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Age Dependent Effects of 5-Hydroxytryptamine Upon Memory Consolidation and Cerebral Protein Synthesis[1]

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ESSMAN, W. B. *Age dependent effects of 5-hydroxytryptamine upon memory consolidation and cerebral protein synthesis.* PHARMAC. BIOCHEM. BEHAV. 1(1) 7–14, 1973.–In four experiments consideration was given to the effect of intracranially administered 5-Hydroxytryptamine (5-HT) upon several age dependent cerebral processes. These included (1) the tissue uptake of exogenously administered intracranial 5-HT, which in 17-day old CF-1S strain mice did not lead to increases in forebrain 5-HT level; (2) when administered in close temporal proximity with a single trial passive avoidance training trial, intracranial 5-HT produced a significant degree of retrograde amnesia in 15, 20, and 30-day-old mice, with a temporal gradient for this amnesic effect measured out to eight min. No significant retrograde amnesic effect for such passive avoidance behavior was produced in 17-day old animals. The amnesic effect appeared to be specific to 5-HT, in that (3) other amines or 5-HT analogs administered intracranially in equimolar concentration, had no amnesic effect. The relationship between age and the effect of intracranially administered 5-HT was considered with regard to regional cerebral protein synthesis. The incorporation of radioactive leucine into proteins of several regions of the brain, including cerebral cortex, basal ganglia and diencephalon, midbrain, and cerebellum, was significantly inhibited within 20 min following 5-HT administration. No significant inhibition of cerebral protein synthesis was observed in 17-day old mice of the same strain given identical treatment. The data from the series of experiments generally supported the view that inhibition of protein synthesis may be effected by increases in the regional concentration of 5-HT, and under circumstances wherein such increases are precluded either because of endogenous metabolic circumstances or tissue uptake properties, protein synthesis inhibition may not occur. Such was the case for the 17-day old CF-1S strain mouse wherein protein synthesis inhibition was attenuated and the retrograde amnesic effect of intracranial 5-HT was antagonized.

5-Hydroxytryptamine Memory consolidation Protein synthesis Age

THERE have been several bases upon which a relationship between cerebral macromolecular synthetic processes and the consolidation, storage, or retrieval of behavior has been formulated. Among the most prominent of those methodological approaches to this issue are those studies in which centrally administered inhibitors of protein synthesis have been shown to exert effects upon memory related capabilities of the organism [1, 3, 12]. Use has been made of potent and toxic antibiotics for both the inhibition of cerebral protein synthesis as well as for modification of memory processes; this approach inherently assumes that the stability or vulnerability of macromolecular synthesis associated with memory processes becomes dependent upon the degree to which anti-metabolite induced protein synthesis inhibition must occur.

The one factor that has emerged from previous studies [4] that holds possible significance for processes related to retrograde amnesia and inhibition of protein synthesis is brain 5-Hydroxytryptamine (5-HT) metabolism. The forebrain level of this amine has been shown to be altered by agents or events that produce a retrograde amnesia in mice; this amine, as well can initiate a significant degree of inhibition of cerebral protein synthesis [5, 8]. These effects of 5-HT have been demonstrated both *in vivo* and *in vitro*. More specifically, it has been shown [4, 9] that the *in vitro* synthesis of proteins maintained by metabolically active synaptosomes isolated from either the cerebral cortex or limbic system was inhibited by approximately 30% at concentrations of 5-HT approximating those necessary to produce significant *in vivo* elevation of this amine in the forebrain.

A very striking characteristic of experimentally induced retrograde amnesia in mice that has been previously reported [4] is an age dependent susceptability to electroconvulsive shock. In this instance it was shown that 17-day old male CF-1S strain mice were highly resistant to the amnesic effects of posttraining ECS treatment and also had an endogenous neural metabolic profile which not only differed from that of mice of other ages, but also was consistent with a metabolic profile suggesting attenuation of biochemical changes normally attending ECS. It was the purpose of this series of experiments to give further

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consideration to the effects of intracranially administered 5-HT upon: (1) tissue uptake of this amine as a function of age; (2) retrograde amnesic effects of 5-HT as a function of age; (3) the specificity of 5-HT induced behavioral effects; and, (4) the relationship between age and the effect of intracranial 5-HT upon cerebral protein synthesis.

