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AGE DEPENDENT CHANGES IN AVERSIVE RESPONSES OF MICE TO ETHANOL AND THEIR CORRELATION WITH LIFE DURATION

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Abstract—Age dependent changes in voluntary responses to an ethanol solution were observed in CF1 male control mice, and an experimental group provided 5% sucrose (w/v) as their only liquid for ten days beginning at the age of four weeks. This was a life duration study that began when the mice were ten weeks of age. An untreated group of C57/B1 mice was similarly observed. A 3% ethanol solution (v/v) was offered the CF1 mice, and for a comparable demonstration of aversion to ethanol in C57/B1 mice, an 18% ethanol solution was required. In all three groups, the curves of decreasing proportions of behaviorally aversive mice that occurs with advancing age closely parallels the survivorship curves.

In the CF1 experimentals a more rapid development of behavioral aversion during the early portion of the life cycle, and an earlier onset of its decline phase occurs. Mean life span was significantly shorter in this group than in controls.

The age dependent changes in another parameter, total fluid intake per gram body weight, were compared for short-lived and long-lived subgroups of the C57/B1 mice. This ratio is relatively high early in life, decreases to significantly lower levels in adulthood, and later increase with advancing age. In the short-lived contingent lowest values are attained around the age of 40 weeks compared to 100 weeks in long-lived mice; and the subsequent phase of increasing values is similarly shifted.

INTRODUCTION

AGE DEPENDENT changes in behavioral aversion to an ethanol solution were observed in prior studies of CF1 and C3H mice (Kahn, 1975). The proportions of subjects aversive to an ethanol solution offered in a free choice situation is relatively low early in life, increases to peak and plateau levels in adulthood, and subsequently decreases with advancing age.

The current studies were undertaken to determine whether there is an association between the decreasing intensity of behavioral aversion to ethanol and the increasing mortality observed with increasing age.

We have reported that provision of five percent sucrose (w/v) as the only liquid source

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during a sensitive period (around the age of four weeks) results in a more rapid development of behavioral aversion to ethanol (Kahn, 1981). This procedure was employed with the CF1 experimentals of this study in an attempt to produce alterations in the pattern of age dependent changes in behavioral aversion to ethanol, and to see whether parallel changes take place in mortality and life span. The C57/B1 mice were included to assess the intensity of behavioral aversion to ethanol in this relatively high ethanol consuming strain, and to see whether age dependent changes in this parameter parallel survivorship in this strain as well.

Ethanol consumption data for the CF1 controls were previously reported (Kahn, 1975). Data relating the decline in behavioral aversion to increasing mortality with increasing age, and the effect of an experimentally imposed condition on both parameters is newly reported here.

METHODS AND PROCEDURES

CF1 and C57/B1 male mice were obtained from Carworth Laboratories, caged individually, and provided Purina food blox and liquids *ad libitum*. The room temperature was kept at 72 ± 2°F, and a 12 hour light-dark cycle maintained.

There were 76 CF1 controls, and 20 experimentals pretreated with five percent sucrose (w/v) as their only liquid source for a period of ten days beginning at the age of four weeks. A three percent ethanol solution (v/v) was offered as the alternative to plain water in the CF1 consumption studies.

95 C57/B1 mice were studied. None were experimentally pretreated. To ensure use of a test solution of sufficient concentration to evoke aversive responses comparable to those of the CF1 mice offered three percent ethanol, randomly selected subgroups were provided different concentrations of ethanol. 20 mice were offered three percent ethanol (v/v), 36 six percent, 20 twelve percent, and 19 eighteen percent ethanol (v/v). The group provided the 18% solution demonstrated a range of age dependent changes in aversive responses similar to those demonstrated by CF1 mice offered three percent ethanol. The C57/B1 subgroups offered the lower concentrations of ethanol did not provide as sensitive a picture of age dependent changes. The data from the subgroup offered 18% ethanol were used to represent all 95 C57/B1 mice in this regard. Mortality data were closely similar for all four C57/B1 subgroups; therefore the mortality data derived from all 95 mice were used in relating this parameter to the ethanol response data of the subgroup offered 18% ethanol.

Consumption data for water and for the ethanol solution, offered at separate sites at the front of the cage, were obtained by weighing the bottles three times weekly for the first month of the study, and at weekly intervals thereafter. The two bottles were site interchanged after each observation period. Consumption of the ethanol solution at preferred and non-preferred sites was expressed as percent of total fluid intake.

