81 van Abeelen, J. H. F., Genotype and the cholinergic control of exploratory behavior in mice, in: The Genetics of Behaviour, pp. 347–374. Ed. J. H. F. van Abeelen. North-Holland, Amsterdam, and American Elsevier, New York 1974.
82 van Abeelen, J. H. F., Genetic analysis of behavioural responses to novelty in mice. Nature *254* (1975) 239–241.
83 van Abeelen, J. H. F., Rearing responses and locomotor activity in mice: Single-locus control. Behav. Biol. *19* (1977) 401–404.
84 van Abeelen, J. H. F., and Boersma, H. J. L. M., A genetically controlled hippocampal transmitter system regulating exploratory behavior in mice. J. Neurogenet. *1* (1984) 153–158.
85 van Abeelen, J. H. F., Ellenbroek, G. A., and Wigman, H. G. A. J., Exploratory behaviour in two selectively-bred lines of mice after intrahippocampal injection of methylscopolamine. Psychopharmacologia *41* (1975) 111–112.
86 van Abeelen, J. H. F., and Gerads, H. J. M. I., Role of hippocampal met-enkephalin in the genotype-dependent regulation of exploratory behavior in mice. J. Neurogenet. *3* (1986) 183–186.
87 van Abeelen, J. H. F., and van den Heuvel, C. M., Behavioural responses to novelty in two inbred mouse strains after intrahippocampal naloxone and morphine. Behav. Brain Res. *5* (1982) 199–207.
88 van Abeelen, J. H. F., and van Nies, J. H. M., Effects of intrahippocampally-injected naloxone and morphine upon behavioural responses to novelty in mice from two selectively-bred lines. Psychopharmacology *81* (1983) 232–235.
89 van Daal, J. H. H. M., de Kok, Y. J. M., Jenks, B. G., Wendelaar Bonga, S. E., and van Abeelen, J. H. F., A genotype-dependent hippocampal dynorphinergic mechanism controls mouse exploration. Pharmac. Biochem. Behav. *28* (1987) 465–468.
90 van Daal, J. H. H. M., Zanderink, H. E. A., Jenks, B. G., and van Abeelen, J. H. F., Distribution of dynorphin B and methionine-enkephalin in the mouse hippocampus: Influence of genotype. Neurosci. Lett. *97* (1989) 241–244.
91 Vinogradova, O. S., Functional organization of the limbic system in the process of registration of information: Facts and hypotheses, in: The Hippocampus, vol. 2, Neurophysiology and Behavior, pp. 3–69. Eds R. L. Isaacson and K. H. Pribram. Plenum, New York 1975.
92 Walker, J. M., Berntson, G. G., Paulucci, T. S., and Champney, T. C., Blockade of endogenous opiates reduces activity in the rat. Pharmac. Biochem. Behav. *14* (1981) 113–116.
93 Warburton, D. M., and Wesnes, K., Drugs as research tools in psychology: Cholinergic drugs and information processing. Neuropsychobiology *11* (1984) 121–132.
94 Weber, E., and Barchas, J. D., Immunohistochemical distribution of dynorphin B in rat brain: Relation to dynorphin A and α-neo-endorphin systems. Proc. natl Acad. Sci. USA *80* (1983) 1125–1129.
95 Yu, O., Ito, M., Chiu, T. H., and Rosenberg, H. C., GABA-gated chloride ion influx and receptor binding studies in C57BL6J and DBA2J mice. Brain Res. *399* (1986) 374–378.

0014-4754/89/090839-07$1.50 + 0.20/0

Using genetically-defined rodent strains for the identification of hippocampal traits relevant for two-way avoidance behavior: a non-invasive approach

H.-P. Lipp[a]*, H. Schwegler[b], W. E. Crusio[c], D. P. Wolfer[a], M.-C. Leisinger-Trigona[a], B. Heimrich[d] and P. Driscoll[e]

[a] *Anatomisches Institut der Universität Zürich-Irchel, Zürich (Switzerland);* [b] *Zentrum für Morphologie, Johann-Wolfgang-Goethe-Universität, Frankfurt (Federal Republic of Germany);* [c] *Institut für Humangenetik und Anthropologie, Universität Heidelberg, Heidelberg (Federal Republic of Germany);* [d] *Physiologisches Institut der Universität Mainz, Mainz (Federal Republic of Germany);* [e] *Laboratorium für Verhaltensbiologie, Eidgenössische Technische Hochschule Zürich, Zürich (Switzerland)*

Summary. Genetically-defined rodent strains permit the identification of hippocampal traits which are of functional relevance for the performance of two-way avoidance behavior. This is exemplified here by analyzing the relationship between infrapyramidal mossy fibers (a tiny projection terminating upon the basal dendrites of hippocampal pyramidal neurons) and two-way avoidance learning in about 800 animals. The necessary steps include 1) identification of structural traits sensitive to selective breeding for extremes in two-way avoidance, 2) testing the robustness of the associations found by studying individual and genetical correlations between hippocampal traits and behavior, 3) establishing causal relationships by Mendelian crossing of strains with extreme structural traits and studying the behavioral consequences of such structural 'randomization', 4) confirming causal relationships by manipulating the structural variable in inbred (isogenic) strains, thereby eliminating the possibility of genetic linkage, and 5) ruling out the possibility of spurious associations by studying the correlations between the hippocampal trait and other behaviors known to depend on hippocampal functioning.

In comparison with the classical lesion approach for identifying relationships between brain and behavior, the present procedure appears to be superior in two aspects: it is non-invasive, and it focuses automatically on those brain traits which are used by natural selection to shape behaviorally-defined animal populations, i.e., it reveals the natural regulators of behavior.

Key words. Mouse; rat; genetic variation; selective breeding; inbred strains; hippocampus; two-way avoidance; learning; neuroanatomy; morphometry; development; hyperthyroidism.

Introduction

Historical notes

In any mammalian species, no brain is like the other, and every individual behaves differently. But is there a link between the variability of the brain and individual talents and propensities? We believe so, sharing the view that had been introduced almost 200 years ago by the phrenological approach of Franz Josef Gall (1743–1826). Perhaps surprising to modern neuroscientists, phrenology, the first psychobiological theory, was based on the combination of three concepts: comparative ethology, modularity of the brain, and connectionism[38, 39]. Behavioral differences between species were attributed to the presence or absence of specific brain modules and their differential connections. Individual differences among the members of a species, however, were thought to arise from the differential development of specific brain parts and their mode of interaction. The phrenological vision was that the overall function of the brain, and of its portions, might be deciphered non-invasively and non-destructively, by means of patient and careful observations of behavioral variation and cerebral covariation. We do not intend to re-establish phrenology, but believe that the underlying concepts and principles, including a reluctance to mutilate animals brains, can still serve as a useful guideline for the analysis of brain and behavior. We shall show here that such an approach is heuristically equivalent and, in some aspects, even superior to the classical invasive techniques. Thus, 'microphrenology' as presented here is nothing new, but 'the' classical approach in neurobiology. Yet, it would have been impossible without the modern methodology of behavior genetics which has been brought to brain research notably by Bignami[6], Bovet[8], Broadhurst[9], Collins[13], McClearn[78], Oliverio[89], Roderick[95], van Abeelen[113], Wahlsten[116], the Wimers[120–122], and their co-workers.

Simple brain factors and behavior

The realization of an experimental approach based on the study of covariation between brain structures and behavior requires a concept of how simple factors can specifically influence mental processes. Such factors may be of genetic or epigenetic origin. A model emphasizing evolutionary aspects of the relations between genes, brain and behavior has been presented elsewhere[66]. Thus, only a brief summary will be presented here.

Studies in behavior genetics in mammals (particularly in mice) demonstrate strong genetic effects on behavior in a variety of behavioral situations[37, 104–108]. The inheritance of such traits seemingly follows the schemes of Mendelian inheritance only rarely[10, 79, 90], but many studies of selective breeding for behavioral divergence show a rapid response[14, 29, 32, 53, 57, 76, 112, 114]. This is no indication for monogenic inheritance, but it clearly shows that there must be some simple factors through which a differential set of alleles can reliably bias behavioral traits, without crude effects on biological fitness.

The preferred brain targets for such genetic influences must be the coordination systems of the brain, i.e., neuronal populations which modulate and coordinate other brain systems. By definition, modulatory systems are hierarchically superimposed on brain systems executing the main sensory and motor procedures. One must expect that allelic variation affecting modulatory systems can exist without destructive side effects, since the mammalian brain has a series of buffering procedures compensating for, and/or masking interventions, be they genetic or non-genetic in origin. These procedures include developmental reorganization, system homeostasis, adult structural plasticity, and learning capacities[54, 55, 63, 66]. In terms of evolutionary theory, these systems serve as reservoirs of genetic variation, permitting rapid genetic adaptation of behavior to specific selective pressure, a process eventually followed by morphological adaptations of the body.

This phenomenon can also be exploited for the investigation of the relations between brain and behavior. By concentrating on the variability of these topranking system levels, one may detect genetic variation in brain traits with a rather direct impact on the control of behavior. These influences must be *probabilistic*, since variations of a modulatory system can only bias probabilities of certain behavioral actions. In addition, they probably will never encode a specific behavior per se, because modulatory systems can operate only by influencing processes executed by subordinated systems. These are certainly different across species, but may differ also within a species. In other words, the effect of such coordination systems are *context-specific*. Thus, one must look for variation in top-ranking systems and for covariation of behavioral measures reflecting choices and decisions, or which demand a high degree of coordination and modulation. But which ones?

The choice of brain structures and behavior

One of the toughest problems in behavioral brain research is to decide which brain variables and which behavioral trait should be investigated. There are countless brain structures, and there are many ways to measure behavior. Matching the correct variables is, therefore, largely a matter of educated guesses or luck. The ideas just outlined provide no solution, but they narrow the choices.

If there is no a priori reason to investigate a given behavior, the most straightforward approach is to select a behavioral trait for which successful selective breeding has been reported. This implies a) that there must be genetic variation in the brain traits causing divergent behavior, b) that these divergences are not associated with overt malfunctioning, and c) that the genetic architecture for the behavioral divergence cannot be too com-

plex, since selective breeding programs are usually abandoned if no rapid responses to selection are observed. The choice of brain systems is more arbitrary. From the theory above, top-ranking coordination systems are preferable. Fortunately, they are not difficult to identify, be this for genes or for neuroscientists, because they ordinarily do not develop and mature in advance of the systems they modulate – a neuroanatomical principle known as Flechsig's rule[33,34]. Thus, system level specificity does not require biochemical but temporal encoding – late-acting genes must necessarily influence the maturation of behavioral coordination systems. This fact greatly facilitates the search for neuronal systems to be examined for genetic variation. It must be noted, however, that developmental time-tables for brain structures preferentially deal with neuronogenesis and less with the more subtle aspects of functional maturation. Nevertheless, the maturational gradients of the mammalian brain currently point to the prefrontal cortex[56], the fascia dentata[1,5,30], the olfactory bulbs[77] and, perhaps, the cerebellum[60,102] as the most promising targets for a neuroanatomical and/or neurochemical investigation aimed at behaviorally relevant genetic variation. Concentration on genetic variability is not a matter of ideology but of practicality: a genetic difference is, by definition, resistant to masking influences due to plasticity or environmental factors, and, thereby, more convenient to work with.

