

Attempts to segregate malformed chromosomes, if they lag on the central spindle, might prolong or prevent cytokinesis. But it is difficult to explain delayed abscission, abnormal myosin II organization, or the mitotic localization pattern of BRCA2 on this basis.

Besides structurally aberrant chromosomes, primary cultures of BRCA2-deficient cells accumulate with 4*N* and greater DNA content during successive passage, consistent with exit from mitosis without cytokinesis (3). Also, aneuploidy often occurs in cancers from BRCA2 mutation carriers (2) and might, as in sporadic cancers, predict poor clinical outcomes. Our findings suggest that both these phenotypes may arise from the inactivation of previously unrecognized functions of BRCA2 in cytokinesis, which is a possible link between cytokinetic abnormalities and the pathogenesis of a human genetic disease as-

sociated with chromosomal instability and cancer predisposition.

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21. We thank J. Pines and L.K. Ferrigno (Cambridge) for helpful comments on this paper. M.J.D. received an AstraZeneca studentship through the Cambridge University M.B., Ph.D. program. Work in A.R.V.'s laboratory is supported by Cancer Research UK and the Medical Research Council.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1102574/DC1
Materials and Methods
SOM Text
Figs. S1 to S4
References and Notes

9 July 2004; accepted 7 September 2004

Published online 16 September 2004;

10.1126/science.1102574

Include this information when citing this paper.

Early-Life Blockade of the 5-HT Transporter Alters Emotional Behavior in Adult Mice

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Reduced serotonin transporter (5-HTT) expression is associated with abnormal affective and anxiety-like symptoms in humans and rodents, but the mechanism of this effect is unknown. Transient inhibition of 5-HTT during early development with fluoxetine, a commonly used serotonin selective reuptake inhibitor, produced abnormal emotional behaviors in adult mice. This effect mimicked the behavioral phenotype of mice genetically deficient in 5-HTT expression. These findings indicate a critical role of serotonin in the maturation of brain systems that modulate emotional function in the adult and suggest a developmental mechanism to explain how low-expressing 5-HTT promoter alleles increase vulnerability to psychiatric disorders.

5-HTT appears to be a critical regulator of emotional function. It is the primary molecular target for many antidepressants, especially the serotonin selective reuptake inhibitors (SSRIs), which are used as a first-line treatment for a number of psychiatric conditions (1). SSRIs increase serotonergic tone, and this effect is thought to mediate their therapeutic actions.

A genetic variant that reduces expression of 5-HTT has been associated with elevated levels of neuroticism, anxiety-like traits, and depressive symptoms in some (2–4) but not

all studies (5). A study including environmental factors in its analysis demonstrated that individuals with one or two copies of the low-expressing 5-HTT allele are more prone to depression and suicidality only after childhood or adulthood stressors (6). Such a gene-environment interaction may explain the variability between studies.

Mice lacking the 5-HTT gene (5-HTT^{-/-}) also exhibit increased depression- and anxiety-related behaviors (7, 8). The emotional and behavioral abnormalities produced by genetically reduced 5-HTT function are paradoxical because in a mature organism, long-term treatments with SSRI antidepressants also produce a reduction of 5-HTT function, yet these agents act to ameliorate anxiety- and depression-related symptoms.

Because 5-HT acts as a trophic factor modulating developmental processes such as neuronal division, differentiation, migration,

and synaptogenesis (9), we hypothesized that the divergent effects of adult pharmacologic and lifelong genetic inhibition of 5-HTT function may be explained by events occurring during early brain maturation (10). Thus, we investigated whether we could mimic the effect of genetic 5-HTT disruption by briefly inhibiting 5-HTT function between postnatal days 4 and 21 (P4 and P21) with the use of the SSRI fluoxetine (FLX) in mice.

Mice heterozygous for the 5-HTT mutation (5-HTT^{+/-}) were crossed to produce a Mendelian mix of 5-HTT^{+/+}, 5-HTT^{+/-}, and 5-HTT^{-/-} offspring (11). Mixed litters were randomly assigned to either saline or FLX (10 mg/kg, intraperitoneally) treatments beginning on P4 and lasting until P21. This design allowed us to directly compare the behavioral effects of transient pharmacological 5-HTT inhibition and constitutive disruption of the 5-HTT gene.

