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Development of Cholinergic Inhibitory Capacities in the Hyperthyroid Mouse

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MURPHY, J. M., AND Z. M. NAGY. *Development of cholinergic inhibitory capacities in the hyperthyroid mouse.* PHARMAC. BIOCHEM. BEHAV. 5(4) 449–456, 1976. – The ontogeny of behavioral arousal and inhibition, as measured by spontaneous locomotor activity, was compared in four experiments for controls and mice injected with thyroxine as neonates. Mice treated with thyroxine at 1–3 days of age had higher activity levels at 10–15 days of age than controls, suggesting potentiation of arousal systems by the hormone treatment. Although thyroxine-accelerated development had no reliable effect upon the age at which peak activity occurred, scopolamine injections increased activity as early as 15 days of age in thyroxine-treated mice, whereas saline-treated or unhandled controls did not show a similar increase until 16–17 days of age. The findings were interpreted as indicating both a potentiation of arousal and a compensatory acceleration of cholinergic inhibitory capacities as a result of the neonatal hyperthyroidism. In addition, the importance of the behaviorally suppressive effects of a novel injection experience in the neonatal mouse was demonstrated.

Neonatal mice Thyroxine Locomotor activity Scopolamine Behavioral arousal and inhibition Cholinergic development

DEPRIVATION of thyroid hormones in neonatal rats causes retarded development and chronic behavioral deficits [6, 17, 22], whereas excess neonatal thyroid hormone levels yield a period of accelerated development during preweaning but behavioral deficits in the adult [7,29]. The relatively brief period of accelerated development affects numerous central nervous system (CNS) processes [16,29], as well as behavioral capacities such as accelerated emergence of motor and reflexive abilities and enhanced performance on some learning tasks [7,29]. Additionally, mice receiving neonatal thyroxine injections show an earlier onset of 24-hr memory capacities for a discriminated escape task [21].

Locomotor activity of altricial rodents is represented by an inverted U-shaped curve as a function of age [2, 23, 25]. This curve presumably reflects the caudal to rostral sequence of CNS development where brainstem excitatory areas are functionally mature prior to the ontogenetic peak, while the subsequent decline in activity toward adult levels is a consequence of a shift to a predominance of later maturing forebrain inhibitory systems which modulate arousal. That the accelerated development induced by early thyroid hormone excess should yield an earlier emergence of behavioral inhibitory capacities would be predicted from the effect of early thyroid hormone levels upon CNS cholinergic systems [13,28], one of the biochemical systems which apparently mediate behavioral inhibition by modulating brainstem excitatory mechanisms [3]. An accelerated development of the inhibitory system would be expected to result in an earlier peak in the ontogenetic activity curve. However, this was not the case in previous studies with rats [8] or mice [20], although Murphy and Nagy [20] did find that thyroxine-treated mice responded at a younger age than did controls to the anticholinergic effects of scopolamine. The latter finding was interpreted as indicating that although the forebrain cholinergic system is accelerated by neonatal thyroxine injections, concurrent potentiation of arousal mechanisms also occurs, as evidenced by consistently higher activity levels during early development. Thus, a more advanced state of inhibitory functioning is apparently necessary to modulate the enhanced arousal.

The present study was conducted to further assess the possible acceleration of cholinergic inhibitory capacities by thyroxine, and to examine the interaction of thyroxine acceleration with experiential variables.

GENERAL METHOD

Animals

All experiments used Swiss-Webster (S-W) mice (*Mus musculus*) born in the Bowling Green State University psychology department mouse colony. Litters were housed in 30.4 × 18 × 12.8 cm polyethylene cages with wire grid tops and wood chips on the floor. Nesting material was provided, and the mothers remained with the pups at all times, except during testing sessions and other experimental procedures. Ad lib food and water were available to the

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mothers. The colony room was maintained at 24 ± 1°C and was on a normal 12 hr light-dark cycle beginning at 0800 hrs.

Apparatus

Activity monitoring cages, 19.4 × 6.4 × 9 cm, were made of clear Plexiglas. Grid floors were constructed of 1 mm dia. stainless steel rods spaced 4 mm center-to-center and extending parallel to the length of the cage. Red-filtered light sources and photocells were spaced 3.4 cm from each end. Activity cages were enclosed in a humidity-controlled environmental chamber, which was maintained at 24 ± 1°C. The ventilation fan for the chamber furnished a 74 db ambient noise level, and two 60 W fluorescent ceiling lights provided illumination for the chamber.

