

sulting from an 11.5 mg/kg dose of *l*-amphetamine (potency ratio of 5:1). That is, our results, unlike those of others (2, 3), suggest an equivalent difference in potency between *d*- and *l*-amphetamine for the two behaviors.

Our data indicate that if two or more different neurochemical systems mediate amphetamine-induced locomotion and stereotypy, then *d*- and *l*-amphetamine have the same relative effects on those systems, the only differences between the isomers being of potency and of temporal course of action. However, it is also conceivable that both behaviors are mediated by the same underlying neurochemical system(s). Because the relative behavioral potencies of *d*- and *l*-amphetamine found in the present study are comparable to most of the results obtained for the relative effectiveness of the two isomers on various DA mechanisms (1), DA pathways in the brain may be involved in mediating both amphetamine-induced behaviors.

We have observed that the basic feature of stereotypy, continuous repetition of certain behavioral elements, is apparent with doses of *d*-amphetamine as low as 0.5 mg/kg, in the form of marked perseveration in the pattern of locomotion. The perseveration may simply become more focused with the greater activation produced by higher doses. Although the relationship between amphetamine-induced stereotypy and locomotion has not yet been elucidated, it is apparent that the temporal pattern of induced behavioral effects must be considered in the interpretation of behavioral and neurochemical studies comparing *d*- and *l*-amphetamine.

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Neural Connections of Sparrow Pineal: Role in Circadian Control of Activity

Abstract. *Surgical and chemical interference with the neural connections of the house sparrow (Passer domesticus) pineal does not abolish the free-running rhythm in constant darkness, unlike pinealectomy. Pineals transplanted to the anterior chamber of the eye are capable of restoring rhythmicity to pinealectomized birds in constant darkness. The avian pineal does not appear to be neurally coupled to other components of the circadian system.*

Surgical removal of the pineal organ abolishes the free-running circadian rhythms of locomotor activity and body temperature in the house sparrow (*Passer domesticus*) as well as the rhythm of migratory restlessness in the white-throated sparrow (*Zonotrichia albicollis*) (1). These and other data (2) suggest that, within the circadian system of birds, the pineal may be acting as a self-sustained oscillator driving the overt rhythms that we are able to measure or, alternatively, as a coupling device between such an oscillator located elsewhere and other components of the system. As a first step toward understanding the function of the avian pineal, we have, in the present study, investigated the routes by means of which circadian information may enter or leave the pineal organ of the house sparrow.

The only documented neural input to the avian pineal is an extensive sympathetic innervation from the superior cervical ganglion (3). Its only known neural output is a tract of numerous, small, unmyelinated, acetylcholinesterase-positive fibers of unknown termination which leave the pineal organ through its stalk (4). In addition, there could be hormonal inputs to or outputs from the pineal which, because of the absence of specialized pineal vasculature (5), might well be carried in the general circulation. We report here effects of three treatments on the free-running rhythm in constant darkness: surgical and chemical interference with the pineal's neural connections, and transplantation of the pineal to the anterior chamber of the eye.

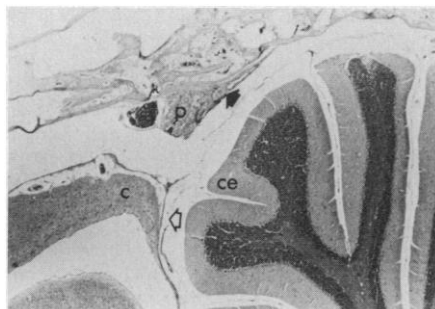


Fig. 1. Sagittal section through the brain after stalk deflection. The solid arrow indicates the deflected stalk of the pineal; the open arrow marks the normal position of the stalk; c, cerebrum; ce, cerebellum; p, pineal.

We have developed a surgical procedure [which we call stalk deflection (6)] that unambiguously disrupts the neural output from the pineal through its stalk. When stalk deflection is successfully performed it causes very little disruption to the body of the pineal but severs all the connections of the stalk, which is freed at its base and deflected onto the dorsal surface of the cerebellum (Fig. 1). Success of the operation is estimated visually at the time of surgery and confirmed histologically at the end of the experiment.

Stalk deflection was performed successfully on 12 sparrows that, except for the brief exposure to light that occurred during surgery, were free-running in constant darkness. In none of these birds was the circadian rhythm abolished: in fact there was no effect whatsoever on the locomotor rhythm except for the phase shifts which are predicted as a consequence of light exposure (7). We conclude that, whatever the pineal's role in the circadian organization of the house sparrow, it does not exert its effect via the nerves that leave it through the stalk. As there is no other known neural output from this organ, our results suggest strongly that its influence on the circadian system is exerted hormonally.

