References and Notes

1. D. Madison, *Anim. Behav.* **17**, 25 (1969).
2. V. Twitty, D. Grant, O. Anderson, *Proc. Nat. Acad. Sci. U.S.* **56**, 864 (1966); *ibid.* **57**, 342 (1967).
3. The movement of an *Ambystoma maculatum* was continuously monitored after it was displaced in daylight 45 m from a breeding pond. It returned directly toward the pond for a distance of 34 m [C. R. Shoop, *Biol. Bull.* **135**, 230 (1968)].
4. *Plethodon jordani* is completely terrestrial (no aquatic larval stage and no breeding migrations), nocturnal, and lungless. It is quite sedentary and restricted to moist environments both because respiration is partly cutaneous and because of the vulnerability of the eggs to desiccation.
5. A Thyac III (Victoreen) scintillation survey meter and a model 489-55 gamma scintillation probe [3.17 by 3.82 cm NaI (Ti) crystal] were used.
6. Seven salamanders (10, 13, 14, 19, 20, 21, 22) served as controls for 1 to 4 weeks. Six were displaced later in the study. Salamander 20 disappeared 1 week after initial capture and thus could not be displaced.
7. Generally checks were made more often soon after the salamanders were released or during evenings when conditions seemed suitable for homing.
8. Data presented here indicate that, occasionally, *P. jordani* climb on tree trunks and other vegetation up to a height of about 2.3 m. Although this behavior has been reported [R. Gordon, J. MacMahon, D. Wake, *Zoologica (New York)* **47**, 9 (1962)], no reason has been given for its occurrence.
9. The method that gives the maximum home range as indicated by isotopes was used to estimate the size of the home area [H. Ambrose III, *Amer. Midland Natur.* **81**, 535 (1969)]. This method makes it possible to include areas of marginal familiarity to the salamander which we wished to include since the salamanders, upon arriving in such areas, could possibly use local landmarks to find the more frequented areas.
10. The size of the home area of this salamander was not determined. It was detected five times within 3 m of the release point during 3 days after release, but was detected only once thereafter 22 m from the release point 6 days after release.
11. Excluding salamanders 15 and 20 which disappeared, we detected salamanders on 588 of 606 attempts. Eight of the failures were for two salamanders which disappeared for 3- and 6-day intervals. The locations of the detection points, therefore, were not biased to include only those in the vicinity of release and home or between these areas.
12. Only those salamanders displaced upon initial capture were used in the course analyses (Fig. 2), thus excluding salamanders 10, 13, 14, 19, 21, and 22. Salamanders 19 and 23 (1 to 24 m) and 31 (8 to 24 m) were omitted from both the course and time analyses because failure to detect them during searches prior to their being detected at home gave unreliable course and time estimates. For the course analysis, any point along the estimated course (dashed line) was considered to be the best estimate of the salamanders' position between the actual (observed) positions.
13. E. Batschelet, *Amer. Inst. Biol. Sci. Monogr. 1* (1965). The Rayleigh, Stephens, and F analyses were used, when appropriate. All courses were examined for possible influence due to distance and direction of displacement. In addition, the observer's position at release, the position of a nearby creek, and the direction uphill and downhill to the release point were studied for their possible effects. In no case were there any noticeable effects on the homing courses.
14. Since *P. jordani* move on the forest litter only after dark, all movement times were considered in terms of cumulative dark hours (1930 to 0600 E.S.T.).
15. For example, male 18 released at 2220 hours, was detected 4.5 m from the release point at 0250 hours and 2 m from the initial capture site at 0530 hours on the same night. The best estimate of his arrival at home is 0410 hours, the midpoint of the interval prior to his being detected at home. Thus the THT was from 2220 to 0410 hours, a time of 5.8 hours. The RTT is 1.3 hours, one half the duration of the period from 0250 to 0530 hours.
16. S. Siegel, *Nonparametric Statistics* (McGraw-Hill, New York, 1956), pp. 116–127 (Mann-Whitney U test, timing analysis); *ibid.*, pp. 42–47 (chi-square one-sample test, climbing analyses).
17. Without the pause components, then, the salamanders displaced 30 m could have made the return trip in 30 minutes.
18. D. Grant, O. Anderson, V. Twitty, *Science* **160**, 1354 (1968); V. Twitty, *Of Scientists and Salamanders* (Freeman, San Francisco, 1966).
19. H. Landreth and D. Ferguson, *Nature* **215**, 516 (1967); D. H. Taylor and D. E. Ferguson, *Science* **168**, 390 (1970).
20. H. Landreth and D. E. Ferguson, *Science* **158**, 1459 (1967). A sun compass is postulated to be a basic orientation mechanism of amphibians.
21. D. Ferguson and H. Landreth, *Behaviour* **26**, 7 (1966). The necessity of wind and its flow in the one direction favorable for information transmission was thought to be relatively inefficient. Wind directions recorded in this study (in the woods) varied by as much as 180 degrees nightly. These variations along with the low wind speeds (typically from 0.1 to 0.8 m/sec) would support the operation of a short-range olfactory-based orientation system. Such a system operates in various animal groups; see, for example, E. O. Wilson, W. F. Blair, G. Tembrock, in *Animal Communication*, T. A. Sebeok, Ed. (Indiana Univ. Press, Bloomington, 1968); M. Jacobson, *Insect Sex Attractants* (Interscience), New York, 1965).
22. Y-axis or compass orientation in amphibians [D. E. Ferguson, in *Animal Orientation and Navigation*, R. M. Storm, Ed. (Oregon State Univ. Press, Corvallis, 1967)].