Activity counts were recorded automatically on apparatus located outside the sound-attenuated chamber. One activity count was registered each time the subject crossed alternate photocell beams. Thus, a mouse had to traverse at least the 12.6 cm distance between the photocells for each activity count.

Procedure

All neonatally-treated mice were selected from litters born on the previous day, with each containing at least 10 pups and 4 of each sex. Mice from each litter were weighed, and 4 males and 4 females were selected with equal or near equal weights. The remaining pups were sacrificed, keeping litter size constant at 8, in order to minimize possible nutritional differences between litters. Animals were toe-clipped for identification purposes, and 2 males and 2 females from each litter were randomly assigned to one of two groups. Hormone-treated animals received IP injections of thyroxine (1 μg/g body weight) at 1, 2, and 3 days of age. The thyroxine was frozen in crystalline form (L-thyroxine, Sigma Chemical Co.) and was freshly prepared in saline suspension immediately prior to injecting litters. The remaining 4 pups in each litter were assigned as controls and were similarly injected with saline.

All activity sessions were conducted between 0800 and 1700 hr, the normal light period of the day-night cycle. Sessions lasted for 2 hr on each test day, and animals were returned to the home nest immediately following testing. Assignment to age groups was according to modified split-litter designs.

EXPERIMENT 1

Ontogenetic activity studies have shown that the amount of experience with the testing apparatus can alter activity levels [24] and possibly the age of the ontogenetic peak [19]. Experiment 1 assessed the potential interaction of thyroxine-accelerated development with differing periods of exposure to the activity testing apparatus.

Method

Animals. Postnatal injections were administered to 72 saline- and 72 thyroxine-treated mice.

Procedure. Nine males and 9 females from each of the postnatal treatment groups were assigned to 1 of 4 groups which began activity testing at 7, 9, 11, or 13 days of age. Animals from each litter were assigned to age groups such that a male and female from each neonatal treatment began testing at one age and the remaining 4 animals began at another age. All animals were tested daily at the same time from the first day of testing through 20 days of age. Body weights were taken daily, and the day when both eyes were first open was also noted.

Results and Discussion

An analysis of variance was conducted upon the body weight data, the factors being group (age at first testing session), hormone, sex, and age. Body weights increased with age, $F(19,2432) = 3697.67$, $p<0.0005$, while no effect was obtained for group or sex, $Fs<1.00$. A significant main effect for hormone, $F(1,128) = 13.18$, $p<0.0005$, and a Hormone × Age interaction $F(19,2432) = 12.56$, $p<0.0005$ reflected the slower rate of weight gain over age by the thyroxine-treated animals. Individual comparisons conducted within the Hormone × Age interaction indicated no significant differences in body weight from Day 1–5, a marginal difference at 6 days ($p<0.08$), and that the saline controls weighed significantly more than thyroxine-treated mice on Days 7–20, $ps<0.05$.

The mean day when both eyes were open for the saline- and thyroxine-treated mice was 14.36 and 12.74 days of age, respectively. Analysis of the eye-opening data indicated that this difference was highly significant, $F(1,128) = 304.21$, $p<0.0005$. The findings of decreased weight gain and earlier eye-opening are in agreement with previous studies that have examined the effects of early thyroxine excess upon the physical development of rats and mice [7, 21, 28].

Figure 1 presents the mean number of crossings during the activity sessions as a function of group, hormone, and age. Hormone, sex, and age were the factors considered in analyses of the activity scores for each of the groups. Activity changed significantly with age for groups which began testing at 7, 9, and 11 days of age (all $ps<0.0005$). The form of the ontogenetic curve is concordant with that reliably observed for altricial rodents in other studies [2,25], with activity being low in the immature mouse up to 10–11 days of age, followed by a large increment to a peak at 14–15 days and a subsequent decline. The period of peak activity is in general agreement with recent studies using S-W mice [23,24]. A significant Hormone × Age

