females were injected with progesterone for 8 days and then paired with normal males, revealed marked aggressiveness on the part of females injected neonatally with testosterone (Table 2); fighting among such pairs was often vicious and usually initiated by the females. Females treated with estradiol also fought with males, but both the incidence and severity of fights were lower. No fighting was noted among pairs in which the female had been injected only with oil in infancy. No vaginal plugs were found in any females receiving steroid neonatally, but 55 percent of the females injected with oil had plugs during the 3 days after pairing.

The effects of neonatal injections of estradiol or testosterone on vaginal cycles and reproductive tracts were similar to previous findings (*2*, *4*) and will be reported here only to an extent necessary for correlation with the behavioral data. Neonatal injections of estradiol in males resulted in decreased body and reproductive organ weights and relative aspermia. Injections of testosterone in infancy also decreased weights of male organs but to a lesser extent than that caused by estradiol (Table 3). All vaginal smears obtained from all females injected neonatally with either steroid contained approximately 80 percent cornified cells and 20 percent leukocytes, and ovaries of such females were polyfollicular and devoid of corpora lutea. Body and uterine weights were increased among females injected neonatally with testosterone.

Androgen is a necessary prerequisite for attack behavior in inexperienced male mice (*9*), whereas estrogen administered during adulthood has no effect on aggressiveness of males (*10*). The reduction in spontaneous aggression shown by males injected with estrogen in our study was correlated with large changes in their reproductive tracts, and secretion of testicular androgen was probably considerably reduced. Weights of reproductive organs were also lower in males given neonatal injections of androgen, but they were as aggressive as control males. These facts suggest that those males injected with androgen neonatally probably had sufficient androgen in their circulation during adulthood to permit a high degree of aggressive behavior, whereas those that received estrogen did not. The amount of fructose in seminal vesicles, a good correlate of androgen titers (*6*), was reduced by 72 percent among males given estradiol in infancy compared to that in controls given oil (Table 3). The comparable figure for males receiving testosterone neonatally was only 21 percent and, hence, the postulate appears reasonably good on this basis.

The low incidence of spontaneous aggression found among control females agrees well with observations of other workers using mice (*11*). Androgen will not increase aggressiveness in either immature or mature gonadectomized females (*12*). However, neonatal injections of testosterone, and to a lesser extent estradiol, increase aggressiveness in females after maturity. These effects were significant in both experiments although more dramatic in the uncontrolled secondary experiment where some previously tested females were paired with naive males in the females' home cages after receiving progesterone to induce estrous cycles. Under such conditions mating did not occur, and the females usually attacked and sometimes wounded males. Wounding was sufficiently severe to cause death in one case. The reasons for the dramatic effects observed in this experiment are not readily obvious because of its uncontrolled nature and the data are presented only as an extreme example of a phenomenon observed in the primary experiment. Two investigators have reported that "masculine or aggressive responses" interfered with normal female sexual behavior when rats were treated with estrogen or testosterone in infancy (*13*) but not to the extent shown in the present study with mice.

A reasonable hypothesis to explain the increased aggressiveness of females treated neonatally with gonadal hormones is the alteration of a neural mechanism whose sexual differentiation is normally regulated by androgen in infancy. Such a concept parallels the conclusions of many studies dealing with either sex behavior or the hypothalamic control of gonadotropin secretion, and some degree of experimental mimicking of androgen by estrogen is well documented in this respect. It does not seem reasonable at this time, however, to suspect the hypothalamus at the expense of other neural structures because the number of brain areas known to function in aggression is relatively large (*14*). Furthermore, as evidenced by changes in body weight in both sexes, the effects of early administration of steroids may be widespread.

