Nongenomic Transmission Across Generations of Maternal Behavior and Stress Responses in the Rat

Darlene Francis, *et al.*

Science **286**, 1155 (1999);
DOI: 10.1126/science.286.5442.1155

cloning. Second, it is sensitive. Activities can be detected in the purified GST-ORF pools that simply cannot be detected in extracts or cells, the starting point of both conventional purification and expression cloning. Because the GST-ORFs are individually expressed at high levels and are largely free of extract proteins after purification, activities can be measured for hours without competing activities that destroy the substrate, the product, or the enzymes.

In addition to the conventional use demonstrated here, this array could be used in two other ways: (i) to determine the range of potential substrate proteins for any protein-modifying enzyme (such as a protein kinase) before genetic or biochemical tests to establish authentic substrates and (ii) to identify genes encoding proteins that bind any particular macromolecule, ligand, or drug. Thus, one could rapidly ascribe function to many presently unclassified yeast proteins, complementing other genomic approaches to deduce gene function from expression patterns, mutant phenotypes, localization of gene products, and identification of interacting partners.

References and Notes

1. H. Simonsen and H. F. Lodish, *Trends Pharmacol. Sci.* **15**, 437 (1994).
2. Plasmid pYEX 4T-1 (Clontech, Palo Alto, CA) was modified by the addition of a 140-nucleotide recombination domain, 3′ of its Eco RI site, linearized within the recombination domain by restriction digestion, and cotransformed with a genomic set of reamplified ORFs that had the same ends as the linearized plasmid [J. R. Hudson Jr. *et al.*, *Genome Res.* **7**, 1169 (1997)] into strain EJ 758 [*MAT***a** *his3-Δ200*, *leu2-3,112*, *ura3-52*, *pep4::URA3*], a derivative of JHRY-20-2Ca (*5*). Transformants obtained on synthetic minimal (SD) − Ura drop-out plates [F. Sherman, *Methods Enzymol.* **194**, 3 (1991)] (>100 in all cases, and more than five times the cut vector in 97% of the cases) were eluted in batch and saved in 96-well microtiter plates. The library contains 6080 ORF-containing strains and 64 strains with vector only.
3. Cell patches were inoculated in SD − Ura liquid medium, grown overnight, reinoculated, and grown overnight in SD − Ura − Leu medium, and then inoculated into 250 ml of SD − Ura − Leu medium, grown to absorbance at 600 nm of 0.8, and induced with 0.5 mM copper sulfate for 2 hours before harvest [I. G. Macreadie, O. Horaitis, A. J. Verkuylen, K. W. Savin, *Gene* **104**, 107 (1991)]. Cells were resuspended in 1 ml of buffer [50 mM tris-HCl (pH 7.5), 1 mM EDTA, 4 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 10% glycerol, and 1 M NaCl] containing leupeptin (2 μg/ml) and pepstatin (1 μg/ml), and extracts were made with glass beads [S. M. McCraith and E. M. Phizicky, *Mol. Cell. Biol.* **10**, 1049 (1990)], followed by supplementation with 1 mM phenylmethylsulfonyl fluoride and centrifugation. GST-ORF fusion proteins were purified by glutathione agarose chromatography in buffer containing 0.5 M NaCl, essentially as described [J. R. Nelson, C. W. Lawrence, D. C. Hinkle, *Science* **272**, 1646 (1996)], followed by dialysis in 20 mM tris-HCl, 2 mM EDTA, 4 mM $MgCl_2$, 1 mM DTT, 55 mM NaCl, and 50% (v/v) glycerol and storage at −20°C.
4. C. L. Greer, C. L. Peebles, P. Gegenheimer, J. Abelson, *Cell* **32**, 537 (1983); E. M. Phizicky, R. C. Schwartz, J. Abelson *J. Biol. Chem.* **261**, 2978 (1986); E. M. Phizicky, S. A. Consaul, K. W. Nehrke, J. Abelson, *J. Biol. Chem.* **267**, 4577 (1992).
5. S. M. McCraith and E. M. Phizicky, *J. Biol. Chem.* **266**, 11986 (1991); G. M. Culver, S. M. McCraith, S. A. Consaul, D. R. Stanford, E. M. Phizicky, *J. Biol. Chem.* **272**, 13203 (1997); S. L. Spinelli, S. A. Consaul, E. M. Phizicky, *RNA* **3**, 1388 (1997).
6. G. M. Culver, S. A. Consaul, K. T. Tycowski, W. Filipowicz, E. M. Phizicky, *J. Biol. Chem.* **269**, 24928 (1994).
7. G. M. Culver *et al.*, *Science* **261**, 206 (1993).
8. R. J. DeLange, A. N. Glazer, E. L. Smith, *J. Biol. Chem.* **245**, 3325 (1970).
9. Crude extract from 430 g of strain JHRY-20-2Ca (*5*) was chromatographed successively on Blue Sepharose, heparin agarose, hydroxyapatite, and Orange A Sepharose to purify cyclic phosphodiesterase activity 4000-fold in relation to crude extract.
10. C. L. Peebles, P. Gegenheimer, J. Abelson, *Cell* **32**, 525 (1983); C. R. Trotta *et al.*, *Cell* **89**, 849 (1996).
11. S. L. Spinelli, R. Kierzek, D. H. Turner, E. M. Phizicky, *J. Biol. Chem.* **274**, 2637 (1999).
12. H. H. Liao and F. Sherman, *J. Bacteriol.* **138**, 853 (1979); H. Morino, T. Kawamoto, M. Miyake, Y. Kakimoto, *J. Neurochem.* **48**, 1201 (1987).

