Pharmacology Biochemistry & Behavior, Vol. 17, pp. 841–845, 1982. Printed in the U.S.A.

Effects of Reinforcement-Blocking Doses of Pimozide on Neural Systems Driven by Rewarding Stimulation of the MFB: A ^{14}C-2-Deoxyglucose Analysis

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Received 1 October 1981

GOMITA, Y. AND C. R. GALLISTEL. *Effects of reinforcement-blocking doses of pimozide on neural systems driven by rewarding stimulation of the MFB: A ^{14}C-2-deoxyglucose analysis.* PHARMAC. BIOCHEM. BEHAV. 17(4) 841–845, 1982.—An analysis by means of ^{14}C-2-deoxyglucose autoradiography of the neural systems unilaterally activated by the reinforcing stimulation used in the two accompanying papers revealed strong and reliable effects in the nucleus of the diagonal band of Broca, in the medial forebrain bundle (MFB) and/or the fornix throughout the diencephalon, and in the part of the anterior ventral tegmentum where the dopaminergic projection to the lateral habenula originates. The terminal fields of the dopaminergic forebrain projections were not affected, but there was bilateral suppression of lateral habenular activity. A second experiment found that the same systems are still activated by (automatically administered) reinforcing stimulation in rats treated with reinforcement blocking doses of pimozide. The only clear effect of pimozide was to reverse the bilateral suppressive effect of the stimulation on lateral habenular activity. Animals treated with pimozide show greatly elevated activity in the lateral habenula, whether or not they receive reinforcing stimulation. The results suggest that pimozide's effect on reinforcement is mediated by the circuitry interconnecting the lateral habenula with the nucleus of the diagonal band of Broca and/or the anterior ventral tegmentum.

Self-stimulation Reinforcement ^{14}C-2-Deoxyglucose autoradiography Pimozide Lateral habenula Diagonal band of Broca Ventral tegmentum

IN the light of the finding that the neuroleptic pimozide blocks the reinforcing but not the motivating effect of rewarding electrical stimulation of the MFB [4,14], we have begun an investigation of the effects of this drug upon the pattern of altered functional activity produced by rewarding stimulation of the MFB. We are using 2-deoxyglucose autoradiography to index alterations in functional activity. In this technique, a radioactively labelled, chemically altered form of glucose (^{14}C-2DG) is incorporated into neural systems at a rate dependent on the functional activity of the system [12]. The preliminary results of this investigation are sufficiently surprising to warrant a brief publication of them in conjunction with the two behavioral papers in this same issue [4,14].

EXPERIMENT 1

METHOD

Four rats used in the behavioral experiments self-stimulated by pressing a lever in a Skinner box during the 45 minute period following the IP [9] injection of 30μCi of ^{14}C-2-deoxyglucose. Each press yielded a train of 66 cathodal pulses, 0.1 msec wide, at 100 Hz and a current intensity of 400 μA, the parameters used in the behavioral experiments. The rats pressed at mean rates of 45–90 per minute. At the end of the 45 minutes allowed for isotope incorporation, the rats were anesthetized by the IP injection of 2 cc of Chloropent and perfused intracardially for 30 seconds with 3.4% Formalin phosphate buffered to a pH of 7.4 [3]. First the electrode was extracted, then the brain was removed, rapidly frozen in liquid Freon at −55°C, allowed to equilibrate in a cryostat at −18° and sectioned in a cryostat at 20 μ. Every 10th section was picked up on a chilled coverslip and dried on a warming tray at 60°C. The coverslips were mounted on cardboard and exposed to Kodak SB 5 X ray film for 10 days, along with calibrated ^{14}C concentration standards. The sections were subsequently stained with thionin [3].