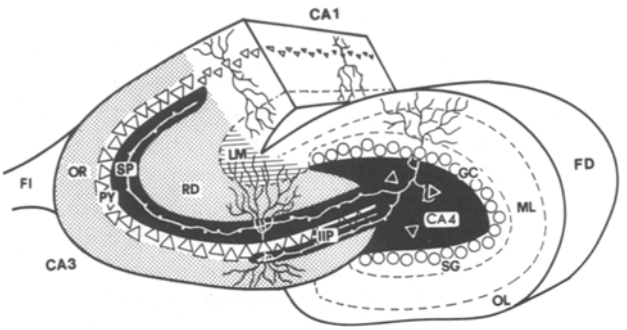

Figure 1. Diagram of the Timm-stainable hippocampal fields in CA3/CA4 as revealed on hippocampal cross-section. Black areas: terminal fields of the mossy fiber projections; stippled areas: terminal fields of the intrinsic associational projections (Schaffer collaterals and commissural projections); hatched area: terminal field of entorhinal projection to CA3. Abbreviations: CA1; regio superior; CA3, regio inferior; CA4, hilus of the fascia dentata; FD, fascia dentata (dentate gyrus); FI, fimbria hippocampi; GC, granule cell layer; IIP, intra- and infrapyramidal mossy fiber projection; LM, stratum lacunosum-moleculare; ML, medial molecular layers in fascia dentata; OL, outer molecular layer; OR, stratum oriens; RD, stratum pyramidale; RD, stratum radiatum; SG, supragranular dentate layer; SP, suprapyramidal mossy fiber projection. Reproduced from Lipp and Schwegler[70], with permission of the publisher.

Methodology

The series of studies reported in this section were initiated in order to investigate possible causes underlying the extreme genetic differences in avoidance acquisition, observed in behavioral genetic studies using mouse strains[8,10]. The decision to investigate avoidance learning was reinforced by the availability of rat lines selectively bred for this task[6,28], and the brain variables of interest were the terminal fields of hippocampal mossy fibers, for which considerable genetic variation had been reported[4].

The neuroanatomical variables (figs 1 and 2)

The rodent hippocampus is characterized by a laminar architecture typical for the archicortex of most mammals. Pyramidal neurons form monolayer sheets, while their dendritic trees are covered with boutons of afferent axons originating within, and outside of, the hippocampus. They terminate at well-defined levels termed synaptic fields. The resulting lamination is visualized with particular clarity by Timm's procedure[48,111], which stains synaptic boutons according to their content of zinc or other heavy metals[47]. In particular, the distribution of the giant boutons of the granule cell axons, or mossy fibers[2], is reliably revealed under a variety of staining protocols[11,23,46]. In mice and rats, these mossy fiber

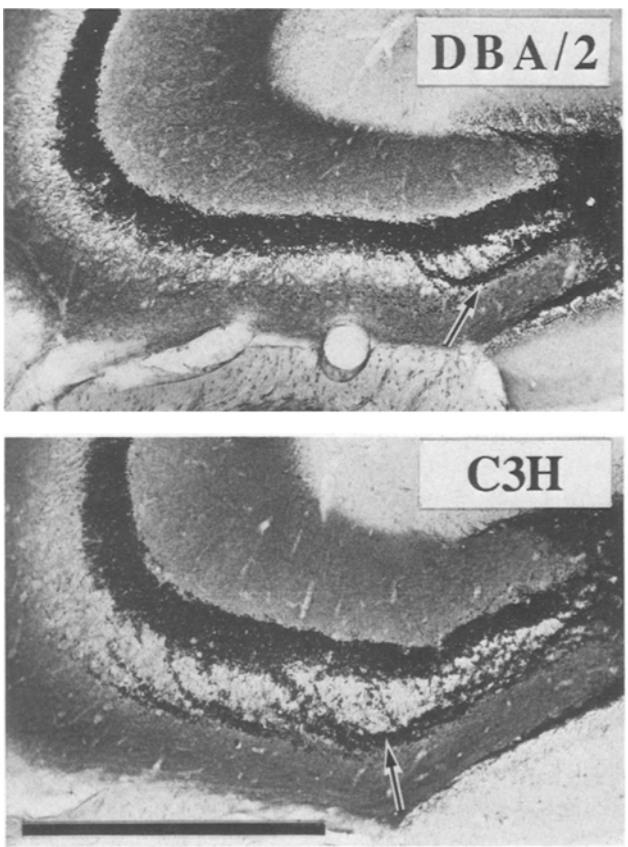

Figure 2. Genetically determined extremes of the infrapyramidal mossy fiber projection (arrow) as seen in the strains DBA/2 and C3H. For details of structures, see fig. 1 Bar: 0.5 mm. Timm's stain, horizontal sections from the midseptotemporal level. Reproduced from Lipp and Schwegler[70], with permission of the publisher.

boutons terminate preferentially in a layer immediately above the pyramidal cells in the regio inferior (CA3). They are designated as *suprapyramidal mossy fibers*. A smaller portion of the terminal fields extends within and below the pyramidal cells, making contact with basal dendrites, the *intra/infrapyramidal mossy fiber projection* (IIP-MF)[4]. Another terminal field of mossy fibers is the hilus of the fascia dentata (CA4), where mossy boutons densely cover dendrites as well as cell bodies ('mossy' cells[3]). The other fields stained reliably by Timm's procedure are stratum oriens, formed by basal dendrites, and stratum radiatum, formed by apical dendrites above the suprapyramidal mossy fiber layer. Both strata are covered with boutons from intrinsic associational systems, the ipsilateral (Schaffer) and commissural axon collaterals of the pyramidal neurons in CA3. Stratum lacunosum-moleculare, finally, is generally poorly stained. It is contiguous with the molecular layers of the fascia dentata, and contains the synapses of axons originating in the entorhinal cortex.

The morphometrical methods used to assess the areas or volumes of these hippocampal terminal fields have been described in detail elsewhere[74,99]. Measurements were always done on 5–10 horizontal sections corresponding to a standard mid-septotemporal level from the longitudinal axis of the hippocampus. Planimetry on this sample of sections was done either manually with the aid of a graphic tablet, or by means of digital analysis of video images. The reader should note that assessing the distribution of mossy fibers gives reliable results for a given technique. However, changes of the morphometrical techniques (for example using differential point lattices, or differential section thickness) can yield different absolute values. A comparison of computer-assisted and manual analysis (by means of drawings and graphic tablet) showed that the manual methods consistently overestimated the IIP-MF projection, whereas computer-assisted analysis gave consistently lower values, probably because of neglecting the distribution of single clusters of Timm-stainable boutons. Therefore, we prefer to give no absolute values for the areas covered by mossy fibers, but use percentages (extent of IIP-MF as percentage of the entire regio inferior, or of the suprapyramidal layer). While the mossy fiber distribution is assessed only from a portion of the hippocampus, comparable strain difference of the IIP-MF projection have been found at septal (rostral) levels of the hippocampus[99].

Two-way avoidance

The behavioral apparatuses used were standard, two-chambered shuttleboxes using light as the conditioning stimulus[10,28]. After the presentation of a warning stimulus, the animals had to cross through a door to the dark side of the shuttlebox in order to avoid an electrical shock. Between trials, the animals could move with impunity between the compartments. The rats were always tested for the number of trials needed in order to attain a criterion of 4 consecutive correct avoidance responses. This criterion had served for the selection of genetically superior or inferior avoidance learners[28]. Mice were always tested over a period of 5 days (about 80 trials per day), since performance at the end of training had been the variable studied in previous behavior genetical experiments.

Statistical analysis

In this review, statistical results are presented mainly as bivariate correlations and scatterplots. The original results are based on multiple regression analysis and other multivariate methods, usually including all other hippocampal subfields of CA3 as well. Scatterplots are usually presented with standardized variables, in which the mean is given as zero, and the individual data points are scaled in units of one standard deviation from the mean[31,127].

Hippocampal mossy fibers and two-way avoidance

Selective breeding for two-way avoidance and its effects upon hippocampal morphology

The most extensively investigated rat strains, selectively bred for differential two-way avoidance acquisition, are the Roman High- and Low avoidance rats (RHA and RLA). Initially developed by Bignami and Bovet at the Istituto Superiore di Sanità in Rome[6], these two strains have been maintained since in several laboratories. The animals used by us were the Swiss sublines, RHA/Verh and RLA/Verh[27,28]. In the original investigation, a sampling of these animals from the 45th generation was used to test whether such selective breeding might have entailed any differentiation of the hippocampal terminal fields as visualized by Timm's stain. The results showed that the hippocampi of the two lines were different in several aspects. The regio inferior of RHA/Verh rats had proportionately larger stratum lacunosum-moleculare and stratum oriens while, in RLA/Verh rats, the three mossy fiber fields appeared to be enlarged[98,99]. Thus, the morphology of regio inferior showed genetic variation which was sensitive to selective breeding.

