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Perspective

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Hormones, genes, and behavior

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ABSTRACT **With assays of hormone-sensitive behaviors, it is possible to demonstrate both direct and indirect actions of genes on mammalian social behaviors. Direct effects of estrogen receptor gene expression and progesterone receptor gene expression figure prominently in well analyzed neuroendocrine mechanisms for sex behavior, operating through a neural circuit that has been delineated. Indirect effects, notably the consequences of sexual differentiation, display complex dependencies. In a human condition, Kallmann syndrome, the data show a clear, indirect genetic influence on an important human social behavior, in which damage at chromosome Xp-22.3 works through at least six discrete steps to affect libido. Altogether, simplistic extrapolations from lower animals, especially during brief summaries for nonscientists, do not appear justified as we discover and conceptualize genetic influences on mammalian brain and behavior.**

In several papers published recently in *Cell*, *Nature*, or *Science*, biologists working with *Drosophila* have reported their discoveries that targeted expression of particular genes or deletion of certain genes can markedly alter *Drosophila* behavior. The very success of these interesting analyses has heightened the danger of some readers who are not in biological or medical fields getting the impression that "genes will organize our thinking" about brain mechanisms for behavior. This is not likely to happen.

More generally, I fear that oversimplified public reactions to discoveries in *Drosophila* sometimes will anticipate a preponderance of straightforward genetic programming in higher animals. Like most scientists, I cannot believe that "one gene–one behavior" formulations will work for mammals. Even in *Drosophila* it is rare to see a single gene underlying a behavioral polymorphism. And, clearly, opportunities for sophisticated physiological integration are simply very limited in invertebrates compared with mammals (Fig. 1).

The best studied examples of mammalian brain–behavior mechanisms show that genes do indeed influence behavior through both direct and indirect routes in higher animals and humans. But the chains of causation from genes to behavior are multiple and complex, defying simple description and demanding, for the systematic unraveling of behavioral mechanisms, some very fine physiology. That is, the most orderly summaries of behavioral mechanisms will be based not in genetics but in physiology.

Therefore, for biomedical scientists who lack the research background of *Drosophila* geneticists and for others who think mainly about humans, clear examples of reasoning from gene to mammalian behavior are given. Well analyzed mechanisms of hormone/brain/behavior relations illustrate how subtle the reasoning can be, even for simple instinctive behaviors.

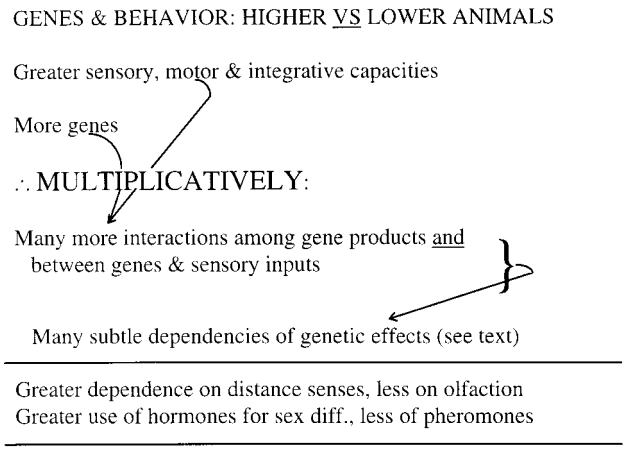

FIG. 1. Causal relationships of genes to behaviors pose a greater analytic challenge in mammals than in *Drosophila* because of the multiplicative nature of sensory, motor, and interneuron combinations and because of the greater variety of intermediate behavioral states.

