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Action of Intrahypothalamic 6-Hydroxydopamine on Motivated Responding for Food and Water in the Rat[1]

G. E. MARTIN AND R. D. MYERS

Laboratory of Neuropsychology, Purdue University, West Lafayette, Indiana 47907

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MARTIN, G. E. AND R. D. MYERS. *Action of intrahypothalamic 6-hydroxydopamine on motivated responding for food and water in the rat.* PHARMAC. BIOCHEM. BEHAV. 2(3) 393–399, 1974. – 6-Hydroxydopamine (6-OHDA) was micro-injected in minute quantities into the hypothalamus of the food- and water-deprived rat that had been trained to lever press for food pellets and water. During a 2-hr period subsequent to a micro-injection, the effect was examined of 0.2, 0.5, 2.0 or 4.0 μg of the drug on lever pressing responses (FR-10 schedule of reinforcement) that were motivated by food and water deprivation. Injected bilaterally, a low dose (0.5 μg) of 6-OHDA significantly disrupted the motivated responding in only one rat. However, after successive injections of 2.0 or 4.0 μg of 6-OHDA, the lever responses for both food and water were suppressed in a dose-dependent fashion in all animals. Similarly, the ad lib consumption of food and water during an additional two-hour period in the home cage was markedly reduced after the highest dose was injected. Directly after the injection was given, there was no significant increase in food or water intake. The possible mechanisms are discussed by which 6-OHDA impairs the rat's performance of an operant task motivated by hunger and thirst.

6-OHDA lesions Hypothalamic injections of 6-OHDA Chemical lesions Food motivated behavior Feeding behavior Drinking Water intake

IN a previous paper, we found that the application of 6-hydroxydopamine (6-OHDA) in a discrete region of the rat's hypothalamus has a protracted inhibitory action on the intake of food [15]. The aphagic effect of 6-OHDA on ingestive behavior [6, 21, 23] has been attributed to an action of the drug in selectively destroying the pre-synaptic elements of catecholaminergic neurons [22], which have already been implicated in the central control of feeding behavior in the rat [7] and primate [10, 16, 20, 26].

Although 6-OHDA may exert a local neurochemical action when injected into cerebral tissue in a quantity as low as 0.2 μg [24], doses ranging from 4.0 to 32.0 μg reliably block ingestive behavior [15, 21, 23]. On the other hand, when 6-OHDA is micro-injected into the hypothalamus of the rat in a dose of 0.1 to 16.0 μg, the animal may also eat [5].

In earlier studies, the food and water intakes under ad lib conditions were studied following the treatment with 6-OHDA. To determine whether this compound may also exert an effect in a condition in which the rat had to emit an instrumental response, the present experiment was undertaken in which the animal had to depress one lever to obtain food and another to obtain water. Such a behavioral task could provide a somewhat more sensitive measure of the effect of 6-OHDA on motivated behavior [9]. Thus, the primary purpose of the present experiment was to establish the minimal doses at which 6-OHDA would cause an alteration in the lever response for food or water in an operant task.

METHOD

Male albino rats of the Sprague-Dawley strain were used which ranged in weight from 360 to 460 g. Each of 10 animals was trained on an FR-10 schedule of reinforcement to depress one of two levers in order to obtain either 0.07 ml of water or a 45 mg Noyes pellet, both of which were delivered by automated dispensers (Lafayette Instrument Co.). Throughout the experiment, the testing cages containing the two levers were kept in sound-attenuated chambers (Lehigh Valley Electronics). The rates and total number of operant responses were recorded simultaneously on cumulative recorders and digital counters. Water intakes were determined from the scale on the liquid dispenser which was calibrated in 1 ml gradations.

Each animal was deprived of food for 20 hr daily and

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was then placed in the operant chamber for a 2 hr period. Immediately after each test session, the animal was returned to its home cage where food pellets (Noyes Co.) and water from a calibrated Nalgene tube were provided ad lib for an additional 2 hr period so that the rat could maintain its body weight at 80% of the pre-deprivation level. After the rate of responding of each animal had reached an asymptote and body weight had stabilized under this regimen, the 10 rats were divided randomly into two groups: 5 controls and an equal number which were given 6-OHDA.

