prism diopters. There is little difference between the ocular dominance distributions for the animals that wore a total of 30 diopters of prism in either the base-in or base-out direction (Fig. 1, D and E).

In addition to the alterations in ocular dominance, two of the prism-reared kittens demonstrated abnormal interocular alignments [as judged by the eye positions during paralysis (*9*)]. The two kittens reared with the 15-diopter prisms mounted base-in in front of the right eye demonstrated inward or esotropic deviations. In both cases these deviations appeared to be symmetrical with respect to the two eyes. The interocular alignments for the other six prism-reared animals were within the range of eye alignments for the normal kittens.

Whereas Maffei and Bisti (*3*) have shown that asymmetrical ocular motility causes a breakdown in cortical binocularity, our results demonstrate that conflicting visual images that do not always give one eye a definite competitive advantage can also disrupt the binocular inputs to the cortex. Even though two of the prism-reared kittens manifested a strabismus, it is unlikely that their oculomotor abnormalities resulted in the decrease in binocular connectivity, because the prism-rearing procedures did not induce an asymmetry in ocular motility. Ocular motility was not encumbered for either eye. The altered eye alignment, however, may have contributed to the differences in ocular dominance noted between the animals that wore a single 15-diopter prism mounted in front of the right eye (Fig. 1, B and C). That monocular units driven by the right and left eyes were encountered in approximately equal numbers in all of the prism-reared kittens supports the notion that neither eye was given a definite competitive advantage and suggests that the animals may have developed an alternating fixation pattern. These results, along with those of others (*1*–*4*), emphasize that the development and maintenance of normal single binocular vision is an extremely complex process requiring a delicate balance in both the motor and sensory processes of vision.

EARL L. SMITH III
MICHAEL J. BENNETT
RONALD S. HARWERTH
*College of Optometry, University of Houston, Houston, Texas 77004*
M. L. J. CRAWFORD
*Sensory Sciences Center, University of Texas Graduate School of Biomedical Sciences, Houston 77025*

**References and Notes**

1. D. H. Hubel and T. N. Wiesel, *J. Neurophysiol.* **28**, 1041 (1965); U. Yinon, E. Auerbach, M. Blank, J. Friesenhausen, *Vision Res.* **15**, 1251 (1975).
2. H. Ikeda and M. J. Wright, *Exp. Brain Res.* **25**, 63 (1976); H. Ikeda, G. T. Plant, K. E. Tremain, *J. Physiol. (London)* **270**, 345 (1977); H. Ikeda and S. G. Jacobson, *ibid.*, p. 367.
3. L. Maffei and S. Bisti, *Science* **191**, 579 (1976).
4. T. N. Wiesel and D. H. Hubel, *J. Neurophysiol.* **26**, 1003 (1963); C. Blakemore, *J. Physiol. (London)* **261**, 423 (1976).
5. The prisms were 1 inch in diameter and were held at a vertex distance of approximately 12 mm. The orientation of the prisms in the goggles is given with respect to the base of the prism. In the "base-in" direction, the base-apex line of the prism was horizontal and the base of the prism was oriented nasally. In the "base-out" direction, the base of the prism was oriented temporally.
6. The procedure included trephining a hole through the skull over the left posterior lateral gyrus, removing the dura, attaching a head restraint to the skull, performing a tracheotomy, and cannulating a femoral vein. The animals were immobilized by infusing Flaxedil intravenously and were artificially respirated. The electrocardiogram was monitored, and the expired $CO_2$ and core temperature were maintained at normal levels throughout the recording session. All the wound margins were infiltrated with a local anesthetic (Xylocaine). The pupils were dilated, and accommodation was paralyzed by the topical application of atropine; the nictating membranes were retracted with Neo-Synephrine. The corneas were protected and the retinas were made conjugate to a rear-projection screen 1 m from the animal with the appropriately powered contact lenses. The reflections of the area centralis and the optic discs were projected and marked on the stimulus screen by illuminating the retina via a fiber optic [R. Fernald and R. Chase, *Vision Res.* **11**, 95 (1971)].
7. D. H. Hubel and T. N. Wiesel, *J. Physiol. (London)* **160**, 106 (1962).
8. Although K. Albus [*Brain Res.* **89**, 341 (1975)] has reported a predominance of monocularly driven cells within the central visual fields, we did not observe any systematic variations in ocular dominance as a function of receptive field eccentricity for either the control or prism-reared kittens. This difference can probably be attributed to our recording from few units with receptive fields within the central 2° to 3° of the retina.
9. S. M. Sherman, *Brain Res.* **37**, 187 (1972).
10. We thank A. Heston for technical assistance. Supported by NIH grants F32 EY015168, K07 EY00052, R01 EY01139, and R01 EY01120.