The presence of differences in a given brain system of psychogenetically selected animals must not necessarily be taken as support for a hypothesis that this brain system is involved in the modulation of the behavior selected for. Due to the relatively small number of animals in selective breeding studies, a corollary selection of traits is quite likely. The most important single factor is genetic drift, that is, unwanted homozygosity[103,126]. This can develop if the majority of the mated animals at a given stage of selection carries, by chance, the same allele, e.g. albinism. Once white has become the dominating coat color, that line will remain white irrespective of the behavioral selection procedure[24]. Other forms of correlative selection (linkage disequilibrium) may also play a

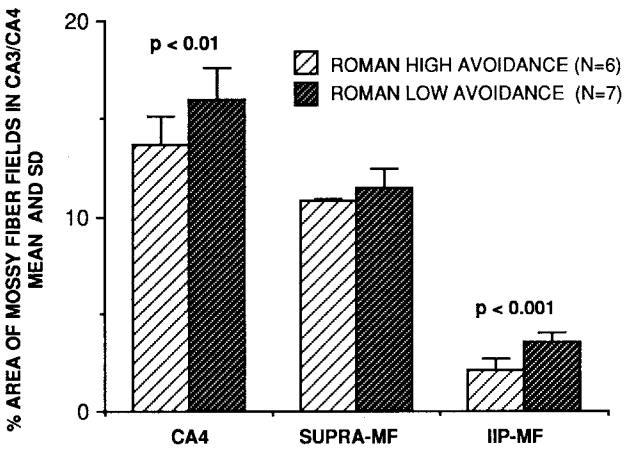

Figure 3. Morphological differentiation of the relative sizes of hippocampal mossy fiber fields as a result of selective breeding for good and poor two-way avoidance acquisition in rats. Note that low-avoidance rats have a significantly larger mossy fiber field in the hilus of the fascia dentata, as well as a larger intra/infrapyramidal mossy fiber projection. Other synaptic terminal fields in CA3/CA4 were different also (see text). Abbreviations: CA4, hilus of the fascia dentata; IIP-MF, infra- and intrapyramidal mossy fiber projection; SUPRA-MF, suprapyramidal mossy fiber projection.

role. The practical conclusion is that some of the observed differences between such selectively-bred lines are irrelevant for behavior. Thus, the manifold differences that have been observed between the Roman lines[17,22,25,28,40,41,43] certainly reflect a diversity of genetically variable factors affecting two-way avoidance learning, but it is difficult to distinguish between behaviorally relevant and irrelevant differences. This requires either replication of selective breeding (which is impractical), or further delineation of important variables.

Testing the robustness of identified associations

a) Genetic correlations using inbred strains. The most convenient way of verifying an association of a brain trait with behavioral performance is the investigation of inbred mouse strains. There is a fairly large body of documented genetic differences in brain or behavior[104–108], and many strains are offered commercially. While strain differences cannot always be replicated across laboratories, presumably due to technical variations or, perhaps, subline differentiation, some mouse strains are known for consistent behavioral peculiarities. Hence, available strains can either be screened for their behavioral performance, and differentially behaving strains can then be further investigated for brain traits, or vice versa.

Two-way avoidance performance had been established[10] for five widely used mouse strains (DBA/2, C3H/He, BALB/c, C57BL/6, and NMRI, the latter partially inbred). The mean performance of the strains ICR and SM/J, the latter being known for large IIP-MF projections[4], was assessed as well. Afterwards, strain means and variances were established for every subfield in CA3, and correlations were calculated between all hippocampal variables and avoidance performance at training day 5. This revealed a strong and negative correlation between the extent of the IIP-MF projection and two-way avoidance ($r = -0.97$, fig. 4a). None of the other hippocampal subfields showed a significant correlation[99]. Therefore, the only variable which showed a correlation in both rats and mice was the extent of the IIP-MF projection which, thereafter, moved into our focus of attention.

This particular study underlined the convenience of using inbred strains for verifying correlations. Technically, this type of correlation corresponds grosso modo to a genetic correlation, since it was computed between the strain means. In ideal cases, intrastrain variability is small, with the strain means barely overlapping, and the within-strain correlations (environmental correlations) show the same sign as the between-strain correlation. Such a result has been observed in a replication of the strain study in which the performance of the mice was individually assessed prior to morphometry[70] (fig. 4b). The use of inbred strains for computing genetic correlations calls for some statistical caveats, however[50]. In most cases, genetic correlations are based on relatively small samples of strains. Without further evidence, the observed presence or absence of correlations must be interpreted cautiously. The other problem is biased (non-randomly drawn) samples. The choice of strains is often restricted by practical considerations such as availability, and known behavioral and neuroanatomical peculiarities of some strains may lead to their unwarranted exclusion from, or inclusion in, a study.

b) Individual correlations observed in a random-bred stock. In randomly bred stocks, genetic heterogeneity is maintained by means of controlled outbreeding schedules with fairly large numbers of animals. In such stocks, one may expect considerable individual variability of traits known to be genetically dependent. The important aspect is that the genetical variability of brain traits be randomized across animals, except for cases of strong linkage disequilibrium. It is thus possible to test the robustness of an observed correlation, because the random distribution of brain traits potentially affecting the same behavior is likely to produce more behavioral 'noise'[95]. For example, individual and genetically caused differences in the content of hypothalamic octopamine may affect two-way avoidance performance[25] as well as variations of the mossy fiber projection. Yet, if these two genetic factors are not tightly linked, a sample of random-bred animals will contain animals with large IIP-MF projections and differential octopamine levels. This may introduce behavioral variation in animals with similar mossy fiber fields. In statistical terms, this means more error and, hence, weaker correlations. If the correlations disappear completely, one may assume that the correlation tested is not robust and can be masked by

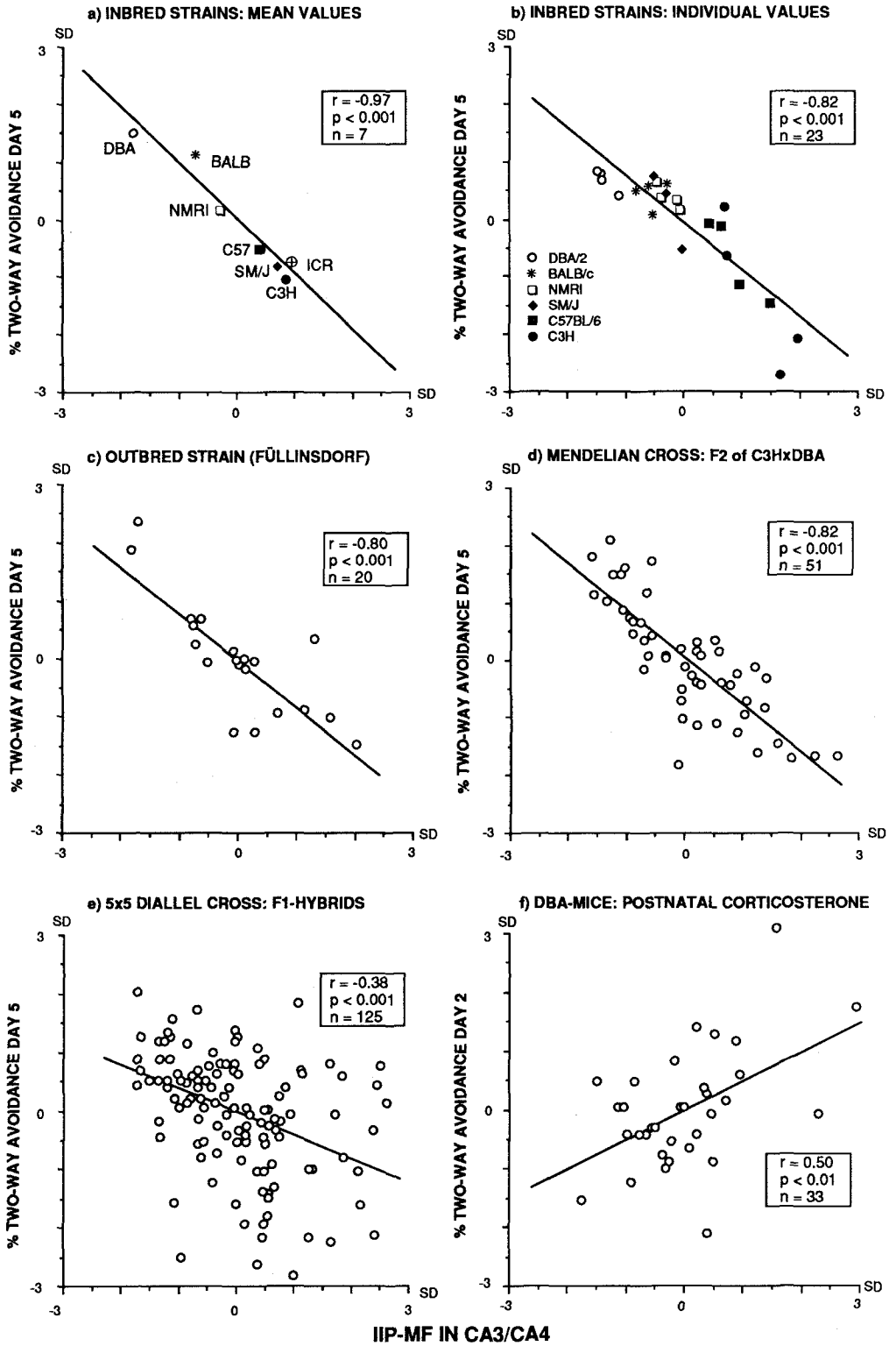

Figure 4. Standardized regression plots of the extent of the IIP-MF and performance of avoidance learning in several samples of mice. Note that the scaling cannot be compared between samples, since it is based on the means and standard deviations of each sample. *a* Negative correlation shown by the mean values of several mouse strains; *b* Negative correlation shown by individual mice from 6 different mouse strains (not the same animals as in 4a); *c* Negative correlation between the individual values of IIP-MF and avoidance learning in outbred mice (Füllinsdorf Albino); *d* Genetically randomized IIP-MF projection (by means of an F_2-cross) and negative correlation with adult avoidance learning; *e* Genetically varied IIP-MF projection (by means of a 5 × 5 diallel-cross) and adult avoidance learning; *f* Developmentally induced variation of the IIP-MF in isogenic animals (DBA/2 mice) and adult avoidance learning after postnatal injections of corticosterone and saline. This is the only positive correlation observed thus far, and it shows that the influence of mossy fibers on two-way avoidance is not unconditionally negative but context-specific. Perhaps due to the postnatal corticosterone injections, the overall performance of all these DBA/2 mice was fairly poor. For further explanations, see text.

unknown factors. If it persists, the brain variable must be robust and have considerable functional strength.
Twenty mice from an outbred stock, Albino Füllinsdorf, were tested for the robustness of the association between the IIP-MF projection and two-way avoidance[99,101]. As shown by figure 4c, a strong negative correlation was again observed (r = 0.82). This implied a surprising robustness and possibly a causal role for a brain factor intimately related to the extent of the IIP-MF projection.