Using methods described previously by Myers [11], guide cannulae fashioned from 22 ga stainless steel tubing were implanted bilaterally one mm above the site of injection in each of 5 rats anesthetized with pentobarbital sodium (40 mg/kg). The intended site of 6-OHDA injection in each instance was: AP = 5.4, Lat. = 2.0, HV = 3.5 [18]. A 10 day recovery period of free-feeding was given before the animal was replaced on the 20 hr deprivation schedule.

After each animal responded reliably for food and water at a rate equal to that observed prior to surgery, bilateral micro-injections of 6-OHDA were made just before the daily 2 hr session in the test cage. Each injection was delivered over a 45 sec interval through a 26 ga injector cannula in a final volume of 0.5 μl. The injector needle was left in place for 45 additional sec to permit infiltration of the injected material into the tissue surrounding the tip [11]. Upon replacement of the stylet, the same procedure was repeated immediately at the contralateral site. Following these bilateral injections the rat was placed at once in its test cage, and the operant responding for food and water was monitored over the next two hours. Then the animal was given food and water ad lib in its home cage for an additional 2 hr.

6-OHDA (6-hydroxydopamine hydrobromide, Sigma Chemical Co.) was dissolved in an artificial CSF solution described previously [12] to which 0.2 mg/ml of ascorbic acid was added to prevent auto-oxidation of the drug. The compound, expressed here as the salt, was administered initially to each animal in doses of 0.2 or 0.5 μg. Thereafter the doses were increased to 2.0 and then to 4.0 μg. Each animal was used as its own control since repeated microliter injections do not alter lever responding [13].

If during a daily test session an animal displayed only a temporary decline in feeding and drinking, the same concentration of 6-OHDA was readministered at a later test session upon recovery of normal eating and drinking. The interval between bilateral injections for each rat was always greater than 24 hr. This period was simply determined by the normality in food and water intake and maintenance of body weight. The non-operated controls were kept on a similar schedule of food and water deprivation. Again, food and water intakes were recorded for the same number of consecutive days for all animals.

At the end of the experiment each rat was anesthetized with an overdose of pentrobarbital sodium given intraperitoneally. Then 0.9% saline followed by 10% buffered neutral Formalin was perfused retrograde through the descending aorta after the heart had been clamped. After fixation, the brain was sectioned at 100 micra on a Lipshaw freezing microtome and stained for cells with cresyl violet according to the method of Wolf [25]. The location of each of the micro-injection sites was identified following standard anatomical procedures.

RESULTS

The motivated responding for food and water was markedly suppressed by 6-OHDA when injected into the brain in a dose of 4.0 μg. Although a less severe and transient disruption of ingestive behavior was observed following the application of the compound in smaller doses, the magnitude of the suppression of food and water intake followed a dose-response relationship. This is shown in Fig. 1. Following the 0.5 μg injection, a complete blockade of food and water intake occurred in only one animal which failed to eat in either the ad lib or the operant test situation; however, this animal did recover the normal pattern of eating and drinking in both situations after 2 days on ad lib food and water for 24 hr. Within 24 hr of the second 4.0 μg injection, the lever pressing of each animal declined almost to the zero level. In 2 of the 5 animals the ad lib food and water intakes recovered partially within 7 days. The other 3 showed the same deficits in ingestive behavior as reported in our earlier study [15]. However, in all 5 rats, the motivated responding for food or water did not recur in that same period.

In most cases, the suppressive effects on ingestion of 6-OHDA appeared almost immediately after its injection. An example of this is illustrated for food intake in Fig. 2. The cumulative record of the rat's rate of responding shows clearly that the intake of food pellets decreased as a function of the concentration of each injection. A similar result was observed for water intake, with little or no lever pressing following the 4.0 μg injection. The data presented in Fig. 2 indicate the immediacy as well as the potency of 6-OHDA's effect. It should also be noted that no significant increase in food consumption was observed following any of the 46 micro-injections of 6-OHDA.

The cumulative effects of the destruction of catecholaminergic nerve terminals is equally noteworthy. The results of sequential doses of the compound given in the dorsolateral hypothalamus are presented for a representative rat in Figs. 3 and 4. Following successive injections of 6-OHDA, there was a slight potentiation of the rat's lever pressing for food. However, following the 4.0 μg injection, the animal's operant response for food was substantially lower. After a second injection of the same dose, both lever responding and ad lib intake declined almost to the zero level, as shown in Fig. 3. This overall lack of response continued until the animal was sacrificed.