13. We thank M. Dumont, M. Gorovsky, and L. Tabak for comments and B. Pinsky for assistance. Supported by the University of Rochester Medical School and the Merck Genome Research Institute (grant 196 to E.M.P. and E.J.G.), NIH (grant GM52347 to E.M.P.), American Cancer Society (grant RPG-95-049-05-MBC to E.J.G.), the National Center for Research Resources (grant P41 RR11823 to S.F.), and the Merck Genome Research Institute (to S.F.). S.F. is an investigator of the Howard Hughes Medical Institute.

26 July 1999; accepted 23 September 1999

Nongenomic Transmission Across Generations of Maternal Behavior and Stress Responses in the Rat

Darlene Francis, Josie Diorio, Dong Liu, Michael J. Meaney*

In the rat, variations in maternal care appear to influence the development of behavioral and endocrine responses to stress in the offspring. The results of cross-fostering studies reported here provide evidence for (i) a causal relationship between maternal behavior and stress reactivity in the offspring and (ii) the transmission of such individual differences in maternal behavior from one generation of females to the next. Moreover, an environmental manipulation imposed during early development that alters maternal behavior can then affect the pattern of transmission in subsequent generations. Taken together, these findings indicate that variations in maternal care can serve as the basis for a nongenomic behavioral transmission of individual differences in stress reactivity across generations.

Individual differences in personality traits appear to be transmitted from parents to offspring. A critical question, however, concerns the mode of inheritance. Concordance studies with mono- and dizygotic twins have provided evidence for a genetic mechanism of transmission even of complex traits (*1*). In addition, parental behavior influences the development of the offspring (*2*) and could therefore serve as a mechanism for a nongenomic behavioral mode of inheritance. In the Norway rat (*Rattus norvegicus*), variations in maternal care are associated with the development of individual differences in behavioral and endocrine responses to stress in the offspring (*3, 4*). In the studies reported here we have examined the possibility that such variations in maternal care might be the mechanism for a behavioral transmission of individual differences across multiple generations.

Mother-pup contact in the rat occurs primarily within the context of a nest bout that begins when the mother approaches the litter, gathers the pups under her, licks/grooms her pups, and nurses her offspring while continuing to occasionally lick/groom the pups, and terminates when the mother leaves the nest (*5*). Naturally occurring variations in maternal licking/grooming and arched-back nursing (LG-ABN) have been associated with the development of individual differences in hypothalamic-pituitary-adrenal (HPA) and behavioral responses to stress in the offspring (*3, 4*). As adults, the offspring of high LG-ABN mothers are behaviorally less fearful and show more modest HPA responses to stress than do the offspring of low LG-ABN mothers. The variation in maternal behavior may thus constitute a mechanism for the nongenomic behavioral transmission of fearfulness from parent to offspring. Alternatively, of course, the differences in fearfulness and those in maternal behavior may both be associated with a common genotype so that the observed continuity of individual differences from mother to offspring is mediated by a genomically based pattern of inheritance.

We found that the female offspring of high LG-ABN mothers showed significantly increased licking/grooming of pups in comparison with those of low LG-ABN mothers (12.9 ± 1.0 versus 6.9 ± 1.1; $P < 0.001$) (*6*),

Developmental Neuroendocrinology Laboratory, Douglas Hospital Research Centre, Departments of Psychiatry and of Neurology and Neurosurgery, McGill University, Montreal, H4H 1R3, Canada.

*To whom correspondence should be addressed. E-mail: mdmm@musica.mcgill.ca

which suggests that individual differences in maternal behavior are transmitted across generations. To determine the mode of transmission we performed a cross-fostering study with the offspring of high and low LG-ABN mothers (*7*). A primary concern here was that the wholesale fostering of litters between mothers is known to affect maternal behavior (*8*). To avoid this problem and maintain the original character of the host litter, no more than 2 of 12 pups were fostered into or from any one litter (*7*). The control groups included (i) the offspring of low LG-ABN mothers fostered to other low LG-ABN mothers as well as offspring of high LG-ABN mothers fostered to other high LG-ABN mothers, (ii) sham-adoption animals, which were simply removed from the nest and fostered back to their biological mothers, and (iii) unmanipulated pups of high or low LG-ABN mothers. The limited cross-fostering design did not affect group differences in maternal behavior. The frequency of pup licking/grooming (Fig. 1A) and arched-back nursing across all groups of high LG-ABN mothers was significantly greater than that for any of the low LG-ABN dams, regardless of litter composition.