9 February 1979

# Testosterone Reduces Refractory Period of Stria Terminalis Neurons in the Rat Brain

Abstract. *The absolute refractory period of neurons projecting from the corticomedial amygdala to the medial preoptic-anterior hypothalamic junction in rats was significantly increased by castration (from 1.01 to 1.61 milliseconds) and decreased again by testosterone (from 1.48 to 0.97 millisecond). Corticomedial amygdala neurons which projected to the capsule of the ventromedial nucleus of the hypothalamus were unaffected. These results demonstrate a specific, direct neuronal effect of testosterone.*

Steroid hormones have effects on behavior which are mediated by the central nervous system. This generalization has been confirmed repeatedly by studies of the uptake of radioactive hormones (*1*) and the effects of hormone implants in the brain (*2*). Electrophysiological investigations have shown that sex hormones alter the level of spontaneous activity in anatomically defined populations of neurons (*3*) and change the responses of peripheral and central pathways to sensory and electrical stimulation (*4*, *5*). We report here a striking effect of testosterone on the refractory period of an electrophysiologically defined group of neurons in the brain of the rat.

In the male rat, the corticomedial amygdala (CMA) and the medial preoptic-anterior hypothalamic junction (MPH) concentrate radioactive testosterone (*6*). The olfactory and accessory olfactory bulbs project to the CMA and the sense of smell is important in the control of sexual behavior in the male rat (*7*). The CMA gives rise in its turn to a large projection to the MPH via the stria terminalis (ST) (*8*). Lesions of the ST and amygdala alter the timing of sexual behavior and the number of responses to ejaculation, and lesions of the MPH abolish sexual behavior entirely (*9*). Further, electrical stimulation of the MPH increases sexual activity (*10*). It seems likely, therefore, that neurons of the CMA-ST-MPH system play a role in the control of sexual behavior in the male rat. These neurons can be distinguished electrophysiologically from other CMA neurons by stimulating them antidromically by an electrode in the MPH. Since mating is usually lost in rats 8 weeks after castration and is reinstated by testosterone propionate (TP) [200 μg/day for 16 days or more (*11*)], we examined the effect of these treatments on the physiological characteristics of CMA neurons projecting to the MPH. Another group of CMA neurons terminates in the capsule of the ventromedial nucleus of the hypothalamus and can be identified by antidromic stimulation from this site. These neurons are probably not involved in sexual behavior, since lesions of the ventromedial nucleus did not alter the sexual behavior of the male rat (*12*). As a control for nonspecific effects of castration, therefore, we also examined its effects on these neurons.

