Inhibition of Flowering by Light in the Short-Day Plant Salvia occidentalis

Abstract. *Long days (continuous light) have inhibitory effects on* Salvia occidentalis *by producing transmissible substances that suppress flowering. Only when the inhibition at the apex is removed can flowering proceed normally.*

Schwabe (*1*), working with the short-day plant *Kalanchoe blossfeldiana*, suggested that long days intercalated in a short-day treatment interfere with the promotive effects of short-day cycles and thus block or limit their effect by producing an inhibitive substance or substances. Such long days would counteract the effect of succeeding, but not of preceding, short-day cycles. Wellensiek (*2, 3*) demonstrated with *Perilla crispa* and *Salvia occidentalis* that continuous light applied during an inductive short-day treatment has a markedly inhibitive effect on processes leading to the induced state, that is, the ability to produce a floral stimulus.

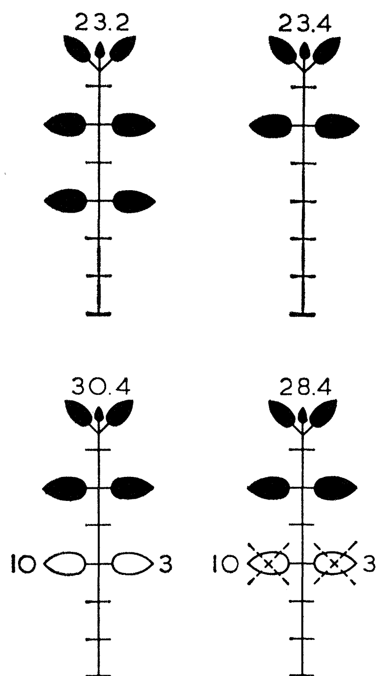

Fig. 1. Two-leaf-pair scheme and transmissibility of light-induced inhibitor. *Black leaves*, permanently exposed to short days. Two plants in upper row are controls. *White leaves*, after 10 short-days (figure at the left), then 3 days of continuous light (figure at the right), followed by short days. *White leaves (crossed)*, same as "white leaves," but removed immediately after the continuous light. Figures above the plants are mean days to budding.

Both authors support the contention that the inhibitory effects of intercalated long days or continuous light are localized within the leaves and thus interfere with the production of the floral stimulus, but not with its action. However, I have presented evidence (*4*) of the production of a transmissible floral bud inhibitor in the leaves of *S. occidentalis*, a qualitative short-day plant with a critical day length of about 13½ hours of strong white light (*5*).

In some experiments where short days were alternated with photoperiods of 12 to 24 hours of light, not all noninductive day lengths were inhibitory, but the flower-inhibiting effect of noninductive cycles, if present, increased with increasing day length. Maximum inhibition was obtained from continuous light. When a period of one or more days of continuous light was intercalated between two series of short days, continuous light had a marked positive action and was not merely passive. Moreover, inhibitory continuous-light cycles showed a marked time effect when applied during an inductive treatment. The short-day treatment of groups of plants was interrupted once by 48 hours of continuous light (high intensity 3500 μw/cm^2, at greenhouse temperature ± 20°C) after 0 to 24 short days (Table 1). The numbers of days necessary for flower bud formation clearly show the time effect of inhibition by continuous light. Continuous light has no inhibitory effect when applied before the short-day treatment, the difference from the uninterrupted short-day control being exactly 2 days, which is the duration of the interruption by continuous light. Only after two or more short-day cycles is continuous light appreciably inhibitive. The effect of light inhibition gradually increases and reaches its maximum after 8 to 10 short days. For more than 10 short days the difference declines, and completely disappears after 20 short days.

When increasing numbers of continuous-light cycles were intercalated after 10 short days in a short-day treatment, the effect of inhibition by continuous light increased for up to at least 3 days of continuous light. Moreover, when the combination of 10 short days and 3 days of continuous light was repeated from one to five times, followed by exposure to short days, all plant groups initiated flower buds simultaneously with the control in 21 to 22 short days. These results strongly suggest that inhibition by continuous light completely erases the effects of ten preceding short-day cycles. Continuous-light inhibition is not negated by 1 to 7 days of subsequent darkness.

Table 1. Inhibitory effect of 2 days of continuous-light interruption during the short-day inductive treatment. Five plants were used for each treatment.

No. short days before continuous-light interruption	Days to flower bud formation	Difference from control
Control	26	
0	28	2
2	30	4
4	32	6
6	34	8
8	37	11
10	37	11
12	32	6
14	31	5
16	31	5
18	30	4
20	28	2
22	28	2
24	26	

When intercalated continuous-light periods were replaced by occasional breaks of light lasting 4 hours during the middle of the dark periods of normal short-day treatments, the inhibitory response was similar to, though quantitatively much smaller than, the response to continuous light. This inhibitory effect of intercalation of 48 hours of continuous light disappears at low light intensity (below 800 μw/cm^2) and low temperature (10°C).

Plants were defoliated, leaving either one or two leaf pairs on each plant (*4*). In a one-leaf-pair scheme, in which the intercalation treatment was given either to the single leaf pair or to the apex, considerable inhibition was found when the continuous-light treatment was given to the leaves. This suggests that the main site of the inhibitory effect of intercalated continuous light is in the leaves and not the apex.