Mice demonstrate "behavioral aversion" to ethanol when its consumption at preferred and non-preferred sites is less than 50% of total fluid intake. In the presence of aversive behavior, consumption of the ethanol solution when offered at the *preferred site* is the index of its intensity by inverse relationship (Kahn, 1969; 1972). "Highly aversive" behavior is demonstrated when the mean consumption of ethanol at the preferred site is less than 30% of total fluid intake.

The proportion of mice in a designated category of aversive behavior (as defined by its intensity) depicts the age dependent changes in this parameter. With CF1 mice much of the age dependent changes appear as movement into or out of the "highly aversive" category. These changes would be missed if the inclusive category of "behavioral aversion" was used as the index of such changes. With C57/B1 mice, even the subgroup provided 18% ethanol as test solution was not as intensely aversive as the CF1 groups offered 3% ethanol. Too few mice demonstrated "highly aversive" behavior to adequately portray the changes taking place in aversive behavior during developmental and aging phases. For these reasons the CF1 groups are reported as the proportions of "highly aversives"; while for the C57/B1 group the inclusive category of "behavioral aversion" is used.

Observations were begun with the CF1 mice when they were ten weeks of age, and with the C57/B1 mice at age 15 weeks, and were continued for their life duration. Body weights were obtained at intervals throughout the study.

Another parameter derived from the data employed in the C57/B1 study is the ratio of total fluid intake per gram body weight. This parameter is known to undergo its own characteristic pattern of age dependent changes (Kahn, 1977), and was used in this study as a basis for comparing long lived and short lived groups of C57/B1 mice. The chi-square, or Student *t* test, was used in appropriate situations for statistical analysis.

RESULTS

The CF1 mice

The proportions of control and experimental mice highly aversive to three percent ethanol and of percent survivorship at different ages are presented in Figure 1.

Although the proportions of highly aversive experimentals are somewhat higher than in controls during the developmental phase, the differences were not statistically significant. A parallelism between the declining proportions of highly aversive mice and the declining proportions of surviving mice is evident in both groups. The phase of rapid decline in the proportions of highly aversive subjects begins about the age of 60 weeks in experimentals, but not until the age of 100 weeks in controls. The mean life span ±S.D. of 20 experimentals was 571 ± 160 days; that of 76 controls, 664 ± 150 days. The difference is statistically significant by the Student t test, $p < 0.025$.

Statistical significance of the decreasing proportions of highly aversive mice is difficult to evaluate when associated with an ongoing mortality that is substantial. However, the reality of a decreasing intensity of behavioral aversion to ethanol, as an aging phenomenon, can be confirmed in the observed behaviors of individual mice. 23 of the 69 control mice (33%) that died after the age of 60 weeks *had* demonstrated highly aversive behavior, succeeded by a period of *non-aversive* behavior prior to death. The remaining 46 mice were still highly aversive when they died. The transition to non-aversive behavior was completed (in those subjects demonstrating this change) at a mean age ±S.D. of 609 ± 146 days. Nine of the 16 experimentals (56%) that died after the age of 60 weeks demonstrated this transition to non-aversive behavior at a mean age of 497 ± 70 days. This is significantly earlier than in controls, $p < 0.01$ (Student t test).

Thus both control and experimental groups display a parallelism between the decline in proportions of highly aversives and the decline in proportions of survivors. The experimentals show a significantly earlier age of transition to non-aversive behavior and a significantly shorter mean life span.

The C57/Bl mice

For the reasons stated in Methods, survivorship data for all mice were compared with the ethanol response data from the subgroup offered 18% ethanol. The data are presented in Figure 2. The peak level in proportions of aversive mice occurs at the age of 60 weeks when 17 of 18 mice (94%) are aversive. By the age of 100 weeks only four of the 16 mice remaining alive (25%) are aversive. As in the CF1 study, the phase of rapid decline in proportions of survivors parallels the decline in proportions of aversive mice (as in Figure 2). The mean life span ±S.D. of the 95 C57/Bl mice was 844 ± 113 days.

In the subgroup offered 18% ethanol, 18 died after the age of 60 weeks (when the peak level of aversive behavior was attained). 14 of these mice (78%) demonstrated a shift from aversive to non-aversive behavior at a mean age of 693 ± 171 days (compared to their mean life span ±S.D. of 844 ± 113 days). Three mice remained aversive throughout the study, and one was non-aversive throughout.