As illustrated in Fig. 4, the intake of water was somewhat more sensitive to 6-OHDA treatment in the same rat. After 0.2 μg were injected, there was no lever response for water in the 2 hr operant session. Following the second 2.0 μg injection, lever pressing as well as the ad lib consumption of water were suppressed. The same pattern can be seen in Fig. 4 for the two high doses of 4.0 μg of 6-OHDA, but after the second dose, the lever response for water was almost entirely absent.

The cannula placements for each animal were verified and the tips were found to be in the dorsal or ventral aspect of the far lateral hypothalamus in the coronal plane corresponding stereotaxically to AP 4.4 to 4.6. All injections were made at the edge of the internal capsule, an area shown previously to be sensitive to 6-OHDA [15].

DISCUSSION

An injection of 6-OHDA in a dose as small as 0.5 μg into

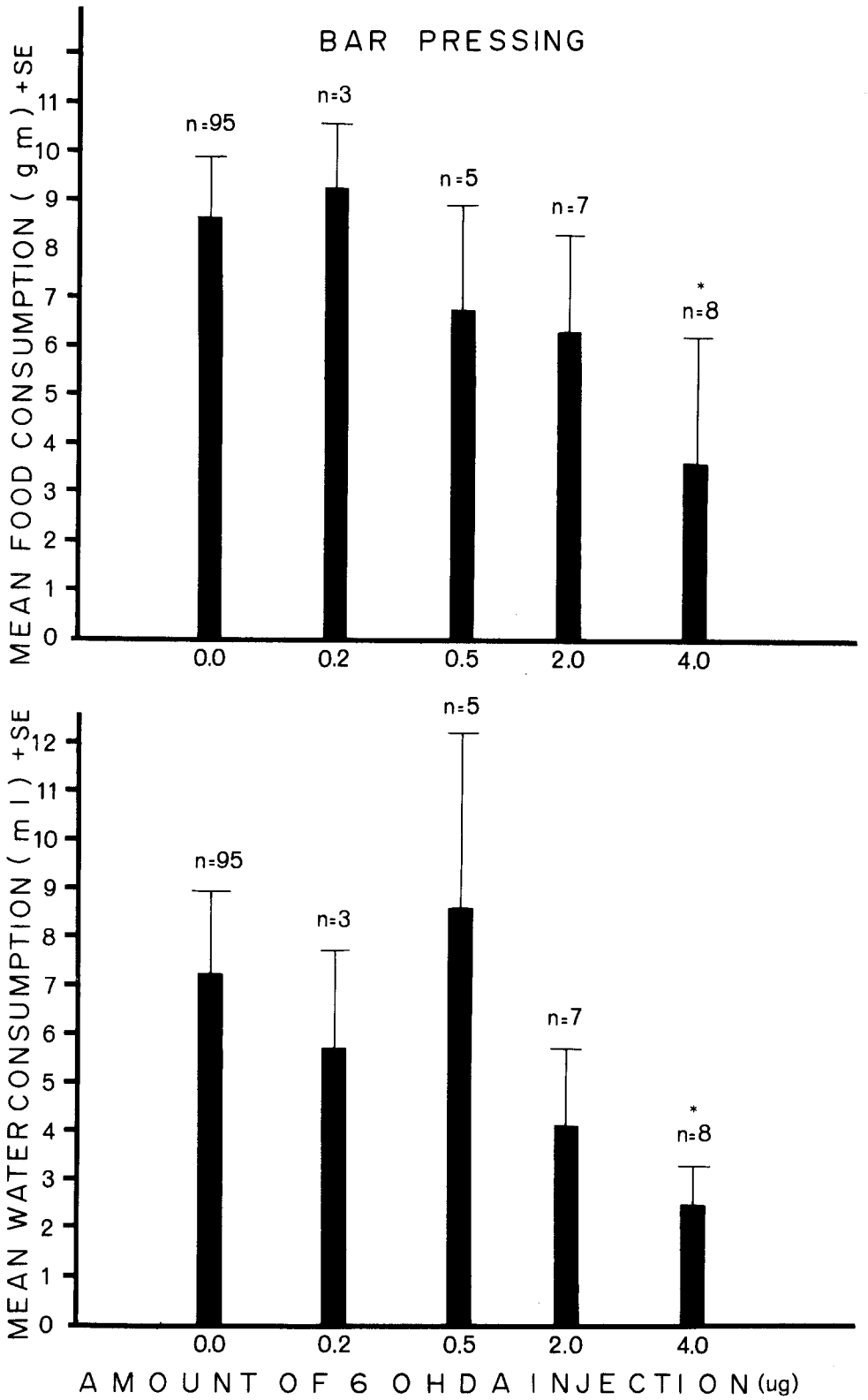

FIG. 1. The group mean values for food (gm) and water (ml) consumption during the 2 hr period following the bilateral micro-injection at sites in the hypothalamus of 6-OHDA at the dose level indicated on the abscissa. The number of bilateral injections made at each dose level is indicated above the appropriate bar. The asterisks (*) denote a value that is statistically significant ($p<0.05$) from the control (0.0) value.

