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2 February 1955

Technique for Assessing the Effects of Drugs on Timing Behavior

Before a drug can safely be recommended for relief of a specific physiological malfunction it must be checked for possible deleterious effects upon other systems. Careful consideration must also be given to the possibility of "toxic" effects upon normal adaptive behavior, particularly in the case of those drugs that are known to produce behavioral changes. In view of the importance of temporal orientation in normal behavioral functions, the effect of drugs upon timing would appear to constitute a primary area of investigation. The purpose of this report is to describe a method, based on earlier work by Skinner (*1*), for producing and measuring timing behavior in experimental animals, and to present some data resulting from the administration of amphetamine and alcohol (*2*).

White rats, deprived of water for 22.5 hours, were placed in a small chamber containing a lever and a mechanism for automatic delivery of a small drop of water. In the first session every depression of the lever by the animal produced the water reinforcement. In all following sessions, each 2 hours long, reinforcement was contingent on lever presses spaced at least 21 seconds apart. That is, a response produced the water only if it followed the preceding lever depression by at least 21 seconds. Programming and recording were accomplished automatically by timers, magnetic counters, and associated relay circuits.

Using this procedure, Wilson and Keller have demonstrated that the rate of lever pressing is inversely related to the length of the required delay between responses (*3*). Since drug-produced general excitatory or depressive effects might themselves alter the rate of lever pressing (*4*), a more useful measurement of the timing is the relative frequency distribution of interresponse times (intervals between successive responses). This distribution, which displays no consistent trend after the animals have been exposed to the experimental procedure for 30 to 60 hours, is illustrated by the "saline" control records of Fig. 1. The large proportion of responses that are spaced less than 3 seconds apart is typical and unexplained, but the remainder of the distribution provides a clear description of the timing behavior. The relative frequencies of interresponse times rise to a peak between 18 and 21 seconds and then display a gradual decline.

The center sections of Fig. 1 illustrate the effects of two relatively large doses of *dl*-amphetamine sulfate administered subcutaneously, 5 minutes prior to the experimental session, in a solution of 1 mg/ml of physiological saline. Increasing doses of this drug tend to move the peak of the distribution progressively toward the short interresponse times. That is, the animals press the lever more frequently before the required interval has elapsed.

In addition to the shifts in the relative frequency distribution, large increases in the total response output occurred (Table 1). In contrast, intraperitoneal injection of 3 ml of a 10-percent ethyl alcohol solution, representing a dose of 1 mg/kg, produced a decline of more than 50 percent in the rate of lever pressing. The relative interresponse time frequencies, however, as shown in the lower section of Fig. 1, displayed little change except for a slight leveling of the total distribution. Although alcohol, in the dose administered, produced a general depression of lever-pressing behavior, there was relatively little effect upon the timing.

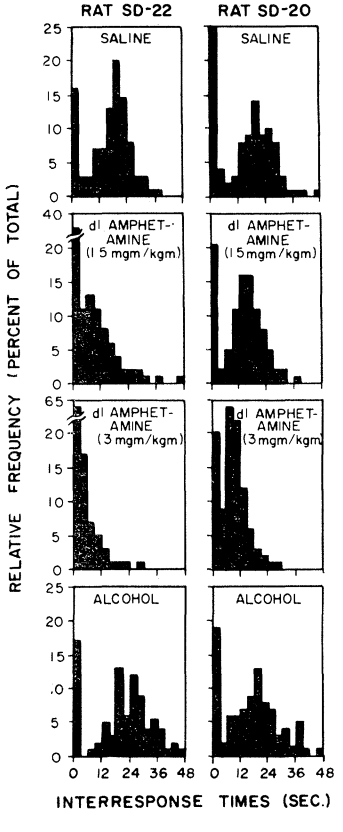

Fig. 1. Relative frequency distributions of the time intervals between successive lever presses. Each distribution represents one animal's performance in a single 2-hour session.

Table 1. Total number of lever presses emitted during each 2-hour session

Rat	Saline	*dl*-Amphetamine		Alcohol
		1.5 mg/kg	3.0 mg/kg	
SD-20	471	534	854	201
SD-22	433	820	1816	176

This technique for generating and measuring timing behavior is applicable to the minimally restrained individual organism, produces stable behavior over long periods of time, has procedural simplicity, and permits automatic programming and recording. The orderly response to drugs, illustrated by this data, indicates the feasibility of including this method in programs designed to screen drugs for their behavioral effects.

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13 June 1955

Altering Juvenility with Auxin

One of the most carefully described instances of the juvenile phase in plant development is that of the Brussels sprouts (*Brassica oleracea gemmifera* L. var. Kolom) (*1, 2*). Plants of this species must reach a certain age—generally 11 weeks—before they are sensitive to the floral inductive experience of low temperature.

The effectiveness of low temperatures on flowering is known for many species of plants (*2–5*). The recent report by Moore (*6*) that auxin applied before low temperature may accentuate the bolting response of cabbage suggests the possibility that auxin levels insufficient for bolting may be a partial basis for juvenility.

This paper (*7*) deals with the influence of auxin on the low temperature induction of floral initiation in Brussels sprouts. It is shown that an application of auxin during the cold treatment has a promotive effect on flowering and can effectively shorten the juvenile phase.

Table 1. Influence of auxin on flowering of Brussels sprouts when applied to plants of various ages. Ages at the beginning of cold treatment are given.