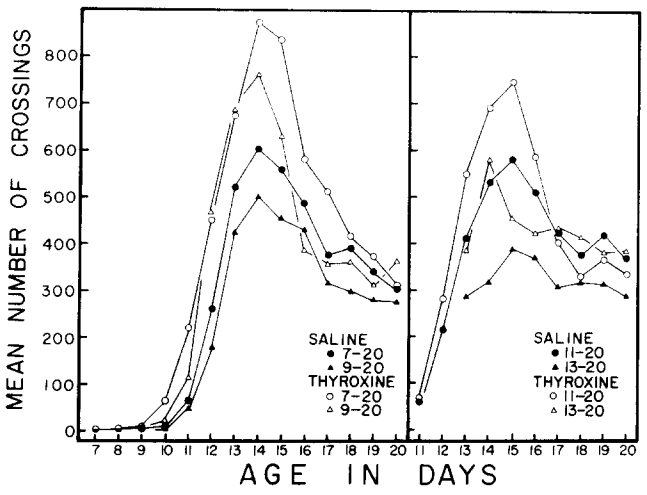

FIG. 1. Mean activity scores as a function of group (age at first testing session), hormone, and age.

interaction was obtained for the 9–20 group, $F(11,352) = 2.32$, $p<0.025$, resulting from the thyroxine group being more active than the saline group at 12–14 days of age ($ps<0.025$), but not differing significantly at 9–11 or 15–20 days.

In order to further assess the potential age-related effects of hormone treatment and experiential influences upon activity, a separate analysis was conducted at each age, the factors being hormone, sex, and group, except for 7 and 8 days of age where only hormone and sex were the factors. There were no significant effects obtained in the analyses of 7–9 or 16–20 days of age. The main effect for hormone was significant at 10–15 days of age, with the thyroxine-treated groups being generally more active throughout this period than saline groups (all $ps<0.05$). Mice that began testing at younger ages tended to have higher activity levels, although significant group effects were obtained only at 11 and 13 days, ($ps<0.05$). Thus, while prior experience with the testing apparatus produced slightly higher activity levels, the finding that thyroxine-treated mice remained consistently more active from 10–15 days of age than their respective control groups suggests a potentiation of arousal systems by the hormone treatments.

Figure 1 shows that there was no appreciable shift in the age of the activity peak for thyroxine or saline groups due to the amount of experience with the apparatus and testing procedure. This replicates a previous finding [24] and indicates that CNS maturation, rather than experience, is the major determinant of the peak. The only suggestion of an earlier peak due to the hormone treatment occurred in the 13–20 groups, where the thyroxine group peaked at 14 days of age and the saline controls at 15 days. In general, however, the results replicate previous findings [8,20] that the accelerated development induced by neonatal hyperthyroidism does not yield an earlier ontogenetic activity peak.

EXPERIMENT 2

Our previous study [20] demonstrated that mice treated with saline or thyroxine as neonates increased activity at 17 days of age when injected with scopolamine, whereas only thyroxine-treated mice increased activity at 15 days of age. However, these subjects were tested for a single session, and activity levels were relatively low compared to subjects tested for repeated days. The present experiment was conducted to determine if adaptation to the testing apparatus would alter the age difference in response to scopolamine as a function of the hormone treatment. A second purpose of the present experiment was to evaluate the effects of the injection experience and of repeated scopolamine administration upon the development of behavioral arousal.

Method

Animals. The animals were 120 S-W mice, half of which were postnatally treated with thyroxine and half with saline.

Procedure. All mice were tested for daily 2-hr sessions from 11–14 days of age. Beginning at 15 days of age, 10 males and 10 females from each of the thyroxine- and saline-injected animals were assigned to 1 of 3 groups such that activity during daily test sessions at 11–14 days of age was as nearly equal as possible for the 3 groups. All animals received IP injections of either saline or scopolamine prior to each of the daily activity sessions from 15–20 days of age. Mice in the first group received scopolamine injections beginning at 15 days of age, while those in the second and third groups were injected with saline beginning at 15 days, but were switched to scopolamine injections at 17 or 19 days of age, respectively, for the remainder of the daily sessions. Scopolamine was prepared fresh daily in saline solution, and the dosage employed (1 mg/kg, scopolamine hydrochloride, Sigma Chemical Co.) was based upon an ontogenetic dose-response study [27], which indicated that 1 mg/kg produced maximal activity increments in young S-W mice.

Results and Discussion

Figure 2 presents the mean activity scores from 11–20 days of age for the saline and thyroxine groups (15, 17, or 19 days of age on the first day of scopolamine injections). The data were analyzed by a 5-way repeated measures analysis of variance with group (day of first scopolamine injection), hormone, age (test day), sex, and 15 min intervals as the factors.