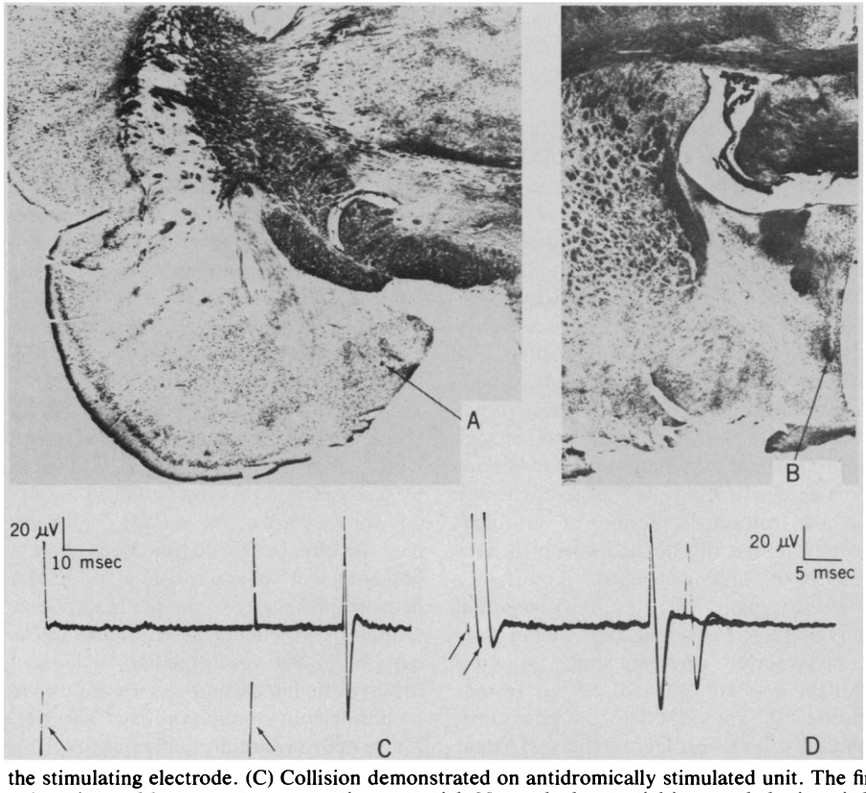
Sexually naïve, adult male Wistar rats


0036-8075/79/0525-0877$00.50/0 

(400 to 650 g) were used. Half, chosen at random, were castrated under ether anesthesia at least 8 weeks before use. For electrophysiological recording the rats were anesthetised with urethane (1.3 g/kg, intraperitoneally). A preliminary experiment showed that the anesthetic dose was the same for intact and castrated animals. The rats were mounted in a conventional stereotaxic frame (Kopf Instruments) and coordinates were calculated from (*13*). A monopolar stainless steel electrode, insulated to within 0.5 mm of the tip, was used to stimulate the MPH or the capsule of the ventromedial nucleus (VMC) with cathodic monophasic pulses (50 to 500 $\mu$A; duration, 0.5 msec). Glass micropipettes filled with 0.5*M* sodium acetate plus 2 percent Pontamine blue were used to make single-unit extracellular recordings from CMA neurons. No recording electrode was used in more than one animal. At the end of each experiment a small current was passed through the stimulating and recording electrodes to facilitate histological localization. The rats were perfused in situ with a 10 percent Formol saline solution to which a small amount of potassium ferrocyanide had been added. Brains were mounted in paraffin wax, sectioned at 15 $\mu$m, and stained in cresyl violet and Luxol fast blue.

In all experiments the criteria for antidromic invasion were (i) for silent neurons, constant latency and following of a stimulus pulse pair above 150 Hz, and (ii) for spontaneously active neurons, these two and collision between a spontaneous action potential and a stimulus-evoked potential. If these conditions were not met, orthodromic stimulation was assumed. Absolute refractory periods were measured by varying the intrapair interval of a stimulus pulse pair until 50 percent following was achieved [as in (*14*)]. The current was increased to two or more times threshold until no further reduction of the refractory period ensued. The latencies and absolute refractory periods of spontaneously active and silent antidromically invaded CMA neurons did not differ significantly within conditions, so the results shown combine the two. For statistical analysis, refractory periods from all neurons recorded from an individual rat were reduced to a single mean, and these means were used in statistical tests. This is because individual electrodes may have different sampling biases and neurons adjacent on an electrode track may have similar properties. Results from individual neurons within an animal ought not to appear, therefore, as data points in statistical analyses based on the assumption that all such data points are independent.

Figure 1 shows examples of the stimulation and recording sites and of neuronal evoked potentials. In the first experiment we determined the refractory period of 85 CMA neurons, stimulated antidromically from the MPH in a total of 25 rats (13 intact, 12 castrated). Results are shown in Fig. 2. The mean refractory period was significantly longer in the castrated animals (Mann-Whitney U, test; U = 10, $P < .002$, two-tailed). In a second experiment castrated rats were treated daily for 18 to 22 days with either TP (200 $\mu$g in arachis oil) or arachis oil alone, given as a 0.1-ml subcutaneous injection. The refractory periods of 133 antidromically invaded CMA neurons were determined from 23 rats (12 treated with TP, 11 oil controls) after stimulation of the MPH. The results in