Other correlative studies

The association between a large IIP-MF projection and poor avoidance learning has been corroborated by correlative findings in other rat lines and mouse strains.

a) Rats selectively bred for self-stimulation. Lieblich had established 4 rat lines selectively bred for their propensity, or not, to self-stimulate the lateral hypothalamus by means of implanted electrodes[61]. In order to test whether selective breeding for self-stimulation would alter hippocampal morphology, the hippocampi of 8 rats per line were analyzed morphometrically[62]. Since a genetic correlation between the propensity for self-stimulation and avoidance learning had already been observed[64], the animals in question were tested for acquisition of two-way avoidance, as well. The IIP-MF projection did not show a response to selective breeding for self-stimulation, the stratum oriens was enlarged in both the LC1-HI and the LC2-HI lines and both LC-2 lines had much larger IIP-MF projections than the two LC-1 lines, probably because of pronounced differences in the mossy fiber projections of the parental stocks[62]. The difference in the mossy fiber projection was reflected in the level of two-way avoidance acquisition, with the LC2-lines performing more poorly. Taken together, the results implied first, a difference in avoidance behavior associated with IIP-MF differences between the progenitor stocks, and second, a selection-dependent difference associated with differential self-stimulation, the strong self-stimulators showing superior-performance.

b) Effects of selective breeding for differential neuromuscular thresholds and differential avoidance behavior. Dimitrieva et al.[26] investigated the hippocampi of four rat lines, two lines being selectively bred for differential two-way avoidance behavior, and two lines for differential thresholds of leg muscle contraction following peripheral electrical stimulation. High- and low-avoidance rats showed a mossy fiber distribution similar to the one observed in the RHA/Verh and RLA/Verh rats, with poor avoiders having larger IIP-MF projections. Rats with high and low neuromuscular excitability differed in two-way avoidance, sensitive rats being superior, but no differentiation of hippocampal mossy fibers was observed. These findings corroborate the notion that two-way avoidance has multiple determinants, some of which must reside in the peripheral nervous system. On the other hand, selective breeding for neuromuscular excitability (a peripheral trait) apparently leaves the morphology of the mossy fibers unaltered. This is a strong argument in favor of site-specific effects of selective breeding. However, it must be noticed that both high- and low-excitable rats had fairly large infrapyramidal mossy fiber fields. Hence, extended IIP-MF projections may be compatible with superior avoidance learning capacities rooted in sensorimotor differences.

c) Mossy fiber projections in mice with differential avoidance learning capabilities. Gozzo and Ammassari-Teule[42] have described differences in the lamination of infrapyramidal mossy fibers in two inbred strains of mice. SEC mice had a fairly short and well-laminated IIP-MF projection which, in C57BL/6 mice, was more extended, frequently invading in its distal parts the pyramidal cell layer. The authors hypothesized that the behavioral difference between the strains (among other things a superior avoidance performance of the SEC mice[88]) might be a function of the orderliness of lamination. While this proposal has never been explicitly tested, it is somewhat contradicted by the relatively good avoidance performance shown by our BALB/c mice, a strain with a clear lamination defect of the hippocampal mossy fiber projection[86].

Testing for causality

While causality is rarely approached in an explicit fashion in biological experiments, criteria are available according to which 'correlational' studies (as those described above) may be differentiated from 'causative' studies. One criterion, manipulability, is psychological, and reflects an almost irresistible penchant of establishing causal relationships through personal interference. Experimentally, this criterion leads to the somewhat illusionary claim of controlling the biological systems under study: a brain variable is said to be under the direct control of the experimenter (usually through physical interference), whereas the other variables are thought to be held constant, or being controlled otherwise (neglected by necessity is perhaps a more appropriate term). While such a conceptual view is clearly mechanistic, it ironically relies, for confirmation, upon statistical techniques. Yet, numerical analysis is purely correlational, and the underlying approach probabilistic. This paradox, however, is rarely perceived by proponents of, in their terminology, causal approaches. They precisely use the same correlational tools but prefer to overlook their conceptual implications. The only valid criterion for distinguishing between biological causality and correlation is statistical: the difference between regression and correlation. In the case of a regression, the interactions between the two variables are not bidirectional, there being

an independent, and a dependent, variable. In correlations, the variables may interact equivalently.

In analyzing brain-behavior relationships, it is possible to distinguish quite clearly between independent and dependent variables. In many cases, a brain trait is the independent, and behavior the dependent, variable. If a brain trait differentiates long before behavioral actions are possible, it cannot be a feedback function of behavior, and if a brain trait is genetically-dependent, it must have some non-plastic properties which make it recognizable as a genetic variation. This is the case for the topographical distribution of mossy fibers in the rodent regio inferior. During their development, but not afterwards, they show a sensitive period during which their distribution can be substantially altered by physical, or hormonal, interference[16, 59, 83, 119]. Slow changes during adulthood may occur[12, 109], but the simple fact of strain-typical mossy fiber distribution at various ages[100] shows that late changes are, at the most, limited. It should be noted, however, that the recurrent mossy fiber projection to the dentate gyrus is quite a different matter[11, 36, 65, 94, 110, 118].

In testing for a causal role for infrapyramidal mossy fibers, we intend to show only that a brain variable which is intimately and spatially associated with the IIP-MF must be of functional relevance, and not some remote system outside of the hippocampus. This hypothesis of a spurious correlation has two main counter-arguments which must be approached experimentally, those being that: 1) the observed relationship between IIP-MF and two-way avoidance may be based on some strange form of linkage disequilibrium, i.e. tightly linked genetic factors, and 2) there is an epigenetic linkage, i.e. the differential expression of infrapyramidal mossy fibers unconditionally produces a change somewhere else in the brain which is ultimately responsible for the observed behavioral variations. Satisfactorily explaining this requires the following experimental steps:

Meiotic randomization of hippocampal morphology

The inbred mouse strains DBA/2 and C3H/He show extreme differences of the IIP-MF projection (fig. 2) and, correspondingly, in two-way avoidance behavior, although there appears to be considerable subline variation[13, 52]. The poor avoidance behavior of C3H mice may be partly due to retinal degeneration[84, 115], although this strain also behaves poorly with acoustic conditioning stimuli. Yet, if variations of hippocampal circuitry were an important determinant of two-way avoidance performance, in our set-up used for behavior-genetical studies, then individual differences of the IIP-MF projection observed in F2-generations of Mendelian crosses between these strains should correlate with adult avoidance performance. In formal terms, the extent of the IIP-MF projection is randomized by means of chromosomal recombination during meiosis. The morphological trait then serves as an independent variable onto which behavioral differences observed in adulthood are regressed. Thus, the approach fulfills the operational criteria of a causal relationship as described above, as in regression analysis the independent variable must not be under direct control of the experimentator. Such a regression is called a regression of type II, and differs from a regression of type I (parametric control of the independent variable) only slightly, in some assumptions about the underlying model[127].

In order to test the prediction, 51 animals from a Mendelian cross between the strains DBA/2 and C3H were tested for their avoidance performance at day 5[51]. Morphometry of the mossy fiber distribution in the adult mice revealed a strong and negative correlation with two-way avoidance (fig. 4d). Yet there was no indication of a monogenic inheritance of the IIP-MF distribution. Thus, a factor strongly associated with the IIP-MF distribution must play a causal role in the modulation of avoidance performance.

The power of this simple experiment was that a simple manipulation, cross-breeding, resulted in individual variations of the IIP-MF distribution without having to damage the brain. We are not aware of any other manipulative procedure which operates under so completely physiological conditions. Moreover, the effects of other genetically variable factors influencing two-way avoidance have been randomized. Statistically, this is an almost ideal situation. Yet, it must be kept in mind that the DBA/2 and C3H strains both share a common ancestry[82]. This does not argue against a causal relationship but, if both strains are genetically fairly similar, randomization is restricted to the few remaining genetic differences. This may lead to an overestimation of the functional impact of mossy fiber variations.

Meiotic control of the IIP-MF distribution

In a 5×5 diallel cross, five inbred strains were crossed so as to produce 25 offspring populations in which each carried a different, yet controlled, proportion of the genetic variation found in the parental strains. This breeding design is commonly used to analyze hereditary and environmental contributions to the phenotypic expression of a particular trait. Based on the strong differences of the IIP-MF distribution in the parental strains, one would expect considerable mossy fiber differences among the 25 hybrid samples. Differences between the hybrid means can be attributed to genetic factors, while the within-sample variability provides a measure of environmental influences on mossy fiber variations. From a practical point of view, this cross-breeding scheme permits a reproducible animal sample in which the extent of the IIP-MF distribution is, at least to some degree, under the control of the experimenter, and in which the potential interference from other genetic factors is reproducibly balanced. Furthermore, this approach permits the application of sophisticated statistical models[18].

Crusio and Schwegler have tested the relationship between IIP-MF distribution and open-field behavior in a 5 × 5 diallel cross involving the strains BA, DBA/2, BALB/c, C57BL/6, and C57BR[18]. The same animals have been tested for two-way avoidance as well (unpublished data). Variations in the extent of the IIP-MF projection in this sample of mice were still negatively correlated with two-way avoidance performance, albeit much weaker (fig. 4e). Thus, the predictions of causality were confirmed again. The weaker correlations indicated a comparatively stronger influence of 'non-mossy-fiber factors' on two-way avoidance. These may have had genetic origins, perhaps reflecting a masking influence of hybrid vigor, or heterosis. For example, strong locomotor activity in the shuttle-box is a factor facilitating two-way avoidance. Most inbred strains are not particularly active in this situation, but many hybrids are, and this may induce an additional behavioral variation unrelated to the mossy fiber distribution.

Another surprising finding in this study was that the genetical correlations between IIP-MF and avoidance (using the mean values of the samples) were significant in the early phases of conditioning, but not afterwards. Correlations based on variability within samples (environmental correlations) were not evident at the begin of conditioning but were significant (about the same coefficient) at day 5. In other words, the genetic architecture of the IIP-MF explained the behavior of the mice in the early phases of conditioning, while the variability of mossy fibers within a given sample appeared to predict the relative changes in behavior during later conditioning, with mice having relatively more IIP-MF being more likely to show poor avoidance performance at the end.

'Chimeric mixing' of the mossy fiber distribution

Mouse chimeras can be produced by merging blastocysts from strains with different behavioral and neuroanatomical traits[85]. Thus, individual variations found in such animal samples are based on a special form of somatic, instead of genetic, randomization of traits. The relation between IIP-MF distribution and two-way avoidance behavior in 35 chimeric mice (C57BL/6 and BALB/C) has been studied by Bär (unpublished results). She found, once again, a negative correlation. It was, however, weak, and significant only for the second day of training (perhaps due also to a temporary technical problem with the shuttle-boxes). As no study with F2-animals from Mendelian crosses between the two strains has been made, it is not clear whether the weak correlation is a result of chimerism itself, or whether crosses between the strains would yield identical results. Further studies may clarify this point.

Thyroxine-induced variability of the IIP-MF and adult two-way avoidance

The distribution of infrapyramidal mossy fibers is sensitive to developmental interference. Lauder and Mugnaini[58] have shown that a transient postnatal hyperthyroidism expands the infrapyramidal mossy fiber field, and possibly the recurrent collaterals to the fascia dentata as well[94]. Thus, developmental expansion of the infrapyramidal mossy fiber projection in strains showing good avoidance acquisition and few IIP-MF should lead to a reduction in avoidance, depending upon the increase in mossy fiber terminals.