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Lysergic Acid Diethylamide: Sensitive Neuronal Units in the Midbrain Raphe

Abstract. *Units in areas of the midbrain rich in neurons containing serotonin respond to parenteral injections of* d-*lysergic acid diethylamide by a reversible cessation of spontaneous activity. The dose required is at or below threshold for gross behavioral effects. An inhibition of neurons containing serotonin after administration of* d-*lysergic acid diethylamide could account for the decreased metabolism of serotonin produced by this drug.*

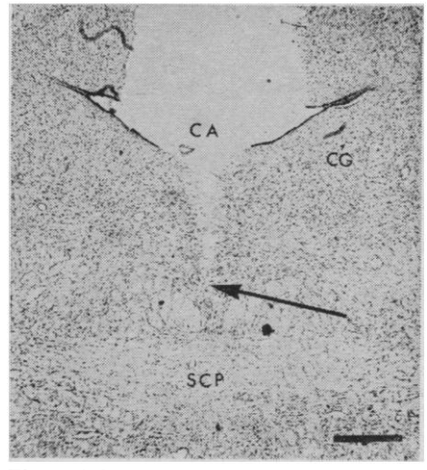
In the caudal midbrain raphe we find neurons whose spontaneous firing is reversibly inhibited by small doses of parenterally administered LSD (*d*-lysergic acid diethylamide). The caudal midbrain raphe is the locus of two large aggregates of neurons containing serotonin (5-hydroxytryptamine) (*1*). Although there have been studies on the effects of LSD applied to single units (*2*), these have not dealt specifically with the effects of LSD on units in the midbrain raphe or other brain regions with neurons containing serotonin.

There has been much speculation about possible interactions between LSD and serotonin in the brain (*3*). The notion that serotonin may be involved in the central effect of LSD is derived from observations that LSD could antagonize or facilitate the effects of serotonin in smooth-muscle preparations (*4*). Within the brain, LSD induces a small but consistent increase in sero-

tonin concentration (*5*), accompanied by a decrease in its metabolite 5-hydroxyindoleacetic acid (5-HIAA) (*6*). Conversely, electrical stimulation of neurons containing serotonin produces a decrease in the concentration of serotonin and a large increase in 5-HIAA (*7*).

These studies indicate that the metabolism of serotonin in the brain is influenced by the activity of the serotonin-containing neurons. Since the overall effect of LSD on the metabolism of serotonin seems to be the opposite of that of stimulation, it has been suggested that LSD may decrease serotonin turnover by depressing the firing rate of neurons containing serotonin (*8*). We have studied the influence of LSD on the activity of neuronal units in areas rich in neurons containing serotonin (that is, the dorsal and median raphe nuclei of the caudal midbrain) by direct extracellular recordings with microelectrodes.

Thirty-two albino male rats (Sprague-Dawley Strain, Charles River) weighing 250 to 350 g were used for observations of single neurons. Animals were anesthetized with chloral hydrate and placed in a sterotaxic instrument. It was determined in a separate group of animals that LSD produced its typical effect on the metabolism of serotonin in the brain (that is, an increase in serotonin and a decrease in 5-HIAA) when they were anesthetized with chloral hydrate. Microelectrodes (tungsten, with tip diameter approximately 1 to 5 μm, insulated with Formvar except for tips) were lowered into the midbrain raphe nuclei and other areas with a hydraulic microdrive system. Electrode signals were fed into a high-impedance preamplifier and displayed on an oscilloscope. An FM tape recorder was used for recordings. Coordinates for the dorsal and median raphe nuclei of the caudal midbrain were respectively (*9*): A (frontal plane), 350 μm; H (horizontal plane), -0.6 mm; L (saggital plane), 0 mm; and A, 350 μm; H, -2.6 mm; L, 0 mm. The activity of a neuronal unit was usually observed for 5 to 10 minutes before administration of LSD. The LSD (*10*), in a solution at a concentration of 100 μg/ml, was given intraperitoneally or intravenously via a tail vein. At the end of each experiment, animals were perfused with fixative (5 percent glutaraldehyde, in 0.3*M* phosphate buffer, *p*H 7.4). Serial sections (50 μm) of the midbrain were cut and stained with cresyl violet, and the location of the tip of the tract produced by the electrode was determined.