The ratio of total fluid intake per gram body weight is a physiologic parameter known to undergo characteristic age dependent changes (Kahn, 1977). Long-lived and short-lived subgroups of the C57/Bl mice of this study were compared in this regard. There were 15

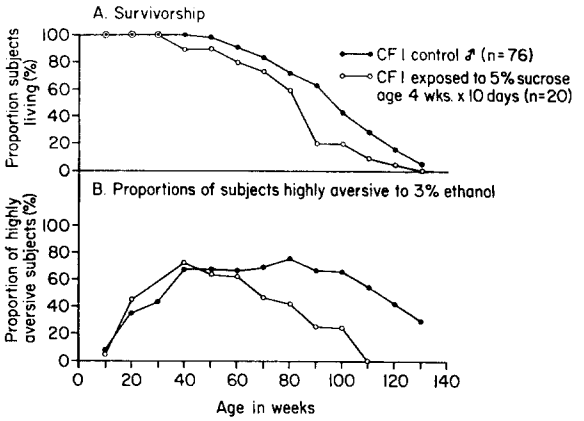

FIG. 1. **A.** Comparison of survivorship in control and experimental CF1 mice pretreated with 5% sucrose (w/v) as only liquid, at age 4 weeks for 10 days.
B. Comparison of proportions of highly aversive mice at successive ages in the same CF1 control and experimental groups.

mice with a life duration under 700 days (mean life span ±S.D. 610 ± 122 days), and 28 with a life duration over 900 days (mean life span 974 ± 43 days). Mean values for this ratio at successive ages are shown in Figure 3.

At the age of 15 weeks these values are closely similar in the two groups; 0.205 ± 0.026 cc/gm (N = 15) in the *short-lived* group, and 0.209 ± 0.031 cc/gm body weight (N = 28) in the *long-lived* group. From these levels significant decreases to low mean values occur in both groups. In the short-lived group a low of 0.133 ± 0.017 cc/gm (N = 14) is attained at the age of 35 weeks, a value significantly lower than that at age 15 weeks, $p < 0.001$ (Student *t* test). In the long lived group a low of 0.132 ± 0.021 cc/gm body weight (N = 27) is attained at the age of 95 weeks, a value significantly lower than at age 15 weeks, $p < 0.001$.

After the low values are attained, they increase significantly with age in both groups. In the long-lived group mean values increase to 0.150 ± 0.021 (N = 27) at the age of 115 weeks, $p < 0.001$ (Student *t* test). They were higher still at the age of 140 weeks (0.233 ± 0.033 cc/gm, N = 9), but the analysis of statistical validity of the difference to the low value at age 95 weeks is rendered difficult by the large number of deaths occurring by the age of 140 weeks.

In the short-lived group the values increase significantly from their low at age 35 weeks, to a peak of 0.207 ± 0.108 cc/gm (N = 11) at the age of 90 weeks ($p < 0.001$). To make sure that the three deaths occurring between these two data points have not distorted the probability calculation, the mean value for age 35 weeks was recalculated to include only those 11 subjects that survive until the age of 90 weeks. This made little difference in the mean, and no change in the statistical validity of the difference between these two values.

Mean values decrease more rapidly in the short lived group, and at the age of 35 weeks is significantly lower than that for the long lived group; 0.133 ± 0.017 (N = 14) versus 0.152 ± 0.018 cc/gm (N = 28) respectively, $p < 0.05$ (Student *t* test). Mean values con-

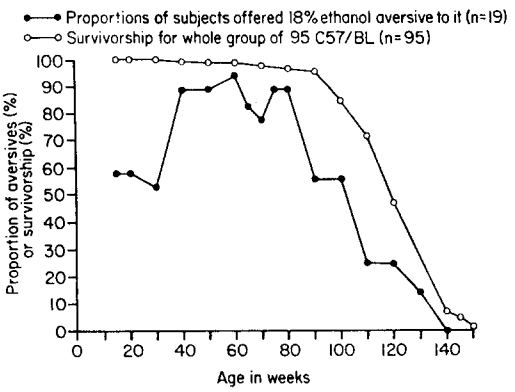

FIG. 2. Progression of the proportions of aversive C57/B1 mice offered 18% ethanol (v/v) as alternative to plain water, compared to survivorship curve of whole population.

