Absence of Effects of Corticosterone Given at 22 Days

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Mice given a single large dose of corticosterone at 2 days of age, and tested when adult, have shown lasting alterations in operant behavior, associated with reductions in cerebral weight and DNA content. When mice were given corticosterone at 22 days, no changes appeared in cerebral weight, DNA, or in operant behavior, but an apparent decrease did occur in open-field activity. The results suggest that the effects of corticosterone treatment at 2 days on the developing brain are mediated by one or more types of sensitivity to the steroid that have been largely lost by 22 days.

Mice or rats, given a single large dose of corticosterone at 2-3 days of age and studied when adult have been found to show a variety of behavioral changes (Howard & Granoff, 1968; Howard, Olton, & Taylor, 1974; Olton, Johnson, & Howard, 1975), notably increased reactivity in certain operant behavior tests and impaired adaptability in making a transition from a schedule requiring a rapid rate of bar-pressing to obtain food to a schedule requiring a slow response rate (Howard, 1973a). Corticosterone given at 2 days also produces a deficit in the desoxynucleic acid (DNA) content of both the cerebrum and cerebellum that persists over a large part of the life span (Howard, 1968; 1973b). This reduction in DNA content is due mainly to an interference with DNA synthesis by the steroid (Cotterrell, Balázs, & Johnson, 1972), though a small amount of destruction of cells already formed may contribute to the reduction (Howard & Benjamins, 1975). Whether the behavioral effects of corticosterone treatment in infancy can be produced by treatment after the brain has largely matured, or if the effects are a response to treatment at a period when the brain is developing rapidly and sensitive to suppression of DNA synthesis, poses a problem of both practical and theoretical concern.

The present report is on the results of behavioral testing in mature mice after corticosterone treatment begun at 22 days of age. At this age cerebral growth and DNA accumulation are approaching completion; hence, little brain stunting was expected. But, whether the behavioral changes after the earlier steroid treatment are due largely to the brain stunting, or whether they are due, at least in part, to localized specific corticosterone effects is not known. At 22 days body growth is still very rapid, hence the reactivity to the steroid at the 2 age periods can be compared in terms of the inhibition of body growth.

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Methods

Methods and materials were as previously described (Howard, 1973a; Howard & Granoff, 1968) unless otherwise mentioned. At 22 days of age mice were implanted, under light ether anesthesia, with a subcutaneous pellet of undiluted corticosterone, .8 mg/g body weight (mean body weight: 10.35 g). The steroid was absorbed more rapidly—completely within 10 days— when undiluted than when given as a 2 mg pellet of corticosterone (40% mixture in a cholesterol vehicle) to the 2-day-old group as in Howard (1973a). The animals were weaned at the time of implantation and housed in pairs consisting of an experimental and litter-mate control until a few days before behavioral testing was initiated, when they were housed individually.

Results and Discussion

The effect on body growth is illustrated in Figure 1. Initially, the mean values were reduced to the same extent in both groups, but the mice given corticosterone at 22 days recovered sooner and eventually achieved body weights not significantly less than those of the controls.

Continuous reinforcement testing (CRF) was initiated at 66 ± 3 days of age, twice weekly for an individual mouse. After 10 days on CRF, the magazines were emptied of food and the response to the extinction test situation observed. The results are presented in Figure 2, together with the previous findings on mice given corticosterone at 2 days of age (Howard, 1973a). The response rate on CRF showed no significant effect of the treatment at either age. In the extinction situation no significant difference appeared in the responses of the mice given steroid at 22 days of age compared with their controls. This was in contrast to the effects of steroid at 2 days of age, in which the treated group

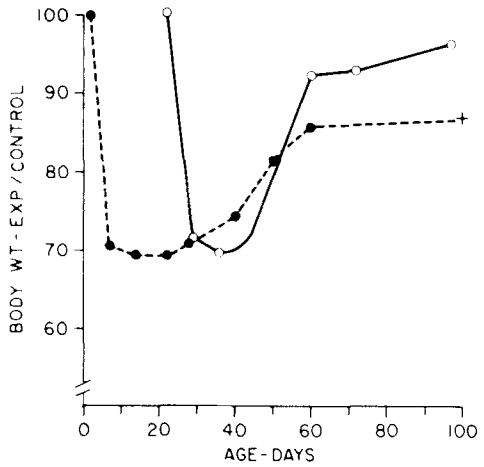

Fig. 1. Comparison of effects of corticosterone on body growth in mice when treatment was initiated at 2 or 22 days of age. Body growth, expressed as the mean of the experimental body weights divided by the body weights of the litter-mate controls, is plotted as a percent against age in days. The steroid dose at both ages seemed close to the maximum that would permit resumption of growth after termination of the treatment.