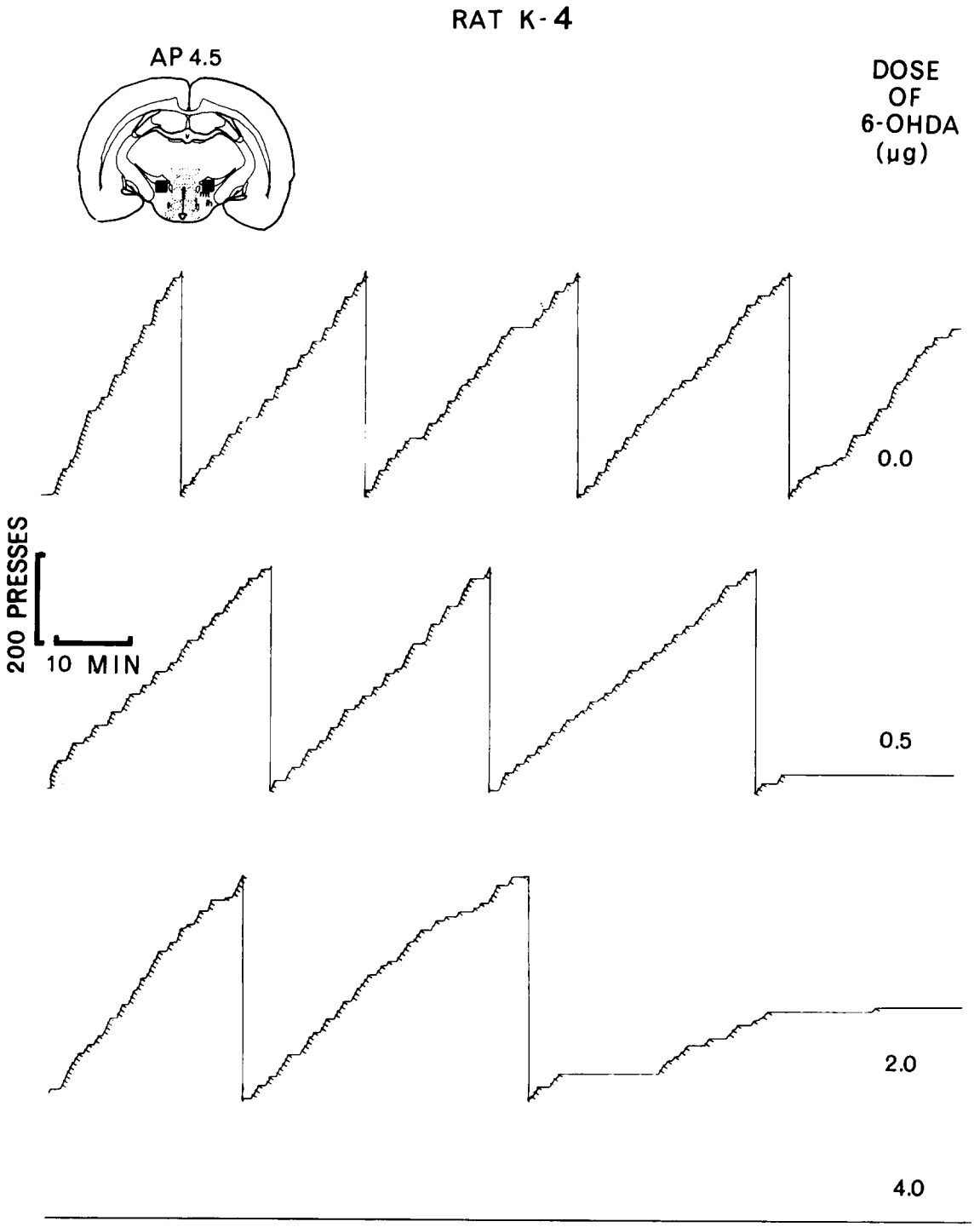

FIG. 2. Cumulative recording of the response for food pellets on an FR-10 schedule for Animal K-4. The two hour bar pressing session followed the bilateral injection of 6-OHDA at the dose level indicated at the right of each tracing. The coronal section of rat brain (upper left) indicates the sites at which the micro-injections were given in this animal. A full excursion of the pen in the vertical direction indicates 500 lever responses.

a given hypothalamic site of the rat can have a profound effect on the food and water obtained by operant responding. This is similar to that reported for the ad lib consumption of food and water [15]. The almost immediate onset of the drug's action is similar to that previously reported for the compound in the cardiovascular system [8]. The blockade of ingestive behavior produced by a dose of 4.0 μg of 6-OHDA persisted for 5 days or longer in each instance. Nevertheless, it is difficult to designate 4.0 μg as a critical dose, since the residual damage to neurons from each previ-