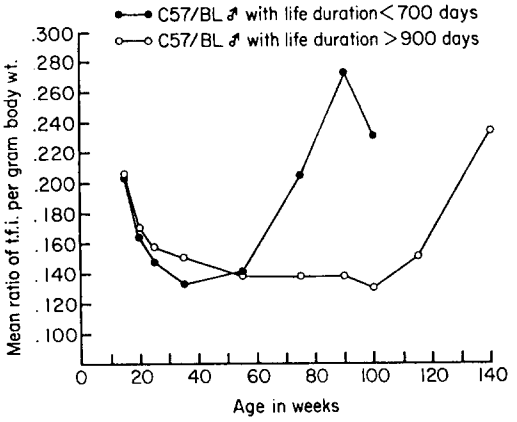

FIG. 3. Total fluid intake per gram body weight in 15 C57/B1 mice with life duration under 700 days, compared to 28 mice with life duration over 900 days.

tinue to decrease in the long lived group as they begin to rise in the short lived group. The two curves cross at a value of 0.140 cc/gm at the age of 55 weeks, as mean values in the long lived group continue to decrease to their low of 0.132 ± 0.022 (N = 28) at the age of 95 weeks. The rising values in the short lived group attain a peak at the age of 90 weeks, just prior to the low point attained by the long lived group. At the age of 90 weeks the mean is significantly higher in the short lived group than in the long lived group; 0.272 ± 0.125 (N = 11) versus 0.135 ± 0.016 cc/gm (N = 28) respectively, $p < 0.05$.

Thus there is a more rapid progression of these age dependent changes in the short lived group than in the long lived group during the early phase of the life cycle. The onset of the phase of increasing mean values begins earlier in the short lived group than in the long lived group; 35 weeks versus 95 weeks of age, respectively. The generally more rapid progression of age dependent changes in the short lived group is apparent in Figure 3.

DISCUSSION

The characteristic sequence of responses to the ethanol solution observed by us in prior studies (Kahn, 1975) was confirmed in this study. It includes an early phase with relatively low proportions of aversive (or highly aversive) mice, increasing to peak and plateau proportions in adulthood, and subsequently decreasing with advancing age. The full sequence of these responses was observed in many, but not all mice. Somewhat more than one third of the control CF1 mice, and somewhat over half of the experimentals demonstrated the shift from aversive to non-aversive behavior prior to death, while 78% of the C57/B1 mice did so. The differences between CF1 and C57/B1 groups in this regard may be attributable to differences in the intensity of the aversive response. Perhaps a more sensitive index for depicting changes in aversive behavior would have demonstrated some decline in those CF1 mice that did not demonstrate the transition to non-aversive behavior.

The basic findings of this study can be stated as follows:

1) The decline in proportions of mice behaviorally aversive to ethanol that occurs with

advancing age parallels the decline in the proportions of surviving mice in all groups studied. A decrease in the intensity of behavioral aversion to ethanol as an aging phenomenon was confirmed in the behavior of individual mice.

2) An experimentally imposed condition that resulted in an earlier mean age of transition from aversive to non-aversive behavior was associated with a reduction in mean life span.

3) Short-lived C57/B1 mice demonstrated a more rapid pace of age dependent changes in a physiologic parameter, total fluid intake per gram body weight, than do long-lived mice.

In regard to the first of these findings, a number of investigators have demonstrated how a decreasing competence of functional processes with age can determine the mortality curve of a population (Sacher, 1956; Strehler and Mildvan, 1960). Although behavioral aversion to ethanol cannot be considered a vital function *per se,* its decline does parallel the mortality curve. The possibility that the determinants of behavioral aversion are in some way related to vital functions should be considered. As a behavioral entity it must be mediated thru the CNS. The brain is a vital organ by virtue of its regulation and coordination of a host of physiologic functions (Hess, 1954; Konorski, 1967). As the brain ages these vital functions can be expected to decline in activity, as would non-vital functions mediated by the brain. Thus the decline in intensity of behavioral aversion to ethanol could be a reflection of generalized aging of the infrastructure of the CNS.

