Genetic control of hippocampal cholinergic and dynorphinergic mechanisms regulating novelty-induced exploratory behavior in house mice

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Summary. Neurobehavioral genetics endeavors to trace the pathways from genetic and environmental determinants to neuroanatomical and neurophysiological systems and, thence, to behavior. Exploiting genetic variation as a tool, the behavioral sequelae of manipulating these neuronal systems by drugs and antisera are analyzed. Apart from research in rats, this paper deals mainly with the genetically-influenced regulation in mice of exploratory behaviors that are adaptive in novel surroundings and are hippocampally-mediated. Special attention is paid to neuropeptidergic, GABAergic, and cholinergic synaptic functions in the mouse hippocampus.

The behaviorally different inbred mouse strains C57BL/6 and DBA/2 show opposite reactions (reductions and increases, respectively, in exploration rates) to peripheral and intrahippocampal injections with agents that interfere with peptidergic, cholinergic, and GABAergic neurotransmission. These findings can be explained by an interdependent over-release of opioids, arrested GABA release, and excess acetylcholine in the hippocampal neuronal network of DBA/2 mice, as compared to C57BL/6 mice where these systems are functionally well balanced. Very similar results have been obtained with the lines SRH and SRL, derived from C57BL/6 and DBA/2, and genetically selected for rearing behavior. Most probably, the opioids act to disinhibit exploratory responses. An additional genetic approach is mentioned, in which four inbred mouse strains and one derived heterogeneous stock are used for estimating genetic correlations between structural properties of the hippocampal mossy fibers and levels of hippocampal dynorphin B, on the one hand, and frequencies of exploratory responses to environmental novelty, on the other.

Key words. Inbred, selected, and heterogeneous mouse and rat strains; genotype; genetic correlations; exploratory behavior; locomotor activity; novelty; disinhibition; hippocampus; mossy fibers; opioid peptides; antibodies; dynorphine B; GABA; acetylcholine; drug treatment.

Introduction

Behavior is a phenotype. The field of investigation called neurobehavioral genetics, which is the subject of the present series of reviews, is primarily concerned with the proximate causation of behavioral differences. It is recognized that the genotype is one important determinant of behavior and its underlying neurophysiological mechanisms. These mechanisms are subject to the action of the genetic information contained in the DNA and to influences from the environment during ontogeny. One might call this the phenogenetic aspect of causation, as distinct from the ultimate phylogenetic aspect which deals with the evolutionary origins of the genetic construction that controls the specific properties of the physical substrata of behavior. The great potential of the genetic approach to neurobehavioral problems has been outlined previously[5, 12]. Behavioral variables may be manipulated indirectly through different kinds of breeding schemes and the concomitant changes in neurotransmitter level and release, fiber and receptor distribution, and enzyme activity can be observed. Alternatively, behavior can be affected more directly, for example by psychopharmacological and biochemical manipulations. This type of experimentation should always utilize animals of different genotypes to take into account or, better still, capitalize on the genetic variable.

One of the first convincing demonstrations of the importance of genotypic influences on a behavioral response to a drug concerned the effects of nicotine, injected i.p., on avoidance conditioning in mice from nine inbred strains and one non-inbred strain[10]. Clear strain-drug interactions emerged in this experiment. Additional examples of such interactions in inbred and selected stocks of mice and rats have been described and commented upon in Broadhurst's impressive survey of comparative psychopharmacogenetics[12]. The present paper briefly and selectively reviews the genotype-dependent involvement of cholinergic and peptidergic neurotransmitter mechanisms in the rodent hippocampus that control exploratory responses to environmental novelty.

Exploration

Exploratory behavior can be defined as follows: it is evoked by novel stimuli and consists of behavioral acts and postures that permit the collection of information about new objects and unfamiliar parts of the environment[22]. Without apparent reward, the animal wanders through its novel surroundings, showing a variety of investigative acts such as rearing-up and scanning (visual exploration), sniffing (olfactory), and leaning against objects (tactile). The opportunity to actively explore an area has an intrinsic rewarding value[6]. The adaptive value of this behavior seems clear: entering and exploring new places promotes dispersion and improves the chances of finding life necessities (food, shelter, escape routes, etc.). As implied by the above definition, the concept of exploration is closely associated with that of novelty. The latter may consist of absolute novelty, which involves some quality never previously experienced, and/or rela-

tive novelty of familiar items arranged in an unfamiliar way[6]. Of course, a gradual difference in novelty between situations that are more or less frequently encountered also exists. Thus, novelty depends on the degree of previous (short-term and long-term) experience of the animal with a particular situation. Exploration can also be seen as a stress-elicited activity because confrontation with unfamiliar circumstances may entail a certain degree of fearfulness (as suggested by the 'freezing' phenomenon, which occurs more frequently in laboratory rats than in laboratory mice). As put forward in a recent review chapter[50], it seems to be impossible to separate empirically a unique effect of novelty on exploration from that of stress.

