. Stimulation parameters consisted of negative square-wave pulses, 100 per second, 0.1 msec long, and either 2.5 to 30 volts from a Tektronix pulse generator or 0.3 to 0.8 ma from a constant current stimulator. Stimulation trains of 10 to 25 seconds were used. Periodically, the stimulation epochs were presented simultaneously with the stretch of the carotid arteries. The resulting interactive responses were compared with control sinus reflexes elicited alone several minutes before and after the interaction. The portion of the inferior olive of special interest was usually localizable as the "silent" area immediately ventral to the depressor-inspiratory apneic area of the ventromedial reticular formation (*5*). As pure a "silent" response as possible was obtained by manipulating the parameters of stimulation intensity. When desired, the point of stimulation was marked with the Prussian-Blue technique.

Interaction between medullary stimulation and carotid stretch was infrequent. Most stimulation points yielding no alteration of heart rate or blood pressure also failed to affect the magnitude, latency, or duration of the carotid sinus reflex. However, at certain locations the stimulation consistently inhibited the bradycardia and depressor components of the sinus reflex (Fig. 1). As illustrated, these points are referable to the medial portion of the inferior olive at the level of the *area postrema*. An example of the inhibitory effect is given in Fig. 2. Stimulation of the more rostral portion of the olive demonstrated little inhibitory interaction.

Although the interaction could be interpreted as a peripherally mediated phenomenon summating algebraically, rather than as a central nervous inhibitory mechanism, some response to stimulation would then be expected if the former were the explanation. It might also be expected that if stimulation of the olive is inhibiting the tonic sinus activity that there should be some increase in rate and pressure to the stimulation. At times such increases were observed, but in order to avoid the possible objection mentioned above, the stimulation parameters were changed until the increases were minimum or absent.

The existence of this inhibitory effect gives some insight into how the higher levels of the cerebrum may govern the cardiovascular system to fit the requirements of the intact, behaving organism. Increased blood pressure during episodes of exercise or emotion would aid in insuring high flow rates to critical structures. This result is probably effected via a diencephalic mechanism (*6*).

If the baroceptor reflexes are left unimpeded to counter the increased blood pressure, they could well reduce perfusion pressure to ineffective levels. It seems reasonable to expect that the diencephalic integrative system provides some mechanism for turning off these reflexes as well as for initiating the increases in rate and pressure during periods of stress. It is suggested that the anatomical pathways descending from the diencephalic and midbrain areas controlling cardiovascular pressor responses and ending (among other places) in the medial portion of the inferior olive serve this function. Preliminary results indicate that the effect is mediated through the cerebellum, as would be suggested by the work of Moruzzi (*7*) and Reis and Cuénod (*8*).

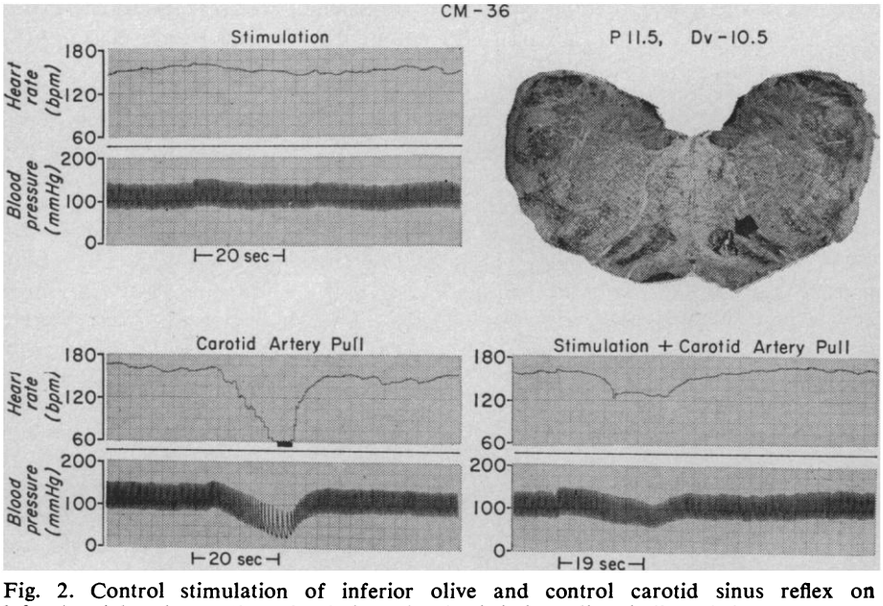

Fig. 2. Control stimulation of inferior olive and control carotid sinus reflex on left. At right above, the stimulation site in inferior olive indicated by arrow. At right below, the inhibitory effect of the inferior olive stimulation on the carotid sinus reflex.

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