The autoradiographic images were analyzed at the Computerized Image Processing and Pattern Recognition Facility of Drexel University using software developed for use in 2DG autoradiography [5]. The system permits precise alignment of the histological and autoradiographic images of a

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section, so the user can outline a structure in the histological image and have the outline superimposed on the radiographic image. From the darkness of the radiographic image within the outline, the system computes an index of functional activity for the outlined neural structure. A normalized index of functional activity, relative optical density, was used in place of an estimate of the rate of glucose utilization. The index is computed as follows: The system digitizes an image by treating it as a matrix of 60 μ square spots called pixels (picture elements). The darkness of each pixel is recorded in a matrix memory. The computer surveys this memory and calculates for each pixel its percentile rank relative to all the other pixels in the image. A rank of 0.80 means that 80% of the pixels are lighter than the pixel in question. The relative optical density of a neural structure is the mean relative optical density of the pixels falling within the outline. Rank-order normalization removes from the data the variance due to the many irrelevant factors that affect the overall darkness of the image. Normalized indices of functional activity are usually preferable to indices based on estimates of rate of glucose utilization [5].

RESULTS

Unilateral effects of unilateral rewarding stimulation of the posterior lateral hypothalamus were found throughout most of the length of the MFB. The strongest and most reliable effects were in the nucleus of the diagonal band of Broca (DBB nucleus), near the anterior end of the MFB, and in the anterior ventromedial tegmentum in or near the origins of the dopaminergic projection to the lateral habenula [10]. The MFB was a focus of activation throughout the diencephalon in three of the subjects, but not in the fourth. In the fourth, the fornix was the focus of activation along its entire length from the mammillary bodies to where it enters the hippocampus. In this animal, as in the others, the DBB nucleus and the anterior ventromedial tegmentum were clearly activated. There was no detectable activation at any of the origins of the noradrenergic projections (in the posterior midbrain and hindbrain). Nor was there any strong and reliable activation in the principal terminal fields of the dopaminergic projections. Neither the caudate, nor the nucleus accumbens, nor the tuberculum olfactorium, nor the lateral habenula reliably showed unilateral activation. The pattern of unilateral activation seen in these four animals corresponds with the pattern reported in [17]. There were also no readily apparent bilateral effects. However, quantitative analysis of these same data in conjunction with the data from Experiment 2 revealed a bilateral suppression of functional activity in the lateral habenula.

EXPERIMENT 2

METHOD

Seventeen male white rats with electrodes in or near the posterior MFB were used. Five self-stimulated during uptake of the 2DG. Before the 2DG session, we had determined the animal's rate of pressing as a function of current intensity in a Skinner box, where each press yielded a 0.5 sec train of cathodal pulses 0.1 msec wide and at 100 Hz. The rats were advanced to autoradiography when their rate-intensity curves were stable to within 0.1 log units on three successive days. During the uptake of 2DG, the stimulating current was at the value derived from the 75% of maximum point on the animal's rate-intensity curve. Five more rats, for whom rate-intensity curves had also been determined were injected IP with 0.75 mg/kg pimozide (in 0.3% tartaric acid) four hours before the injection of the 2DG. This is a reinforcement-blocking dose [4], hence these animals would not self-stimulate. They were matched to a mate in the first group on the basis of rate-intensity curves and given rewarding stimulation during the 2DG uptake at the same rate at which the animal's mate self-administered it. An eleventh animal, for which a rate-intensity curve had also been determined was automatically administered rewarding stimulation in the absence of pimozide. Two rats were given pimozide but no stimulation and four more rats served as controls, receiving an injection of the drug vehicle and no stimulation. The rats that received no stimulation were left in the self-stimulation box during the period of 2DG uptake. The autoradiographic procedure was the same as in the first experiment.

RESULTS

All the rats receiving rewarding stimulation of the MFB, whether self-administered or automatically administered, and whether or not the animal had been injected with a reinforcement-blocking dose of pimozide, showed the same pattern of unilateral activation observed in Experiment 1 and in [17]. Figure 1 shows by means of color windows that in each self-stimulating (SS) rat and its pimozide treated mate receiving administered stimulation (PS), the nucleus of the diagonal band of Broca, the MFB and/or fornix, and the anterior ventral tegmentum were activated on the side of stimulation.