The biological female offspring of low LG-ABN dams reared by high LG-ABN mothers were significantly less fearful under conditions of novelty (*9*) than were any of the female offspring reared by low LG-ABN mothers, including the biological offspring of high LG-ABN mothers (Fig. 1B). This was also observed for male offspring (*10*). A separate group of female offspring was then mated, allowed to give birth, and observed for differences in maternal behavior (*6*). The effect on maternal behavior followed the same pattern as that for differences in fearfulness. As adults, the female offspring of low LG-ABN dams reared by high LG-ABN mothers did not differ from normal, high LG-ABN offspring in the frequency of pup licking/grooming (Fig. 1C) or arched-back nursing (*10*). The frequency of licking/grooming and arched-back nursing in animals reared by high LG-ABN mothers was significantly higher than in any of the low LG-ABN groups, including female pups originally born to high LG-ABN mothers but reared by low LG-ABN dams.

Postnatal handling of pups is known to increase the frequency of maternal licking/grooming and arched-back nursing (*11*) and to decrease the response to stress in the offspring (*12*). Postnatal handling should alter the phenotype of the low LG-ABN offspring, and the behavioral transmission hypothesis would suggest that these effects should then be transmitted to the next generation. To see whether an experimental manipulation that alters maternal behavior would influence the transmission of these individual differences in behavior in subsequent generations, female offspring (F_1) of high or low LG-ABN mothers were mated (*6*), and the pups (F_2) in one-half of the litters in each group were exposed daily to brief sessions of handling (*11*). The female offspring of high LG-ABN mothers showed significantly more licking/grooming (Fig. 2A) and arched-back nursing than did the offspring of low LG-ABN mothers. Thus, as observed in our earlier study, individual differences in maternal behavior were transmitted across generations. The handling of the pups significantly increased the frequency of maternal licking/grooming and arched-back nursing in the offspring of low LG-ABN mothers but had no effect on the offspring of high LG-ABN mothers (Fig. 2A). Thus, the effects of maternal behavior of the low LG-ABN mothers with handled pups was indistinguishable from that of the high LG-ABN mothers. The maternal behavior of the adult female offspring (F_2) showed the same pattern (Fig. 2A), and this result is consistent with the transmission of individual differences in maternal behavior across generations. As adults, the handled female offspring of low LG-ABN mothers did not differ from the offspring of high LG-ABN dams in the frequency of maternal licking/grooming and arched-back nursing.

The next question concerned the effective transmission of the individual differences in behavioral responses to stress in the unmanipulated offspring (F_3) of these females (F_2). The level of fearfulness under conditions of novelty in the male or female offspring of handled, low LG-ABN mothers, which did not differ from high LG-ABN mothers in measures of maternal behavior, was comparable to that of the offspring of high LG-ABN mothers (Fig. 2B). The postnatal handling results suggest that environmental events that affect maternal behavior can alter the pattern of transmission of individual differences in stress reactivity and maternal behavior from one generation to the next.

The effects of variation in maternal care on the development of stress reactivity are mediated by changes in the levels of expression of specific genes in brain regions that regulate behavioral and endocrine responses to stress (*3, 4, 13*). In comparison to the offspring of low LG-ABN mothers, the adult offspring of high LG-ABN dams showed increased hippocam-