Inbred, selected, and heterogeneous strains of mice

Among the hundreds of inbred mouse strains, a veritable cornucopia, C57BL/6 and DBA/2[60,77] belong to the oldest and most widely used. They differ with regard to a number of behaviors in a novel open-field. C57BL/6 mice are characterized by their high locomotor activity (horizontal activity) and their high frequencies of rearing (vertical activity) and object-sniffing, as compared to the low-scoring DBA/2 strain, whereas DBA/2 mice show more grooming behavior[81]. These strains have also been found to react differentially to peripheral or intracerebral administration of various kinds of drugs and antisera against neuropeptides (see below). From an F_2 cross between the two strains, the inbred selection lines SRH (selection for rearing: high) and SRL (selection for rearing: low) have been derived by a process of bi-directional selection of male mice for rearing frequency in an open-field[77,82]. In the first four generations of selective breeding, these males were backcrossed with DBA/2 females; after this, the selection was combined with inbreeding by sib-mating over more than 50 generations. The backcrossing procedure was employed to ensure that C57BL/6-alleles responsible for high rearing scores were transferred to a DBA/2 background.

The resultant divergence in rearing between SRH and SRL, which has been tested repeatedly and has been found to be large and reproducible, was accompanied by similar changes in locomotor activity. This correlated response to selection, in conjunction with the results of quantitative-genetic analyses, provided evidence that the line differences in both behaviors are due to segregation at a single genetic unit, or perhaps a few closely linked loci, conceivably involving a common neurophysiological mechanism[52,82,83]. SRH and SRL differ also in their behavioral responses to psychoactive drugs (see below) and, hence, represent excellent experimental material for neurobehavioral-genetic research. Using the unrelated inbred strains C57BL/6, DBA/2, BLN[77], and CPB-K (listed in 'Mouse News Letter 79', 1987), a four-way cross has been made, resulting in a genetically heterogeneous line maintained by random breeding over, at present, 20 generations, and denoted HL. These five populations, together, create an opportunity to determine genetic correlations[43] between neurobiological and behavioral variables so that any pleiotropic gene effects upon these variables, and surmised causal relationships between the variables, can be elucidated (van Daal et al., in preparation).

Hippocampus

One of the major functions of the mammalian hippocampal complex is the regulation of exploratory and orientational activities, novelty recognition, and memory formation through the processing of spatial and temporal information in its neuronal networks[45]. This brain region acts as a comparator of incoming novel information and stored information and constitutes a multidimensional map of other brain areas[17,40,62,66,68,72,74,76,79,91]. The hippocampal network receives its inputs from several brain regions, among which the most extensive ones are the input through mainly cholinergic neurons from the septum, innervating pyramidal cells, and the input through glutamatergic neurons from the entorhinal cortex, innervating granule cells; its output is via the glutamatergic pyramidal cells[56,78]. This output ultimately gives rise to visual, olfactory, and tactile exploration.

Essential in the coupling of the hippocampal input to the hippocampal output of the above neuronal network is the dentate granule cell that, through mossy fibers, innervates the pyramidal cell in excitatory glutamatergic terminals (see fig. 1). There is evidence that the mossy fibers also innervate GABAergic interneurons[1,32]; these basket cells act to inhibit the pyramidal cells (see review Corrigall[19]). In this function, the mossy fibers probably

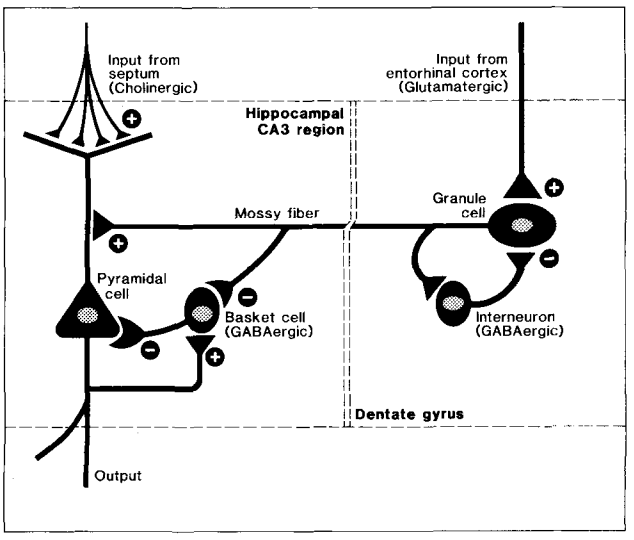

Figure 1. Schematic representation of part of the hippocampal circuitry involved in the regulation of behavioral responses to environmental novelty. The (+) and (−) symbols indicate stimulatory and inhibitory terminal fields, respectively. (Elaborated after Frotscher[32]).