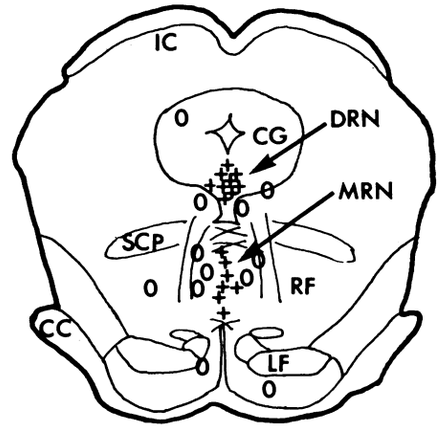


Fig. 1 (left). Histology of an electrode tract in dorsal raphe nucleus of caudal midbrain [frontal plane A 350 μm, after König and Klippel (*9*)]. The tip of tract (arrow) is seen within a cluster of cells in the dorsal raphe nucleus. This nucleus, termed "B7" by Dahlström and Fuxe (*1*), is composed of a closely packed group of serotonin-containing neurons. The unit that had been observed in this experiment responded to a dose of LSD (25 μg/kg, intravenous) by a 5-minute cessation of spontaneous activity. *CA*, cerebral aqueduct; *CG*, central gray; *SCP*; decussation of superior cerebellar peduncle; scale, 0.5 mm. Fig. 2 (right). Summary of correlations between electrode site and unit response after parenteral administration of LSD; +, sites at which units stopped firing after LSD; 0, units that either did not respond or accelerated in rate after LSD. *CC*, cerebral crus; *DRN*, dorsal raphe nucleus; *IC*, inferior colliculus; *LF*, longitudinal fasciculus; *MRN*, median raphe nucleus.

Thirty-two neuronal units were observed in various sites of the midbrain; 17 stopped firing after injection of LSD, 3 increased in rate, and 12 showed no change. The type of response was strictly associated with the anatomic locus of the electrode tip. Without exception, those units responding to LSD by a cessation of spontaneous firing were situated in or near the midline of the dorsal and median raphe nuclei of the caudal midbrain (Figs. 1 and 2). These sites correspond precisely to the location of the serotonin-containing neuronal perikarya termed "B7" and "B8," respectively, by Dahlström and Fuxe (*1*). All but two of the raphe units (15 out of 17) responding to LSD were characterized by an extremely slow spontaneous rate of firing (20 to 40 spikes per minute) and by biphasic extracellular potentials (a positive then negative wave). Conversely, only one of the units observed outside the raphe (1 out of 15) showed these characteristics; the remainder primarily exhibited a negative potential and usually had spontaneous rates in excess of one spike per second. Sites not illustrated in Fig. 2 include the pons and the midbrain at the level of the interpeduncular nucleus. In these regions the only units that stopped firing after administration of LSD were located near the midline above the caudal third of the interpeduncular nucleus; the "B8" nucleus of serotonin-containing cells extends into this area.

Initially the LSD was given in a dose of 200 μg per kilogram of body weight either by intravenous or intraperitoneal injection. Raphe units ceased firing within 1 to 2 minutes after completion of intravenous injections and within 5 minutes after the intraperitoneal ones. These delays correspond to the times required for entry of LSD into the brain and for the onset of behavioral effects in rats (*11*). Once inhibited, units did not return to original firing rates for at least 20 to 30 minutes, indicating that the dose of LSD used was considerably above threshold. By means of very slow intravenous injections, an approximate threshold dose for inhibition was established in eight raphe units. In these experiments firing stopped when a dose of 25 to 50 μg per kilogram of body weight had been given. When LSD is given by the intravenous route, a dose of 50 μg/kg corresponds to a threshold level for gross behavioral effects in the rat. After a threshold dose, spontaneous activity usually began to return within 5 minutes. Such a rapid return of activity is compatible with the short biologic half-life of LSD (20 minutes) in the rat (*12*). After recovery of spontaneous activity, a cessation of firing could again be produced by administration of additional LSD. However, when LSD was given within a few minutes after the onset of recovery only 10 to 15 μg/kg

was required to again stop firing; presumably a subthreshold concentration of the drug was still present in the tissues. Equimolar doses of chlorpromazine did not affect the firing of the LSD-sensitive units in the raphe.