EXPERIMENT 1

Procedure

Male CF-1S strain mice, bred under laboratory conditions wherein litters were reduced at three days of age to five, were used in all experiments. A total population of 20 animals per group were used in all experiments. A total population of 20 animals per group were used at either 15, 17, 20, or 30 days of age, where they were given a unilateral intracranial injection of 8.5×10^{-5} M of 5-Hydroxytryptamine, creatine sulphate salt, administered in 5λ of a 153.9 μEq/ml solution of sodium chloride. Groups of control animals at each of these ages were injected with the NaCl carrier alone. 5-HT and saline control solution were given using a 30 gauge stylette mounted needle on a Hamilton microliter syringe. Injections were made into the area of the medial hippocampus, approximately 1.5 mm lateral to the midline, 3.0 mm posterior to the orbital margin, and approximately 1.5 mm below the surface of the skull. Independent assessment of the injection site was provided through histological observations of sections through an injection site following introduction of equivalent volumes of dilute dye. Any indication of a marked change in electrocortical activity caused by either experimental treatment or control injection was obviated by independent electrocorticographic assessment of the injection sequalae. These data clearly indicated only a 20% voltage reduction with no frequency alteration; there was no evidence of seizure activity or slow-wave components.

Brain tissue was obtained from all animals following cervical dislocation at 15 min posttreatment, the forebrain tissue between the rhinal fissure and the posterior colliculus was homogenized in 0.1 N HCl and then processed by solvent extraction for the subsequent fluorometric determination of 5-HT concentration, in accord with established procedures [14]. A slight modification from this procedure consisted of alkinization after extraction from heptane and measurement of the final extract at 295 mμ excitation and 340 mμ emission. The 5-HT concentrations obtained as a function of age which were used to constitute baseline levels were derived from the NaCl treated control mice.

Results

The data obtained have been summarized in Fig. 1. It may be observed that whereas mice of 15 days of age treated intracranially with 5-HT showed approximately a 60% increase in forebrain 5-HT level ($F=32.00$; $p< 0.02$), and mice of 20 and 30 days of age showed almost a 100% increase in the level of this amine ($F=26.87$, 23.17; $p< 0.05$, respectively), the 17-day old animals only showed an increase of less than 20% in forebrain 5-HT content ($F=2.70$; $p>0.20$). This result is highly suggestive of reduced uptake and retention of exogenous 5-HT by forebrain tissue in 17-day old CF-1S strain mice.

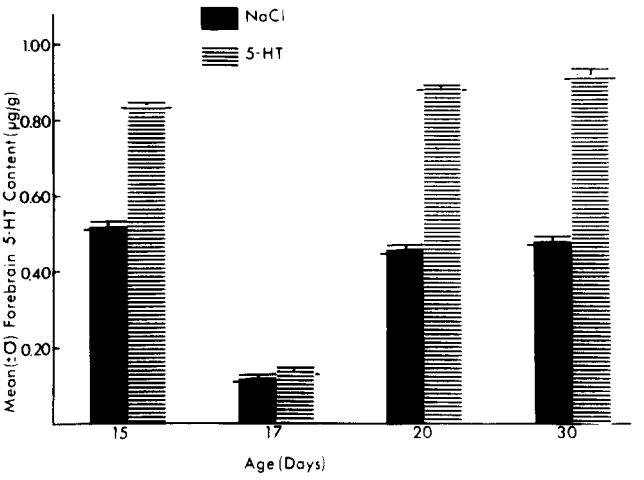

FIG. 1. Mean (±σ) forebrain 5-Hydroxytryptamine concentration in mice of several ages injected intracranially with NaCl or 5-HT.

EXPERIMENT 2

In order to assess the age related retrograde amnesic effects of intracranially administered 5-HT, a similar experimental sequence as that described in the previous experiment was followed. The rationale for this study rested, in part, upon our observation that reduced uptake and retention of cerebral 5-HT in 17-day old mice should provide for a reduced central effect and extent to which this amine possesses amnesic properties. We have previously observed [4,6] that intrahippocampal 5-HT in young adult mice in close temporal proximity with avoidance training can result in a significant degree of retrograde amnesia for the avoidance response, when subsequently evaluated.