Activity levels changed significantly with age, $F(9,972) = 48.96$, $p<0.0005$, and decreased over 15 min intervals, $F(7,756) = 90.40$, $p<0.0005$. The Group × Age × Interval interaction and two-way interactions of Group × Age, Group × Interval and Age × Interval (all $ps<0.0005$) reflected a pattern of increased activity when groups were injected with scopolamine on Days 16–20, with most of the activity increase occurring during the initial part of the sessions and decreasing over the 2 hr testing period. However, Hormone × Age × Interval and Group × Hormone × Age × Interval interactions ($ps<0.05$) indicated some differential in age of response to scopolamine, with thyroxine groups generally more active than saline groups, especially on the first day of injection with scopolamine.

Examination of Fig. 2 indicates a substantial decrease in activity from 14–15 days of age for all groups, which may represent the normal decrease following the peak in the ontogenetic activity curve. However, comparison with the

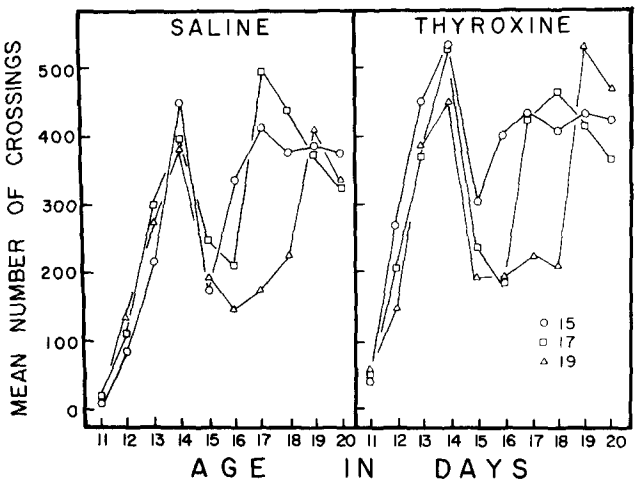

FIG. 2. Mean activity scores as a function of age for groups treated with saline or thyroxine as neonates. One group within each neonatal treatment condition was injected with scopolamine prior to activity testing from 15–20 days of age, whereas the other two groups received saline injections beginning at 15 days but were switched to scopolamine injections at 17 or 19 days.

activity levels in Experiment 1 suggests that much of this decrease may be due to a marked suppression of activity at 15 days as a result of the initial injection experience. By 16–17 days of age, considerable activity increments are present in response to scopolamine compared to vehicle controls, suggesting that cholinergic control over behavioral arousal is emerging during this period for the S-W mouse.

The only evidence for thyroxine-accelerated forebrain cholinergic development was the relatively smaller suppression of activity for thyroxine animals injected with scopolamine at 15 days of age. Perhaps one reason for the failure to observe a substantial scopolamine effect at 15 days of age for the thyroxine-treated animals was that this period of accelerated development may be the beginning of a minimal forebrain cholinergic functional capacity to modulate behavioral arousal, and the suppressive effects of the novel injection may have tended to counteract any potential drug effect. If this is the case, then failure to adapt animals to the injection experience may decrease the behavioral expression of the drug response during the initial phase of cholinergic control over behavioral arousal.

EXPERIMENT 3

Since the initial injection experience may have produced a marked suppressive effect upon activity which interfered with the behavioral expression of the disinhibitory drug effect, it was decided to attempt to adapt subjects to the injection experience, or to at least diminish the effects of that experience, by beginning injections at a younger age. Additionally, since 15 days of age was the earliest at which mice were injected in Experiment 2, it did not rule out the possibility that thyroxine-treated mice may show some response to scopolamine at an even younger age. Thus, a second purpose of the present experiment was to assess that possibility. Finally, control groups receiving the quaternary drug methylscopolamine were also employed in order to substantiate the central origin of the scopolamine effect.

Method

Animals. Sixty S-W mice injected with thyroxine and an equal number injected with saline were used.

Procedure. Mice were tested daily for activity from 11–18 days of age. Prior to testing at 13 days of age, 10 males and 10 females from each of the thyroxine and saline treatments were assigned to 1 of 3 groups such that activity scores were as nearly equal as possible within each neonatal treatment condition for the first two days of testing. Groups were subsequently designated according to neonatal treatment and drug injection conditions: Saline-saline (S-S); Thyroxine-saline (T-S); Saline-scopolamine (S-D); Thyroxine-scopolamine (T-D); Saline-methylscopolamine (S-M); Thyroxine-methylscopolamine (T-M). Animals were injected immediately prior to testing at 13–18 days of age with either saline, scopolamine (1 mg/kg), or an equimolar dose of methylscopolamine (scopolamine methyl nitrate, Sigma Chemical Co.).