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Amphetamine: Differentiation by d and l Isomers of Behavior Involving Brain Norepinephrine or Dopamine

Abstract. d-*Amphetamine is markedly more potent an inhibitor of catecholamine uptake by norepinephrine neurons in the brain than is* l-*amphetamine, whereas the two isomers are equally active in inhibiting catecholamine uptake by the dopamine neurons of the corpus striatum. In behavioral studies,* d-*amphetamine is ten times as potent as* l-*amphetamine in enhancing locomotor activity, while it is only twice as potent in eliciting a compulsive gnawing syndrome. This suggests that the locomotor stimulation induced by amphetamine involves central norepinephrine, while dopamine neurons play an important role in the induced compulsive gnawing behavior. Assessment of differential actions of* d- *and* l-*amphetamine may be an efficient method to differentiate behaviors involving norepinephrine or dopamine in the brain.*

The behavioral effects of many drugs are thought to involve the catecholamines norepinephrine or dopamine in the central nervous system. Investigators have had only limited success in ascertaining whether particular effects are attributable to interactions with norepinephrine or dopamine, or both (*1*). By comparing and contrasting the biochemical and behavioral actions of *d*- and *l*-amphetamine, we have developed a method for the differentiation of behaviors mediated by norepinephrine or dopamine.

Amphetamine produces several types of behavioral effects in animals. It enhances locomotor activity, an action which may be a model for the central stimulant effects of amphetamine in man, and it provokes a compulsive gnawing sydrome. Both of these actions are thought to involve dopamine or norepinephrine, or both. Some studies implicate the corpus striatum, an area which contains the bulk of brain dopamine, in mediating the compulsive gnawing syndrome (*2*). Although indirect evidence suggests that norepinephrine tracts are important in effecting the enhancement of locomotor activity by amphetamine (*3*), some workers have favored a preponderant role for dopamine in eliciting this behavior (*4*).

Brain dopamine and norepinephrine are stored in distinct neuronal tracts (*5*). The corpus striatum contains very high concentrations of dopamine and low concentrations of norepinephrine, while in most other brain regions norepinephrine is the predominant catecholamine. In nonstriatal brain regions, *d*-amphetamine is ten times as potent as *l*-amphetamine in inhibiting catecholamine uptake by synaptosomes (pinched off nerve endings), while in the corpus striatum the two amphetamine isomers are equally active (*6*). This uptake system is thought to reflect reuptake of catecholamine released at synapses

Table 1. Inhibition of [^{3}H]norepinephrine uptake into different regions of rat brain by *d*- and *l*-amphetamine. Rats were given prior treatment with *d*- or *l*-amphetamine (10 mg/kg; subcutaneously) 1 hour before the intraventricular injection of 20 μc (20 μl) of [^{3}H]norepinephrine. All rats were decapitated 5 minutes after the administration of [^{3}H]norepinephrine; their brains were removed and dissected; and concentrations of endogenous and [^{3}H]norepinephrine were determined. Control animals received 0.9 percent sodium chloride solution in place of amphetamine. Each value is the mean ± S.E.M. for eight rats.