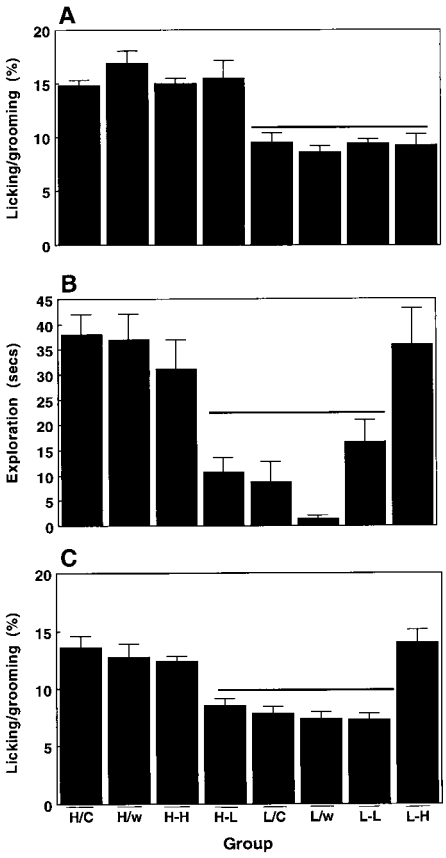

Fig. 1. (**A**) Mean ± SEM percentage frequency of licking/grooming in high LG-ABN and low LG-ABN mothers (n = 6 to 8 per group), collapsed over the first 10 days postpartum in the adoption study (*6, 7*). The biological offspring of high LG-ABN or low LG-ABN mothers were (i) left undisturbed with their mothers, high/control (H/C) and low/control (L/C); (ii) cross-fostered back onto their own mothers, high/w (H/w) and low/w (L/w); (iii) cross-fostered to mothers of the same group, high-high (H-H) and low-low (L-L); and (iv) cross-fostered across groups, high-low (H-L) and low-high (L-H). No more than two pups were cross-fostered from any one litter. The ANOVA revealed a significant group effect (F = 12.67; P < 0.0001). Post-hoc analysis revealed that the frequency of licking/grooming was significantly higher in each of the high LG-ABN groups as compared to any one of the low LG-ABN groups (P < 0.05; differences are indicated by a solid horizontal line). (**B**) Mean ± SEM time in seconds spent in the inner area of a novel open field (exploration) (*9*) in the adult female offspring from the cross-fostering study (n = 6 to 8 per group). The ANOVA revealed a significant effect (F = 3.37; P < 0.05) of the mother, a significant effect of cross-fostering (F = 11.88; P < 0.0001) and a significant mother × cross-fostering interaction effect (F = 7.39; P < 0.001). Post-hoc analysis revealed that the time spent in inner area exploration was significantly (P < 0.01) higher in the biological offspring of low LG-ABN mothers reared by high LG-ABN mothers (L-H) than in the offspring of high LG-ABN mothers reared by low LG-ABN mothers (H-L). Groups lying below the solid line differ significantly from those above the line. (**C**) Mean ± SEM percentage frequency of licking/grooming, collapsed over the first 10 days postpartum in the adult female offspring from the cross-fostering study (n = 5 to 7 per group). The ANOVA revealed a significant effect (F = 26.28; P < 0.0001) of the mother, a significant effect of cross-fostering (F = 13.56; P < 0.0001) and a significant mother × cross-fostering interaction effect (F = 8.13; P < 0.001). Post-hoc analysis revealed that the frequency of maternal licking/grooming was significantly (P < 0.001; solid line) higher in the biological offspring of low LG-ABN mothers reared by high LG-ABN mothers (L-H) than in offspring of high LG-ABN mothers reared by low LG-ABN mothers (H-L).

pal glucocorticoid receptor (GR) mRNA expression, increased central benzodiazepine (CBZ) receptor levels in the central and basolateral nuclei of the amygdala, and decreased corticotropin-releasing factor (CRF) mRNA in the paraventricular nucleus of the hypothalamus (PVNh) (*3, 4*). As adults, the offspring of handled, low LG-ABN mothers showed hippocampal GR mRNA levels that were comparable to those observed in the offspring of either handled (H) or nonhandled (NH) high LG-ABN mothers and were significantly higher than those in the offspring of NH/LG-ABN females (Fig. 2, C and D) (*14*). Moreover, the offspring of the H/low LG-ABN females showed significantly reduced CRF mRNA levels in the paraventricular nucleus of the hypothalamus in comparison to the offspring of the NH/low LG-ABN mothers (Fig. 2, C and E) (*14*). CRF mRNA levels in these animals were comparable to those of the offspring of H or NH high LG-ABN mothers. In previous studies, we also found that the offspring of high LG-ABN mothers show increased CBZ receptor binding in the amygdala in comparison with the offspring of low LG-ABN mothers (*3, 4*). As expected, the adult offspring of H/low LG-ABN mothers showed CBZ receptor levels in the central and basolateral nuclei of the amygdala that were comparable to those observed in the offspring of either H or NH high LG-ABN mothers and were significantly