Transmissibility was tested in a two-leaf-pair scheme (Fig. 1). The upper leaf pair and the apex were always kept under short-day conditions, whereas the lower leaf pair received a treatment of 10 short days, followed by 3 days of continuous light, and then permanent short days. The lower leaf pair was either retained or removed after the continuous light. Inhibitory response was marked when the leaf inhibited by continuous light was removed immediately after the 72

hours of continuous light. Inhibition of flowering is possible by the action of continuous light on leaves which are situated well below the leaves treated with short days. The inhibitor induced by continuous light must migrate from its origin toward the apex soon after it is produced. Inhibition by continuous light is not localized in the leaves; an inhibitor is transmitted to the apex without interfering with either the production or the translocation of the floral stimulus, but prevents flower bud formation at the growing point. The dominant role of this inhibition at the apex disappears after a certain time so that the stimulus can express itself; only then can the flowering processes proceed normally.

On the basis of these results and other work to be reported elsewhere (*6*), I suggest that the morphogenetic changes at the apex are regulated through competition between the flower-inhibiting and flower-promoting substances, both arising in the leaves and both acting at the apex.

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References and Notes

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Spreading Depression and Recovery from Lateral Hypothalamic Damage

Abstract. *Spreading cortical depression reinstates aphagia and adipsia in rats recovered from lateral hypothalamic lesions. We suggest that cortical activity facilitates and maintains recovery by enhancing the activity of depressed, but intact, tissue adjacent to the lesions.*

Lateral hypothalamic lesions produce in rats an aphagia and adipsia from which they gradually recover (*1*). Such rats, at first, do not eat or drink, lose weight, and will die unless kept alive by intragastric tube-feeding. Then they progress to a stage of anorexia and adipsia, in which they eat wet and palatable foods, but not enough to maintain their weight. They still refuse to drink water, and die unless tube-feeding is continued. In a later stage of recovery, they regain their ability to regulate their caloric intake and maintain their weight but they remain adipsic—they still refuse to drink water. Eventually, however, they drink water, eat dry food, and appear completely recovered (*2*).

We do not fully understand how this recovery of function occurs. The tissue adjacent to the original lesions appears to be important: after complete recovery, additional lesions adjacent to the original lesions will again cause aphagia and adipsia (*2*). Therefore, it is possible that, in addition to the total destruction caused by a lesion, a temporary depression of activity is produced in the tissues adjacent to it. Such a temporary depression could be caused by local physical trauma—edema, partial poisoning of cells through deposition of metallic ions by the direct current used to produce our lesions (*3*), or by gliosis. In addition, a phenomenon such as "spinal shock" may occur; destruction of one region may cut off facilitating impulses to adjacent or related tissues, which are then unable to function (*4*). After some time, the depressed tissues recover their excitability and the symptoms disappear. By this reasoning, the activity of nervous tissue adjacent to lateral hypothalamic lesions is essential for the recovery of feeding and drinking.

The cerebral cortex appears to be important in maintaining lateral hypothalamic activity. During spreading cortical depression induced by KCl, the activity of cells in the lateral hypothalamus is greatly decreased (*5*). Spreading depression also eliminates self-stimulation in the lateral hypothalamus (*6*). Because the lateral hypothalamic areas that lie in the positive reward system are the same ones in which electrical stimulation induces feeding (*7*), it follows that spreading cortical depression should also decrease the activity of the feeding system. As shown by Bureš and his coworkers (*8*), aphagia and adipsia are produced in normal animals during spreading depression. In rats which have recovered from lateral hypothalamic lesions and which possess less remaining functional tissue in the feeding system, spreading depression should reinstate aphagia and adipsia, and the effect should be more pronounced than in normal animals. This is what we show in this report.

Two groups of Long-Evans hooded male rats each weighing 250 to 300 g were prepared. The experimental group consisted of six rats, fully recovered from lateral hypothalamic lesions (with the skull held level, the stereotaxic coordinates were: A 6.0, L 2.0, 8.0 mm down from cortex; anodal direct current, 1 ma, applied for 20 seconds). The average rate of recovery was 26 days (range 20 to 33 days), and recovery terminated an average of 15 days before the onset of spreading depression. Five control animals were used.

In both groups of animals, using the method developed by Bureš (*8*), we exposed the dura of both cerebral hemispheres by drilling a 5-mm hole in the skull directly over the midline suture, or in some animals, two such holes as close as possible to the midline. If a piece of filter paper large enough to fill the hole in the skull is soaked in 25 percent KCl and is placed directly on the dura, waves of depolarization are produced which spread over the entire cerebral cortex and diminish spontaneous cortical activity for 3 to 4 hours (*8*). The depression is believed to be largely confined to the cerebral cortex of that hemisphere (*8*) although there are reports of it spreading into the striatum (*9*). Animals trained previously to avoid shock do not do so during spreading depression (*8*). This is so reliable an indicator that we routinely monitored the effectiveness of cortical depression by testing the animal's ability to turn a wheel to avoid shock. After 3 to 4 hours, all animals regained this ability. To allow repeated applications of KCl in the same animal, in a method similar to that of Russell and Ochs (*10*), we placed stainless steel tubes (5 mm inside diameter and 6 to 7 mm long) on the skull surrounding each hole and fixed them permanently to the skull with screws and dental cement. Each tube was threaded internally to receive a stainless steel screw so that the opening in the skull could be capped tightly to prevent drying of the dura.

To insure that they would eat readily at the time of testing, all animals received no food for one day and limited food thereafter to keep their weight at approximately 85 percent of normal. Filter paper was placed on the dura and was then soaked with 0.05 ml of 25 percent KCl dripped into each dural cap with a hypodermic syringe.