All of the pimozide treated animals, whether or not they received rewarding stimulation, showed a dramatic bilateral elevation of functional activity in the lateral habenula. Figure 2 shows the difference in lateral habenula activation between

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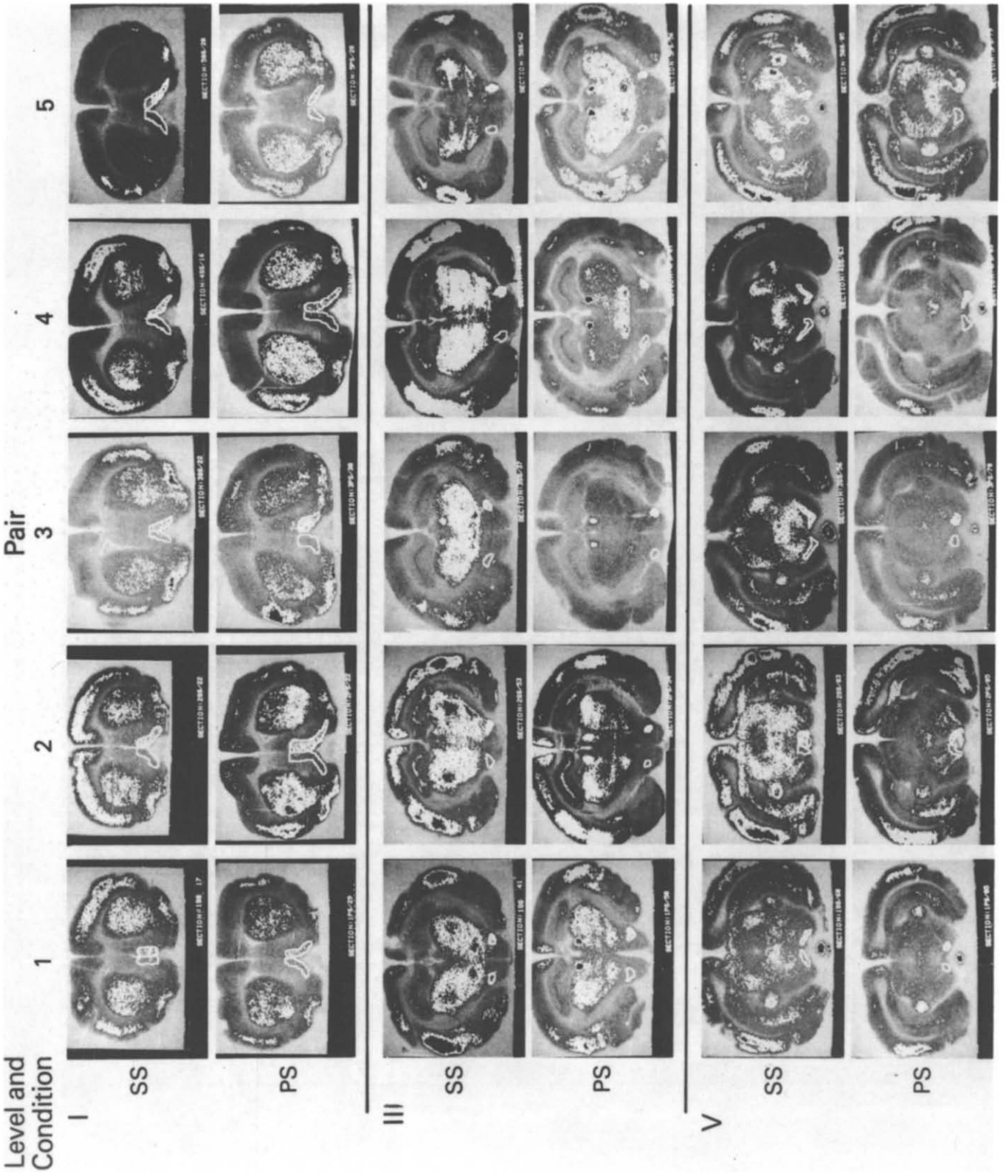
FIG. 1. Representative autoradiographs from Levels I, III, and V for five pairs of animals that received reinforcing stimulation of the posterior MFB during uptake. The SS member of each pair self-stimulated. The PS member was treated with pimozide and had the stimulation administered automatically. The stimulation was on the right in all cases. The activating effects of the stimulation were highlighted by setting a yellow color window, which photographs white, to pick out the darkened area on the stimulated side, outlining the area picked out by the window and then the comparable area on the unstimulated side. Note that in all cases there is a considerably higher density of colored in pixels within the outline on the right. At Level I, one sees activation of the nucleus of the DBB; at Level III, of the MFB and/or fornix; at Level V, of the origins of the dopaminergic projection to the lateral habenula.