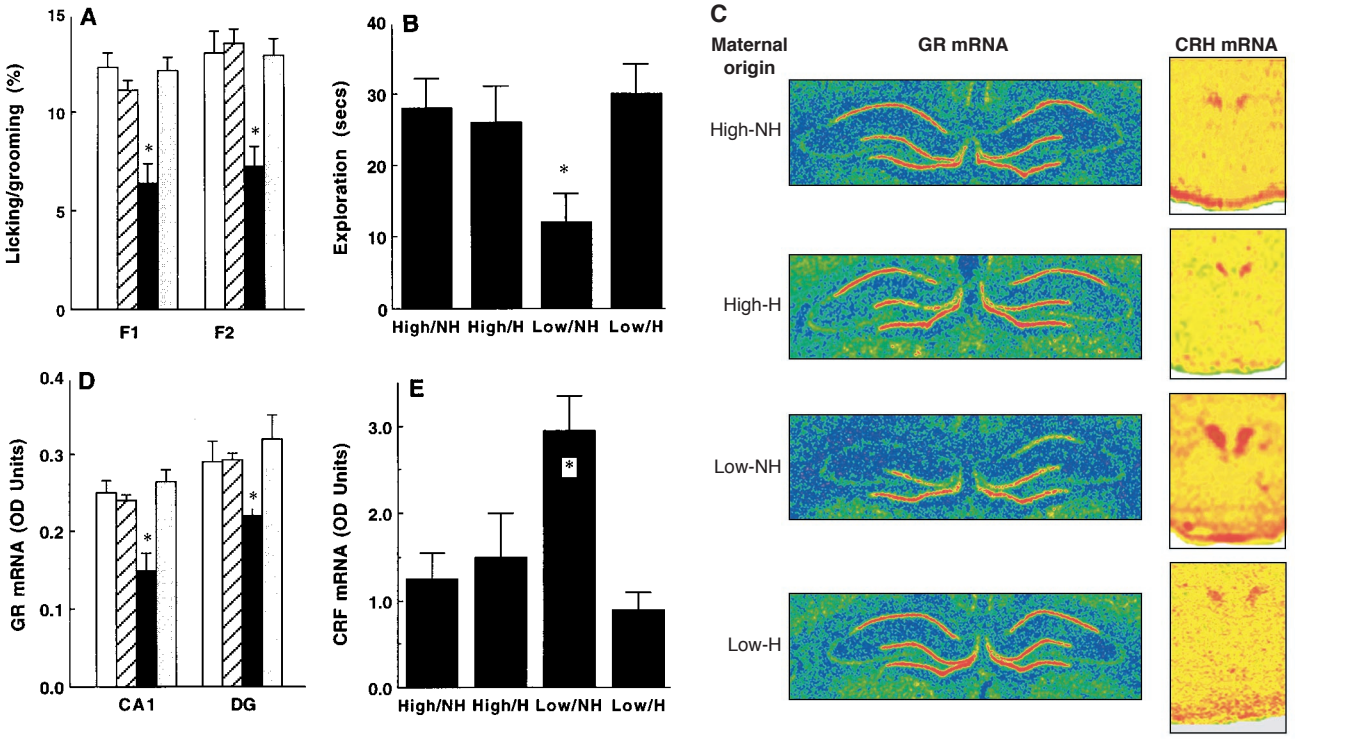

Fig. 2. (**A**) Mean ± SEM frequency (as a percentage of total observations) of licking/grooming, collapsed over the first 10 days postpartum in high and low LG-ABN mothers (F_1), with handled (H) or nonhandled (NH) pups (n = 5 to 7 per group). The ANOVA revealed a significant group × pup treatment interaction effect (F = 7.67; df = 1, 19; $P < 0.01$). Post-hoc analysis showed that low LG-ABN mothers with nonhandled offspring showed significantly (*, $P < 0.01$) less licking/grooming than any other group, including low LG-ABN mothers with handled offspring. The same group × pup treatment interaction effect (F = 9.78; df = 1, 24; $P < 0.001$) in pup licking/grooming was apparent in the lactating female offspring (F_2) of these mothers. Open bar, High-NH; striped bar, High/H; black bar, Low/NH; gray bar, Low/H. (**B**) Mean ± SEM time in seconds spent in the inner area of a novel open field (exploration) (*9*) in the unmanipulated adult female offspring (F_3) of H or NH, high or low LG-ABN (F_2) mothers (n = 8 to 10 per group). The ANOVA revealed a significant group effect (F = 3.39; df = 3, 31; $P < 0.05$). Post-hoc analysis revealed that the time spent in inner area exploration was significantly lower in the offspring of the low LG-ABN/NH animals than in any other group (*, $P < 0.05$). (**C**) A pseudocolor image of representative sections showing relevant brain regions from in situ hybridization studies examining GR mRNA expression in the dorsal hippocampus and CRF mRNA expression in the PVNh in the unmanipulated adult female offspring (F_3) of high LG-ABN/NH, high LG-ABN/H, low LG-ABN/NH, and low LG-ABN/NH (F_2) mothers (n = 4 per group). (**D**) Mean ± SEM levels of GR mRNA (arbitrary optical density units using [^{35}S]-labeled standards) (*14*) in Ammon's Horn (CA1) and the dentate gyrus (DG) in the unmanipulated adult female offspring (F_3) of high LG-ABN/NH, high LG-ABN/H, low LG-ABN/NH, and low LG-ABN/NH (F_2) mothers (n = 4 per group). The two-way ANOVA (group × region) revealed a significant group effect (F = 7.74; df = 3, 12; $P < 0.01$). Post-hoc analysis showed that for both the DG ($P < 0.05$) and the CA1 ($P < 0.002$) regions, GR mRNA levels were significantly lower (*) in the offspring of the low LG-ABN/NH animals than in any other group. Bar shading is the same as in (A). (**E**) Mean ± SEM levels of CRF mRNA (arbitrary optical density units using [^{35}S]-labeled standards) (*14*) in the PVNh in the unmanipulated adult female offspring (F_3) of high LG-ABN/NH, high LG-ABN/H, low LG-ABN/NH, and low LG-ABN/NH (F_2) mothers (n = 4 to 5 per group). The ANOVA revealed a significant group effect (F = 4.11; df = 3, 15; $P < 0.05$). Post-hoc analysis revealed that in both regions mRNA levels were significantly highly in the offspring of the low LG-ABN/NH animals than in any other group (*, $P < 0.05$). (**F**) Mean ± SEM levels of CBZ receptor binding (femtomoles per milligram protein) (*15*) in the basolateral nucleus (BLnA) and central nucleus (CnA) regions of the amygdala and the ventromedial nucleus of the hypothalamus (VMH) of unmanipulated, adult female offspring (F_3) of high LG-ABN/NH, high LG-ABN/H, low LG-ABN/NH, and low LG-ABN/NH (F_2) mothers (n = 4 per group). The two-way ANOVA (group × region) revealed a marginal group effect (F = 3.04; df = 3, 12; $P < 0.10$) and, more important, a significant group × region interactions effect (F = 3.18; df = 6, 24; $P < 0.02$). Post-hoc analysis showed that for both the basolateral ($P < 0.05$) and the central ($P < 0.002$) regions of the amygdala, CBZ receptor levels were significantly (*, $P < 0.05$) lower in the offspring of the low LG-ABN/NH animals than in any other group. Bar shading is the same as in (A).