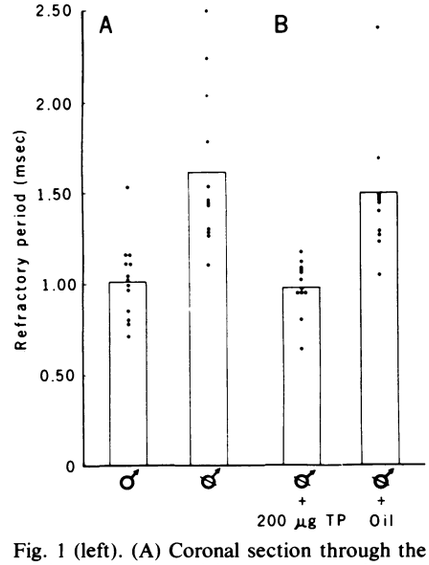




Fig. 1 (left). (A) Coronal section through the corticomedial amygdala, approximately corresponding to A3990 in (*13*). The arrow indicates the location of the tip of a recording electrode. Most neurons from which records were made were posterior to this. (B) Typical stimulation site in the medial preoptic-anterior hypothalamic region; coronal section, approximately corresponding to A6360 in (*13*). The arrow indicates the location of the tip of the stimulating electrode. (C) Collision demonstrated on antidromically stimulated unit. The first stimulus artifact (left arrow) marks a stimulus pulse triggered by a spontaneous action potential. No evoked potential is recorded, since it has collided with the spontaneous potential. The second stimulus artifact (right arrow) marks a second stimulus pulse about 50 msec later, which produced an evoked potential as usual. (D) Absolute refractory period determination. The second stimulus pulse of two or more times threshold current produced an evoked action potential half the time. Arrows indicate the stimulus artifacts of the two stimulus pulses. Fig. 2 (right). (A) Absolute refractory period of CMA neurons projecting to the MPH in intact (♂) and castrated (♂) rats [overall mean and individual means (dots)]. Results are for 43 neurons from 13 intact rats and 42 neurons from 12 castrated rats ($P < .002$, two-tailed). (B) Absolute refractory period ot CMA neurons projecting to the MPH in castrated rats treated with testosterone propionate (80 neurons from 12 rats) or with oil alone (53 neurons from 11 rats) ($P < .002$, two-tailed).

Fig. 2 show that the mean absolute refractory periods of CMA neurons were significantly shorter in castrated rats after treatment with TP ($U = 5$, $P < .002$, two-tailed). Comparisons of refractory periods between the first and second experiments show no significant difference between the mean refractory period of intact (experiment 1) and testosterone-treated (experiment 2) animals ($U = 71$, $N = 12$ and 13, not significant). This experiment shows, therefore, that testosterone reverses the effect of castration observed in the first experiment.

In a third experiment we measured refractory periods of CMA neurons stimulated antidromically not from the MPH but from the VMC. Refractory periods were determined for 57 neurons from 13 intact rats and 59 neurons from 13 castrated rats. The results show no significant difference in mean refractory period between the two groups. Means and ranges are 1.23 and 0.84 to 2.11 msec for intact rats and 1.21 and 0.97 to 1.79 msec for castrated rats ($U = 81$, not significant).

All the neurons for which results are given were located in the cortical and medial nuclei of the amygdala as defined in (*13*). In accordance with anatomical evidence (*8*), the neurons driven from the MPH and VMC were encountered in the same caudal region of the CMA. The refractory period is altered, therefore, in amygdala neurons projecting via the dorsal stria to the MPH, but not in adjacent amygdala neurons projecting via the dorsal stria to the VMC. So the effect of castration is restricted to the pathway known on other grounds to be involved in the control of sexual behavior. The effect of castration must be due to the removal of testosterone, since it is reversed by testosterone injections. Testosterone must be affecting these neurons directly, since it is the refractory period that is altered, and a change in refractory period implies a membrane change.

The change in refractory period that we have demonstrated would alter the output of the pathway to high-frequency inputs. Presumably these inputs would be olfactory. This interpretation receives support from a previous study (*4*), which shows that testosterone does alter preoptic neuron responses both to electrical stimulation of the olfactory bulb and to natural sexual odors.