Procedure

Male CF-1S strain mice, derived from laboratory bred litters, as previously described, were trained at either 15, 17, 20, or 30 days of age, in groups of 20, to acquire a passive avoidance response which was provided through training on a single trial in an apparatus modified from previous description [11]. This consisted essentially of placing a mouse into an open transparent vestibule adjoining a larger, darkened chamber, access into which was provided by a 5 cm hole. The larger chamber had a floor consisting of stainless steel rods wired in series through a cam-operated grid scrambler to a 400 V power supply. Contact through the paws of the animal between any two adjacent grids completed the circuit and provided for a foot shock of 3 mA maintained for three sec.

All animals were given a single training trial, as described, and were then removed from the chamber in which footshock was given. An injection of either 8.5×10^{-5} M of 5-HT in 5λ of a 153.9μEq/ml solution of NaCl (experimental) or the NaCl alone (control) was given into the region of the medial hippocampus at either 20 sec, or 2, 4, 8, 16, or 32 min following the training trial. Between the training trial and the intracranial injection, the animals were kept in holding cages. After intracranial injection they were returned to their home cages. For the training trial a latency to respond, i.e., enter the darkened chamber from

the outer vestibule, was measured for each animal. On a testing trial, given 24 hr following training, the retention of the passive avoidance response, i.e., avoidance of the larger chamber by failure to enter for 120 sec or more, or retrograde amnesia, i.e., entry into the larger chamber within 10 sec, were measured.

Results

A summary of the incidence of conditioned response retention, evaluated for each mouse on the basis of a testing trial latency of 120 sec or more, is presented in Table 1. Although there were no statistically significant differences between the number of animals showing conditioned response retention for training given at 15, 20, or 30 days of age for any of the training trial 5-HT treatment conditions ($\chi^2=6.50$; $p>0.70$), there was a clear indication in all of these age groups of a short temporal gradient for 5-HT induced retrograde amnesia. As the interval between the training trial and the intracranial injection of 5-HT was increased, the retrograde amnesic effect of this treatment was decreased. It was apparent that 5-HT injection given 960 sec after the training trial did not appreciably alter the incidence of conditioned response retention measured 24 hr after the training trial ($\chi^2=3.00$; $p>0.30$). All mice at each age, with exception of the 17-day old animals, showed a significant incidence of 5-HT related retrograde amnesia, when such treatment was given either 10 ($\chi^2=29.70$; $p<0.001$), 120 ($\chi^2=43.70$; $p<0.001$), 240 ($\chi^2=30.75$; $p<0.001$), or 480 (χ^2-21.00; $p<0.001$) sec after training.

The notable exception to these data is represented by those mice trained and treated at 17 days of age. It is apparent that even with a training treatment interval as short as 10 sec, the incidence of 5-HT induced retrograde amnesia did not reach a statistically acceptable level of significance ($\chi^2=4.00$; $p>0.10$), and certainly by 120 sec or more after training 5-HT treatment did not have any effect upon the retention of the conditioned avoidance response as measured 24 hr later. It is clear, therefore, that whereas intracranially administered 5-HT at a dose sufficient to effect significant increases in brain 5-HT level in mice of 15, 20, or 30 days of age, the same dose of this amine in 17-day old animals did not lead to significant forebrain 5-HT elevation, and correspondingly did not effect a retrograde amnesia for the passive avoidance response to which these animals were trained.

TABLE 1

PER CENT INCIDENCE OF CONDITIONED RESPONSE RETENTION FOR MICE OF SEVERAL AGES GIVEN INTRACRANIAL 5-HT AFTER TRAINING

Time Between Training Trial and 5-HT (sec)	Age (Days) 15	17	20	30
10	25	80	20	10
120	30	100	30	15
240	30	100	40	40
480	40	100	50	70
960	90	100	90	90
1920	100	100	100	100