higher than those in the offspring of NH/LG-ABN females (Fig. 2F) (*15*).

These findings suggest that individual differences in the expression of genes in brain regions that regulate stress reactivity can be transmitted from one generation to the next through behavior. The studies of Denenberg (*16*) in rodents suggested that individual differences in behavioral fearfulness to novelty could be transmitted from parent to offspring through a nongenomic mechanism of inheritance. The results of the present study support this idea and suggest that the mechanism for this pattern of inheritance involves differences in maternal care during the first week of life. In humans, social, emotional, and economic contexts influence the quality of the relationship between parent and child (*17*) and can show continuity across generations (*18*). Our findings in rats may thus be relevant in understanding the importance of early intervention programs in humans.

References and Notes

1. R. Plomin, J. C. De Fries, G. E. McClearn, M. Rutter, *Behavioral Genetics* (Freeman, New York, ed. 3, 1997).
2. J. Bowlby, *Maternal Care and Mental Health* (World Health Organization, Geneva, 1951); H. F. Harlow, *Am. Psychol.* **13**, 673 (1958); S. J. Suomi, *Br. Med. Bull.* **53**, 170 (1997); M. Rutter, *Am. Psychol.* **52**, 603 (1997); S. Levine and E. B. Thoman, in *Postnatal Development of Phenotype*, S. Kazda and V. H. Denenberg, Eds. (Academia, Prague, 1970).
3. D. Liu *et al.*, *Science* **277**, 1659 (1997).
4. C. Caldji *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 5335 (1998).
5. J. R. Alberts and C. P. Cramer, in *Handbook of Behavioral Neurobiology*, vol. 9, E. M. Blass, Ed. (Plenum, New York, 1989); J. S. Rosenblatt, *Acta Paediatr. Suppl.* **397**, 3 (1994); J. M. Stern, *Dev. Psychobiol.* **31**, 19 (1997).
6. The animals were derived from Long-Evans hooded rats obtained from Charles River Canada (St. Constant, Québec), mated with males drawn randomly from our colony breeding stock, and maintained under previously described conditions (*3, 4*). In cases where the offspring of high or low LG-ABN mothers were used in studies, no more than two animals per group were drawn from any single mother. All procedures were performed according to guidelines developed by the Canadian Council on Animal Care and the protocol was approved by the McGill University Animal Care Committee. Mothers and their litters were housed in 46 cm by 18 cm by 30 cm Plexiglas cages and maternal behavior was scored [M. M. Myers, S. A. Brunelli, H. N. Shair, J. M. Squire, M. A. Hofer, *Dev. Psychobiol.* **22**, 55 (1989) and (*3, 4*)] for six 100-min observation periods daily for the first 10 days postpartum by individuals unaware of the origin of the animals. The following behaviors were scored: mother off pups; mother licking/grooming any pup; and mother nursing pups in either an arched-back posture, a "blanket" posture (in which the mother lies over the pups), or a passive posture (in which the mother is lying either on her back or side). The data were analyzed as the percentage of total observations (frequency per total observations × 100) in which animals engaged in the target behavior (*3, 4*). In order to define populations, we observed the maternal behavior in a cohort of 32 mothers and devised the group mean and standard deviation for each behavior over the first 10 days of life. High LG-ABN mothers were defined as females whose frequency scores for both licking/grooming and arched-back nursing were greater than 1 SD above the mean. Low LG-ABN mothers were defined as females whose frequency scores for both licking/grooming and arched-back nursing were more than 1 SD below the mean. As in our previous reports (*3, 4*), the frequency of licking/grooming and arched-back nursing were highly correlated ($r > +0.90$). The adult female offspring of high and low LG-ABN dams were then mated and observed for maternal behavior with the use of the same procedures described above over the first 10 days postpartum. As previously reported (*3, 4*), there were no differences in the percentage of total observations in which the offspring of high or low LG-ABN mothers were observed to be in contact with their pups (53 ± 5 versus 51 ± 4%; NS). Variations in licking/grooming or arched-back nursing were not related to differences in time spent with pups.