In our hands it has proved impossible to interrupt the neural input to the pineal organ of sparrows by surgical removal of the superior cervical ganglia. We have, however, performed chemical sympathectomies on a large number of birds with 6-hydroxydopamine (6-OHDA) (8). A single injection of 6-OHDA (75 to 100 mg per kilogram of body weight, a dose close to that lethal for 50 percent of sparrows) depletes norepinephrine fluorescence in the pineal within 24 hours (9). Five weeks after the administration of such a dose, norepinephrine fluorescence is still undetectable in the pineals of some sparrows while a few faint fibers can be seen in others. We have determined that although sparrows do not tolerate a single dose of 6-OHDA much larger than 75 to 100 mg/kg, they tolerate multiple administrations at this level if injections are given approximately every 48 hours.

Sparrows that were free-running in constant darkness were given single doses of 6-OHDA (9 birds) or multiple doses (18

birds, three or four injections per bird). In no case was the free-running rhythm abolished. In a few cases there appeared to be a slight effect on the stability of the period of the free-running rhythm, but this effect, if

indeed it is real, is difficult to interpret because of the very general physiological effects of chemical sympathectomy. Even with the very large doses of 6-OHDA that we have employed, it is difficult to support

the claim that we have completely abolished neural input to the pineal. We may not have managed to destroy every sympathetic fiber and, in addition, there may be as yet unrecognized neural inputs of other kinds. Nevertheless, our experiments with 6-OHDA suggest that the role of the pineal within the circadian system of sparrows does not depend on its being neurally coupled to other oscillators. This suggestion is supported by the results of our transplant experiments.

We performed both stalk deflection and chemical sympathectomy on 12 sparrows. The combination of these two procedures provides the most complete neural isolation of the pineal in situ of which we are presently capable. Eleven of the birds were essentially unaffected (Fig. 2); the 12th became arrhythmic for 11 days after a series of four 6-OHDA injections, but the rhythm then reappeared and persisted.

Our results indicate that the avian pineal is not neurally coupled with other components of the circadian system. This suggestion is supported by the work of Binkley (10) and is in marked contrast to the well-documented situation in mammals (11). We tested this hypothesis directly by attempting to reestablish rhythmicity in pinealectomized birds by transplantation of donor pineals into the anterior chamber of the eye.

Previously we had tantalizing but limited success in such transplantation attempts. When replaced in its normal site the pineal is usually destroyed [but see (2)]. Our initial attempts to establish pineal transplants in the anterior chamber also failed in most cases (12). Möller (13) suggested to us that in order to improve gas exchange, we should make a series of small cuts in the pineal body before inserting it into the anterior chamber. This slight modification in technique has dramatically improved our results. We now remove the pineal, with its stalk, from the donor bird and make about three small cuts before inserting it into the anterior chamber of the eye of an arrhythmic bird through a slit in the cornea. When successful, transplants are visible within the anterior chamber and are extensively revascularized.

Using this technique we were able to reestablish rhythmicity in 12 sparrows (about 30 percent of our attempts) that had previously been rendered arrhythmic by pinealectomy (Fig. 3). Control implants, with pieces of cerebellum, uniformly failed to reestablish rhythmicity. When the transplant is successful, rhythmicity appears rapidly after surgery; in seven cases it was evident within 24 hours (Fig. 3). Because the implant is in the anterior chamber, it is possible to perform a control procedure by simply removing the entire eye that bears