Level and Condition
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1
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V
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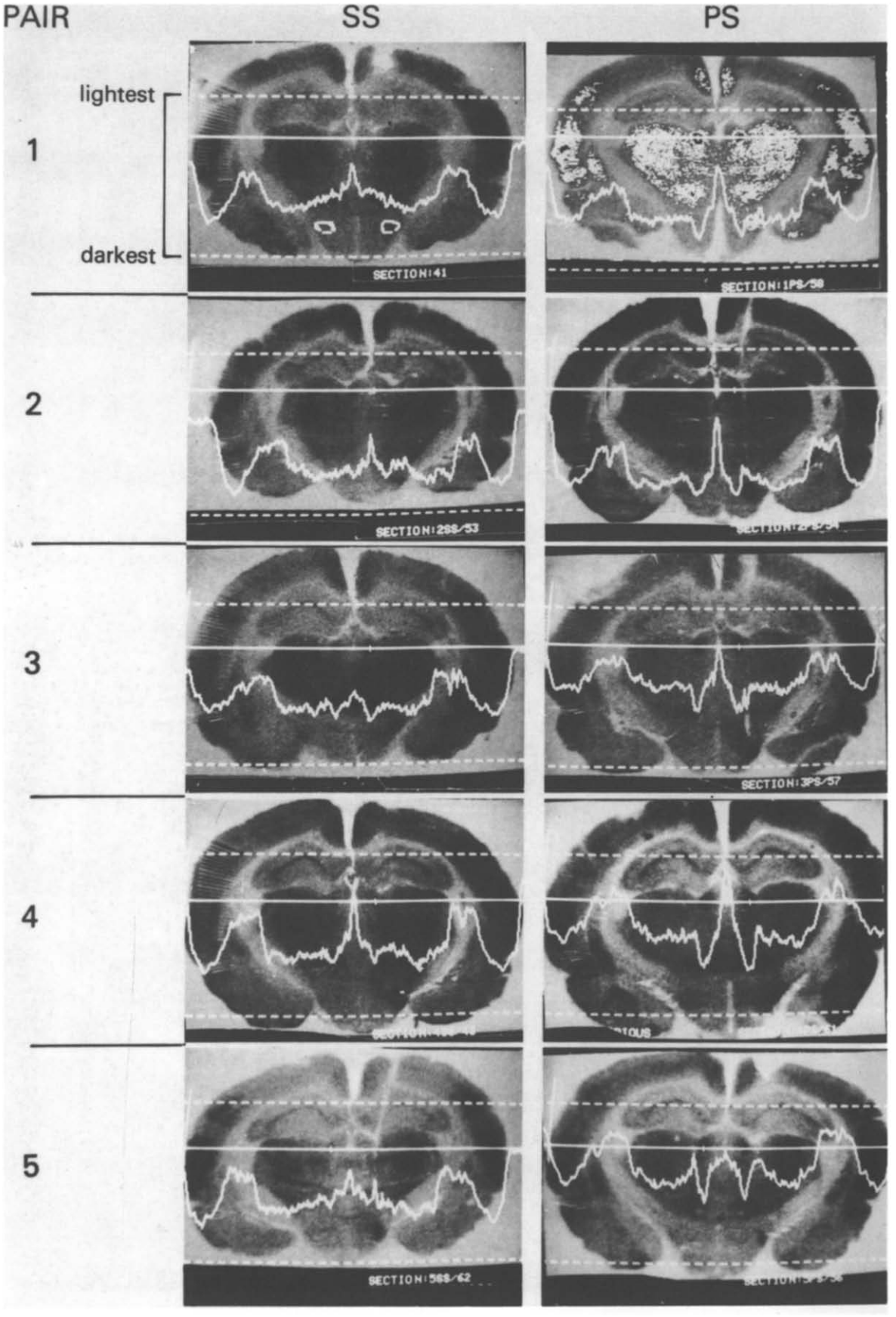
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SECTION:2SS/53
SECTION:3PS/57