Our results suggest that those units which respond to injections of LSD by a cessation of spontaneous firing are serotonin-containing neurons since their location corresponds precisely to those areas of the midbrain where these cells are clustered. The fact that cells in the raphe are inhibited after injections of LSD is in accord with expectations based on the biochemical data showing a decreased turnover of serotonin after administration of this drug. However, since there may also be some neurons of other types in the dorsal and median raphe nuclei, a definitive demonstration of specificity must await studies which combine the methods of fluorescence histochemistry and the identification of an electrode tip within the area of a single cell. Nevertheless, it is important to note that an inhibitory effect on neuronal firing is not universal after the small parenteral doses of LSD used; in fact, none of the cells observed outside the raphe ceased firing in response to the drug.

It should not be assumed that the effect of LSD on raphe units is a direct one. It has been suggested that LSD may affect serotonin-containing neurons by an indirect, neuronal feedback mechanism (*8*). This speculation is based in part on the similarity between certain behavioral effects of LSD (*13*) and those seen after stimulation of serotonin-containing neurons (*14*). In both circumstances there is a failure of habituation to repetitive sensory stimuli. One explanation for this common behavioral effect would be that LSD acted "like" serotonin at a postsynaptic site. This could result in an indirect inhibition of the serotonin-containing neurons by a compensatory neuronal feedback mechanism. The inhibition of raphe neurons could also be due to complex actions of LSD at other neuronal sites. In any event, our data do not discriminate between the possibilities of a direct or indirect action of LSD on the serotonin-containing neurons.

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Taste Stimuli: A Behavioral Categorization

Abstract. *The selective patterns of generalization to various chemicals, obtained in rats after radiation-induced gustatory-avoidance conditioning against single chemicals, were used to evaluate qualitative similarities among taste stimuli.* DL-*Alanine, glycine, and sodium saccharin were classed together, but not with* D-*glucose or potassium chloride. Groupings such as these may serve as a basis for determining the dimensions along which taste quality is represented.*

In contrast to what is known about other sensory stimuli, knowledge of how taste stimuli should be classified is limited. For example, in audition the physical dimension of frequency bears an orderly relation to judgments of tonal quality and to the neural responses of the auditory receptor system. Although limited correlations have been proposed between the physical characteristics of chemicals and human description of taste quality (*1*), the appropriate dimensions with which to scale taste stimuli are not known (*2*). Neurophysiological data suggest that taste quality may be represented along several dimensions (*3*). The available behavioral data, although few in number, support these neural observations (*3, 4*).

We now present a method which gives information on how animals classify taste stimuli. Ionizing radiation is used to create a strong and sustained gustatory aversion toward any one of a variety of chemicals (*5*). Animals are conditioned to reject a selected concentration of a specific chemical, the primary conditioning solution (PCS) (*6*). We assume: (i) the PCS becomes the quality standard against which the animals compare other solutions; (ii) the test solutions will be aversive, that is, associated with the PCS, as long as their taste to the animal is qualitatively similar to the PCS (*7, 8*); (iii) the magnitude of rejection indicates the degree of similarity in taste between the test solution and the PCS (*9*). When animals are tested for rejection of solutions of the same chemical as the PCS, the pattern of rejection over the concentration range tested constitutes an intrachemical generalization function.

We evaluated the qualitative similarity between two PCS's by cross-generalization studies in which the intrachemical generalization function for each PCS is compared with another function, the interchemical generalization function. The interchemical generalization function is obtained by measuring the rejection of the same solutions of one PCS chemical in animals conditioned to reject the other PCS. The more the functions of each generalization pair resemble one another, the closer the qualities of the two PCS's will be (Fig. 2).

Male Sprague-Dawley rats (Charles River, 150 to 200 g) were trained to drink their normal daily supply of fluid during a 30-minute session during which distilled water was offered every 40 seconds for 20-second periods. Fluid consumption was measured by counting licks. Avoidance was conditioned only after the animals demonstrated a high (>100 licks per 20 seconds) and sustained drinking level throughout the session. The conditioning was done dur-