Treatment	Endogenous norepinephrine (μg/g)	Endogenous dopamine (μg/g)	[^{3}H]Norepinephrine (count min^{-1} g^{-1}) × 10^4
	Striatum		
Saline	0.09 ± .01	4.44 ± .25	159 ± 4
d-Amphetamine	0.08 ± .01	4.36 ± .30	79 ± 6*
l-Amphetamine	0.09 ± .01	4.69 ± .32	86 ± 4*
	Thalamus-hypothalamus-midbrain		
Saline	0.61 ± .03		221 ± 9
d-Amphetamine	0.44 ± .04*		160 ± 8*
l-Amphetamine	0.59 ± .03		211 ± 12
	Cerebellum		
Saline	0.35 ± .01		383 ± 17
d-Amphetamine	0.20 ± .01*		198 ± 23*
l-Amphetamine	0.33 ± .01		348 ± 20
	Brainstem		
Saline	0.42 ± .04		160 ± 27
d-Amphetamine	0.33 ± .02†		99 ± 23†
l-Amphetamine	0.44 ± .02		160 ± 27

* $P < .001$. † $P < .05$.

in the brain accounting for the inactivation of synaptically released amine (*7*). Drugs that inhibit catecholamine uptake should potentiate its synaptic actions so that *d*-amphetamine should be markedly more active than *l*-amphetamine at synapses of norepinephrine while the two isomers would be equal at synapses of dopamine. Accordingly, behavior mediated by brain norepinephrine might be affected considerably more by *d*-amphetamine than by *l*-amphetamine, whereas behavior mediated by dopamine should be affected similarly by the two isomers. We have confirmed by experiments in vivo the differential action of *d*- and *l*-amphetamine on catecholamine uptake by several nonstriatal brain regions and the equal activity of the two isomers in the striatum. We also report that *d*-amphetamine is ten times as potent as *l*-amphetamine in enhancing locomotor activity, while it is only twice as active in provoking compulsive gnawing behavior.

We used male Sprague-Dawley rats (150 to 200 g). For biochemical studies rats were given injections of *l*-[^{3}H]-norepinephrine (20 μc in 20 μl of Merles solution; 2.3 c/mmole; Amersham Searle) into the left lateral ventricle. Rats were decapitated after 5 minutes and their brains removed; the brains were dissected into different regions, and the concentrations of tritiated and endogenous norepinephrine were determined in each region (*8*). The rats were injected subcutaneously with 10 mg of *d*- or *l*-amphetamine per kilogram of body weight 1 hour before the administration of [^{3}H]norepinephrine.

For behavior studies, rats were given prior treatment with iproniazid (150 mg/kg), injected intraperitoneally 16 hours before the administration of amphetamine; iproniazid inhibits monoamine oxidase and thus facilitates the production of compulsive gnawing behavior (*2*). Control animals were injected with 0.9 percent NaCl. Rats injected with iproniazid alone did not differ from controls in locomotor activity and showed no evidence of compulsive gnawing behavior.

In behavioral studies rats were kept in individual cages with a wire grid floor and equipped with a light source and a photocell connected to a digital counter. Activity was measured as the number of times that a rat crossed the beam of light during a 30-minute session. Rats were placed in these cages 30 minutes before administration of amphetamine, and photocell recordings were initiated 5 minutes after injection of amphetamine. Compulsive gnawing behavior was assessed every 30 minutes after drug treatment. The compulsive gnawing syndrome was considered to be present when the rats were gnawing, chewing, or licking the grid floor of the activity cage, and when, after the rats were lifted from the grid and then replaced, they resumed gnawing within 10 seconds (*9*). At different doses total gnawing behavior was scored as the number of rats in groups of six that exhibited gnawing behavior. No attempt was made to "grade" the "degree of gnawing" or to score sniffing or stereotyped movements associated with this syndrome, because such scoring was markedly susceptible to observational errors. For behavioral studies, groups of six rats received amphetamine at 15 dose concentrations from 0.1 to 20 mg/kg. Each group of rats received only one dose of amphetamine, and a total of 180 rats were used.

In the cerebellum, the hypothalamus, thalamus, and midbrain, and the brainstem, *d*-amphetamine caused a marked reduction in the accumulation of the [^{3}H]norepinephrine while *l*-amphetamine had no effect (Table 1). Because rats were killed soon (5 minutes) after injection of [^{3}H]norepinephrine, the effect of amphetamine on amine accumulation is probably related to inhibition of neuronal membrane uptake rather than to effects on granular retention or amine release (*10*). *d*-Amphetamine but not *l*-amphetamine lowered endogenous norepinephrine concentration in these areas. In the corpus striatum, however, both *d*- and *l*-amphetamine caused marked reductions in accumulation of [^{3}H]norepi-

Table 2. Effects of *d*- and *l*-amphetamine on locomotor activity and compulsive gnawing behavior of rats. Rats were given prior treatment with iproniazid (150 mg/kg; intraperitoneally), and were then placed in individual photocell activity cages for 30 minutes before the injection of amphetamine. Five minutes after amphetamine was injected, locomotor activity was recorded for 30 minutes. Compulsive gnawing behavior was assessed every 30 minutes as an all or none effect (see text). The number of rats exhibiting compulsive gnawing behavior and locomotor activity after being treated with *d*- or *l*-amphetamine, expressed as percentage of maximum response, was plotted against the amphetamine dose on log-probit graph paper (*16*). The effective dose (ED-50 ± S.E.M.) for both types of behavior was calculated from this graph as the dose that produced 50 percent of maximum enhancement of locomotor activity or produced compulsive gnawing behavior in 50 percent of the rats.

