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Postnatal Modification of Hippocampal Circuitry Alters Avoidance Learning in Adult Rats

Abstract. *In rats and mice, the genetically mediated extent of the mossy fiber projection that synapses on the basal dendrites of hippocampal pyramidal cells is inversely correlated with rate of two-way avoidance (shuttle-box) learning. Postnatal hyperthyroidism, induced in 51 rat pups, resulted in marked variations of this infrapyramidal mossy fiber projection. The number of trials required for criterion performance of these rats in adulthood remained correlated with the neuroanatomical trait (r = 0.74, $P < 0.0001$).*

The ability of rats and mice to learn a two-way avoidance task (shuttle-box) is inversely correlated with a discrete and nonpathological variation in the intrinsic circuitry of the hippocampal formation: the more mossy fibers (efferents of the granule cells of the fascia dentata) terminating on the basal dendrites of hippocampal pyramidal cells, the poorer the animal's capacity for shuttle-box learning (*1*). The extent of this intra- and infrapyramidal subdivision of the mossy fiber projection (IIP-MF) is genetically mediated (*2*). To test the prediction that shuttle box learning would remain correlated with an experimentally altered mossy fiber distribution, we injected rat pups with thyroxine—a treatment that produces a variable hyperplasia of the IIP-MF projection that persists into adulthood (3). We now report that such developmental manipulation of neuronal circuitry can change the expression of an inherited talent for avoidance learning. This phenomenon is unlikely to reflect a pathological process but appears to manifest the action of a simple developmental factor that controls the graded and correlated expression of both a neuroanatomical trait and performance in an avoidance learning situation.

We studied rats of a strain that has been selectively bred for more than 40 generations for superior two-way avoidance (Roman High Avoidance, RHA/Verh) (*4*), the behavioral trait being correlated with a modest IIP-MF projection (*1*). One might expect that in these animals a thyroxine-induced hyperplasia of the IIP-MF projection fibers would impair the acquisition of a two-way avoidance response. Although attempting to impair performance is often a questionable strategy, it seemed appropriate for this experiment. A possible dynamic relation between the extent of the IIP-MF projection and two-way avoidance cannot be verified by reducing the IIP-MF in a poorly performing strain. Even with correlated improvement of shuttle-box learning, it does not seem possible to dissociate such an effect from the consequences of possible nonspecific damage inflicted on hippocampal circuitry, for almost any damage of the hippocampus results in an improvement in two-way avoidance. This paradoxical effect reflects a lesion-induced hyperreactivity that is beneficial in a test situation characterized by conflicting cues. The underlying mechanisms, however, are unknown, although several explanatory hypotheses have been offered (*5*). We thus preferred an experimental approach that predicts behavioral effects opposite to those seen after hippocampal lesions. This does not imply an attempt to "correct" a hippocampal malfunction (for

Table 1. Mean (and standard deviation) morphological and behavioral variables in adult RHA rats postnatally treated with saline or thyroxine. Thyroxine values are the pooled means of all treatment groups. Brain weight refers to fixed and trimmed brains before cutting. Data were analyzed with one-tailed t-tests; N.S., not significant.

Variable	Saline controls (n = 24)	Thyroxine (n = 51)	P
Body weight (g)	261 ± 52	238 ± 47	*
Brain weight (mg)	1438 ± 97	1458 ± 111	N.S.
Midseptotemporal volume of CA3-CA4 (mm^3)	0.93 ± 0.08	0.95 ± 0.08	N.S.
Volume ratio (%) of IIP-MF in CA3-CA4	2.9 ± 0.7	4.8 ± 1.3	**
Volume ratio (%) of remaining MF fields and stratum lacunosum–moleculare	32.3 ± 1.3	33.7 ± 1.8	**
Avoidance score (trials to criteron)	16.8 ± 10.2	21.2 ± 9.3	*
Latency to change compartment (shock delivered after 5 seconds)	5.7 ± 1.3	6.0 ± 2.4	N.S.

*$P < 0.05$. **$P < 0.001$.

which no evidence has been found in RHA rats). Our goal was to manipulate a hippocampal trait within the limits of its natural range and to observe a possible covariation with two-way avoidance that would remain dissociable from nonspecific hippocampal damage. Any further comparison with hippocampal lesions would be inappropriate since their behavioral consequences necessarily reflect the joint activity of the remaining brain parts and therefore cannot provide any information about the possible functional relevance of structural variations within the hippocampus itself.