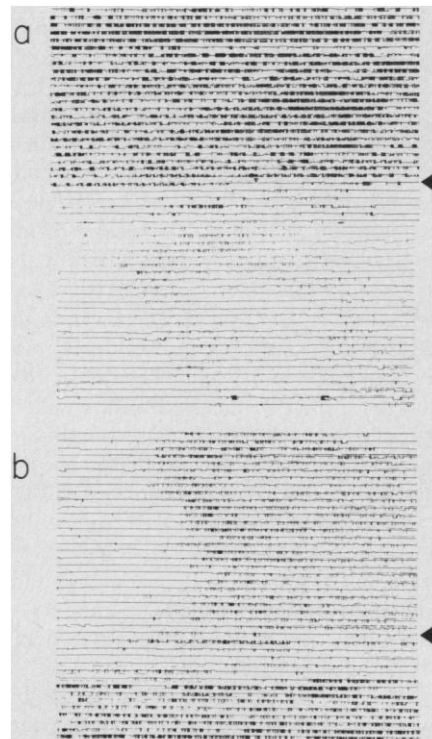
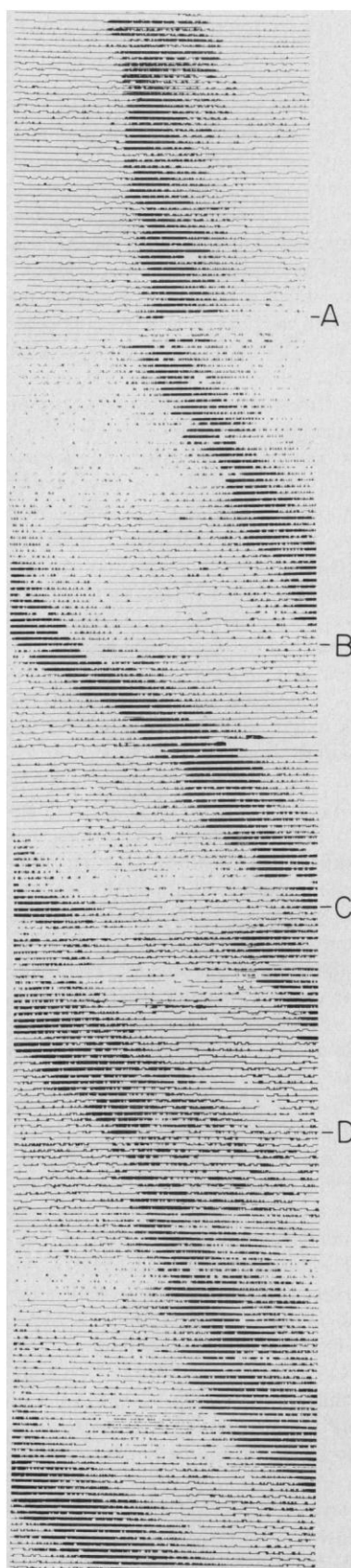


Fig. 2 (left). The effect of 6-OHDA injection and stalk deflection on circadian locomotor rhythmicity in *Passer domesticus*. Time reads from left to right and top to bottom, with each horizontal line representing 24 hours of activity. The bird was in constant darkness throughout the record except for brief light periods at the time of injection or surgery. (A) A single injection of 6-OHDA reduced activity temporarily without disrupting the free-running rhythm. (B) The first of a series of four saline injections was given, with subsequent injections at approximately 48-hour intervals. (C) The pineal stalk was deflected. Either the surgical procedure or the light exposure caused a phase advance but rhythmicity persisted. (D) The first of a series of three 6-OHDA injections was given, with subsequent injections at approximately 48-hour intervals. Chemical sympathectomy following stalk deflection disrupts both neural input and output of the pineal without abolishing the free-running rhythm of activity. Fig. 3 (right). The effect on locomotor activity of transplanting a pineal to the anterior chamber of the eye of a previously pinealectomized house sparrow. (a) The bird was arrhythmic in constant darkness before the pineal transplant, which was performed in the middle of the day indicated by the arrow. The bird was free-running for approximately a month before being exposed to a light-dark cycle. Entrainment to the light cycle was normal; this segment of activity has been omitted to shorten the record. (b) When the bird was returned to constant darkness, the phase of the rhythm had been shifted by the light cycle and the level of activity had increased when compared to that during the earlier free-running period. At the time indicated by the arrow, the eye bearing the transplanted pineal was removed and arrhythmicity recurred.

the donor pineal. We have done this in 4 of our 12 successful cases and all 4 birds became arrhythmic as a result (Fig. 3).

Tissue transplanted to the anterior chamber is often reinnervated by sympathetic fibers from the superior cervical ganglion which normally supply the iris. In our view, however, the rapidity with which the reestablishment of rhythmicity follows the introduction of a pineal organ into the anterior chamber precludes an interpretation of our results which assigns a major role to such reinnervation. This markedly strengthens our belief, based initially on the results of chemical sympathectomy, that the avian pineal does not require sympathetic input, or any neural input, for the performance of its role in the birds' circadian organization [the situation in mammals is discussed in (10, 11)].

The success of the transplant experiments also confirms the conclusions that we reached on the basis of stalk deflection: the output of the pineal which affects other components of the circadian system must be hormonal and, further, it is probably capable of exerting its effect when released into the general circulation.