Age (wk)	Plants flowering (%)					
	Auxin before induction (ppm)		Auxin during induction (ppm)		Auxin after induction (ppm)	
	0	50	0	50	0	50
15	100	100	100	100	100	100
13	100	100	100	100	100	80
11	70	70	60	80	80	40
9	0	0	0	60	10	0
7	0	0	0	0	0	0
5	0	0	0	0	0	0

Six groups of plants of different ages, respectively 5, 7, 9, 11, 13, and 15 weeks old, were simultaneously given a cold treatment (5°C) of 9 weeks duration. To each group, auxin (0, 50, or 500 parts per million naphthaleneacetic acid in lanolin) was applied over a period of 10 days either before, during, or after the cold treatment to the debladed petiole stumps of the 2 youngest mature leaves. Each treatment was given to 10 plants. The plants were grown during the whole experiment (September 1954 to April 1955) in the greenhouse.

The percentages of plants that flowered in response to the cold treatment are given in Table 1. Just as expected, control plants younger than 11 weeks at the commencement of cold treatment did not flower except for one plant in the third series, which flowered at 9 weeks of age. However, plants 9 weeks of age treated with 50 parts per million of auxin during the cold treatment flowered in 60 percent of the cases. The auxin applications before or after the cold treatments were essentially ineffective in shortening the juvenile phase.

It is interesting to note that Stokes and Verkerk (*1*) have reported that earliest flowering occurs in this species at about the thirtieth node. Our data agree in that the average node of first flower of the 11-week-old plants was 31.8. However, the induced 9-week-old plants flowered at 19.3 nodes.

Not only did auxin paste treatment bring about flowering of apparently juvenile plants, and at a node lower than normal, but the 50-parts-per-million treatment also hastened floral initiation *in time* when it was applied during the cold treatment. The effects on time of flowering are shown in Fig. 1. Auxin applied during the cold treatment hastened the appearance of flower buds by 3 to 4 days. There was little effect resulting from treatment before the cold, and no effect with the aftertreatment. The effect of the high auxin application (500 parts per million) was much less pronounced. The question arises whether a lower concentration than the ones that were used would be still more effective.

The lengths of flower stalks yielded an interestingly similar response, as shown in Table 2. It can be seen that stalk elongation was increased by auxin applied during the cold induction. This increase ranged from 16 to 52 percent. Auxin applied before or after the induction had no apparent effect on length of flower stalks.

The data presented here indicate that under the test circumstances, auxin applications during cold treatment can permit floral initiation of otherwise juvenile plants of Brussels sprouts, at an unusually low node, with an acceleration evident both in the 9- and 11-week-old plants, and with a resultant increase in length of the flower stalks as well.

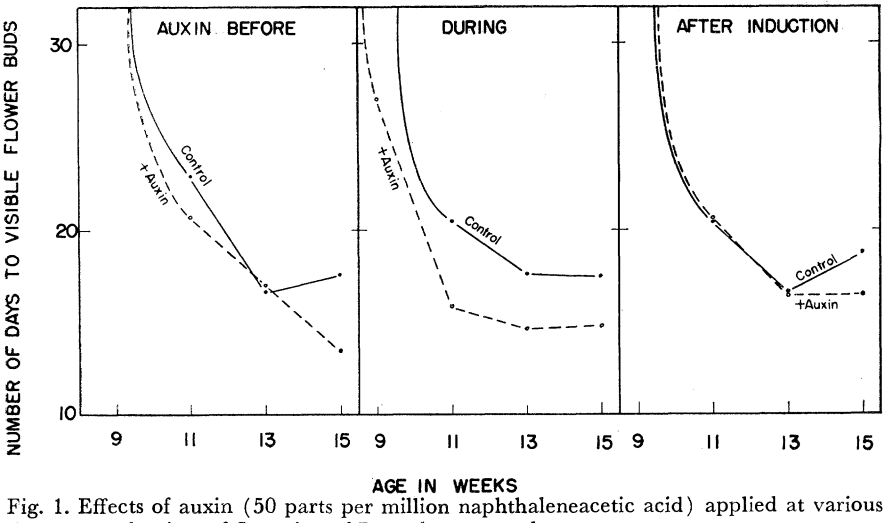

Fig. 1. Effects of auxin (50 parts per million naphthaleneacetic acid) applied at various times upon the time of flowering of Brussels sprouts plants.

Table 2. Influence of auxin applied during induction on the length of the flower stalk of Brussels sprouts. Ages of plants at the beginning of cold treatment are given.

Age (wk)	Length of flower stalk (cm)		
	Auxin concentration (ppm)		
	0	50	500
15	46.0	51.5	40.0
13	34.6	50.2	43.0
11	26.0	34.3	26.6
9		5.2	4.7
7			
5			

The results of these experiments extend the growing body of evidence that auxin treatments associated with low temperatures are promotive of flowering. Previous studies have reported such auxin promotions for species sensitive to photoperiods, to vernalization, and for indeterminant species (*8*). In the present experiments, auxin treatment resulted in flowering of otherwise juvenile plants. It is suggestive that the completion of the juvenile phase may be in part the accumulation of a sufficient auxin level at the apical meristem to bring about the condition receptive to cold.

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Production of Toxin by Resting Cells of Cl. Parabotulinum Type A

The toxin of *Cl. parabotulinum* type A is a high molecular (*1*) protein distinguished by its extremely high biological potency (*2*). It appeared therefore that this system might provide an interesting model for a study of protein biosynthesis. Toxin formation by these cells is a rela-