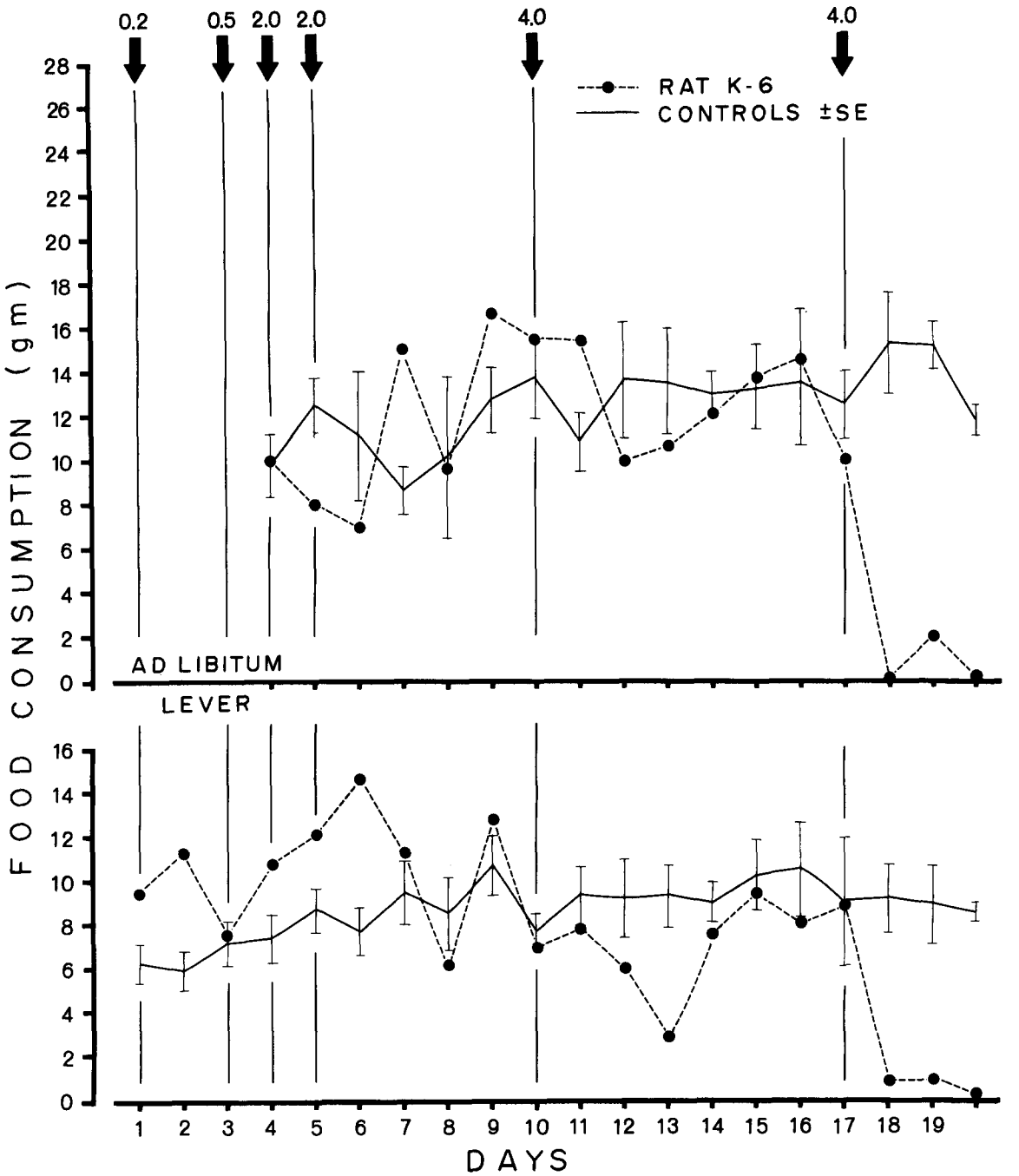

FIG. 3. The daily food consumption expressed in grams for both the 2 hr lever-pressing session and the 2 hr ad lib situation for animal K-6. The solid line (———) indicates the mean consumption, ± the standard error, of 5 control animals under the same conditions. The dotted line (– – – –) indicates the food intake of Rat K-6. The vertical arrows indicate the days on which 6-OHDA was injected at the specified dose.

ous injection could contribute to the overall deficit in eating and drinking. In this connection, Breese *et al.* [3] have shown that successive injections of 6-OHDA produce an additive reduction in self-stimulation behavior.

That the intracerebral micro-injection of norepinephrine in the normal rat does not induce a motivated response for eating after 24 hr of food deprivation has been reported [4]. Hence, one could assume the the depletion of endogenous norepinephrine would not affect motivated activity to obtain food. In the present experiment, 6-OHDA did depress significantly the lever response for food when it was injected into the hypothalamus. Thus, a catecholamine would seem to play at least a partial role in the modulation of the motivated behavior to obtain food. Further, Myers