In an initial study, 51 RHA/Verh rats were injected with varying doses of thyroxine during the first three postnatal weeks[71]. 21 control animals received injections of saline. The animals were then tested at the age of 90 days for acquisition of two-way avoidance. Somewhat surprisingly, the treated animals showed only a slightly impaired avoidance learning as compared to the controls. Within the control animals, however, some animals had strain-atypical poor avoidance scores. This riddle was solved by morphometry. The variability of the avoidance scores, i.e., the deviations from the strain-typical superior avoidance performance, was strongly related to the extent of the IIP-MF projection: the larger it was, the more trials the rats took to reach a criterion of 4 correct responses. This was not only the case in the thyroxine-treated animals but, unexpectedly, also in the saline controls: the poor avoiders in this group had the largest IIP-MF projections[74].

The study was replicated using mouse strains[74]. 18 BALB/c mice served as pilot animals, and a total of 75 DBA/2 mice were investigated in the main study. Both strains are known for scanty IIP-MF distribution and superior avoidance performance. In this experiment, we tried to evaluate the influence of different dosages of thyroxine more precisely than in the rats. The results largely confirmed the findings of the rat study. In both the BALB/c mice and the thyroxine-treated DBA/2 mice, a negative correlation was found between the extent of the IIP-MF projection and avoidance performance on day 5 (DBA/2: $r = -0.75$, $p < 0.001$, $n = 75$; BALB/c: $r = -0.77$, $p < 0.01$, $n = 18$).

An analysis of the dosage effects showed that differential doses of thyroxine had not much influence on the adult distribution of the mossy fibers, although high doses had a tendency to yield larger projections. The prominent effect was a net increase of the IIP-MF, but there was considerable individual variability in response to the treatment. This was probably not due to technical problems involving the subcutaneous injections, as brain weight showed a clear-cut dose-dependency, with higher doses leading to smaller brains. In terms of correlations, higher doses of postnatal thyroxine led to progressively poorer correlations: the best within-dosage correlation was -0.90 in the group receiving the lowest doses, while the correlation in the highest dosage group dropped to a coefficient of -0.58 (fig. 5). Interestingly, the within-group variability of the untreated mice was also negatively correlated with two-way avoidance, albeit non-significantly. A surprisingly strong correlation ($r = -0.95$)

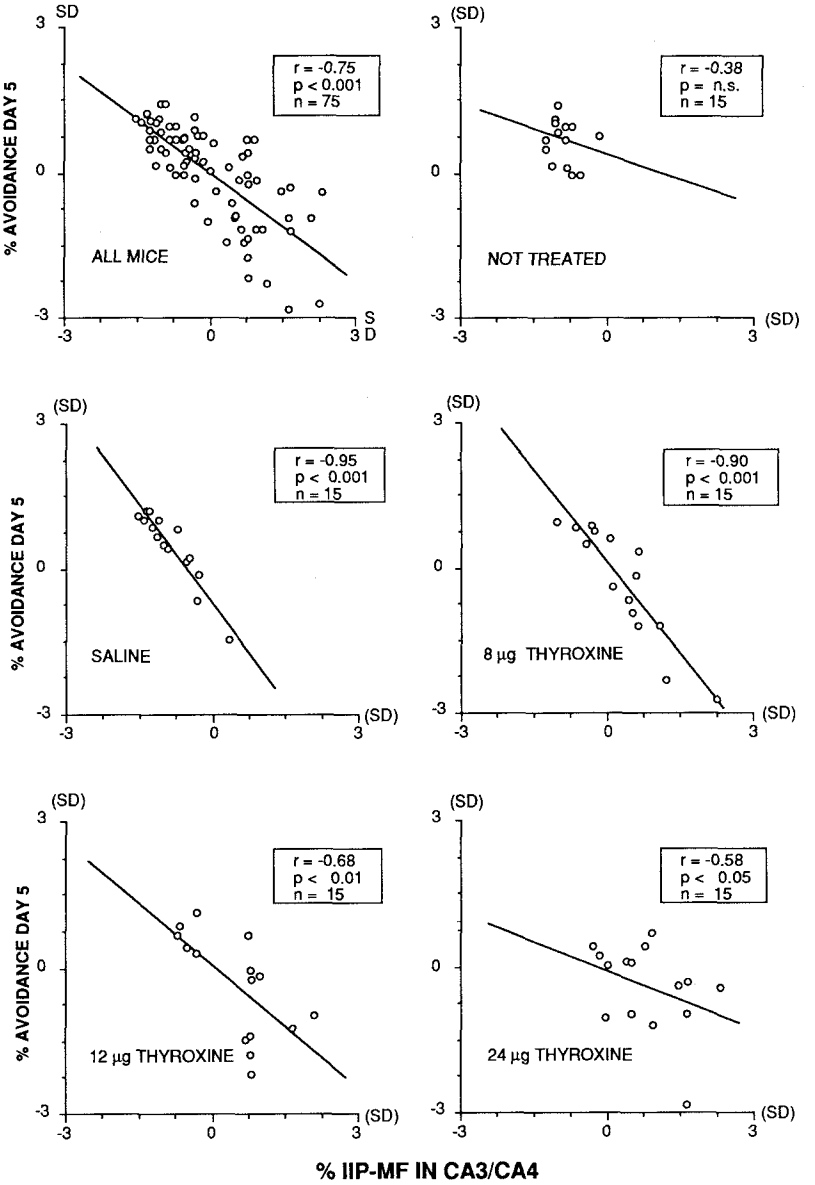

Figure 5. Thyroxine- and saline-induced variability of the IIP-MF projection (by means of postnatal injections) and adult two-way avoidance performance of DBA/2 mice. The standardized plot with all mice gives the scaling for the remaining subsamples in which the position of the mice from the various treatment groups has been extracted. These subsamples thus show the within-treatment variability with respect to the entire sample.

was found in the mice treated with saline alone (fig. 5). In this experiment, postnatal thyroxine levels did not precisely determine the extent of the IIP-MF projection. Rather, the treatment resulted in a predictable randomization of the IIP-MF projection: given a constant dosage, there was always considerable individual variation. In contrast to the crossbreeding studies, however, this randomization occurred in a constant genotype, the structural and behavioral covariations being produced only by the non-specific effects of thyroxine. Since the treated mouse strains were inbred, the mossy fibers were varied while the genetic architecture remained unaltered. In physiological terms, this corresponds to a ceteris paribus situation, which permits recognition of even the most subtle behavioral effects of a brain trait, a concept proposed by Wahlsten [116, 117].

These results totally eliminate the hypothesis of a linkage disequilibrium underlying a spurious correlation between mossy fiber variations and behavior. They do not eliminate the hypothesis of an 'epigenetical balance', but they weaken it considerably: the data showed that the variations in mossy fibers were strongly correlated with behavior irrespective of the procedure which caused the variations. Thus, the hypothetical system in balance with the mossy fibers must either share the same developmental timing properties and biochemical sensitivities, or be a perfect covariate. If this were not the case, it would be impossible to observe quasi-linear relationships, for a

marker system cannot yield better correlations than the true system. Also, there is not the slightest evidence for an extrahippocampal system being a perfect covariate of individual differences in mossy fiber distribution.

Effects of postnatal corticosterone treatment

Whereas postnatal corticosterone treatment did not produce visible differences in the IIP-MF projection of DBA/2 mice, it yielded considerable variability and bilateral asymmetries of the mossy fiber distribution, suggesting a developmental disturbance of the hippocampus which might be related to changes in the hippocampal distribution of corticosterone receptors. In any case, a surprising result was that a positive correlation of the IIP-MF with two-way avoidance performance on the second day of training was seen (fig. 4f), and variation of the suprapyramidal mossy fiber projection was positively correlated with avoidance performance at five days of training[125].

Correlations with other hippocampus-dependent behaviors

IIP-MF, open-field behavior and exploratory activities

In mice, selective breeding for differential open-field behavior entails a differentiation of the IIP-MF projection. This has been observed in lines selectively-bred for rearing[20], and in the Boulder lines selectively bred for high and low locomotor activity[24,68]. Also, Crusio and Schwegler found a weak covariation between habituation and the extent of the IIP-MF projection using a 5×5 diallel cross design[19]. In contrast, the Naples rat lines, bred for differential activity in a Làt maze (essentially a square alley)[96], did not show consistent differences in their IIP-MF projections[73]. The relations between IIP-MF and various open-field measures will be evaluated in a forthcoming review by Crusio et al.

IIP-MF and radial maze performance

Radial maze performance is severely hampered by hippocampal lesions[45,91–93], although it is quite sensitive to the destruction of other brain systems, as well[44,123]. By studying a sample of mouse strains known for differential IIP-MF distributions, Crusio et al. found a strong and *negative* genetic correlation with a simple measure of performance errors (number of re-entries into an arm previously visited[21]).

IIP-MF and swimming navigation

A recently-developed task for assessing hippocampal functions is swimming navigation, as introduced by Morris[80,81]. Thus far, at least two studies have revealed positive correlations between the IIP-MF and behavioral measures of swimming navigation in mice[75,124]. Another study, using a conventional water maze and factor analytical procedures, found that the IIP-MF projection of mice correlated negatively with swimming time, the mice with a large IIP-MF projection requiring more time to escape from the water[97]. The size of the IIP-MF projection, however, also showed a second loading on the number of errors at turning points, the animals making less errors having more IIP-MF.

IIP-MF and 'non-hippocampal' tasks

According to theories envisioning the hippocampus as a structure which encodes spatial relationships, there ought to be behaviors for whose execution the hippocampus is not crucial[87]. One of these behaviors is discrimination learning, reportedly insensitive to hippocampal lesions. However, by testing mice in which the distribution of mossy fibers was varied by means of systematic crossbreeding ('meiotic randomization'), we found strong positive correlations with the individual capacity for (aversively-motivated) discrimination learning in a Y-maze[72]. Here, the extent of the IIP-MF covaried positively with a non-spatial strategy. Yet, in another study we found a positive genetic correlation between side-perserverance and IIP-MF[35,70], a covariation with a spatial strategy. To complicate matters, a possible correlation between IIP-MF and cerebral lateralization has been found as well. Mice selectively bred for strong or weak paw preference[14,15] in a food retrieval task show clear differences in their IIP-MF distribution[49]. Whether this behavior is a true measure of hemispheric specialization is not yet clear. It is, however, neither learned nor does it require spatial processing.

IIP-MF and two-way avoidance behavior: discussion

These studies demonstrate that the extent of infrapyramidal mossy fibers in the rodent hippocampus, or of a strongly associated intrahippocampal variable (structural and/or biochemical), must influence processes important for the acquisition and performance of two-way, active avoidance. The correlation is expressed most strongly when the influence of other genetic factors is removed, as was the case in crosses between genetically similar strains (e.g., the F2-generation from DBA/$2 \times$C3H), or in the studies based on within-strain variations. Nevertheless, a negative influence of the IIP-MF factor on two-way avoidance performance has been observed in a variety of other studies as well. Hence, it must have appreciable functional strength, as the number of factors known to influence two-way avoidance behavior is large[69].