Results and Discussion

Mean activity scores for the daily sessions are illustrated in Fig. 3 as a function of hormone, drug, and age. The factors considered in a repeated measures analysis of variance of the data were drug group, hormone, age, sex, and 15 min intervals. All main effects were significant:

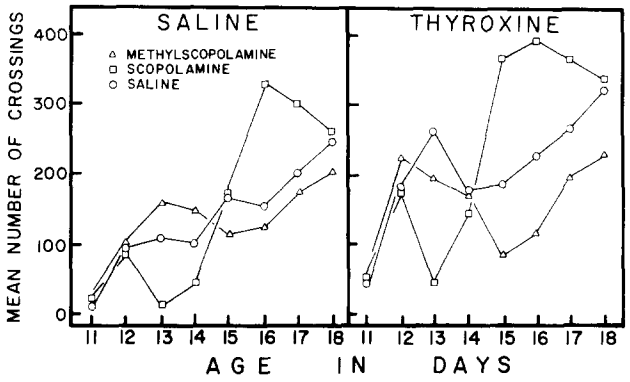

FIG. 3. Mean activity scores for saline and thyroxine groups as a function of age and drug. Saline, scopolamine, or methylscopolamine was injected prior to daily testing sessions from 13–18 days of age.

Group, $F(2,108) = 3.95$, $p<0.025$); Hormone, $F(1,108) = 15.45$, $p<0.0005$; Age, $F(7,756) = 45.18$, $p<0.0005$; Sex $F(1,108) = 5.91$, $p<0.025$; Intervals, $F(7,756) = 36.23$ $p<0.0005$.

A significant Group × Age interaction, $F(14,756) = 13.18$, $p<0.0005$, can be seen in Fig. 3. Relative to the S-S and T-S groups, the S-D and T-D groups showed a suppression of activity at younger ages and the expected elevation of activity at older ages. The S-M and T-M groups had essentially the reciprocal pattern relative to the S-S and T-S groups, with little or no initial activity increase at younger ages and a subsequent suppression. Since the suppressive or potentiating drug effects generally occurred in the first four or five 15 min intervals and the differences tended to increase at the older ages, significant Group × Interval, Age × Interval, and Group × Age × Interval interactions (all $ps<0.0005$) were obtained.

Figure 4 shows the activity scores on days 14–17 as a function of hormone, drug group, and 15 min intervals. The figure illustrates that thyroxine groups were more active overall than saline-treated groups, but this difference decreased over intervals and increased with age resulting in Hormone × Interval ($p<0.005$) and Hormone × Age × Interval ($p<0.0005$) interactions. However, the drug response of the T-D and T-M groups was also greater and occurred at a younger age than for the S-D and S-M groups, as reflected by the significant Group × Hormone × Age × Interval interaction ($p<0.05$).

Individual comparisons between the group session means indicated a substantial attenuation of activity for scopolamine groups at 13 days of age, the first day of injection, and the S-D group continued to be less active than the S-M group at 14 days ($ps<0.05$). The reason for the suppressive effect of scopolamine at younger ages is not clear but is consistent with past findings [1,12]. In the adult animal, the CNS anticholinergic effects of scopolamine occur mainly in the cerebral hemispheres (see [4]), and evidence indicates that CNS muscarinic receptors predominate in forebrain areas [35]. However, in the pre-juvenile mouse, where forebrain cholinergic systems are still in an immature state, it may be that the anticholinergic effects of scopolamine show a relatively greater affinity for ascending cholinergic arousal systems which would be functioning at a more mature level during this period than forebrain systems. In addition, the suppression of activity

by scopolamine in the younger animal does not appear to be a result of peripheral effects since methylscopolamine does not show a parallel action.