EXPERIMENT 3

The foregoing data, while they have essentially been in agreement with previous observations suggesting that 5-HT, when intracranially administered, can bring about a retrograde amnesia, have not given consideration to the possibility of the extent to which this effect is specific to 5-HT. It has been clear that whereas 5-HT in 0.9% NaCl will bring about a retrograde amnesic effect in mice younger or older than, but not of 17 days of age, the NaCl carrier alone did not exert any effect upon the apparent retention of the response. One basis upon which a relationship between 5-HT, cerebral protein synthesis, and conceptual views of the memory consolidation process have been considered and interrelated concerns the interaction of 5-HT and related indoles with tRNA [6, 7]. The posited relationship has held that indole derivatives that are more strongly bound by tRNA exert synaptic effects that are related to the affinity coefficients for such molecular interactions; the greater this affinity, the greater the effectiveness of the indole in inhibiting such protein synthesis as might be tRNA dependent. The affinity, for example, of tRNA for 5-HT is greater (7.7×10^{4}) than for other 5-HT analogs or derivatives such as N-acetyl-5-hydroxytryptamine (5.9×10^{3}) or 5-methoxy-1-methyltryptamine (1.2×10^{4}). Such affinity coefficients have been derived as the product of the number of binding sites and the association constant of the molecule from photometric binding studies. Based upon our earlier hypothesis, a reduced affinity of the indole derivative for the macromolecule would imply a reduced likelihood of direct synaptic effects. Under such circumstances, one could further postulate a reduced amnesic effect if the amnesic effect is contingent upon a disruption of macromolecular directed synaptic processes that are necessary ingredients of the memory consolidation event.

The specificity of posttraining intracranially administered 5-HT was assessed experimentally in comparison with either physiological saline or the two 5-HT derivatives mentioned above.

Procedure

Four groups of 20 male 30-day old CF-1S strain mice each were given a single passive avoidance conditioning trial as described in the previous experiment. Following the administration of the conditioning footshock each mouse was given an intracranial injection, into the region of the medial hippocampus, of either 7.7×10^{-5} Equ sodium chloride, 8.5×10^{-5} M of 5-Hydroxtryptamine, creatine sulphate salt, dissolved in 153.9 μEq/ml of NaCl, or an equimolar amount of the hydrochloride salts of either norepinephrine (NE), N-acetyl-5-hydroxytryptamine (N-A-5-HT), or 5-methoxy-1-methyltryptamine (5-M-MT). The total volume for each unilateral injection was 5λ and the injection was given at 10 sec following footshock and removal from the apparatus.

A single testing trial was given to all animals 24 hr following training, with the interval between placement into the vestibule and entry into the chamber in which prior footshock was given being measured. A failure to respond by entry into the larger chamber after 120 sec had elapsed constituted the end of the testing trial, with the animal being removed from the apparatus and scored as having shown complete retention of the conditioned passive

avoidance response. An entry from the vestibule into the larger chamber within 10 sec of placement into the former constituted the criterion by which retrograde amnesia for the conditioned response was defined.

Results

Following the posttraining injections there was no evidence whatsoever of any motor or muscular impairment among animals in any of the treatment conditions. A mild locomotor excitation was observed for NE treated mice, but this did not persist beyond five min following treatment. On the day on which the testing trial was given there were no differences overtly manifested among any of the mice in the various treatment groups.

A summary of the data for this experiment has been presented in Table 2. The results indicate quite clearly that no significant retrograde amnesic effect was brought about when posttraining intracranial treatment was given either with NaCl, NE, N-A-5-HT, or 5-M-MT ($\chi^2=1.63$; $p>0.30$). Those mice given posttraining intracranial injection of 5-HT showed a significantly increased incidence of retrograde amnesia as compared with those mice within other treatment conditions ($\chi^2=8.32$; $p<0.02$).

It is apparent from these data that for those analogs of 5-HT evaluated in equimolar concentration with 5-HT, the retrograde amnesic effect upon single trial avoidance conditioning in mice was not brought about, whereas for the latter, a significant retrograde amnesic effect was once again confirmed.

TABLE 2

INCIDENCE OF CRITERION RETROGRADE AMNESIA MEASURED ON A TESTING TRIAL FOR MICE GIVEN POSTTRAINING INTRACRANIAL TREATMENTS

Posttraining Intracranial Treatment	PerCent Incidence of Retrograde Amnesia
Sodium Chloride	0
5-Hydroxytryptamine	80*
Norepinephrine	20
N-acetyl-5-Hydroxytryptamine	0
5-methoxy-1-methyltryptamine	5