With which of the possible psychological factors might the IIP-MF be involved? Variations of the mossy fibers are probably not related to a genuine learning capacity, since the majority of the correlations were based on performance variations in the later stages of conditioning. The frequently observed correlations in other behavioral paradigms show that the mossy fiber effect is not restricted to avoidance learning and its associated processes

such as emotionality, sensitivity to shock and other factors associated with punishment. Furthermore, the correlations are difficult to explain in terms of memory processes. The explanatory constructs which would fit a majority of the results are that the IIP-MF may correlate with a) behavioral predictability, b) spatial processing, or c) the capacity of solving conflicting or difficult tasks. In each case there are data sets, however, which contradict a unitary hypothesis [67, 70, 73].

Prospects and limitations of 'microphrenology'

The aim of these studies was to demonstrate that the old phrenological approach of correlating behavioral with structural variation is still valid. Its modern form, 'microphrenology' is, at least in some aspects, superior to crudely manipulative techniques. This superiority is evident on two points: the procedure is noninvasive, and it identifies the natural regulators of behavior.

Most approaches in research on brain and behavior try to link a structure or system with psychologically defined processes such as memory, learning, emotionality, or spatial behavior. In contrast, microphrenology is rather empirical. Before going any further, it tries to identify a portion of the brain in which a given behavior is biased by variations of that structure. The nature of the behavior and the location of the brain system are not relevant at that stage. In the present case, they happened to be two-way avoidance and the hippocampus, not for theoretical reasons, but simply because genetic variation has been documented for both. Some luck was involved because the first structural variation studied, the infrapyramidal mossy fiber projection, proved to be rewarding. It would appear that an economical approach to behavioral brain research (and the one requiring a minimum of luck) would be a systematic screening of the brain for systems showing strong genetic variation. This would pinpoint the loci and system components which are used by evolutionary processes to ensure the behavioral variation 'necessary' for natural selection. Replicated selective breeding for any behavior of interest will eventually disclose those systems whose variability is of prime importance for that behavior.

By taking such a correlational approach, one can identify the natural regulators of behavior, but the underlying processes must be elucidated by other means. With regard to two-way avoidance, we can only speculate as to how mossy fiber variations are translated into behavior. Nevertheless, there is now overwhelming evidence that a fairly specific variation in this part of the rodent hippocampus must have profound physiological consequences: a target has been singled out for investigation. Contemporary brain research has amassed an impressive battery of analytical techniques, all purportedly developed for elucidating the workings of the brain. However, there is no corresponding sophistication in formulating experimental questions for employing these tools for the analysis of brain and behavior. Not surprisingly, the ultimate function of the brain – the orchestration of behavior – is still poorly understood. The 'microphrenological' approach cannot solve the problem by itself, but it should prove to be a most effective tool in selecting the relevant variables to be considered for brain and behavior research.

Acknowledgments. The studies reviewed here were supported by the Swiss National Foundation for Scientific Research (NF 3.041-0.84, 3.286-0.88) and the Julius-Klaus-Stiftung für Genetik (to H.P.L), by the Deutsche Forschungsgemeinschaft (to H.S.) and by a NATO Science Fellowship (from the Netherlands Organization of Pure Research, ZWO) and an Alexander-von-Humboldt stipend (to W.E.C).

* Address for correspondence: Dr. H.-P. Lipp, Institute of Anatomy, University of Zürich-Irchel, Winterthurerstrasse 190, CH-8057 Zürich, Switzerland.

1 Altman, J., and Das, G. D., Postnatal neurogenesis in the guinea pig. Nature *214* (1967) 1098–1101.
2 Amaral, D. G., A Golgi study of cell types in the hilar region of the hippocampus in the rat. J. comp. Neurol. *182* (1978) 851–914.
3 Amaral, D. G., and Dent, J. A., Development of the mossy fibers of the dentate gyrus: I. A light and electron microscopic study of the mossy fibers and their expansions. J. comp. Neurol. *195* (1981) 51–86.
4 Barber, R. P., Vaughn, J. E., Wimer, R. E., and Wimer, C. C., Genetically-associated variations in the distribution of dentate granule cell synapses upon the pyramidal cell dendrites in mouse hippocampus. J. comp. Neurol. *156* (1974) 417–434.
5 Bayer, S. A., Yacke, S. W., and Puri, P. S., Neurons in the rat dentate gyrus granular layer substantially increase during juvenile and adult life. Science *216* (1982) 890–892.
6 Bignami, G., and Bovet, D., Experience de sélection par rapport à une réaction conditionnée d'évitement chez le rat. C. r. Acad. Sci. (Paris) *260* (1965) 1239–1244.
7 Bignami, G., and Michalek, H., Cholinergic mechanisms and aversively motivated behavior, in: The Psychopharmacology of Aversively Motivated Behavior, pp. 173–255. Eds H. Anisman and G. Bignami. Plenum Press, New York 1978.
8 Bovet, D., Bovet-Nitti, F., and Oliverio, A., Genetic aspects of memory and learning in mice. Science *163* (1969) 139–149.
9 Broadhurst, P. L., Experiments in psychogenetics: applications of biometrical genetics for the inheritance of behavior, in: Experiments in Personality, vol. 1, Psychogenetics and Psychopharmacology, pp. 1–102. Ed. H. J. Eysenck. Routledge and Kegan, London 1960.
10 Buselmaier, W., Vierling, T., Balzereit, W., and Schwegler, H., Genetic analysis of avoidance learning by means of different psychological testing systems with inbred mice as model organisms. Psychol. Res. *43* (1981) 317–333.
11 Cassell, M. D., and Brown, M. W., The distribution of Timm's stain in the nonsulphide-perfused human hippocampal formation. J. comp. Neurol. *222* (1984) 461–471.
12 Coleman, P. D., Flood, D. G., and West, M. J., Volumes of the components of the hippocampus in the aging F344 rat. J. comp. Neurol. *266* (1987) 300–306.
13 Collins, R. L., Inheritance of avoidance conditioning in mice: a diallel study. Science *143* (1964) 1188–1190.
14 Collins, R. L., Selective breeding for the degree of functional lateralization in mice. Behav. Genet. *9* (1979) 443–444.
15 Collins, R. L., On the inheritance of direction and degree of asymmetry, in: Cerebral Lateralization in Nonhuman Species, pp. 41–71. Ed. S. D. Glick. Academic Press, New York 1985.
16 Cook, T. M., and Crutcher, K. A., Extensive target cell loss during development results in mossy fibers in the regio superior (CA1) of the rat hippocampal formation. Brain Res. *353* (1985) 19–30.
17 Cooper, S. J., and Webb, Z. M., Microstructural analysis of chlordiazepoxide's effects on food preference behavior in roman high-, control- and low-avoidance rats. Physiol. Behav. *32* (1984) 581–588.
18 Crusio, W. E., Genthner-Grimm, G., and Schwegler, H., A quantitative-genetic analysis of hippocampal variation in the mouse. J. Neurogenet. *3* (1986) 203–214.

19 Crusio, W. E., and Schwegler, H., Hippocampal mossy fiber distribution covaries with open-field habituation in the mouse. Behav. Brain Res. *26* (1987) 153–158.

20 Crusio, W. E., Schwegler, H., Brust, M., and Van Abeelen, J. H. F., Genetic selection for novelty-induced rearing behavior in mice produces changes in hippocampal mossy fiber distribution. J. Neurogenet. *5* (1989) 87–93.

21 Crusio, W. E., Schwegler, H., and Lipp, H.-P., Radial-maze performance and structural variation of the hippocampus in mice: a correlation with mossy fibre distribution. Brain Res. *425* (1987) 182–185.

22 D'Angio, M., Serrano, A., Driscoll, P., and Scatton, B., Stressful environmental stimuli increase extracellular DOPAC-3 levels in the prefrontal cortex of hypoemotional (Roman High Avoidance) but not hyperemotional (Roman Low Avoidance) rats. An in vivo voltammetric study. Brain Res. *451* (1988) 237–247.

23 Danscher, G., Histochemical demonstration of heavy metals. Histochemistry *70* (1981) 1–16.

24 DeFries, J. C., Gervais, M. C., and Thomas, E. A., Response to 30 generations of selection for open-field activity in laboratory mice. Behav. Genet. *8* (1978) 3–13.

25 Delacour, J., Coulon, J. F., David, J. C., and Guénaire, C., Brain octopamine and strain differences in avoidance behavior. Brain Res. *288* (1983) 169–176.

26 Dimitrieva, N. I., Gozzo, S., Dimitriev, Y., and Ammassari-Teule, M., Mossy fiber distribution in four lines of rats: a correlative study with avoidance abilities and excitability thresholds. Physiol. Psychol. *12* (1984) 30–34.

27 Driscoll, P., Roman high- and low-avoidance rats: present status of the Swiss sublines, RHA/Verh and RLA/Verh, and effects of amphetamine on shuttle-box performance. Behav. Genet. *16* (1986) 355–364.

28 Driscoll, P., and Baettig, K., Behavioral, emotional and neurochemical profiles of rats selected for extreme differences in active, two-way avoidance performance, in; Genetics of the Brain, pp. 95–123. Ed. I. Lieblich. Elsevier, Amsterdam 1982.

29 Dudek, B. C., and Abbott, M. C., A biometrical genetic analysis of ethanol response in selectively bred long-sleep and short-sleep mice. Behav. Genet. *14* (1984) 1–20.

30 Eckenhoff, M. F., and Rakic, P., Nature and fate of proliferative cells in the hippocampal dentate gyrus during the lifespan of the Rhesus monkey. J. Neurosci. *8* (1988) 2729–2747.

31 Edwards, A. L., Multiple Regression and the Analysis of Variance and Covariance. W. H. Freeman, San Francisco 1979.

32 Elkins, R. L., Separation of taste-aversion-prone and taste-aversion-resistant rats through selective breeding: implications for individual differences in conditionability and aversion-therapy alcoholism treatment. Behav. Neurosci. *100* (1986) 121–124.

33 Flechsig, P., Die Leitungsbahnen im Gehirn und Rückenmark des Menschen auf Grund entwicklungsgeschichtlicher Untersuchungen. Wilhelm Engelmann, Leipzig 1876.

34 Flechsig, P., Anatomie des menschlichen Gehirns und Rückenmarks auf myelogenetischer Grundlage. Georg Thieme, Leipzig 1920.