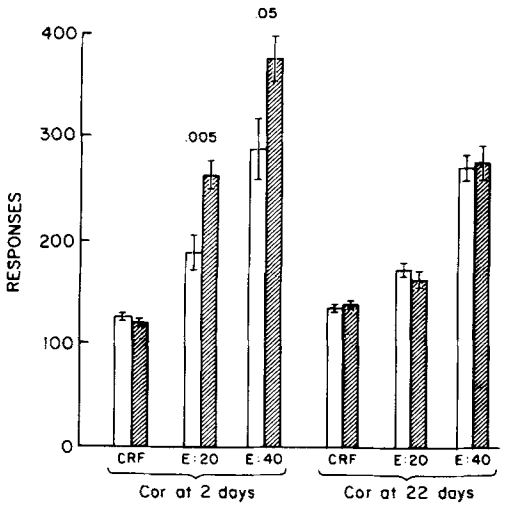

Fig. 2. Bar-pressing responses on the 1st day of the extinction test in mature mice given corticosterone at 2 days (Cor at 2) compared with responses of mice given corticosterone at 22 days (Cor at 22); open columns, controls; shaded columns, corticosterone treated. The 1st pair of columns in each set represents the mean responses for 40-min sessions on the last 6 days of continuous reinforcement training (CRF); E:20, responses during first 20 min of extinction; E:40, responses during total test time of 40 min. Standard errors of means are indicated by the vertical lines, for 11 pairs in the 2-day group and 17 pairs in the 22-day group.

significantly over-responded relative to their controls. The response rates of the controls for the 2 groups were very similar even though the tests were carried out over 1 yr apart. The figure further illustrates that normal mice do not reduce their rate of bar-pressing on the 1st day of extinction, but rather increase it.

The mice were then tested on a series of fixed ratios (FR) where the number of bar-presses required to obtain a pellet was successively 4, 8, 16, 32, 64, and 99, with 2 test days on each ratio. In contrast to the mice given corticosterone at 2 days, that had worked harder to obtain food than their controls, particularly on FR-64, the present group treated at 22 days showed no tendency to over-respond on any of these fixed ratios. On FR-8 they significantly under-responded, but the number of pellets obtained per gram body weight was not significantly reduced relative to their controls.

After the tests on FR-99, the mice were transferred to a differential reinforcement of low rate (DRL) schedule, where they were required to refrain from bar-pressing for 20 sec before a bar-press would produce a pellet (DRL-20). Here again, in contrast to the results after steroid treatment at 2 days when the mice showed an impaired ability to make the adaptive transition, the mice treated at 22 days showed no significant difference from the controls. If anything, the mice treated at 22 days tended to be somewhat more efficient than the controls in this test.

Finally, when the animals were 7 months of age, they were tested for their ability to maintain their stance on a slowly rotating plastic cylinder, and for their activity in an open field. Mice given steroid at 2 days had shown clearly inferior ability to maintain themselves on the rotating cylinder, and also reduced activity in the open field (Howard & Granoff, 1968). When corticosterone was given at 22 days, the treated mice tended to

TABLE 1. Brain Weight and DNA Content in Adult Mice Given Corticosterone at 22 Days.

		Cerebrum		Cerebellum	
Group	n	Weight (mg)	DNA (μg)	Weight (mg)	DNA (μg)
Controls	15	359 ± 3.1[a]	542 ± 6.3	69.1 ± 1.3	534 ± 9.5
Corticosterone	15	355 ± 4.4	539 ± 12.7	68.0 ± 1.6	534 ± 12.3

[a]Mean ± standard error of the mean.

be less efficient on the rotating cylinder, but the difference from the controls was not significant in the 10 pairs tested. However, in the open field the treated group was significantly less active than the controls in the number of squares entered in 10 min, the means being 285 squares for the treated group and 340 for the controls. The control member of a pair showed greater activity than the treated litter-mate in 10 of 12 pairs tested (Sign test: $p = .04$). This rather minor difference requires confirmation, but suggests that open-field activity should be studied at various intervals after treatment. If confirmed, an interesting effect of corticosterone on emotionality, distinct from the DNA deficit produced when 2-day-old mice are given the steroid, might be indicated.

After the behavioral tests, the mice were sacrificed at 7-8 months and the brains analyzed for the DNA content, with the results given in Table 1. No significant effects of corticosterone treatment at 22 days on cerebral or cerebellar DNA content were found, in contrast to the earlier findings after treatment at 2 days when the reductions of 15% in cerebral (including olfactory bulbs) DNA and 27% in cerebellar DNA were highly significant. Although little DNA synthesis takes place in the mouse brain after 22 days, that which may occur appears to escape irreversible suppression by the steroid treatment. No evidence exists of degeneration in cells formed prior to treatment. When the steroid is given at 2 days, a reduction occurs in cerebral gangliosides that suggests a considerable interference with the growth of neuronal processes (Howard, Benjamins, 1975). The reduction in gangliosides was proportional to the cerebral weight reduction. Because corticosterone treatment at 22 days produced no reduction in cerebral weight, no overall reduction in gangliosides would be expected under the conditions of this experiment.

The absence of effect on brain DNA content after corticosterone treatment at 22 days may be due largely to the low level of DNA synthesis going on at this time, rather than to any change in a less well defined sensitivity factor. However, the absence of most of the behavioral changes in the present experiment leaves open the question of whether the behavioral alterations after corticosterone at 2 days are due largely to the brain dwarfing, with its many possible sequelae, or whether they are in part due to specific localized effects of corticosterone on the neonatal brain. If the latter is in part the case, it would appear to be due to a type of of sensitivity to corticosterone that has been largely lost by 22 days.

Note

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