All comparisons were significant for thyroxine groups at 15 days of age, ($ps<0.025$), confirming that the T-D group was more active at this age than the control T-S and T-M groups, while the T-M group was less active than the other two groups. The S-S, S-D, and S-M groups did not differ at 15 days of age, $Fs(1,756) \leq 1.71$. The first evidence of increased activity for the S-D group relative to the S-S and S-M groups occurred at 16–17 days of age, and the respective differences for the thyroxine groups also continued to be significant ($ps<0.025$). The T-M group was less active than the T-S group at 15, 16, 18 days of age ($ps<0.05$), with a marginal difference at 17 days ($p<0.10$). While the pattern of the S-M group was consistent with that of the T-M group, no differences were reliable at any age when S-M and S-S groups were compared, $Fs(1,756) \leq 1.62$. Thus, the findings indicate that compared to controls the thyroxine-treated animals showed both an exaggerated and maturationally accelerated response to the anticholinergic drug scopolamine. Figure 3 shows that by 18 days of age there is an apparent development of tolerance to the repeated administration of scopolamine, an effect which has previously been reported for adult animals (see [41]), since neither the S-S and S-D nor the T-S and T-D groups differed at this age, $Fs(1,756) \leq 1.00$.

The results are compatible with the interpretation that early thyroxine excess accelerates the development of forebrain cholinergic inhibitory systems. Thyroxine-treated animals increased activity in response to scopolamine by 15 days of age, at least one day earlier than saline-treated controls. Moreover, the neonatal thyroxine treatment may actually yield an acceleration of up to two days. Figure 4 shows that 15- and 16-day-old T-D animals responded more like older animals, with a high peak activity of approximately 1 hr duration, than the S-D animals which did not respond in a similar fashion until 17 days of age.

The T-M group showed a suppression of activity at 15–18 days of age relative to the T-S group during the same period when the T-D group showed potentiation of activity. The S-M group showed a similar suppression of activity, but of lesser magnitude, relative to the S-S group at 16–18 days of age. These findings suggest that there are some age-dependent anticholinergic effects of this dosage of methylscopolamine which are mildly incompatible with increased locomotor activity. Most important, however, is the demonstration that any peripheral effects of scopolamine are negligible or, if anything, are opposite to the CNS activational effects. Since the peripheral effects cannot account for the scopolamine-induced activity increments in the present experiment, it may be concluded that these activity increments reflect a central effect of the drug.

In Experiment 2, it was concluded that a novel injection experience immediately prior to testing may act to counter any drug response. The comparison of Days 11–14 in Fig. 2 and 3 is consistent with this conclusion since groups in Experiment 3 appear less active on injection days 13–14, regardless of the injected substance, than groups in Experiment 2 which were not injected on these days, whereas groups from both experiments showed essentially the same activity levels at 11–12 days when no injections were administered. These observations were confirmed by an analysis of variance upon the activity scores from both experiments on Days 11–14, with the factors being group,

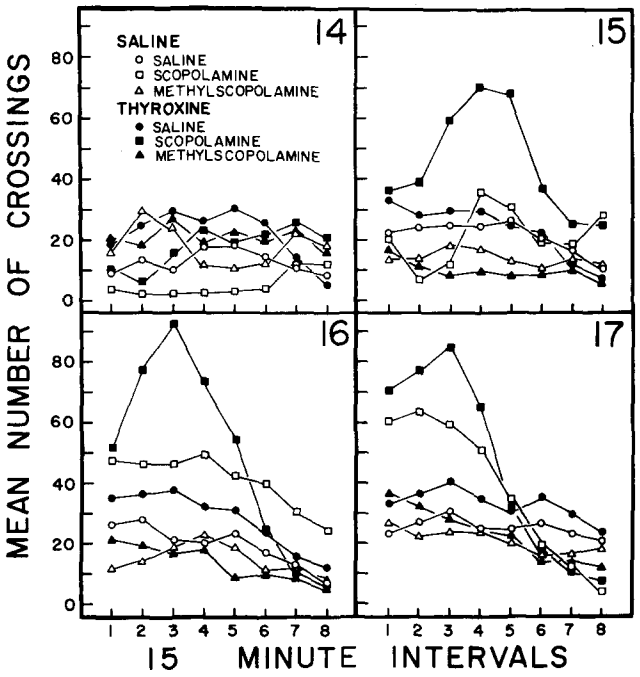

FIG. 4. Mean activity scores on Days 14–17 as a function of hormone, drug group, and 15 min intervals.

hormone, age, and sex. All main effects proved to be significant ($ps<0.005$), but of central interest was the Group × Age interaction, $F(15,648) = 13.34$, $p<0.0005$). Individual comparisons conducted within this interaction indicate no difference in activity levels for all group comparisons at 11–12 days of age, $Fs(1,648) \leq 1.01$, whereas at 13–14 days all groups not injected at these ages for Experiment 2 were more active than injected groups from the present experiment ($ps<0.005$). Thus, the conclusion drawn from Experiment 2 that the suppressive effect of the injection experience can be an important factor was substantiated. The present experiment further demonstrates that two successive days of injection experience are sufficient to diminish the relative effects of that experience, since the T-D group at 15 days of age showed a marked potentiation of activity relative to T-S and T-M groups.