35 Flühmann, S., Lipp, H.-P., and Schwegler, H., A possible role of infrapyramidal mossy fiber terminals in aversively motivated decision making of mice in a Y-maze. Neuosci. Lett. Suppl. *7* (1981) S237.

36 Frotscher, M., and Zimmer, J., Lesion-induced mossy fibers to the molecular layer of the rat fascia dentata: identification of postsynaptic granule cells by the Golgi-EM technique. J. comp. Neurol. *215* (1983) 299–311.

37 Fuller, J. L., and Thompson, W. R., Foundations of Behavior Genetics. C. V. Mosby, St. Louis 1978.

38 Gall, F. J., Sur les Fonctions du Cerveau et sur Celles de chacune de ses Parties, vol. 1–6. Baillière, Paris 1822.

39 Gall, F. J., Franz Josef Gall, Naturforscher und Anthropologe; Ausgewählte Texte. Ed. E. Lesky. Hans Huber, Bern 1979.

40 Gentsch, C., Lichtsteiner, M., and Feer, H., 3H-diazepam binding sites in Roman high- and Roman low-avoidance rats. Experientia *37* (1981) 1315–1316.

41 Gentsch, C., Lichtsteiner, M., and Feer, H., Regional distribution of 3H-imipramine-binding sites in the CNS of Roman high and low avoidance rats. Eur. J. Pharmac. *88* (1983) 259–261.

42 Gozzo, S., and Ammassari-Teule, M., Different mossy fiber patterns in two inbred strains of mice: a functional hypothesis. Neurosci. Lett. *36* (1983) 111–116.

43 Guénaire, C., and Delacour, J., Differential acquisition of a 'working memory' task by the Roman strains of rats. Physiol. Behav. *34* (1985) 705–707.

44 Hall, R. D., and Macrides, F., Olfactory bulbectomy impairs the rat's radial-maze behavior. Physiol. Behav. *30* (1983) 797–803.

45 Harley, C. W., Arm choices in a sunburst maze: effects of hippocampectomy in the rat. Physiol. Behav. *23* (1979) 283–290.

46 Haug, F. M. S., Electron microscopical localization of the zinc in the hippocampal mossy fibre synapses by a modified sulfide silver procedure. Histochemie *8* (1967) 355–368.

47 Haug, F. M. S., Heavy metals in the brain. A light microscopic study of the rat with Timm's sulphide silver method. Methodological considerations and cytological and regional staining patterns. Adv. Anat. Embryol. Cell. Biol. *47* (1973) 1–71.

48 Haug, F. M. S., Light microscopical mapping of the hippocampal region, the pyriform cortex, and the cortico-medial amygdaloid nuclei of the rat with Timm's sulphide silver method. I. Area dentata, hippocampus and subiculum. Z. Anat. Entw. Gesch. *145* (1974) 1–27.

49 Hausheer-Zarmakupi, Z., Lipp, H.-P., Wolfer, D. P., and Leisinger-Trigona, M.-C., Selective breeding for a non-spatial behavior of mice entails a morphological differentiation of the hippocampal mossy fibers. Eur. J. Neurosci. Suppl. *1* (1988) 252.

50 Hegmann, J. P., and Possidente, B., Estimating genetic correlations from inbred strains. Behav. Genet. *11* (1981) 103–114.

51 Heimrich, B., Schwegler, H., and Crusio, W. E., Hippocampal variation between the inbred mouse strains C3H/HeJ and DBA/2: a quantitative-genetic analysis. J. Neurogenet. *2* (1985) 389–401.

52 Heimrich, B., Schwegler, H., Crusio, W. E., and Buselmaier, W., Substrain divergence in C3H inbred mice. Behav. Genet. *18* (1988) 671–674.

53 Hyde, J. S., and Sawyer, T. F., Selection for agonistic behavior in wild female mice. Behav. Genet. *10* (1980) 349–359.

54 Katz, M. J., and Lasek, R. J., Evolution of the nervous system: role of ontogenetic mechanisms in the evolution of matching populations. Proc. natl Acad. Sci. USA *75* (1978) 1349–1352.

55 Katz, M. J., Lasek, R. J., and Kaiserman-Abramof, I. R., Ontophyletics of the nervous system: eyeless mutants illustrate how ontogenetic buffer mechanisms channel evolution. Proc. natl Acad. Sci. USA *78* (1981) 397–401.

56 Kolb, B., and Nonneman, A. J., Functional development in prefrontal cortex continues into adolescence. Science *193* (1976) 33–36.

57 Lagerspetz, K. Y. H., Tirri, R., and Lagerspetz, K. M. J., Neurochemical and endocrinological studies of mice selectively bred for aggressiveness. Scand. J. Psychol. *9* (1968) 157–160.

58 Lauder, J. M., and Mugnaini, E., Early hyperthyroidism alters the distribution of mossy fibres in the rat hippocampus. Nature *268* (1977) 335–337.

59 Laurberg, S., and Zimmer, J., Lesion-induced rerouting of hippocampal mossy fibers in developing but not in adult rats. J. comp. Neurol. *190* (1980) 627–650.

60 Leiner, H. C., Leiner, A. L., and Dow, R. S., Does the cerebellum contribute to mental skills?. Behav. Neurosci. *100* (1986) 443–454.

61 Lieblich, I., Cohen, E., and Beiles, A., Selection for high and for low rates of self-stimulation in rats. Physiol. Behav. *21* (1978) 843–849.

62 Lieblich, I., Driscoll, P., and Lipp, H.-P., Genetic relation between the readiness to self-stimulate lateral hypothalamus, two-way avoidance learning and the proportions of hippocampal synaptic fields in regio inferior. Behav. Genet. *17* (1987) 427–438.

63 Lipp, H.-P., Brain complexity enhances speed of behavioral evolution. Behav. Brain Sci. *2* (1979) 42.

64 Lipp, H.-P., Differential hypothalamic self-stimulation behaviour in Roman high-avoidance and low-avoidance rats. Brain Res. Bull. *4* (1979) 553–559.

65 Lipp, H.-P., Postnatal development of hippocampal mossy fiber circuitry and freezing behavior in the guinea-pig, Neurosci. Lett. Suppl. *22* (1985) S332.

66 Lipp, H.-P., The forebrain as a playground of mammalian evolution, in: Intelligence and Evolutionary Biology. NATO ASI Series Vol. G 17, pp. 109–118. Eds H. J. Jerison and I. Jerison. Springer, Berlin/Heidelberg 1988.

67 Lipp, H.-P., Is the hippocampus a cerebellum of the limbic system?, Verh. Anat. Ges. (1989) in press.

68 Lipp, H.-P., Leisinger-Trigona, M.-C., and Schwegler, H., Selective breeding for differential open-field activity of mice entails a differentiation of hippocampal mossy fibers. Neurosci. Lett. Suppl. *26* (1986) S109.

69 Lipp, H.-P., and Schwegler, H., Hippocampal mossy fibers and avoidance learning, in: Genetics of the Brain, pp. 325–364. Ed. I. Lieblich. Elsevier, Amsterdam 1982.

