ence is not obvious from the available data, but potential explanations include species difference and the possibility that 25 units of 25-HCC in the dog may have represented a dose greater than physiologic. The achievement of vitamin D depletion and its absence in our previous dog experiments may have been important, as suggested by earlier workers (*8, 10*). The time lapse which characterized the development of reduced phosphate excretion in response to the combined administration of 25-HCC at 1 unit per hour and PTH at 0.2 unit per hour (whereas neither agent was effective alone) lends additional credence to the concept that at this minimal dosage level both substances are required for the biological effect of the metabolite to be expressed. This delay could reflect either the time required for further metabolic conversion or the necessity for transcription and new protein formation to occur, or both. The necessity for the presence of PTH most likely results from the hormone's ability to act as a trophic agent (*22*) for the conversion of 25-HCC to that derivative of the vitamin which is directly active at the renal tubular cell level. This hypothesis would also explain the lack of effect of large doses (up to 25 units per hour) of the metabolite in the absence of PTH. The phosphaturia which occurred with massive doses of 25-HCC may well have reflected alterations induced in bone (*23*) or in the renal tubular cell (*24*) which, at this dosage level, may not require further metabolism of the 25-HCC (*23*).

As to the necessity for some form of the vitamin being present if PTH is to effect a phosphaturia, the existent experimental observations are conflicting. Harrison and Harrison described only an equivocal alteration in phosphate excretion after PTH in D-depleted animals, whereas repletion with 500 units of vitamin D 18 hours prior to hormone injection restored the (substantial) phosphaturic effect of the PTH (*5*). Similar conclusions were reached by Suh, Frasier, and Kooh, on the basis of improved renal responsiveness to PTH after vitamin D_2 administration in a patient with pseudohypoparathyroidism (*10*). Rasmussen and his colleagues originally reported that vitamin D was not required for the production of a phosphaturia by PTH (*3, 5*) although they later demonstrated that vitamin D–repleted rats did exhibit greater phosphate excretion in response to a standard dose of hormone, as was the case also when D-deficient animals were fed a high calcium diet (*5, 25*). An important reason, as well as the most likely explanation for this apparent discrepancy between their studies and ours presented here, is the fact that the dose of PTH used by Rasmussen's group (5 μg per hour, approximately equivalent to 10 units per hour of our hormone preparation) must be considered to be above the physiologic range.

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26. Supported in part by PHS grants AM 14708, AM 17575, and 5S01-RR-05709, by a grant-in-aid from the Health Research Services Foundation, Pittsburgh, Pennsylvania, and by institutional research funds from the Allegheny General Hospital (886), Pittsburgh, and the VA Hospital, Philadelphia, Pennsylvania. J.B.P. was a clinical investigator of the Veterans Administration and A.J. was supported by the American Research Hospital in Poland, Indiana, and was sponsored by the Maternal and Child Health Service of the Department of Health, Education, and Welfare. We thank D. Sylk, K. Loy, M. Kuhrman, H. Gaudiosi, D. Gallo, and C. Thomas for their assistance in the performance of the chemical analyses.

24 February 1975; revised 14 June 1975

Behavioral Characterization of *d*- and *l*-Amphetamine: Neurochemical Implications

Abstract. *Various doses of* d- *and* l-*amphetamine affect the temporal pattern of rat behavior in the following ways: First, the patterns of activity produced by* d- *and* l-*amphetamine are similar but out of phase; that is, the response to* d-*amphetamine has a relatively shorter latency whereas the effects of* l-*amphetamine persist for longer periods of time. Second,* d-*amphetamine is approximately five times as potent as* l-*amphetamine in its effects on both the total amount of locomotor activity and the duration of stereotypy. Both amphetamine-induced locomotion and stereotypy may be mediated by the same neurochemical mechanisms.*