Rat pups of both sexes (n = 51) received a standard dose of L-thyroxine, injected in variable intervals that started at birth and ended at day 17 (*3, 6*). Control subjects (n = 24) were given saline (*3*). The thyroxine treatment resulted in the classical signs of hyperthyroidism—acceleration of physical and behavioral development (*7*). Adult body weight was reduced in animals given maximum doses of thyroxine. There were no dead rat pups, however, and no reduction of adult brain weight—a phenomenon observed after excessive hyperthyroidism (*8*) (Table 1). The animals were tested in the shuttle-box at the approximate age of 90 days for the number of trials required to achieve a criterion of four consecutive avoidance responses (*9*); thus, the higher the score, the poorer the learning. Rats failing to reach criterion within 35 trials were given a score of 35. There were no signs of motor impairment during conditioning as indicated by almost identical escape latencies between controls and thyroxine animals (Table 1), and no rat receiving electric shocks was observed to freeze (an immobility reaction frequently seen in poor shuttle-box performers).

The brains of the animals were processed and stained with Timm's silver sulfide stain, which is particularly effective for the hippocampal mossy fiber system (*10*). Since afferent projections to the hippocampus terminate in individual layers on the apical and basal dendrites of the neurons, the volume ratios of the synaptic fields in a given target region can be estimated by means of planimetry on histological cross sections (*1*) (Fig. 1). Planimetric analysis (*11*) was done on five horizontal sections per animal, taken from an intermediate portion of the long axis of the hippocampus. The morphometric variables reported here are the midseptotemporal volume of the hippocampal subregion CA3-CA4, the volume ratio of the IIP-MF projection in this target zone, and, for comparison, a pooled volume ratio that includes the volumes of synaptic fields of other subdivisions of the mossy fiber projection and that of stratum lacunosum–moleculare. Together, the two volume ratios represent the proportion of synaptic fields in CA3-CA4 conveying input of cortical origin to the pyramidal cells (Fig. 1).

In comparison with the saline controls, the thyroxine treatment resulted in a significant expansion of the synaptic fields of both the IIP-MF projection and the other subdivisions (Table 1). The degree of this volume increase, however, was only loosely related to the doses of thyroxine. As predicted, only the thyroxine-dependent variation of the IIP-MF projection remained significantly correlated with the shuttle-box scores of the adult animals (r = 0.74, $P < 0.0001$) (*12*). Practically no correlation with behavior (r = 0.05) was found for the volume ratio of the other afferent projections (remaining mossy fibers and stratum lacunosum–moleculare), despite their considerable thyroxine-induced variability. Therefore, the relation between IIP-MF and two-way avoidance does not simply reflect a generalized trophic action of thyroxine on extrinsic afferents to CA3-CA4: among the hippocampal fiber systems we examined, it was only the mossy fiber projection on the basal dendrites of the pyramidal cells whose volume and spread were related to two-way avoidance.

Since two-way avoidance is influenced by several factors (*13*), it was not surprising that the treatment did not transform the animals into poor avoiders; although the animals were subnormal for this strain, their performance was still superior to that characterizing a parallel line selectively bred for inferior shuttle-box learning (the Roman Low Avoid-