These results, however interesting, do not allow us to distinguish between the alternative hypotheses concerning the pineal's role in avian circadian organization outlined at the beginning of this report. Although our results may perhaps lend support to the idea that the avian pineal is an endogenously rhythmic driving oscillator, they do not preclude the possibility that it functions as a coupling device. The resolution of this question awaits the demonstration that it is (or is not) possible to transfer some specific property of the donor bird's rhythmicity (such as phase) along with its pineal organ.

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The Müller-Lyer Figure and the Fly

Abstract. In the Müller-Lyer illusion two horizontal line segments of equal length are perceived by humans as unequal. The gaze of a fly presented with Müller-Lyer figures corresponds to human eye movements and human (illusory) evaluations of the segment lengths. It is suggested that a theory similar to the phenomenological theory which accounts for the fly's gaze may account for the human eye's movements during an observation of Müller-Lyer figures.

Everybody looking at Fig. 1a will perceive the top horizontal segment as shorter than the bottom one, although an actual measurement shows that they are of equal length. This is the Müller-Lyer illusion (1), probably one of the best known of all geometric illusions. This illusion is still perceived with a stabilized retinal image (2, 3), so eye movements are not necessary for it.

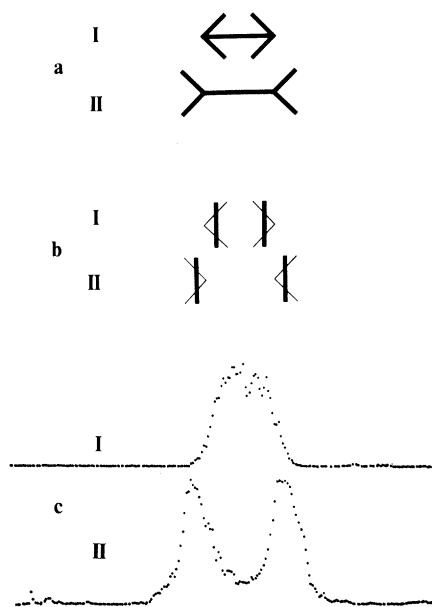


Fig. 1. (a) The Müller-Lyer illusion. In the experiment represented here, the angular length of the horizontal line, as seen by the fly's eye, is 60°. (b) Patterns equivalent to those in (a) for the orientation behavior of the fly (on its horizontal plane), according to theory (4, 6). (c) Histograms of the fly's gaze on the two patterns, aI and aII, respectively. The histograms extend from -180° to +180°; 0° in the histograms gives the fraction of time the fly gazed on the midline of the horizontal segments (a). The appearance of two maxima depends on the angular separation between the vertical segments in (b), and therefore on the (angular) dimensions of the Müller-Lyer patterns. As can be predicted (4, 6), there are two maxima in the histograms when the angular separation in (b) is more than about 40°. Below this value only one maximum appears.

However, "many experiments ... show that the [human] subject's subjective evaluation may always be judged from the eye movements made during comparison of distances." This sentence of Yarbus (2) suggests a correlation between oculomotor response and subjective length evaluation, and in fact the visual evaluation of length and the amplitude of eye saccades during an observation of the Müller-Lyer figure are in mutual agreement (2). Thus, eye movements induced by Müller-Lyer figures may be connected to the psychophysical illusion; although the nature of this relation remains unclear, it prompted us to examine the oculomotor response of flies to Müller-Lyer figures. It is well known that flies orientate toward (fixate) black objects such as vertical stripe segments (if presented to the lower half of the compound eyes). This behavior has been much analyzed (4) and a phenomenological theory (4-6) can quantitatively describe it. Tracking of moving objects (7) as well as of other flies (8) can also be essentially explained in the same way.

We used this orientation behavior to compare the fly's gaze with the known human eye movements when viewing Müller-Lyer figures. A tethered flying fly (head fixed to the thorax) was capable of controlling the rotation of a cylindrical "panorama" through its torque (around its vertical axis) and an electronic simulation of the flight dynamics. This setup, described elsewhere (4), simulates a free-flight situation in which the fly is free to orientate toward a stationary pattern on its horizontal plane.

A fly was presented successively with the two patterns shown in Fig. 1a, on the cylindrical panorama in two sessions of 3 minutes each; the histograms in Fig. 1c show the fraction of time the fly gazed at any part of the two patterns. Figure 1cI is the histogram of the gaze on the pattern in Fig. 1aI and Fig. 1cII is that of the pattern in Fig. 1aII. The histograms show clearly