Compound	Dose range (mg/kg)	Animals (No.)	Effective dose (ED-50)	
			Locomotor activity (mg/kg)	Compulsive gnawing behavior (mg/kg)
d-Amphetamine	0.1 to 20	84	0.9 ± 0.2	2.1 ± 0.4
l-Amphetamine	0.1 to 20	96	8.8 ± 0.9*	4.4 ± 0.9†

* Differs from *d*-amphetamine, $P < .001$. † Differs from *d*-amphetamine, $.05 < P < .1$.

nephrine without affecting endogenous dopamine or norepinephrine concentrations (Table 1). These findings confirm in vivo our earlier findings in vitro that in nonstriatal synaptosomes *d*-amphetamine is a much more potent inhibitor of catecholamine accumulation than is *l*-amphetamine. We also confirm in vivo that *d*- and *l*-amphetamine inhibit to the same degree the accumulation of catecholamines into striatal synaptosomes.

d-Amphetamine was ten times as potent as *l*-amphetamine in enhancing locomotor activity (Table 2). With increasing doses of amphetamine there was an enhancement of locomotor activity up to a dose of 1.5 mg of *d*-amphetamine per kilogram or a dose of 12 mg of *l*-amphetamine per kilogram. After these doses of *d*- or *l*-amphetamine, equal peak locomotor activity was recorded. Further increases in dose resulted in decreased locomotor activity. This tenfold difference between the potency of the two isomers on locomotor activity closely parallels the tenfold difference of their potency in inhibiting catecholamine uptake by cerebral cortical synaptosomes (*6*).

There have been many theories to explain the stimulant action of the amphetamines in the brain, including synaptic release of norepinephrine (*11*), inhibition of its reuptake (*12*), inhibition of monoamine oxidase (*13*) and direct action on receptors (*14*). Our results suggest that inhibition of norepinephrine uptake may be a major mechanism of action. We also found that *d*-amphetamine was much more potent than the *l*-isomer in lowering endogenous norepinephrine concentrations. Because the doses of *l*-amphetamine required to decrease norepinephrine concentrations would be toxic, it is not possible to compare the potencies of *d*- and *l*-amphetamine in decreasing brain norepinephrine; therefore we cannot rule out the possibility that there also exists a close relation between the differential potency of *d*- and *l*-amphetamine in depleting norepinephrine and in stimulating locomotor activity, respectively. It is also unclear whether norepinephrine depletion is a result of inhibition of reuptake or is due to synaptic release of catecholamine by amphetamine.

d-Amphetamine was only about twice as potent as *l*-amphetamine in evoking the compulsive gnawing syndrome, and the differences between groups had only borderline statistical significance. The greater similarity of the two amphetamine isomers in eliciting gnawing than in stimulating locomotor activity, together with biochemical evidence in vivo and in vitro, of effects of the two isomers on uptake of catecholamine in the corpus striatum, suggests that gnawing behavior is related to an action of amphetamine on striatal dopamine neurons. Presumably, if gnawing were determined solely by dopamine tracts, *d*- and *l*-amphetamine should have been equal in their effects. The twofold difference in potency of these isomers indicates that norepinephrine neurons may participate to a limited extent in production of this behavior, possibly as a triggering mechanism.

Because there are a large number of norepinephrine-containing tracts in the brain, pharmacologic criteria, such as the different potencies of *d*- and *l*-amphetamine in producing locomotor stimulation, cannot readily delineate which isomer is involved in eliciting a given behavior. By contrast, there are only a few dopamine-containing tracts in the brainstem. Besides the nigrostriatal tract, which appears to be related to the gnawing behavior induced by amphetamine, other dopamine tracts arising in the brainstem have terminals in the olfactory tubercle and the nucleus accumbens (*5*). A dopaminergic tract originating in the arcuate nucleus of the hypothalamus and terminating in the median eminence has beeen implicated in regulating the synthesis and release of pituitary trophic hormones (*15*). Experiments with *d*- and *l*-amphetamine may help to elucidate the functions of these tracts.