Attempts to Systematize

Despite a long history of brilliant work on genetically amenable organisms such as *Drosophila*, we still cannot easily trace the causal routes and mechanisms by which genes could influence behavior. There are at least four reasons for this state of affairs. First, the pleiotropy of genetic actions (1) dictates that any one gene may have many effects, relations among which may be difficult to discern. Second, overlap among functions of different genes precludes a simple demonstration that a given gene contributes to a given behavior. Third, incomplete and variable penetrance of a dominant allele render the statistical analysis of behavioral results harder to explain. Fourth, and most important, any map of possible mechanistic routes, direct and indirect, from genes to behavior must be as complex as the physiology of the organs contributing to the behavior. Because these routes will always include the central nervous system, the complexity of the mammalian brain guarantees that the task of discerning gene/neuron/behavior relations will not be finished quickly.

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	Incidence of female reproductive behavior	Incidence of female - female aggression
Wild type	normal	2/21 mice
Estrogen Receptor KnockOut	none	10/25 mice*

FIG. 2. Behaviors of wild-type and ERKO female mice. ERKO females would not show lordosis behavior. Summarized from data in Ogawa *et al.* (4). *, Aggression exhibited by ERKO females mainly offensive attacks typical of intermale aggression.

Examples from Genes for Nuclear Receptors

Direct effects of genes on behavior during adulthood are clearly illustrated by estrogens and progestins working through nuclear estrogen and progesterone receptors to control female reproductive behaviors. Early work indicated that the estrogen receptor (ER) drives lordosis behavior, a component of female reproductive behavior in many higher organisms (2), and this was confirmed with ER antagonists (3). Recently, the ER knockout (ERKO) mouse has proven that classical ER gene expression is indeed required for estrogenic effects on lordosis (Fig. 2) (4). Indeed, ERKO yields a female mouse that is more *masculinized*, behaving less like a genetic female and actually treated as a male by other mice (4).

Likewise, the progesterone receptor (PR) gene is required for progestin effects on female reproductive behavior (5). Fluctuations of PR mRNA and PR binding levels in the hypothalamus are well correlated with lordosis behavior, and in fact the ability to reduce female reproductive behavior with hypothalamic PR antisense DNA administration (6) perfectly anticipated the behavior of PR knockout mice (Fig. 3).