K. M. KENDRICK
R. F. DREWETT*
*Department of Psychology, University of Durham, Durham, England*

**References and Notes**

1. R. P. Michael, *Br. Med. Bull.* **21** (No. 1), 87 (1965); W. E. Stumpf, *Science* **162**, 1001 (1968); B. S. McEwen, R. E. Zigmond, J. L. Gerlach, in *Structure and Function of Nervous Tissue*, G. H. Bourne, Ed. (Academic Press, New York, 1972), vol. 5, pp. 205–291.
2. R. D. Lisk, *Acta Endocrinol.* **41**, 195 (1962); G. W. Harris and R. P. Michael, *J. Physiol. (London)* **171**, 275 (1964); J. M. Davidson, *Endocrinology* **79**, 783 (1966).
3. B. A. Cross and R. G. Dyer, in *The Hypothalamus*, L. Martini, M. Motta, F. Frashini, Eds. (Academic Press, New York, 1971), pp. 115–122.
4. D. W. Pfaff and C. Pfaffman, *Brain Res.* **15**, 137 (1969).
5. B. R. Komisaruk, N. T. Adler, J. Hutchison, *Science* **178**, 1295 (1972); L. M. Kow and D. W. Pfaff, *Neuroendocrinology* **13**, 299 (1973/4); R. F. Drewett *et al.*, *Neuroscience* **2**, 1033 (1977).
6. M. Sar and W. E. Stumpf, *Endocrinology* **92**, 251 (1973).
7. L. Heimer and K. Larsson, *Physiol. Behav.* **2**, 207 (1967); K. Larsson, *Brain Behav. Evol.* **4**, 463 (1971).
8. J. S. deOlmos, *Adv. Behav. Biol.* **2**, 145 (1972).
9. V. H. Harris and B. D. Sachs, *Brain Res.* **86**, 514 (1975); L. Heimer and K. Larsson, *ibid.* **3**, 248 (1967); G. W. Giantonio, N. L. Lund, A. A. Gerall, *J. Comp. Physiol. Psychol.* **73**, 38 (1970); D. E. Emery and B. D. Sachs, *Physiol. Behav.* **17**, 803 (1976).
10. H. Van Dis and K. Larsson, *Physiol. Behav.* **6**, 85 (1971); C. W. Malsbury, *ibid.* **7**, 797 (1971); A. Merari and A. Ginton, *Brain Res.* **86**, 97 (1975).
11. D. Pfaff, *J. Comp. Physiol. Psychol.* **73**, 349 (1970); M. J. Baum and J. T. M. Vreeburg, *Science* **182**, 283 (1973).
12. B. Olivier, thesis, State University at Groningen, Netherlands (1977), pp. 29–31.
13. J. F. R. König and R. A. Klippel, *The Rat Brain* (Williams & Wilkins, Baltimore, 1963).
14. E. T. Rolls, *Physiol. Behav.* **7**, 297 (1971).
15. Supported by Science Research Council grant 76300492. We thank D. Harper for his help with the histology. Some initial experiments on this project were carried out by D. J. Sanders [honours dissertation, University of Durham (1977)].
* Reprint requests should be sent to R.F.D.

24 July 1978; revised 20 November 1978

## Physical Factors Affecting the Mutagenicity of Fly Ash from a Coal-Fired Power Plant

Abstract. *The two finest, most respirable coal fly ash fractions collected from the smokestack of a power plant were more mutagenic than two coarser fractions. Mutagenicity was evaluated in the histidine-requiring bacterial strains TA 1538, TA 98, and TA 100 of* Salmonella typhimurium. *Ash samples collected from the hoppers of an electrostatic precipitator in the plant were not mutagenic. The mutagens in coal fly ash were resistant to x-ray or ultraviolet irradiation, possibly as a result of stabilization by fly ash surfaces. All mutagenic activity is lost with heating to 350°C.*

In 1977, fly ash produced and collected by coal-fired power plants was considered to be the sixth most abundant mineral in the United States (*1*). Of the 50 million metric tons of fly ash produced, 13 percent was reused commercially, predominantly as a partial replacement for cement or as fill material in construction; of the remainder, most was deposited in landfills. Electrostatic precipitators (ESP's) are used in most coal-fired power plants for in-plant collection of aerosolized particulate matter. The total mass collection efficiencies of the ESP's vary from 95 to 99.5 percent, with the lowest relative efficiency for collecting respirable, submicron-sized particles (*2*). Because vast quantities of fly ash are produced in the generation of electricity, detailed studies are required of the potential public and occupational health hazards of fly ash released from smokestacks and collected in power plants.

We have demonstrated that extracts from a stack-collected, respirable fly ash sample were mutagenic in the Ames bacterial assay (*3*); moreover, because of the correlation between mutagenicity and carcinogenicity (*4*), our study indicated that fly ash is potentially carcinogenic. We describe here physical factors affecting the mutagenicity of (i) four size-classified, stack-collected fly ash samples; (ii) one size-classified, ESP-collected sample; and (iii) one unsized, ESP-collected sample.

The stack sampling was performed at 95°C; two measurements of the stack gas temperature during the 30-day sampling period varied from 107° to 115°C (*5*). The volume median diameters (VMD's) of these four size-classified samples, fractions 1 through 4, are 20, 6.3, 3.2, and 2.2 $\mu$m, respectively. All have geometric standard deviations ($\sigma_g$) of approximately 1.8.

We also collected fly ash from the power plant's ESP hoppers (*6*). Three measurements of the temperature of the ESP's during 2 days of collection varied from 104° to 107°C. Because the ESP fly ash is predominantly composed of relatively large particles, it was aerodynamically size-classified to obtain a fraction with a VMD of 2.3 $\mu$m and $\sigma_g = 1.4$. The physical and chemical properties of the stack-collected samples and of similarly collected unsized ESP ash have been reported (*7*).

We evaluated the relative mutagenicity of serum filtrates from the four stack-collected fractions using TA-1538 because our previous studies indicated it


0036-8075/79/0525-0879$00.50/0 