7. R. McCarty and J. H. Lee, *Physiol. Behav.* **59**, 71 (1996). Female offspring of high or low LG-ABN dams were mated and allowed to give birth. Within 12 hours of birth, dams were removed from the home cage and two animals per litter were cross-fostered. The cross-fostered pups, along with two native pups, were labeled with a permanent marker (Codman pens, Johnson & Johnson) until day 10 of life and were identified by individual differences in the pattern of pelage pigmentation thereafter. All litters were culled to 12 pups. Subsequent studies showed that marking the pups had no effect on maternal licking/grooming; marked pups are licked/groomed no more or less frequently than unmarked pups (*10*). The dam was returned once the foster pups were introduced into the new litter. The entire procedure took less than 15 min. The data were analyzed using a two-way analysis of variance (ANOVA) (mother × cross-fostering condition).
8. S. Maccari *et al.*, *J. Neurosci.* **15**, 110 (1995).
9. Fearfulness under conditions of novelty was studied by means of an open-field test of exploration as previously described (*4*). Animals were placed one at a time, in a novel, circular open field 1.6 m in diameter for 5 min. The critical measure of exploration was the time (*s*) spent in the inner area of the novel arena (that is, entire body of the animal being >10 cm away from any wall (>10 cm) enclosing the open field.
10. D. Francis and M. J. Meaney, unpublished data.
11. The handling procedure involved removing the mother and then pups from their cage, placing the pups together in a small container, and returning the animals and their mothers to their cage 15 min later. The manipulation was performed daily for the first 14 days of life, and the animals were tested as fully mature adults. Nonhandled animals were left completely undisturbed until day 12 of life, at which time normal cage maintenance was initiated. Mothers of handled pups consistently showed an increased frequency of maternal licking/grooming [M. H. S. Lee and D. I. Williams, *Anim. Behav.* **22**, 679 (1974); S. A. Barnett and J. Burn, *Nature* **213**, 150 (1967); S. Levine, in *Society, Stress and Disease*, L. Levi, Ed. (Oxford Univ. Press, London, 1975); W. P. Smotherman and R. W. Bell, in *Maternal Influences and Early Behavior*, R. W. Bell and W. P. Smotherman, Eds. (Spectrum, New York, 1980); M. B. Hennessy, J. Vogt, S. Levine, *Physiol. Psychol.* **10**, 153 (1982); (*3*).
12. S. Levine, *Science* **135**, 795 (1962); ———, G. C. Haltmeyer, G. G. Karas, V. H. Denenberg, *Physiol. Behav.* **2**, 55 (1967); J. L. Hess, V. H. Denenberg, M. X. Zarrow, W. D. Pfeifer, *Physiol. Behav.* **4**, 109 (1969); M. X. Zarrow, P. S. Campbell, V. H. Denenberg, *Proc. Soc. Exp. Biol. Med.* **356**, 141 (1973); M. J. Meaney, D. H. Aitken, Ch. Van Berkel, S. Bhatnagar, R. M. Sapolsky, *Science* **239**, 766 (1988); M. J. Meaney, D. H. Aitken, S. Sharma, V. Viau, A. Sarrieau, *Neuroendocrinology* **5**, 597 (1989); M. Vallée *et al.*, *J. Neurosci.* **17**, 2626 (1993); S. Bhatnagar, N. Shanks, M. J. Meaney, *J. Neuroendocrinol.* **7**, 107 (1995).
13. A. J. Dunn and C. W. Berridge, *Brain Res. Rev.* **15**, 71 (1990); M. J. Owens and C. B. Nemeroff, *Pharmacol. Rev.* **43**, 425 (1991); P. M. Plotsky, *J. Neuroendocrinol.* **3**, 1 (1991); M. F. Dallman *et al.*, *J. Neuroendocrinol.* **4**, 517 (1993); G. F. Koob, S. C. Heinrichs, F. Menzaghi, E. M. Pich, K. T. Britton, *Semin. Neurosci.* **6**, 221 (1994); S. L. Lightman and M. S. Harbuz, *Ciba Found. Symp.* **172**, 173 (1994); J. Schulkin, B. S. McEwen, P. W. Gold, *Neurosci. Biobehav. Rev.* **18**, 1 (1994); J. P. Herman and W. E. Cullinan, *Trends. Neurosci.* **20**, 78 (1997); J. A. Gray, *The Psychology of Fear and Stress*, (Cambridge Univ. Press, Cambridge, 1987); A. L. Malizia *et al.*, Eds., *GABAA Receptors and Anxiety: From Neurobiology to Treatment* (Raven, New York, 1995); P. Roy-Byrne, D. K. Wingerson, A. Radant, D. J. Greenblatt, D. S. Cowley, *Am. J. Psychiatry* **153**, 1444 (1996).