use the opioid peptide dynorphin, a highly specific ligand of the kappa receptor, as neurotransmitter [89]. This mechanism has been termed 'disinhibition', that is, inhibition of the inhibitor [19, 89]. It has been firmly established that in rats [16, 18, 33, 44, 58, 59, 94], and in mice [34, 90], dynorphin is confined to the mossy fibers, which project onto the CA3 area. Apart from the above, at least four other neurotransmitters and at least six neuropeptides participate in the hippocampal neurochemical network for behavior [26, 46, 56, 61, 70]. Interestingly, genetically-determined structural variations have been demonstrated in the terminal fields of the mossy fibers in mice [4, 21, 28, 38, 55, 73], and in rats [55, 73]. This applies in particular to the sizes of the projection zones of the infra- and intrapyramidal mossy fibers (iip-MF) [21, 55, 73], which make synaptic contact with the basal dendrites of the CA3 pyramidal cells and which can be visualized by means of the Timm staining technique. Moreover, these authors have found morphobehavioral correlations between these heritable neuroanatomical variations, on the one hand, and open-field activity and performance in different learning tasks, on the other.

Genetic selection in rats for avoidance learning (lines RHA and RLA) [73], and in mice for locomotor activity (lines HI and LO) [54], and rearing frequency (lines SRH and SRL) [20], has resulted in alterations in the sizes of the iip-MF terminal fields. It has also been possible to reveal substantial genetic correlations between mossy fiber distribution and various exploratory behaviors, employing a diallel-crossbreeding design with five inbred mouse strains [21] and the heterogeneous mouse stock HL with its four inbred ancestor strains (van Daal et al., in preparation), respectively. In the latter case, immunohistochemical techniques for staining of dynorphin have been used. It is evident, morphologically and neurochemically, that the hippocampus forms an attractive and intriguing object for neurobehavioral studies that incorporate the genetic component. The remainder of the present paper concentrates mainly on the cholinergic and dynorphinergic parts of the system.

Cholinergic control

Drugs frequently used for interfering with acetylcholinergic (ACh) neurotransmission include the anticholinergic agent scopolamine, the acetylcholinesterase (AChE) inhibitor physostigmine, and their respective quaternary analogs methylscopolamine and neostigmine; the latter two pass the blood-brain barrier only with great difficulty. Generally, peripheral administration of scopolamine prolongs and intensifies exploratory behavior, suggesting that the drug may disrupt the processing of sensory information which normally familiarizes an animal with its surroundings as the result of inspection [7, 63, 93]. It is known that scopolamine impairs the ability to habituate to novelty in rats [24, 41, 53] and mice [24, 39, 63], and that it can produce learning deficits and amnesia in rats [9, 23, 93] and mice [13]. All these behavioral processes are probably hippocampally mediated.

The involvement of hippocampal cholinergic activity in memory formation has found further support in hippocampal electrical stimulation studies in three inbred mouse strains (BALB/c, C57BL/6, and C57BR) which, moreover, differed behaviorally and neurochemically; performance in the BALB/c mice was superior to that in the others, they improved more after stimulation, and they showed higher choline acetyltransferase (ChAt) activities that temporarily went up even higher after stimulation [47, 48]. As mentioned before, the inbred mouse strains C57BL/6 and DBA/2 differ consistently in their levels of several exploratory acts in a novel environment; the former rates high and the latter rates low. When injected peripherally with scopolamine (1.25, 2.5, 5, and 10 mg/kg), this situation was altered drastically due to a peculiar strain-treatment interaction. The scores of the C57BL/6 strain were depressed, while those of the DBA/2 strain were enhanced, resulting in either a reversal or an elimination of the original strain differences at particular dose levels [81]. Equal treatments yielded opposite effects. A similar phenomenon has been observed for locomotor activity in the same strains, with the same drug, at dosages of 2.5 and 5 mg/kg [64] and 1, 3, 5, and 10 mg/kg [2]. Injection of physostigmine (18.75, 37.5, 75, and 150 µg/kg) reduced the exploratory behavioral components in both strains [81]; this resembled the effects of this substance (150 and 300 µg/kg) on locomotor activity in the same strains [64]. Peripheral administration of equimolar doses of methylscopolamine or neostigmine failed to affect exploration in C57BL/6 and DBA/2 animals, but injections with the first-mentioned (7.8 µg per mouse) or the second (0.07 and 0.14 µg per mouse) into the left dorsal hippocampus mimicked the results obtained previously (opposite effects with methylscopolamine, reductions with neostigmine) [81]. Comparable psychopharmacogenetic influences have been found in the derived inbred selection lines SRH and SRL; intrahippocampal methylscopolamine (1 and 2 µg per mouse) decreased exploration in the high line and prolonged it in the low line [85].