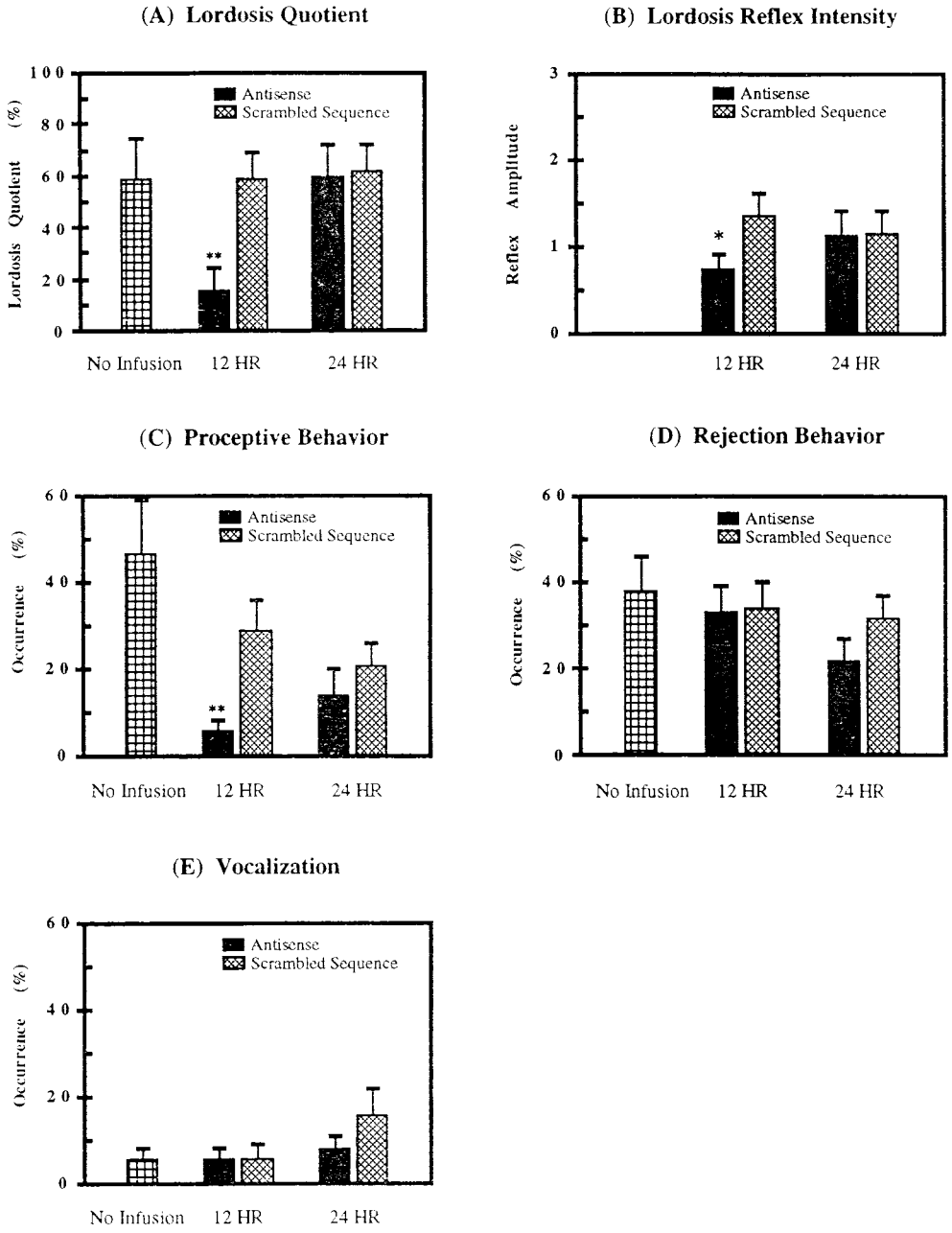

FIG. 3. Administration of antisense DNA directed against PR mRNA directly to the ventromedial hypothalamus significantly reduced lordotic behavior in female rats (*A* and *B*). The effect was even larger on courtship ("proceptive") behaviors, which are known to depend heavily on progesterone. (*C*). In contrast, rejection behaviors were not affected (*D*), nor were vocalizations (*E*). From Ogawa *et al.* (6).

Males. The plot thickens when one looks at the genetic male with an ER knockout. Surprisingly, ERKO males show virtually no intromissions or ejaculations even though several indices of their sexual motivation appear normal (7). Overall, such estrogen-deficient males show marked decreases in a subset of their masculine-typical behaviors and a trend toward a more *feminine-type* of behavioral profile. That is, ERKO males achieved fewer intromissions and virtually no ejaculations. Their aggressive behaviors were dramatically reduced, and in particular they showed absolutely no male-typical offensive attacks. Their emotional responses to the open-field test were demasculinized. Thus, we are left with the situation in which the ERKO gene alteration renders genetic female mice more masculine (see above), yet renders genetic male mice more feminine. *The lesson: As far as a gene like that for the ER is concerned, its effect on the development of behavior depends on the sex of the animal in which it is expressed*.

Finally, analyses of hormone-dependent behaviors also show how the effect of a gene on a specific behavior depends on exactly where and exactly when that gene is expressed. Even as the ERKO female (above) is more *masculinized* in its behavior (4), temporary antisense DNA interruption specifically of hypothalamic ER mRNA during neonatal testosterone administration to females actually *prevents* full masculinization of the rat brain (8). That is, McCarthy *et al.* (8) showed that ER gene product disruption limited to the hypothalamus and applied only on one neonatal day actually reduced the masculinization of forebrain and behavior due to experimentally administered testosterone. In contrast, the ER gene disruption in ERKO females is limited neither in time nor in space, and this genetic maneuver allowed more masculinized behavior (4). *The lesson: The effect of a genetic alteration is a function of exactly where and for how long it is applied*.