EXPERIMENT 4

The results of Experiment 3 did not rule out the possibility that some seemingly innocuous part of the procedure, such as early handling, may also have accelerated cholinergic development to some extent in saline controls if these animals were compared to unhandled controls [29]. Conversely, early saline injections may retard or have some other deleterious effect upon development [36,37], while thyroxine excess may simply counteract these deleterious effects, thus yielding only an apparent acceleration. The purpose of the present experiment, therefore, was to assess the development of scopolamine responsiveness in mice neither handled nor injected during the neonatal period.

Method

Animals. The animals were 48 S-W mice from litters that

had been culled to 8 pups on the day following birth. Care was taken to prevent any handling of the remaining neonates during the culling procedure, and neither mothers nor litters were subsequently disturbed until the beginning of testing. Housing conditions were as described in the general method.

Procedure. Animals were tested over repeated days as in Experiment 3, but only from 13–18 days of age in order to minimize all experiential factors prior to that period. Beginning at 13 days of age, 2 males and 2 females were selected from each litter, and then one of each sex was assigned to either an experimental or control group. Experimental animals were injected IP with scopolamine (1 mg/kg) immediately prior to testing each day, whereas controls were injected with saline. Animals were identified by ear-punching following the first test session and were returned to the home cage following each of the daily 2 hr sessions.

Results and Discussion

Activity scores were analyzed by a 4-way analysis of variance with two repeated measures, the factors being group, sex, age, and 15 min intervals. Figure 5 presents the mean scores as a function of age and group. Activity generally decreased over intervals, $F(7,3089) = 11.74$, $p<0.0005$, and the decrease was greater with increasing age, especially for the group receiving scopolamine, resulting in significant Age × Interval and Group × Interval interactions ($ps<0.0005$).

A significant increase in activity levels with age and the Group × Age interaction, $Fs(5,220) \geq 4.23$, $ps<0.005$, can be seen in Fig. 5. Individual comparisons within the Group × Age interaction indicated that saline and scopolamine groups did not differ from 13–15 days of age, $Fs(1,220) < 1.00$, a statistically marginal difference occurred at 16 days ($p<0.08$), and that the scopolamine group was significantly more active than saline controls at 17 and 18 days of age ($ps<0.01$).

The results indicate that forebrain cholinergic modulation of behavioral arousal is beginning to function by 16–17 days of age in the S-W mouse. Subjects in the present experiment received no handling or experience with the testing apparatus prior to 13 days of age, but the saline and scopolamine groups showed essentially the same age-related activity differences as the S-S and S-D groups in Experiment 3. Thus, it appears that the 16–17 day onset of the drug effect obtained in Experiment 3 was not affected by the neonatal saline treatments or handling.

On the other hand, control and experimental groups in the present experiment continued to differ at 18 days of age, whereas the S-S and S-D groups in Experiment 3 no longer differed significantly by this age. Since the latter finding was interpreted as being a possible development of tolerance to the effects of scopolamine, the continued difference at 18 days of age in the present experiment might indicate that the development of tolerance is to some extent affected by early stimulation. This possibility, however, does not negate the fact that the first day of the drug response remained the same regardless of whether the animals were handled during development, and it may be concluded that animals treated with saline during the neonatal period are comparable to normally reared S-W mice with respect to forebrain cholinergic modulation of behavioral arousal.