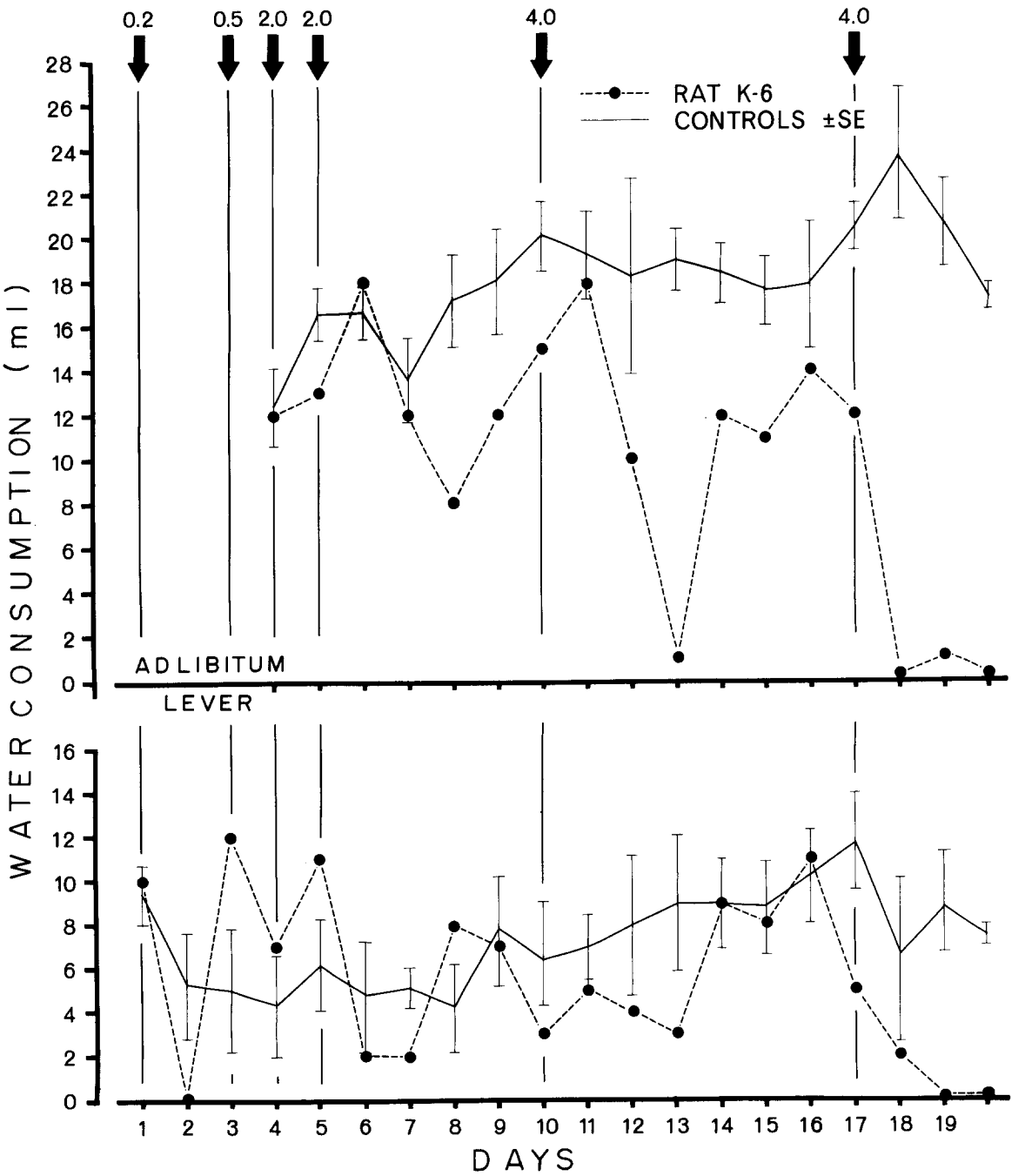

FIG. 4. The daily water consumption recorded for Rat K-6 during both the 2 hr lever-pressing and the 2 hr ad lib situation. The solid line (———) indicates the mean fluid consumption, ± the standard error, of 5 control animals under the same conditions. The dotted line (– – – –) indicates the water consumption of the Rat K-6. The vertical arrows denote when 6-OHDA was injected at the specified dose.

and Bender [14] have observed intense lever responding for food pellets following an injection of norepinephrine into the rat's cerebral ventricle.

A transient increase in the fluid consumption of the rat has been recorded subsequent to an intracerebral injection of 6-OHDA [15,23], but long term deficits have also been found [21,23]. The results of the present experiment indicate that water intake is affected just as severely as food intake on the day that 6-OHDA is administered. Neither eating nor drinking was significantly suppressed by the lower doses of 6-OHDA, but both were reduced from the control value at the higher doses.

Although the precise mechanism of action of 6-OHDA is not clear, it is also possible that the intakes of food and

water are attenuated secondarily to a fall in the rat's body temperature, which would lower the metabolic rate as well. Following an injection of the drug intraventricularly or intracisternally, the animal becomes hypothermic at the normal or at a reduced ambient temperature [2,17]. Moreover, some important experiments have revealed that 6-OHDA may not selectively destroy catecholamine neurons [19]. That is, 6-OHDA injected at sites throughout the upper brainstem causes necrosis of all of the neural and supporting tissue including cell bodies, fibers and neuroglia. Therefore, caution ought to be exercised in the interpretation of any results related to the neurochemistry of the hypothalamic substrates involved in ingestive behavior. Equally important is the anatomical locus of action of a 6-OHDA lesion. 6-OHDA may cause hyperphagia and obesity if infused into the ventrolateral tegmentum of the rat's mesencephalon [1], which would support the view of its action as a non-specific degeneration-inducing substance.

In conclusion, 6-OHDA seems to act on the motivational system involved in the regulation of hunger and thirst in a manner almost identical to that seen when the animal does not have to emit lever responses for food and water. At the specific loci of injection in the lateral hypothalamus, 6-OHDA does not evoke feeding at any dose, but rather exerts an inhibitory effect on food and water intakes of similar magnitude.

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