Recent reports (*1*) have suggested relative potencies for *d*- and *l*-amphetamine that differ significantly from earlier estimates (*2*). The later results indicate that *d*-amphetamine is approximately four to six times more potent than *l*-amphetamine with respect to various indices of dopamine (DA) function in brain areas where DA is the predominant catecholamine (CA), and that the two isomers of amphetamine are approximately equal in their effects on norepinephrine (NE) mechanisms in brain regions where NE is the predominant CA. Similarly, conflicting results have been reported for the relative potency of *d*- and *l*-amphetamine in eliciting stereotypy (which refers to the continuous repetition of behaviors such as sniffing, licking, and gnawing). Scheel-Kruger (*3*) found that, rather than being approximately equivalent to *l*-amphetamine in eliciting stereotypy, *d*-amphetamine is four to six times more potent. These discrepancies may be due, at least in part, to an incomplete characterization of the effects produced by amphetamine. In most studies the time pa-

rameters for neurochemical and behavioral measurement appear to have been chosen arbitrarily. It is conceivable, however, that the temporal patterns of action for *d*- and *l*-amphetamine are substantially different (*4*). If they are, potency ratios determined over a relatively restricted interval would not adequately represent the relative effects of the two isomers.

A second problem in interpretation involves the apparently competitive interaction between amphetamine-induced locomotion and stereotypy. Since amphetamine appears to facilitate both behaviors, the behavior expressed during any interval of time may reflect the net effect of competitive interaction between the two behaviors. However, the fact that locomotion and stereotypy have generally been studied separately makes it difficult to assess their possible interaction.

In this study, the effects of *d*- and *l*-amphetamine on locomotion, rearing, and stereotypy were characterized over 6 hours. Adult male Sprague-Dawley rats, weighing 300 to 350 g each, were obtained from Carworth Farms. After being housed for 1 week under standard laboratory conditions, the rats were placed individually in sound-attenuating chambers (30 by 30 by 38 cm). Food and water were continuously available, and a 12-hour bright light (6 a.m. to 6 p.m.) and 12-hour dim light cycle was maintained. Movements from one quadrant to another (crossovers) were automatically counted through contacts in the floor of the chamber. Rearings were counted by touchplates set 12.7 cm above the floor. Both measures of locomotion were continuously monitored by a Nova 1200 computer. Viewing lenses in each experimental chamber and a closed-circuit videotape system allowed animals to be observed without being disturbed. In previous studies we had observed that moderate to high doses of amphetamine produced stereotypy, preceded and followed by periods of enhanced locomotion and rearing (*5*). The frequency of crossovers and rearings was observed to be inversely related to the intensity of stereotypy. Also, the qualitative features of stereotypy are similar with *d*- and *l*-amphetamine. For these reasons, changes in the frequency of locomotion and rearing were used as the index of onset and duration of stereotypy.

Twenty-four hours after being placed in the experimental chambers the rats were injected subcutaneously with different doses of *d*- or *l*-amphetamine (Smith Kline and French Laboratories) or with isotonic saline. Their behavior was monitored continuously over 12-minute intervals for 4 hours after injection and over 1-hour intervals after the fourth hour. Each group contained at least ten animals. The free base was used to calculate the drug dose.

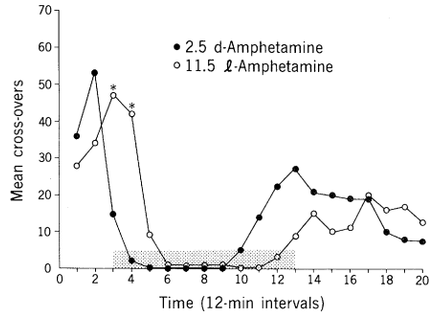

Fig. 1. Comparison of the time course of behavioral effects produced by a 2.5 mg/kg dose of *d*-amphetamine and a 11.5 mg/kg dose of *l*-amphetamine. These doses produce essentially the same total amount of locomotion (mean crossovers ± S.E.M. for 6 hours after injection, 353 ± 70 and 358 ± 52, respectively) and duration of stereotypy (approximately 1.5 hours). However, the temporal patterns are shifted. Shading indicates the presence of intense stereotypy during which most rats are not engaged in any forward locomotion or rearing (*significantly greater than corresponding *d*-amphetamine values, $P < .01$).