the SS and PS animals. By contrast, in self-stimulation (undrugged) animals, the functional activity of the lateral habenula was suppressed bilaterally relative to controls. The bilateral mean relative optical density of the lateral habenulae in the seven pimozide treated rats (PS and P) was 0.90 with a standard deviation of ±0.03; in the control (C) animals, it was 0.83±0.01; and in the self-stimulating animals, it was 0.66±0.12. The bilateral relative optical density of the lateral habenulae in all five self-stimulating rats was below that in any of the controls. The same proved to be true for three of the four self-stimulating rats in Experiment 1. The significance of these differences was verified by *t*-tests (for the 9 SS rats versus the 4 C rats, $p<0.02$; for the 7 P and PS rats versus the C rats, $p<0.01$).

DISCUSSION

The mesohabenular dopamine pathway originates in the anterior ventromedial tegmentum in an area coinciding with the area where the strongest and most reliable unilateral midbrain effects of MFB stimulation are seen [10]. This pathway projects to the lateral habenula. Also projecting to the lateral habenula are non-dopaminergic neurons originating in the nucleus of the diagonal band of Broca, the area where the strongest and most reliable unilateral forebrain effects of MFB self-stimulation are found. Reinforcement-blocking doses of pimozide do not alter the pattern of the unilateral activating effects of reinforcing stimulation, in which the forebrain focus of activation and the midbrain focus are linked by activation in the MFB and/or the fornix throughout the diencephalon. The only bilateral effect of self-stimulation that we have so far detected is a suppression of lateral habenular activity. The only effect of pimozide we have so far found is in this same structure. Pimozide greatly elevates lateral habenular activity, as does haloperidol [8]. Unilateral lesions of the dopamine cell bodies in the anterior ventral tegmentum with 6-OHDA also elevates lateral habenular activity bilaterally [16]. On the other hand, amphetamine, which enhances the reinforcing effect of stimulation [1, 2, 11], is like stimulation in that it suppresses lateral habenular activity [15]. These findings lead us to wonder whether the explanation of pimozide's reinforcement-blocking effect is to be sought in the circuits that interconnect the lateral habenula with the anterior ventromedial tegmentum and the nucleus of the diagonal band [6,7]. It is unlikely that the lateral habenula forms part of the pathway that actually carries the rewarding signal in MFB self-stimulation, because lesioning the lateral habenula improves rate of self-stimulation on electrodes in the MFB ipsilateral to the lesion [13].

ACKNOWLEDGEMENTS

This research was supported by NIH Grant NS 14935 to C. R. Gallistel, P. Hand and M. Reivich, and by two supplemental grants from the Biomedical Research Support Grant from NIH to the University of Pennsylvania (RR 07083). The authors are grateful to the directors and staff of the CIPPR Facility at Drexel University for Assistance in analyzing the images.

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FIG. 2. Single line scans across the lateral habenulae in the five pairs of SS and PS animals. The line of scan is the solid horizontal line passing through the lateral habenulae in each section. The gray value at each point along this line of scan is indicated by the jagged line (down=darker; up=lighter). The dashed lines indicate the limits of the gray scale. In the upper right panel, a color window has been used to create a white background against which the lateral habenulae stand out like two black eyespots in the dorsomedial thalamus. Note the pronounced valleys in the PS scans as they traverse the lateral habenulae, valleys that are missing or less pronounced in the SS scans.

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