*$\chi^2 = 46.20$, $df = 4$, $p<0.001$

EXPERIMENT 4

At least one of the remaining issues upon which the preceding experiments have touched concerns the relationship between age, intracranial 5-HT treatment, and cerebral protein synthesis. Whereas it has become apparent from previous findings [4, 6, 8] that cerebral protein synthesis can be inhibited by intracranial doses of 5-HT sufficient to cause a significant elevation in tissue 5-HT level and effect a retrograde amnesia for passive avoidance behavior, it remains to be shown that age related determinations of tissue amine uptake and amine induced behavioral effect are also consistent with the effects upon cerebral protein synthesis. Since reduced tissue uptake and retention of exogenous 5-HT has been shown in the present experimental series for 17-day old mice, and animals given posttraining intracranial 5-HT at this age also showed a markedly attenuated incidence of retrograde amnesia, it is possible to speculate upon the age-specific relationship between tissue amine retention, tissue protein synthesis, and the occurrance of retrograde amnesia; such speculation could well incorporate the hypothesis that inhibition of cerebral protein synthesis by exogenous 5-HT is contingent upon tissue retention of this amine. Similarly, the retrograde amnesic effect of intracranial 5-HT, in addition to being temporally related to the training situation, also appears dependent upon the ability of 5-HT to induce inhibition of cerebral protein synthesis. These hypotheses were tested indirectly by an experiment in which the effects of intracranial 5-HT upon *in vivo* protein synthesis as inferred from the rate of incorporation of a labeled amino acid into cerebral proteins, were investigated for several regions of the brain in mice of several ages.

Procedure

Male CF-1S strain mice were selected and assigned to two groups of ten each at ages 15, 17, 20, and 30 days of age. Utilizing the intracranial injection procedure previously described, one group of mice at each of these ages was treated with 2λ of 5-HT in 5λ of 0.9% NaCl and the other group at each age was treated intracranially with the NaCl vehicle only. Immediately following intracranial experimental or control treatments an intraperitoneal injection of C^{14}-leucine (0.1 μc of 165 μc/μM/g body weight) was given. The C^{14}-leucine was injected with cold leucine in a final concentration of 0.66 m M to provide an excess of an estimated 10 μg/g free leucine pool present in the mouse brain. In this way the dilution of the brain leucine pool by the experimental treatments was obviated. At 20 min following the injection of the radioactive amino acid the animals were killed by cervical dislocation and the brain tissue was rapidly removed. The tissue was hand dissected on a cold stage of a dissecting microscope, and separated into several major regions consisting of: cerebral cortex, basal ganglia and diencephalon, midbrain, and cerebellum. Each of these regions was placed into a solution of cold 0.25 M sucrose, 0.05 M tris-HCl buffer, pH 7.5, 0.025 M KCl, and 0.002 M $MgSO_4$; the mixture was homogenized in glass homogenizer tubes to which 1.0 g/l of cold leucine had been added in order to preclude the possibility of any significant incorporation of the radioactively labeled amino acid during the preparative procedures.

Trichloroacetic acid (TCA) was added to a final concentration of 5% in order to precipitate the proteins from the prepared tissue. After centrifugation (1×10^6 g min) the precipitate was double washed with 5% TCA and heated for 15 min at 90°C in 5% TCA in order to hydrolize the RNA. A further double TCA wash and an ethanol:ether (2:1) wash were given and a 97% solution of formic acid was used to dissolve the pellet in a final volume of 0.5 ml. Further solubilization was achieved in liquid scintilation fluid and the radioactivity was determined from readings on a Beckman scintillation counter. The method of Lowry *et al.* [13] was used to determine total protein concentration,

which was then used as a base upon which the molar concentrations of incorporated radioactive leucine were expressed.

Results

The rates of cerebral protein synthesis for mice treated intracranially with 5-HT and the extent to which these compared with baseline levels for saline treated mice, are summarized for each respective region of the brain in which these *in vivo* effects were assessed in Figs. 2, 3, 4, and 5.

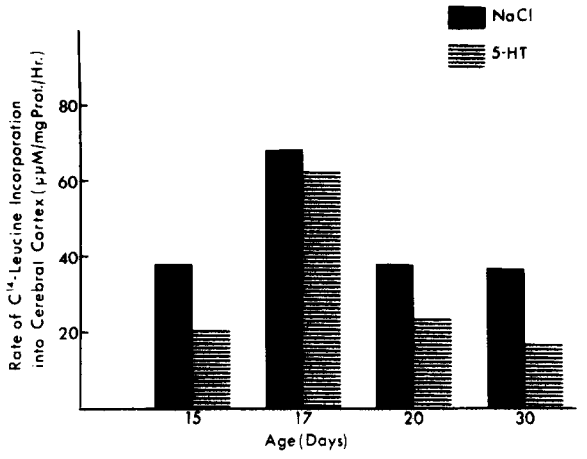

FIG. 2. Protein synthesis in mouse cerebral cortex after *in vivo* treatment with NaCl or 5-HT.