The prominent feature of the response induced by low doses of amphetamine is enhanced locomotor activity lasting up to 4 hours (*d*-amphetamine: 0.25, 0.50, and 1.00 mg/kg; *l*-amphetamine: 1.0, 1.5, 2.5, 3.0, 4.0, and 5.5 mg/kg). Linear regression analysis reveals a significant correlation between total cumulative activity and dose size of both *d*-amphetamine ($r = .909$; $P < .001$) and *l*-amphetamine ($r = .853$; $P < .001$). The relationship between dose size and activity can be expressed as CL (cumulative locomotion) = 378 × dose (mg) for *d*-amphetamine and as CL = 73.7 × dose (mg) for *l*-amphetamine. Although the extent of linearity between the two dose-response curves was not significantly different, the difference between the corresponding regression coefficients was statistically significant ($t = 14.4$; $P < .001$). Both slopes reflect increases in activity relative to saline controls. These data indicate a *d*- to *l*-potency ratio of approximately 5 : 1 for locomotion.

However, analysis of the temporal pattern of increased motor activity indicates that *d*-amphetamine has a more rapid onset and shorter duration of action than *l*-amphetamine. For example, during the first ½ hour after injection, *d*-amphetamine (1.0 mg/kg) produces significantly more activity than does *l*-amphetamine (5.5 mg/kg) (the mean crossovers ± S.E.M. were 68 ± 7 and 42 ± 8, respectively; $P < .05$). The opposite relationship is observed during the third and fourth hours (40 ± 6 and 110 ± 22, respectively; $P < .01$). For doses of *d*- and *l*-amphetamine that produce comparable total levels of activity over 4 hours, the peak 12-minute activity levels, although shifted in time, are of the same magnitude. It is apparent that a behavioral potency ratio based on an arbitrary interval of activity would not accurately represent the difference in potency between the two isomers. Furthermore, the temporal pattern of activity may be a more important variable than total activity in interpreting differences between *d*- and *l*-amphetamine effects on various neurochemical substrates.

We previously reported that with progressively higher doses of *d*-amphetamine, the latency of the onset of stereotypy becomes shorter, and the stereotypy persists for a longer time, as does the poststereotypy phase of enhanced locomotion (*5*). Recently we observed the same pattern with increasing doses of *l*-amphetamine. However, differences in the temporal pattern of effects described above with the low dose range are also apparent with higher doses of the two isomers (Fig. 1). For example, *d*-amphetamine doses of 2.5 mg/kg and *l*-amphetamine doses of 11.5 mg/kg produce essentially the same total amount of locomotor activity (mean crossovers ± S.E.M.: 353 ± 70 and 358 ± 52, respectively), and the duration of intense stereotypy is the same (approximately 1.5 hours). However, the temporal patterns differ. With *d*-amphetamine doses of 2.5 mg/kg the rats begin displaying marked stereotypy within ½ hour after injection, whereas with *l*-amphetamine (11.5 mg/kg) intense stereotypy is not apparent in most of the animals until about 1 hour after injection. In contrast, while animals injected with *d*-amphetamine begin to emerge from stereotypy about 2 hours after injection, those injected with *l*-amphetamine exhibit stereotypy for an additional ½ hour. During this ½ hour few animals engaged in any forward locomotion, whereas most of the rats injected with *d*-amphetamine were actively locomoting. Furthermore, although animals treated with *d*-amphetamine entered the third stage of enhanced locomotor activity sooner, the activity of the animals injected with *l*-amphetamine was significantly higher during the last part of the third phase (mean crossovers ± S.E.M. during the fifth and sixth hours after injection were 31 ± 11 and 80 ± 17, respectively; $P < .02$). This general relationship between the effects of *d*- and *l*-amphetamine is observed throughout the high dose range tested, further supporting the hypothesis that the behavioral patterns produced by the two isomers are similar although displaced in time.

A 2.5 mg/kg dose of *d*-amphetamine produces total locomotor activity and duration of stereotypy comparable to that re-

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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10 June 1975

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.

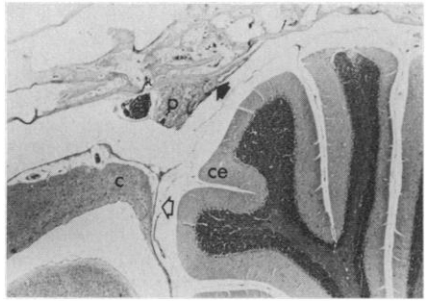

Fig. 1. Sagittal section through the brain after stalk deflection. The solid arrow indicates the deflected stalk of the pineal; the open arrow marks the normal position of the stalk; *c*, cerebrum; *ce*, cerebellum; *p*, pineal.

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