It may be concluded that there exists a genotype-dependent cholinergic mechanism, its site of action located in the hippocampus (the septo-hippocampal pathway), that controls exploratory behavior in mice. In C57BL/6 and SRH animals a functionally well-balanced ACh/AChE ratio seems to promote efficient synaptic transmission, thereby producing high exploration scores. Any injection with drugs that cause an imbalance in this ratio in either direction will thus result in a decline in exploration. In DBA/2 and SRL mice a disequilibrium in the ACh/AChE ratio is postulated, to the effect that ACh is in excess, leading to low levels of exploratory behavior. This imbalance can be restored by the anticholinergics, resulting in higher scores, but it will be aggravated by the anticholinesterases which produce even lower scores. The

above conclusions go charmingly with the finding that oxotremorine, a muscarinic cholinergic agonist, reduces open-field activity in C57BL/6, as well as in DBA/2 mice[57].

Opioid control

That neuropeptidergic mechanisms may participate in the regulation of behavioral processes in a genotype-dependent way follows, in the first place, from the observation that strains C57BL/6 (locomotor type) and DBA/2 (analgesic type), among others, exhibit strain-specific, dose-related responses to peripheral administration of the opiate μ-receptor agonist morphine[11,29,30,65,67,69,75,80]. These divergent responses pertain to running activity, pain inhibition, reaction to stressful situations, drug tolerance, and proneness to addiction. Much of this interesting material has been adequately covered by another contribution to the present series[31]. In the second place, genetic selection for high and low antinociceptive response to the potent opiate μ-receptor agonist levorphanol has been successful[8]. Other frequently-used pharmacological tools are the opiate antagonists naloxone and naltrexone that show a high binding affinity with the μ-receptor and less pronounced affinities with the delta and kappa receptors. Generally speaking, peripheral injections of these substances diminish, in a dose-dependent manner, exploratory and ambulatory activity under novel conditions in rats[3,25,49,50,71,92] and mice[15,42,51], possibly because they are memory-enhancing drugs[27]. Again, the genotype appears to play its role in the effects of opioid blockade on those behaviors that depend on hippocampal function: in mice, a number of strain-drug interactions have become apparent with regard to novelty-induced behaviors[37] and water-maze discrimination learning after pretraining[14].

A clearer picture emerged when the following intrahippocampal microinjections were applied. C57BL/6 and DBA/2 mice reacted in opposite directions to naloxone (0.5 μg per mouse); that is, exploration decreased in the normally high-scoring C57BL/6 mice and increased in the otherwise low-scoring DBA/2 animals, while morphine (1 μg per mouse) depressed it in both strains[87]. Very similar observations have been made in SRH and SRL mice under the influence of intrahippocampal naloxone (0.3 μg) or morphine (1 μg)[88]. These findings suggest that the opioid mechanism involved operates efficiently in C57BL/6 and SRH animals exposed to environmental novelty; treatment with either antagonist or agonist will upset its optimally balanced peptidergic function, thereby depressing exploration. In contrast, DBA/2 and SRL mice seem to possess a less efficient mechanism characterized by an excess release of hippocampal opioids which is, naturally, not alleviated by treatment with an agonist but can be attenuated by an appropriate inactivating agent; the latter thus augmenting exploratory behavior.

The pattern of the influence of naloxone and of morphine upon the exploratory scores resembles the results, obtained for the same behaviors in the same four strains, of direct pharmacological interferences with cholinergic hippocampal pathways, as described above. This indicates that the (anti-)opiate drugs affect the peptidergic modulation of the cholinergic control of exploration. This is consistent with the view that one or more hippocampal opioids are released upon exposure to novelty and then disinhibit this behavior[89]. As far as the SRH and SRL mice are concerned, one might speculate that the single genetic unit responsible for the line difference in rearing, and perhaps other behavioral components, is a regulatory gene causing a structural alteration in a repressor enzyme for transmitter synthesis, which results in a relative surplus of neuropeptide levels in SRL (and, possibly, in DBA/2). Molecular-genetic investigations are needed to clarify that point. Among the hippocampal opioids, methionine-enkephalin (met-enk, a pentapeptide) and dynorphin B (dyn B, a tridecapeptide also called rimorphin) are likely candidates. Immunoreactivities have been observed for met-enk in rats[35] and mice[34,90], particularly, but not exclusively, in the mossy fibers, and for dyn B in rats[16,18,33,44,58,59,94] and mice[34,90], strictly limited to the mossy fibers.