Additional cautions. Even slight increases in the complexity of the behavior analyzed can lead to corresponding complexity of interpretation, as illustrated by maternal behaviors, whose susceptibility to oxytocin *depends exquisitely on the precise conditions of assay* (9). Female rats were not sensitive to oxytocin when they were unstressed or severely stressed, but at intermediate levels of mild stress oxytocin facilitated their maternal behavior (9).

More generally, few would expect mammalian behaviors to depend only on a small number of genes—they are the perfect examples of multigenic traits. Thus, we should expect to see, for any given knockout mouse, influences of genetic background on the magnitude of the effect of any given gene (10).

In summary, the gene product for the nuclear hormone receptor for estradiol has both direct and indirect (developmental) effects on reproductive behaviors. Further, even though masculinization and feminization typically are viewed as naming "opposite ends" of a continuum in reproductive biology, normal expression of the gene for the ER is necessary both for a full pattern of masculine behavior and for a full pattern of feminine behavior.

Kallmann Syndrome

A human syndrome provides an example of how subtle and indirect the relationships between genes and social behavior can be. Kallmann syndrome, hypogonadotropic hypogonadism, afflicts men with a striking behavioral change: absence of libido as part of a disinterest in the opposite sex. Causation of X-linked Kallmann syndrome is now understood in light of the surprising findings that neurons producing gonadotropin-releasing hormone (GnRH) are not born as expected near brain ventricular surfaces, but instead are born in the olfactory epithelium. That is, they must migrate from the nose to the brain. In fact, X-linked Kallmann syndrome caused by genetic damage at Xp-22.3 has been correlated with a failure of migration of GnRH neurons—they were dammed up in the olfactory apparatus and never reached the brain (11, 12). In turn, this neuron migration disorder is rationalized by the fact that damage at Xp-22.3 disrupts a specific gene (13, 14) whose damage causes X-linked Kallmann syndrome, and this gene codes for a cell surface protein present during migration of GnRH neurons into the brain.

Putting these and related facts in a logical order leads to a clear example of complex participation by an individual gene in human behavior through its actions during neural development. That is, in males suffering from X-linked Kallmann syndrome, behavioral libido is reduced *because* of low testosterone levels, in turn *because* of reduced gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which in turn are low *because* there is no GnRH coming from the brain to enter the pituitary, *because* there are no GnRH cells in the brain, *because* GnRH neuronal migration has failed, *because* of the absence of the protein produced by a gene at Xp-22.3.

Thus, these data prove a genetic influence on an important human social behavior but also illustrate the complicated and indirect nature of that effect. Simplistic extrapolations from lower animals would be as unjustified here as they would be for the mammalian behaviors described above.

Implications

In summary, specific genes clearly contribute to the causation of specific mammalian social behaviors and in the case of certain hormone-dependent behaviors, neurochemical mechanisms operating in known neural circuits can be specified (reviewed in ref. 2). Nevertheless, the multiple determinants of the sizes and directions of these genetic effects and the many indirect routes of causation make it seem virtually impossible that simple charts of gene/behavior relationships can be drawn. Interactions between genes and environment as well as among gene products ensure that gene/behavior relations will be neither linear nor modular.

Under these circumstances, the safest theoretical approach is to flip the problem upside down. After all, biological systems are organized according to physiological function, not gene by gene. Therefore, instead of looking for elegant, systematic thinking "starting with the gene" and moving out, modern neurobiologists will do better to start with each obviously essential, "axiomatic" biological function and, in a "geometric" fashion, deduce how neural and, ultimately, molecular mechanisms satisfy that biological function. As part of that effort, illustrated for hormone-dependent behaviors above, genetic contributions both during development and in adulthood to each clearly defined biological function can be woven into the fabric of neurophysiological and neurochemical mechanisms as data accumulate.

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