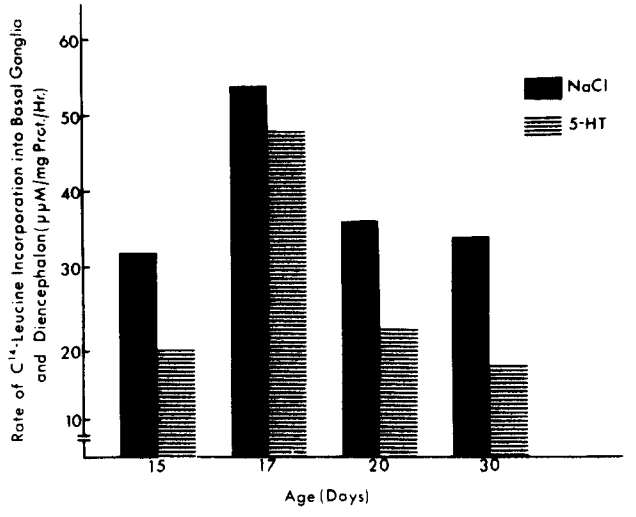

FIG. 3. Protein synthesis in mouse basal ganglia and diencephalon after *in vivo* treatment with NaCl or 5-HT.

The radioactivity of the incorporated amino acid for each of the samples at each age and treatment condition was expressed as the number counts per min per mg of protein. These specific activities were compared for control and 5-HT-treatment conditions and a difference in specific activity was obtained as a measure of the degree to which, at each age, 5-HT effected a reduced rate of leucine

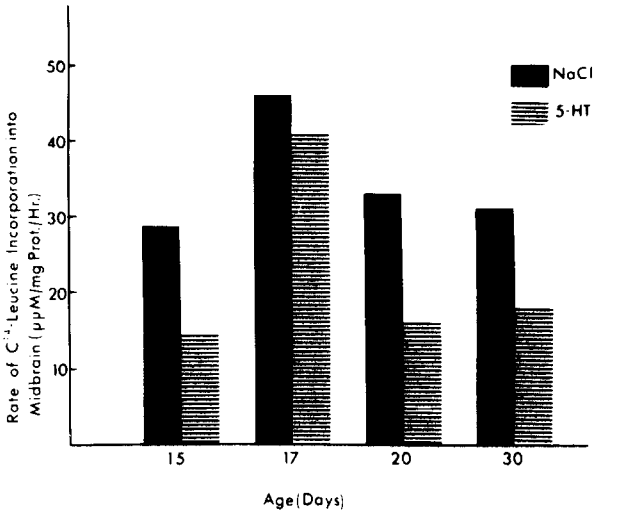

FIG. 4. Protein synthesis in mouse midbrain after *in vivo* treatment with NaCl or 5-HT.

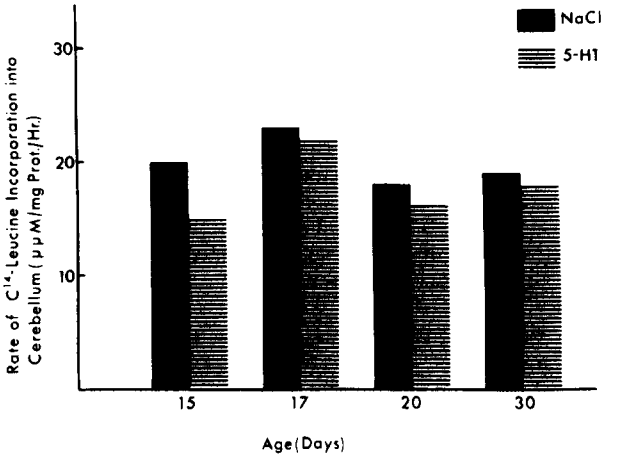

FIG. 5. Protein synthesis in mouse cerebellum after *in vivo* treatment with NaCl or 5-HT.

incorporation into regional proteins. Such a reduction in the rate of amino acid incorporation into cerebral proteins without the reduction of the free leucine pool or dilution of the isotope, has been taken as the index of inhibition of protein synthesis. These specific activity difference scores were analyzed for each brain region from which they were derived in a chi square design. A summary of the results derived from these analyses has been presented in Table 3.