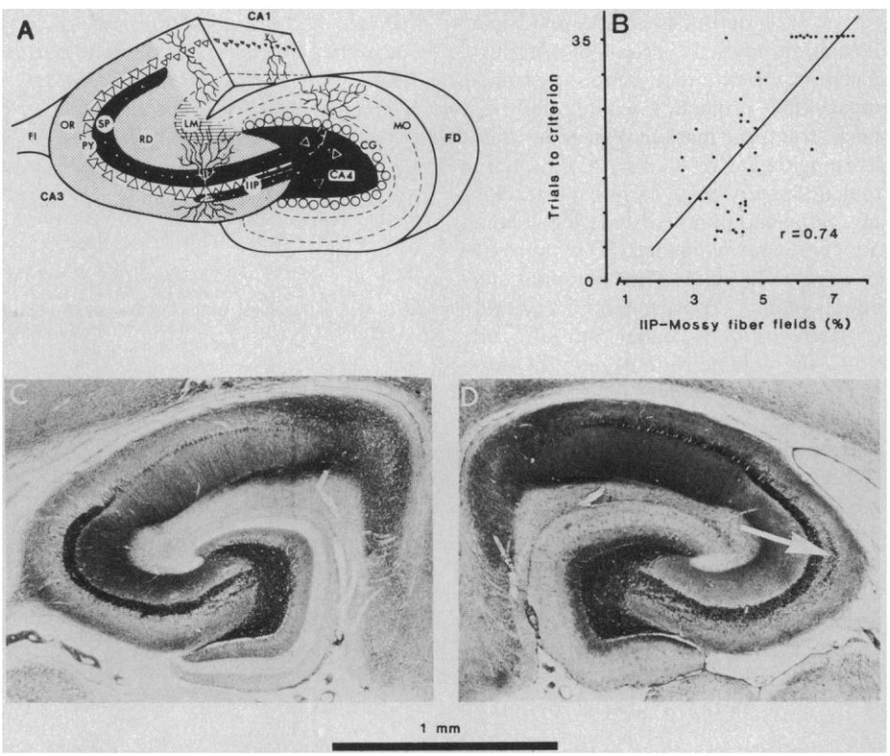

Fig. 1. Morphology and behavior. (A) Diagram of a Timm-stained cross section of the hippocampus. The hippocampal subregion CA3-CA4 (the area of morphometry) is indicated by black, stippled, and hatched areas. Black areas: Suprapyramidal (*SP*), intra- and infrapyramidal (*IIP*), and hilar (*CA4*) mossy fiber terminal fields, originating from the dentate gyrus. Stippled areas: stratum oriens (*OR*) and radiatum (*RD*), the terminal fields of intrinsic hippocampal projections. Hatched areas: stratum lacunosum-moleculare (*LM*), receiving afferents from entorhinal cortex. Abbreviations: *CA1*, subregion of the hippocampus without mossy fibers; *FI*, fimbria hippocampi; *FD*, fascia dentata; *MO*, molecular layers of the fascia dentata (receiving entorhinal projections). (B) Scatter plot of the relation between the extent of the IIP-MF projection and trials to criterion in a shuttle-box learning task. Since testing was terminated after 35 trials, the behavior scores are truncated at this value (*12*). The IIP-MF score indicates the proportion of the synaptic target space within CA3-CA4 that is occupied by mossy fibers synapsing on the basal dendrites of the pyramidal neurons. Its variation corresponds to shifts in the balance of afferents along the basal dendrites. (C) Timm-stained hippocampus of an adult RHA rat in which postnatal hyperthyroidism resulted in a modest hyperplasia of the IIP-MF distribution. The rat's behavioral score was eight trials to criterion. (D) Same region of an RHA rat in which the treatment induced a marked proliferation of infrapyramidal mossy fibers. The animal did not attain criterion within 35 trials. The white arrow points at typical IIP-MF hyperplasia in the distal portion of CA3. Scale bar, 1 mm.

ance rats) (*4*). This indicates that the treatment did not affect all mechanisms responsible for a naturally low capacity for shuttle-box learning. Specifically, the propensity for shock-related freezing so typical for RLA rats was not increased. Yet, the hyperthyroidism apparently acted on a cerebral mechanism strong enough to express itself against other genetic factors promoting two-way avoidance (*14*). Therefore, this brain mechanism seems to play an important role in some form of emotional or cognitive processing that codetermines the capacity for shuttle-box learning.

These data show that a developmental factor controls the graded and correlated expression of both infrapyramidal mossy fiber development and avoidance learning. This thyroxine-sensitive factor presents a target for either genetic influences or developmental interference (*15*). We cannot rule out the possibility that the behaviorally relevant target system is situated outside the hippocampal formation and its proximal circuitry. Even so, the extent of the infrapyramidal mossy fiber projection would still be the best structural marker of a measurable learning behavior found so far, a trait that ultimately may permit the tracking of a hitherto unknown system comediating two-way avoidance. The thyroxine treatment, in spite of its potential side-effects and its crudeness, mimics a natural correlation. As under natural conditions, the volume ratio of only the infrapyramidal mossy fiber projection was significantly correlated with length of time to learn a shuttle-box response, despite considerable variation of other synaptic fields in CA3-CA4 (*1*). Neuroanatomically, the IIP-MF projection is strategically located in a bottleneck of hippocampal circuitry (*16*) that is itself important for mediating two-way avoidance (*17*), and it forms part of a fiber system capable of "plastic" rearrangement only during a critical period in development (*3*, *18*). This IIP-MF circuitry thus seems to be in a commanding position and to have the necessary adult invariance to form a structural bias affecting shuttle-box learning throughout life. It is thus tempting to think that either the variation of the infrapyramidal mossy fibers or that of a closely related structure inside the hippocampal formation (*19*) is a determinant of the capacity for two-way avoidance learning.

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