The second basic finding deals with the effects of providing five percent sucrose (w/v) as the only liquid source during a sensitive period around the age of four weeks. As to the mode of action of this pretreatment, we have observed that 1) the rate of development of aversive behavior to ethanol is *increased* when the five percent sucrose is provided at the age of four weeks, but not when offered at the age of 12 weeks (Kahn, 1981); 2) the rate of development of aversive behavior is *delayed* when pretreatment is provided during gestation (unpublished data); and 3) the effects of pretreatment provided during either of these sensitive periods on *other processes,* such as the development of fighting behavior, can be of *opposite sign* from that on the development of aversion to ethanol (unpublished data). For these reasons it appears unlikely that we are dealing with some general biochemical effect of five percent sucrose as provided, but with the reactions to this treatment by the regulatory mechanisms involved with age dependent activities of specific processes.

Alterations in the course of development evoked by conditions imposed during a sensitive period have been reported for many biologic processes (Levine and Lewis, 1963; Faris and Campbell, 1981; MacLusky and Naftolin, 1981). We have reported that such early pretreatment can affect later phases of the pattern of age dependent changes as well as the development phase (Kahn, 1968).

It is our hypothesis that the entire pattern of age dependent changes in activity of functional processes observed during a life cycle are predetermined by control mechanisms, alterable during a sensitive period early in life. Such a determinant is in fact demonstrated by the CF1 experimental group.

The third basic finding demonstrates a relationship between the rate at which age dependent changes proceed and life duration. Age dependent changes in the parameter, total fluid intake per gram body weight, proceeds more rapidly in the short-lived than in the long-lived group (Figure 3). A rate factor also appears to be in effect in the CF1 group experimentally pretreated with five percent sucrose. There is a somewhat more rapid development of behavioral aversion to ethanol, and an earlier onset of the decline phase (Figure 1B), suggesting a more rapid rate of age dependent changes throughout the life cycle, and associated with a decrease in mean life span. A correlation between a relatively

rapid rate of progression of age dependent changes and a relatively short life span, and vice versa, is both reasonable and in keeping with the data.

Goldschmidt (1938), among others, reported many instances where experimentally induced changes in rates of development are produced by sensitive period effects. Based on evidence that the life span in poikilotherms is inversely proportional to the ambient temperature, Pearl hypothesized that life duration depends upon the "rate of living" (Pearl, 1928). In mammalia, caloric restriction results in a retardation of growth and an increase in life duration (McCay *et al.*, 1935). Our studies indicate that factors other than temperature manipulation in poikilotherms, or growth retarding caloric restriction in mammalia, can affect the rate at which age dependent changes proceed in particular systems; with associated effects on life duration.

The kinds of changes produceable in the pattern of age dependent changes during a life cycle are in all likelihood not confined to simple alterations of the overall rate of change. We have found that hemoglobin concentration develops more rapidly, and is maintained at higher plateau levels for a longer duration in mice exposed to partial asphyxiation during gestation, than in controls (Kahn, 1968).

It is apparent that much remains to be learned about the kinds of alterations in the pattern of age dependent changes that can be evoked by the early imposition of experimental conditions. Extension of our knowledge in this regard might well provide us with a comparable measure of control of life duration.

SUMMARY

The expression of behavioral aversion to ethanol in mice demonstrates a characteristic pattern of age dependent changes in which its intensity is relatively low early in life, increases to peak and plateau levels in adulthood, and subsequently decreases with advancing age. In CF1 control and experimental groups, and in a group of C57/B1 mice, a parallelism was apparent between the decreasing proportions of behaviorally aversive subjects and the decreasing proportions of surviving subjects during the late phase of the life cycle.

The experimental CF1 group was provided five percent sucrose (w/v) as the only liquid source for ten days beginning at the age of four weeks. This resulted in a somewhat more rapid development of behavioral aversion to ethanol, and an earlier onset of the decline phase of this parameter relative to controls.

Short lived (life duration <700 days) and long lived (life duration >900 days) subgroups of C57/B1 mice were compared in regard to age dependent changes in another physiologic parameter, total fluid intake per gram subject. Age dependent changes in this parameter proceeded at a more rapid rate in the short lived than in the long lived subjects.

The hypothesis that regulatory mechanisms, alterable during a sensitive period, determine the subsequent pattern of age dependent changes of biologic processes, was advanced and discussed. The relationship between a relatively rapid rate of the progression of age dependent changes and a relatively short life span, and vice versa, was noted and discussed.

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