14. For all in situ hybridization studies, animals were killed under resting-state conditions directly from the home cage. After rapid decapitation, brains were removed and quickly frozen in isopentane maintained on dry ice. Brains were blocked, and 15-μm cryostat sections were mounted onto poly-D-lysine–coated slides, desiccated under vacuum, and stored at −80°C. CRF mRNA in situ hybridization was performed with a [^{35}S]ATP-labeled 48–base pair (bp) oligonucleotide sequence (CAG TTT CCT GTT GCT GTG AGC TTG CTG AGC TAA CTG CTC TGC CCT GGC) obtained from the Sheldon Biotechnology Center (Montréal, Canada) as previously described (*3*). Preparation and description of GR riboprobes as well as the in situ hybridization procedure have been described (*3*). The GR cRNA was transcribed from a 674-bp Pst I–Eco RI fragment of the rat GR cDNA (steroid binding domain, R. Meisfield, University of Arizona), linearized with Ava I, and transcribed with T7 RNA polymerase. The hybridization signal within the parvocellular subregion of the PVNh (CRF mRNA) or the dorsal hippocampus (GR mRNA) was quantified by means of densitometry with an image analysis system (MCID, Imaging Research, Inc., St. Catherines, Ontario). The data are presented as arbitrary optical density (absorbance) units after correction for background. The anatomical level of analysis was verified with the Paxinos and Watson rat brain atlas (*19*) and Nissl-staining of sections after autoradiography. The hippocampal GR mRNA data were analyzed with a two-way ANOVA (group × region). The CRF mRNA data were analyzed with a one-way ANOVA (group).
15. CBZ receptor binding was measured with in vitro receptor autoradiography as previously described [M. H. Bureau and R. W. Olsen, *J. Neurochem.* **61**, 1479 (1993)]. Brain sections were prepared as described above (*14*) and incubated with [^{3}H]flunitrazepam (84.5 Ci/mmol, New England Nuclear, Boston, MA), with or without 1 μM clonazepam. The sections were left to dry overnight and were then apposed to ^{3}H-sensitive Ultrafilm (Amersham Canada, Montréal, Canada) along with ^{3}H microscales for 14 days. Autoradiograms were analyzed by obtaining optical densities (expressed as mean ± SEM in femtomoles per milligram of protein) that were determined with computer-assisted densitometry using an MCID image analysis system and low activity ^{3}H standards of (*19*). Autoradiographic data were analyzed with a two-way ANOVA (group × region).
16. V. H. Denenberg and K. M. Rosenberg, *Nature* **216**, 549 (1967); V. H. Denenberg, *Psychol. Rev.* **71**, 335 (1964).
17. L. Eisenberg, in *Mammalian Parenting: Biochemical, Neurobiological, and Behavioral Determinants*, N. A. Krasnegor and R. S. Bridges, Eds. (Oxford Univ. Press, New York, 1990); T. Field, *Prev. Med.* **27**, 200 (1998); A. S. Fleming, *Psychoneuroendocrinology* **13**, 189 (1988).
18. M. H. Van Ijzendoorn, *Dev. Rev.* **12**, 76 (1992); K. E. Grossman and K. Grossman, in *Attachment Across the Life Cycle*, C. M. Parkes, J. Stevenson-Hinde, P. Marris, Eds. (Routledge, New York, 1993); R. J. Noone, *Fam. Sys.* **2**, 116 (1995); L. M. Fairbanks, *Adv. Study Behav.* **25**, 579 (1996); L. Miller, R. Kramer, V. Warner, P. Wickramaratne, M. Weissman, *J. Am. Acad. Child Adolesc. Psychiatry* **36**, 1134 (1997).
19. G. Paxinos and D. Watson, *The Rat Brain in Stereotaxic Coordinates* (Academic, New York, 1982).

20. Supported by grants from the Medical Research Council of Canada (MRCC) and the National Institute of Mental Health to M.J.M., as well as an MRCC graduate fellowship (D.L.) and an MRCC Senior Scientist award (M.J.M.). The authors thank B. S. McEwen and M. Hofer for comments on an earlier draft of this paper.

24 June 1999; accepted 23 September 1999