Highly significant differences occurred for each brain region among all age group intercomparisons as a consequence of intracranial 5-HT treatment. Except for some moderate differences between the magnitude of reduced amino acid incorporation observed in the cerebellum of 15-day old mice (22%), which probably accounted for the significant differences observed between 15, 20, and 30 day old animals for that region, no other such differences were observed between these ages for other regions of brain tissue sampled. The differences between the significantly

TABLE 3

SUMMARY OF STATISTICAL ANALYSIS OF DIFFERENCES IN COUNTS PER MIN PER Mg PROTEIN FOR 5-HT AND CONTROLTREATED MICE AT SEVERAL AGES

Brain Region	STATISTICAL COMPARISON χ^2		
	Between Age Groups (3)	Between Ages 15/20 and 30 Days (2)	Between 17 Days and Other Ages (1)
Cerebral Cortex	59.16*	4.80	54.36*
Basal Ganglia and Diencephalon	79.24*	4.97	74.27*
Midbrain	48.97*	2.16	46.81*
Cerebellum	18.13*	12.37†	5.76‡

*$p<0.001$
†$p<0.01$
‡$p<0.05$
Numbers in parentheses indicate *df*.

reduced rate at which intracranial 5-HT treatment inhibited regional protein synthesis in 17-day old mice, and the magnitude by which this inhibition was apparent in those mice of other ages becomes quite apparent in the statistical analysis. Again, in the case of cerebellar tissue, only a very slight degree of inhibited protein synthesis (5%) was observed for 5-HT-treated 17-day old animals. The fact that 15 and 20-day old animals both showed somewhat higher rates of inhibition after 5-HT treatment (22% and 13% respectively) probably accounts for the small, but statistically significant, effect observed.

The one striking observation that clearly emerges in these data is that the endogenous rates of protein synthesis, at least as noted for cerebral cortex, basal ganglia and diencephalon, and midbrain, were appreciably higher among 17-day old animals, as compared with either younger or older mice of the same strain. It would appear that two factors bear a close age specific similarity with one another in these findings.

In reference to the data obtained in Experiment 1, it is clear that 17-day old mice had endogenous forebrain 5-HT levels that were about 20% of those observed in younger or older mice and this perhaps is relevant to the hypothesis that the regulation of cerebral protein synthesis is dependent, not only upon tissue uptake of an exogenous indole amine, but also may rest upon the endogenous content and metabolism of this time.

DISCUSSION

The data which have been summarized for the series of four experiments described have paralleled, in several respects, previous indications that an age specific antagonism of experimentally induced retrograde amnesia may be observed for 17-day old CF-1S strain mice [4]. In the present investigation the retrograde amnesic effect has been observed following the intracranial administration of 5-Hydroxytryptamine (5-HT), an indole amine which has been shown to be elevated and for which altered brain turnover has been observed as a consequence of other amnesic agents such as electroconvulsive shock [5, 8]. It has been previously observed [4, 6] that intracranial injection of 5-HT in mice can produce both a retrograde amnesia for passive avoidance behavior, as well as an inhibition of cerebral protein synthesis [9, 10]. In the present series of experiments the age specific antagonism of 5-HT induced retrograde amnesia and its inhibition of protein synthesis has been observed in 17-day old mice.

It was apparent that the 17-day old animal showed an endogenous level of forebrain 5-HT that was appreciably and very significantly lower than the endogenous levels of this amine observed in 15, 20, or 30-day old animals. This finding was consistent with earlier observations [5] indicating lower brain 5-HT levels in 17 and 18-day old male CF-1S strain mice. The inability of the forebrain tissue to take up, retain, or incorporate exogenous 5-HT when administered intracranially is clearly evident from the findings reported for Experiment 1. Although no specific attempt has been made at this point to explain this unusual, age specific phenomenon, some explanation may reside in an extremely high turnover rate of cerebral 5-HT and very short turnover time for this amine that has been previously reported as being specific to 17-day old CF-1S strain mice [4]. Another possibility may relate to the diffusion of the amine from its injection site, the medial hippocampus; possible age specific differences in diffusion may exist, although this issue has not been given specific attention.