Returning once again to the C57BL/6 and DBA/2 strains, intrahippocampal (CA3 region) administration of specific antibodies raised against met-enk (in an

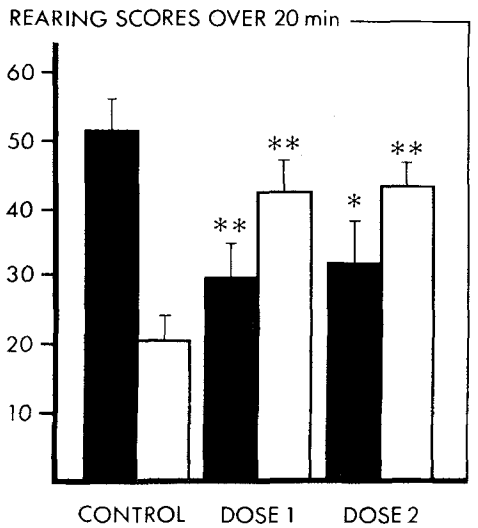

Figure 2. Effects of intrahippocampal microinjections with anti-dynorphin B antiserum, given in doses that can inactivate 9 and 18 pg of the opioid, respectively, upon the frequencies of exploratory rearing in mice from the inbred strains C57BL/6 (filled columns) and DBA/2 (open columns) observed over 20 min in a novel environment. Columns represent means, bars represent SEM. Treatment groups (n = 20) differing significantly from the preimmune serum controls (n = 20) are marked by asterisks: * $p < 0.01$; ** $p < 0.001$. The original strain difference between the controls ($p < 0.001$) was significantly reversed after treatment with the antibody ($p < 0.01$ and 0.05, respectively).

amount capable of inactivating 36 pg of this peptide[86]) or against dyn B (in amounts capable of inactivating 9 and 18 pg of it[89]) resulted in the expected, opposite effects upon exploration rates (C57BL/6: decreases; DBA/2: increases; see fig. 2). Apparently, a dysfunctional over-release of neuropeptides in DBA/2 can be neutralized in part by minute amounts of antibody so that a more efficient disinhibition of responses to novelty becomes possible. In this connection, it is worthy of note that radioimmunoassays have shown that the very strains in which over-release of dyn B is postulated, DBA/2 and SRL, exhibit a lower (unilateral) hippocampal content of it immediately after having explored, as compared to naive controls (van Daal et al., in preparation). The same authors, employing strains C57BL/6, DBA/2, BLN, CPB-K, and HL, and using quantitative-genetic methods[43] adapted for the purpose, arrived at estimates showing considerable genetic correlations between tissue levels of dyn B and frequencies of various exploratory acts. This adds to the evidence that these variables are causally related.

A GABAergic link between cholinergic and opioid control

As stated before, there are strong indications that the opioids exert their exploration-facilitating action through a disinhibitory mechanism by blocking inhibitory hippocampal GABAergic[1, 78] basket cells, thereby activating pyramidal neurons. Although it has been claimed that the γ-aminobutyric acid (GABA) system is not an important mediator of differences in mouse locomotor activity[36], a defective GABAergic function has been suggested in DBA/2 mice, as opposed to C57BL/6 animals, entailing, in that case, a higher susceptibility to audiogenic seizures[95]. In experiments applying intrahippocampal injections (CA3 region) with the $GABA_A$ receptor agonist muscimol (0.5 μg per mouse), the C57BL/6 and DBA/2 animals once again reacted differentially, i.e. reductions in exploration and locomotion occurred in the former and rises occurred in the latter[84]. This result suggests that the opioid modulation of the hippocampal cholinergic mechanism regulating exploration is effectuated indirectly through an inhibitory GABAergic system which depends on the genotype. In C57BL/6 this system seems to function efficiently but in DBA/2 it is unbalanced, probably because of an arrested GABA release that can be made up for by muscimol.

Thus it is possible to construct a concept of a genetically-controlled, interconnected, triple neurotransmitter system in the hippocampus, that acts to regulate the highly adaptive exploratory responses to environmental novelty in house mice (see fig. 1). The author is conscious of the fact that this story is not yet finished, however, and believes that researchers in neurobehavioral genetics are at present maneuvering toward additional, most interesting achievements in the field.

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

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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.