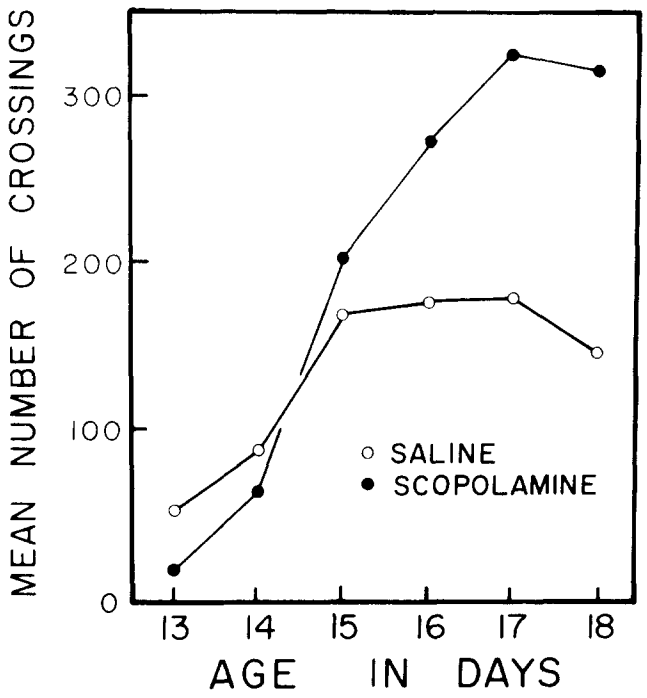

FIG. 5. Mean activity scores for saline- and scopolamine-injected groups as a function of age.

GENERAL DISCUSSION

Thyroxine-treated animals were generally more active than saline-treated controls and showed an extended or elevated locomotor arousal response to the CNS anticholinergic effect of scopolamine. The explanation for the failure to obtain an earlier ontogenetic activity peak as a result of neonatal thyroid hormone injections may lie in the effects of thyroid hormones upon noradrenergic arousal mechanisms. The brainstem noradrenergic system is well established as being primary in the mediation of behavioral arousal [15, 30, 32], and receptor sensitivity is apparently an important factor [14, 31, 33, 34]. One consequence of experimental hyperthyroidism in rats and mice appears to be increased synthesis and turnover of norephinephrine as well as increased receptor sensitivity [9, 10, 11]. Since the noradrenergic brainstem system is rapidly developing and functional in the neonatal rat and mouse [5, 18, 26, 39, 40], it seems that thyroid hormone excess during this critical neonatal period may chronically increase receptor sensitivity, in addition to the well documented accelerated development of many CNS processes. Therefore, it may be the case that the earlier onset of forebrain cholinergic inhibitory functioning is being masked by potentiated and developmentally accelerated brainstem arousal mechanisms in the thyroxine-treated mouse.

The findings of the present series of experiments suggest that cholinergic modulation of behavioral arousal may be advanced by 1–2 days in the thyroxine-treated mouse, as evidenced by the earlier response to scopolamine. The results do not completely eliminate the possibility that thyroxine may have in some way potentiated functioning of existing developing systems, rather than accelerating their development. The findings of the present study, nevertheless, suggest that the thyroxine-treated mouse has,

during the preweaning stage of development, a greater capacity for cholinergic inhibitory functioning. Since the ontogenetic activity peak may best be described as a shift in arousal-inhibitory balance [24,27], the failure to find an earlier peak for the thyroxine-treated mouse appears to be a consequence of the fact that a more advanced state of cholinergic functioning would be necessary to modulate potentiated brainstem arousal mechanisms.

Numerous other behavioral capacities have been found to be affected by early thyroid hormone excess. Motor and reflexive abilities are generally accelerated, and evidence of accelerated or superior learning capacities in the immediate preweaning period for the neonatally, thyroxine-treated rat has been found on active avoidance training, whereas deficits have been obtained on passive avoidance tasks [28,38]. These findings might be interpreted as indicating that only locomotor ability or activity is accelerated by early hormone excess. However, Murphy and Nagy [21] found an earlier onset of 24 hr retention capacities on a discriminated escape task for the thyroxine-treated mouse at 9–10 days of age compared to the onset for controls at 11–12 days, whereas a performance deficit resulted from the thyroxine treatment as early as 13–14 days on this task.

Consideration of these early learning studies yields two relevant points. First, the deficit obtained by Murphy and Nagy [21] approximates the peak activity period and the period where the greatest enhancement of behavioral arousal was obtained for thyroxine-treated groups in the present study. Second, higher activity levels, better preweaning performance on active avoidance tasks, and deficits on passive avoidance for thyroxine-treated rats may all be the results of enhanced behavioral arousal. Thus, the early acceleration produced by neonatal thyroxine treatment results in apparent behavioral deficits where enhanced arousal is not conducive to performance of the task.

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