One possible relationship between the failure of 17-day old animals to show increased 5-HT levels in forebrain following intracranial treatment may be related to the failure of these animals to show any significant degree of 5-HT induced retrograde amnesia for a conditioned passive avoidance response. The observation that 15, 20, and 30-day old animals show a characteristic temporal gradient for 5-HT induced retrograde amnesia, with some amnesic effect of such posttraining treatment still observed by at least eight min after training, further supports the hypothesis that the process by which the memory trace is consolidated

may be disrupted by an increase in cerebral 5-HT level. We have independently observed, on the basis of autoradiographic studies (unpublished data), that 5-HT following intrahippocampal injection is diffused into at least all those brain regions considered in the present investigation, and that the consequences of such treatment do not include seizure activity, cortical depression, or overt behavioral disturbances. This would generally preclude the possibility that intracranial 5-HT injection exerted an amnesic effect by initiating cerebral changes which, in other investigations, have been utilized as methodologies by which the memory consolidation process has been studied.

The specificity of 5-HT for the retrograde amnesic effect observed has been further supported by data indicating that at least one other biologically significant amine, norepinephrine, did not provide for any retrograde amnesic effect, and two structurally similar derivatives of 5-HT similarly did not produce amnesic effects. In the case of the latter two compounds and their lack of behavioral effect, some further support is additionally provided for our previous hypothesis [6, 7] suggesting that the synaptic effects regulating memory consolidation and site specific proteins may be dependent upon a nucleic acid-5-HT interaction. Inasmuch as the same two indole derivatives which failed, in equimolar amounts, to produce a retrograde amnesia following intracranial administration to mice have also been shown to have appreciably lower affinities for tRNA binding, some support has been further provided for the previously cited hypothesis.

The high endogenous rate at which protein synthesis in several regions of the mouse brain occurs in 17-day old mice, as compared with younger or older animals of the same strain, may be one basis upon which the failure of intracranial 5-HT to effect a significant reduction in such protein synthesis may possibly be explained. Another alternative to such explanation rests upon the observation that endogenous levels of 5-HT were not increased in 17-day old mice following intracranial injection of a dose of this amine sufficient to almost double the measured level in the forebrain of younger or older mice. Therefore, if 5-HT induced inhibition of cerebral protein synthesis is indeed dependent upon the uptake, incorporation, and deposition of 5-HT and further perhaps upon the binding of this amine by nucleic acids important to the mediation of protein synthesis, then a failure of tissue uptake might well explain the absence of any protein synthesis effect.

It is difficult to make direct comparisons between either the endogenous rates at which protein synthesis was observed for different regions of the mouse brain and for this reason all observational as well as statistical comparisons were limited to age differences for synthesis within specific regions and experimental effects for age comparisons within specific regions. The present findings are consistent with data for forebrain protein synthesis as a function of age [9] and also provides support for the view that protein synthesis can be regulated by 5-HT. This view has been presented largely on the basis of *in vitro* studies [2, 4, 7]; however, recent findings [9, 10] as well as the data presented in the present paper indicate that cerebral protein synthesis on a regional level can be inhibited by 5-HT treatment, with the notable exception that this effect is absent in 17-day old CF-1S strain mice.

The age dependency of the relationship between increases in intracranial 5-HT and the inhibition of cerebral protein synthesis by such treatment has been previously emphasized [9] by the indication that 5-HT uptake was significantly correlated with reduced amino acid incorporation into protein. Furthermore, it has been observed that the greater the endogenous rate of cerebral protein synthesis as a function of age, the less the inhibitory effect of intracranially administered 5-HT upon cerebral protein synthesis. Whether the process of memory consolidation depends in part upon the synthesis of proteins which underlie synaptic alterations, remains a question that lies beyond the scope of the present paper. It may, however, be pointed out that inhibition of regional protein synthesis in the brain by 5-HT appears to constitute an important determinant of the susceptability of treated animals to interruption of the memory consolidation process, eventuating in a retrograde amnesia. The inhibition of cerebral protein synthesis after intrahippocampal injection of 5-HT has been shown to be age-dependent, to the extent that at least one age group of the strain of mouse used failed to show both uptake of the amine and interference thereby with protein synthesis. This could reflect an age specific limitation upon the interaction of 5-HT at those sites specific for protein synthesis, and a failure, thereby, to observe interference with memory consolidation.

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