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References and Notes

1. J Glowinski, J. Axelrod, L. L. Iversen, *J. Pharmacol. Exp. Ther.* **153**, 30 (1966); K. E. Moore, *Biochem. Pharmacol.* **18**, 1627 (1969); L. L. Butcher and N.-E. Anden, *Eur. J. Pharmacol.* **6**, 255 (1969); H. H. Wolf, D. E. Rollins, C. R. Rowland, T. G. Reigle, *Int. J. Neuropharmacol.* **8**, 319 (1969).
2. A. Ernst, *Psychopharmacologia* **10**, 316 (1967); N.-E. Anden, K. Fuxe, T. Hokfelt, A. Rubenson, *J. Pharm. Pharmacol.* **19**, 627 (1967); J. Scheel-Kruger and A. Randrup, *Life Sci.* **6**, 1389 (1967); R. L. Fog, A. Randrup, H. Pakkenberg, *Psychopharmacologia* **11**, 179 (1967).
3. A. Carlsson and M. Lindqvist, *Eur. J. Pharmacol.* **2**, 187 (1967); L.-M. Gunne and D. J. Reis, *Life Sci.* **11**, 804 (1963); F. Sulser, M. Owens, M. Norvich, J. Dingell, *Psychopharmacologia* **12**, 322 (1968).
4. J. Van Rossum, *Biochem. Pharmacol.* Abstr. 740, Suppl. **12**, 210 (1963).
5. K. Fuxe, *Acta Physiol. Scand.* **64**, 37 (1965); N.-A. Hillarp, K. Fuxe, A. Dahlstrom, *Pharmacol. Rev.* **18**, 727 (1966); O. Hornykiewicz, *ibid.*, p. 925.
6. J. T. Coyle and S. H. Snyder, *Science* **166**, 899 (1969); *J. Pharmacol. Exp. Ther.* **170**, 221 (1969).
7. J. Axelrod, *Recent Progr. Hormone Res.* **21**, 597 (1965); L. L. Iversen, *The Uptake and Storage of Noradrenaline in Sympathetic Nerves* (Cambridge Univ. Press, New York, 1967).
8. R. Laverty and K. M. Taylor, *Anal. Biochem.* **32**, 269 (1968); K. M. Taylor and R. Laverty, *J. Neurochem.* **16**, 1361 (1969).
9. A. Ernst, *Acta. Physiol. Pharmacol. Neerl.* **15**, 141 (1969).
10. S. Schanberg, J. J. Schildkraut, I. J. Kopin, *Biochem. Pharmacol.* **16**, 393 (1967).
11. J. Glowinski and J. Axelrod, *J. Pharmacol. Exp. Ther.* **149**, 43 (1965); K. E. Moore, *ibid.* **142**, 6 (1963); L. Stein, *Fed. Proc.* **23**, 863 (1964).
12. G. Hertting, L. T. Potter, J. Axelrod, *J. Pharmacol. Exp. Ther.* **134**, 146 (1961); A. Carlsson, M. Lindqvist, A. Dahlstrom, K. Fuxe, D. Masuoka, *J. Pharm. Pharmacol.* **17**, 521 (1965); J. Glowinski, L. L. Iversen, J. Axelrod, *J. Pharmacol. Exper. Ther.* **151**, 385 (1966).
13. J. Glowinski, J. Axelrod, L. L. Iversen, *J. Pharmacol. Exp. Ther.* **153**, 30 (1966); H. Blashko, D. Richter, H. Schlossman, *Biochem. J.* **31**, 2187 (1937).
14. C. B. Smith, *J. Pharmacol. Exp. Ther.* **147**, 96 (1965).
15. K. Fuxe, T. Hokfelt, O. Nilsson, *Neuroendocrinology* **5**, 107 (1969); K. Fuxe and T. Hokfelt, in *Frontiers of Neuroendocrinology*, W. F. Ganong and L. Martini, Eds. (Oxford Univ. Press, New York, 1969), p. 47; I. A. Kamberi, R. S. Mical, J. C. Porter, *Science* **166**, 386 (1969).
16. L. Miller and M. Tainter, *Proc. Soc. Exp. Biol. Med.* **57**, 261 (1944).
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Neural Symbolic Activity: A Psychophysical Measure

Abstract. *When a subject views a grating which is partially blocked from view by a cube, adaptation (decrease in contrast of the grating) occurs not only to the visible portions of the grating, but also to those portions blocked from view. This may indicate the existence of a neural mechanism which conveys the information "in back of."*

When a grating is viewed for a prolonged period, discrimination thresholds to the same and similar gratings are subsequently raised, or the apparent contrast of the same and similar gratings is depressed, whereas thresholds to and apparent contrast of gratings differing in orientation or size remain unchanged (*1*). This is called adaptation, and indicates that different popu-