70 Lipp, H.-P., and Schwegler, H., Structural variations of the hippocampal mossy fiber system and avoidance learning, in: The Hippocampus – New Vistas, pp. 295–307. Eds C. Köhler and V. Chan-Palay. Alan Liss Inc. New York 1989.
71 Lipp, H.-P., Schwegler, H., and Driscoll, P., Postnatal modification of hippocampal circuitry alters avoidance learning in adult rats. Science *225* (1984) 80–82.
72 Lipp, H.-P., Schwegler, H., and Hausheer-Zarmakupi, Z., The infrapyramidal mossy fiber projection in the hippocampus of the mouse: a positive correlation with Y-maze avoidance learning. Soc. Neurosci. Abstr. *12* (1986) 751.
73 Lipp, H.-P., Schwegler, H., Heimrich, B., Cerbone, A., and Sadile, A. G., Strain-specific correlations between hippocampal structural traits and habituation in a spatial novelty situation. Behav. Brain Res. *24* (1987) 111–123.
74 Lipp, H.-P., Schwegler, H., Heimrich, B., and Driscoll, P., Infrapyramidal mossy fibers and two-way avoidance learning: developmental modification of hippocampal circuitry and adult behavior of rats and mice. J. Neurosci. *8* (1988) 1905–1921.
75 Lipp, H.-P., Schöpke, R., Wolfer, D. P., Schwegler, H., and Leisinger-Trigona, M.-C., Hippocampal mossy fiber variations and swimming navigation in mice. Behav. Brain Res. (1989) in print.
76 Lynch, C. B., Response to divergent selection for nesting behavior in *Mus musculus*. Genetics *96* (1980) 757–767.
77 Mair, R. G., Gellman, R. L., and Gesteland, R. C., Postnatal proliferation and maturation of olfactory bulb neurons in the rat. Neurosci. *7* (1982) 3105–3116.
78 McClearn, G. E., The genetics of mouse behavior in novel situations. J. comp. physiol. Psychol. *52* (1959) 62–67.
79 Messeri, P., Oliverio A., and Bovet, D., Relations between avoidance and activity: a diallel study in mice. Behav. Biol. *7* (1972) 733–742.
80 Morris, R. G. M., Development of a water-maze procedure for studying spatial learning in the rat. J. Neurosci. Meth. *11* (1984) 47–60.
81 Morris, R. G. M., Garrud, P., Rawlins, J. N. P., and O'Keefe, J., Place navigation impaired in rats with hippocampal lesions. Nature *297* (1982) 681–683.
82 Morse, H. C. III, Historical perspective on the development of inbred mice, in: Origins of Inbred Mice. Ed. H. C. Morse III. Academic Press, New York 1979.
83 Nadler, J. V., Perry, B. W., Gentry, C., and Cotman, C. W., Fate of the hippocampal mossy fiber projection after destruction of its postsynaptic targets with intraventricular kainic acid. J. comp. Neurol. *196* (1981) 549–569.
84 Nagy, Z. M., and Misanin, J. R., Visula perception in the retinal degenerate C3H mouse. J. comp. physiol. Psychol. *72* (1970) 306–310.
85 Nesbitt, M. N., Spence, M. A., and Butler, K., Behavior in chimeric mice combining differently behaving strains. Behav. Genet. *9* (1979) 277–287.
86 Nowakowski, R. S., The mode of inheritance of a defect in lamination in the hippocampus of BALB/c mice. J. Neurogenet. *1* (1984) 249–258.
87 O'Keefe, J., and Nadel, L., The Hippocampus as a Cognitive Map. Clarendon Press, Oxford 1978.
88 Oliverio, A., and Castellano, C., Postnatal brain maturation and learning in the mouse. In: Genetics, Environment and Intelligence, pp. 118–131. Ed. A. Oliverio. Elsevier, Amsterdam 1977.
89 Oliverio, A., Eleftheriou, B. E., and Puglisi-Allegra, S., A genetic approach to behavioral plasticity and rigidity, in: Theoretical Advances in Behavior Genetics, pp. 139–168. Eds J. R. Royce and L. P. Mos. Sijthoff and Noordhoff, Alphen-Germantown 1979.
90 Oliverio, A., and Messeri, P., An analysis of single gene effects on avoidance, maze, wheel-running and exploratory behavior in the mouse. Behav. Biol. *8* (1973) 771–783.
91 Olton, D. S., Becker, J. T., and Handelmann, G. E., Hippocampus, space, and memory. Behav. Brain Sci. *2* (1979) 313–365.
92 Olton, D. S., and Werz, M. A., Hippocampal function and behavior: Spatial discrimination and response inhibition. Physiol. Behav. *20* (1978) 597–605.
93 Reinstein, D. K., DeBoissiere, T., Robinson, N., and Wurtman, R. J., Radial maze performance in three strains of mice: role of the fimbria/fornix. Brain Res. *263* (1983) 172–176.
94 Represa, A., Tremblay, E., and Ben-Ary, Y., Aberrant growth of mossy fibers and enhanced kainic acid binding sites induced in rats by early hyperthyroidism. Brain Res. *423* (1987) 325–328.
95 Roderick, T. H., Wimer, R. E., and Wimer, C. C., Genetic manipulation of neuroanatomical traits: in: Knowing, Thinking, and Believing, pp. 143–178. Eds L. Petrinovich and J. L. McGaugh. Plenum Press, New York 1976.
96 Sadile, A. G., De Luca, B., and Cioffi, A., Long-term habituation to novel environment: amnesic and hyperamnesic effects of various postexposure treatments. Abh. Akad. Wiss. DDR *5* (1978) 203–218.
97 Schwegler, H., Crusio, W. E., Lipp, H.-P., and Heimrich, B., Water-maze learning in the mouse correlates with variation in hippocampal morphology. Behav. Genet. *18* (1988) 153–166.
98 Schwegler, H., and Lipp, H.-P., Is there a correlation between hippocampal mossy fiber distribution and two-way avoidance performance in mice and rats?. Neurosci. Lett. *23* (1981) 25–30.
99 Schwegler, H., and Lipp, H.-P., Hereditary covariations of neuronal circuitry and behavior: correlations between the proportions of hippocampal synaptic fields in the regio inferior and two-way avoidance in mice and rats. Behav. Brain Res. *7* (1983) 1–39.
100 Schwegler, H., Lipp, H.-P., and Crusio, W. E., The NZB mouse: hippocampal mossy fiber patterns and behavioral profiles of young and older animals. Drug Dev. Res. *15* (1988) 297–305.
101 Schwegler, H., Lipp, H.-P., Van der Loos, H., and Buselmaier, W., *Individual hippocampal mossy fiber distribution in mice correlates* with two-way avoidance performance. Science *214* (1981) 817–819.
102 Schüz, A., and Hein, F. M., Comparison between the developmental calendars of the cerebral and cerebellar cortices in a precocial and an altricial rodent, in: Cerebellar Functions, pp. 318–321. Ed. E. Bloedel. Springer, Berlin/Heidelberg 1984.
103 Smith, R. H., Behavioral measures of drift in *Mus musculus*. Behav. Genet. *15* (1985) 483–497.
104 Sprott, R. L., and Staats, J., Behavioral studies using genetically defined mice. A bibliography. Behav. Genet. *5* (1975) 27–82.
105 Sprott, R. L., and Staats, J., Behavioral studies using genetically defined mice. A bibliography (July 1973–July 1976). Behav. Genet. *8* (1978) 183–206.
106 Sprott, R. L., and Staats, J., Behavioral studies using genetically defined mice. A bibliography (July 1976–August 1978). Behav. Genet. *9* (1979) 87–102.
107 Sprott, R. L., and Staats, J., Behavioral studies using genetically defined mice. A bibliography (August 1978–July 1979). Behav. Genet. *10* (1980) 93–104.
108 Sprott, R. L., and Staats, J., Behavioral studies using genetically defined mice. A bibliography (August 1979–July 1980). Behav. Genet. *11* (1981) 73–84.
109 Stanfield, B. B., and Trice, J. E., Evidence that granule cells generated in the dentate gyrus of adult rats extend axonal projections. Exp. Brain Res. *72* (1988) 399–406.
110 Tauck, D. L., and Nadler, J. V., Evidence of functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats. J. Neurosci. *5* (1985) 1016–1022.
111 Timm, F., Zur Histochemie der Schwermetalle, Das Sulfid-Silber-Verfahren. Dtsch. Z. Ges. Gericht. Med. *46* (1958) 706–711.
112 Tolman, E. C., The inheritance of maze-learning ability in rats. J. comp. Psychol. *4* (1924) 1–18.
113 Van Abeelen, J. H. F., Effects of genotype on mouse behavior. Anim. Behav. *14* (1966) 218–225.
114 Van Abeelen, J. H. F., Van der Kroon, P. H., and Bekkers, M. F., Mice selected for rearing behavior: some physiological variables. Behav. Genet. *3* (1973) 85–90.
115 Wahlsten, D., Contribution of the genes (c) and retinal degeneration (rd) to a strain-by-training procedures interaction in avoidance learning in mice. Behav. Genet. *3* (1973) 303–316.
116 Wahlsten, D., Heredity and brain structure, in: Genetics, Environment and Intelligence, pp. 93–115. Ed. A. Oliverio. Elsevier/North Holland, Amsterdam 1977.
117 Wahlsten, D., Genes with incomplete penetrance and the analysis of brain development, in: Genetics of the Brain, pp. 367–391. Ed. I. Lieblich. Elsevier, Amsterdam 1982.
118 West, J. R., and Dewey, S. L., Mossy fiber sprouting in the fascia dentata after unilateral entorhinal lesions: quantitative analysis using computer-assisted image processing. Neurosci. *13* (1984) 377–384.
119 West, J. R., and Hodges Savola, C. A., Permanent hippocampal mossy fiber hyperdevelopment following prenatal ethanol exposure. Neurobehav. Toxic. Terat. *5* (1983) 139–150.
120 Wimer, C. C., Correlates of mouse brain weight: a search for component morphological traits, in: Development and Evolution of Brain Size, pp. 147–162. Eds M. E. Hahn, C. Jensen and B. C. Dudek. Academic Press, New York 1979.
121 Wimer, C. C., Wimer, R. E., and Wimer, J. S., An association between granule cell density in the dentate gyrus and two-way avoid-

ance conditioning in the house mouse. Behav. Neurosci. *97* (1983) 844–856.
122 Wimer, R. E., and Wimer, C. C., Animal behavior genetics: a search for the biological foundations of behavior. A. Rev. Psychol. *36* (1985) 171–218.
123 Wirtshafter, D., and Asin, K. E., Impaired radial maze performance in rats with electrolytic median raphe lesions. Exp. Neurol. *79* (1983) 412–421.
124 Wolfer, D. P., Lipp, H.-P., Leisinger-Trigona, M.-C., and Hausheer-Zarmakupi, Z., Correlations between hippocampal circuitry and behavior: infrapyramidal mossy fibers and navigation in a Morris water maze. Acta anat. *128* (1987) 346.
125 Wolfer, D. P., Lipp, H.-P., Schwegler, H., Brust, M., and Leisinger-Trigona, M.-C., Hippocampal mossy fibers and avoidance learning in mice treated postnatally with corticosterone: correlations with extent and left/right asymmetry. Soc. Neurosci. Abstr. *13* (1987) 225.
126 Wright, S., Evolution in Mendelian populations. Genetics *16* (1931) 97–159.
127 Zar, J. H., Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, N. J. 1974.

0014-4754/89/090845-15$1.50 + 0.20/0

Reviews

Reflections on the ambivalent helix

J. W. Galloway

Director of Public Relations for the Cancer Research Campaign, 2 Carlton House Terrace, London SW1Y 5AR (England)

Summary. The helix is nature's favourite shape. Because of its elementary geometry and distinctive appearance it is also the clearest instance of an enantiomorphic object – a helix and its mirror image are identical in all respects except their screw sense. This is a distinction that can be ignored from the points of view of pure geometry and pure group theory[18] but any helical structure is actually available as either or both hands.
Whether in nature helices do occur as just one hand, or both, is one of the best – perhaps the best – puzzles of the science of form. In this short review I look at a few examples of naturally occurring helices, some where only one hand is found, some where both are commonly found, and perhaps the most interesting examples in biological terms – those where both are found but one hand is very much rarer than the other. I review what mechanisms – physico-chemical, genetic, evolutionary – underlie the different manifestations of left- and right-handedness.
Key words. Helix; handedness; enantiomer.

It is no accident that (Alice) Through the Looking Glass is filled with references to mirror reversals and asymmetric objects. The helix itself is mentioned several times....
Martin Gardner (The Wasp in a Wig, 1977)[33]

A helix "goes the other way in a mirror" – to use Alice's own words. In theory at least then, any helix is one of a pair of identical 'twins', identical that is except that one is left-handed and the other right – they are enantiomers, mutual mirror images that cannot be superposed. Whether a helix possesses an actual twin (or even a counterpart) is I believe one of the best questions of the science of form – possibly its deepest (fig. 1.).
The potential for having a mirror-reversed twin is not, it should hastily be added, peculiar to helices with their screw symmetry. It is characteristic of any object that possesses no inverse symmetry elements. Many molecules exist as enantiomers. A few are helical. Most are not. The most familiar instance of enantiomorphism is that of a pair of hands, which is why 'handedness' (see appendix 2) is attributed to mutual non-superposable mirror images. However the helix is geometrically elementary and readily recognised. It should therefore, reveal most clearly the principles underlying the existence (or not) of mutual mirror images. Conklin[15], for instance, said in his paper *Causes of Inverse Symmetry* that "inversion of symmetry [i.e. production of a mirror image] in animals, with its profound implications for embryology, is clearly seen in gastropods [which are roughly helical] though doubtless taking place in other animals where it is obscured".

The helix: nature's favourite shape

The helix turns out to be nature's favourite shape – its agreeable economy making it the preferred solution for innumerable problems of growth, form and function in living things. Because it is so common, found at every anatomical level across about 9 orders of magnitude (table 1), it is possible to disentangle to some extent the mechanical or structural design principles behind the helix from the large number of ways the design can be realised. One reason for the popularity of the helix can be found in Needham's[57] rather apt description of biology as "largely the study of fibres". Add to this the idea articulated by Crane[19] that "any structure which is straight or rodlike [a category that includes fibres when the length greatly exceeds the diameter] is probably a structure having a repetition along a screw axis", i.e. a helix, and the crucial and central role played by the helix