We chose to use FLX to pharmacologically block 5-HTT function because of its common use in humans and its extended half-life. Our dosing regimen produced therapeutically relevant blood levels (FLX: 360 ± 123 ng/ml; norfluoxetine: 708 ± 168 ng/ml) and had no gross effects on viability or growth (fig. S1, A and B). Although FLX has high selectivity for 5-HTT, it is reported to exhibit weak activity at other transporter and receptor sites (12). The specificity of FLX was monitored in 5-HTT^{-/-} mice because these mice allowed us to distinguish between 5-HTT-mediated and 5-HTT-independent effects of FLX.

Starting at 12 weeks of age (9 weeks after the last injection of FLX), we tested mice in the open field and in the elevated plus-maze. In comparison to saline-treated pups, postnatal-FLX (PN-FLX) treatment decreased exploratory behavior in both 5-HTT^{+/+} and 5-HTT^{+/-}

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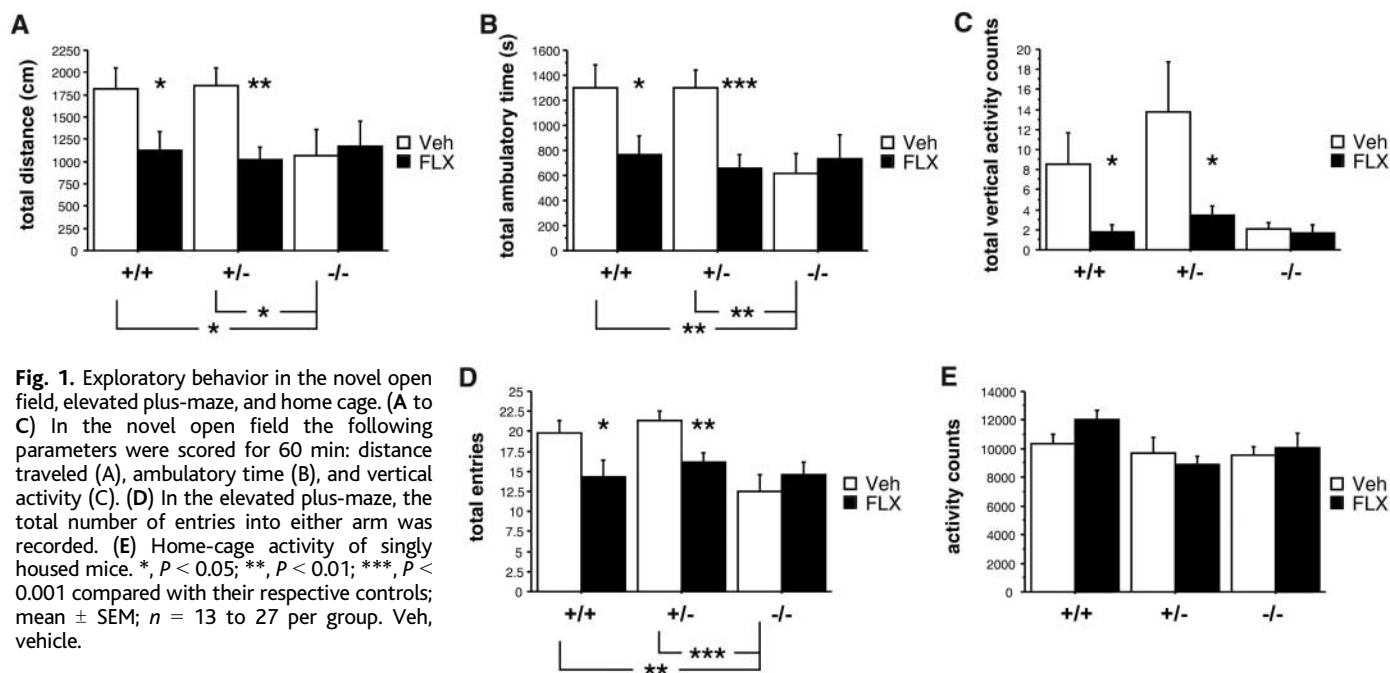


Fig. 1. Exploratory behavior in the novel open field, elevated plus-maze, and home cage. (A to C) In the novel open field the following parameters were scored for 60 min: distance traveled (A), ambulatory time (B), and vertical activity (C). (D) In the elevated plus-maze, the total number of entries into either arm was recorded. (E) Home-cage activity of singly housed mice. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ compared with their respective controls; mean \pm SEM; $n = 13$ to 27 per group. Veh, vehicle.

mice, as demonstrated by a reduction in the total distance traveled (Fig. 1A), time spent ambulating (Fig. 1B), and rearing in the open field (Fig. 1C), and a decrease in the total number of arm entries in the elevated plus-maze (Fig. 1D). In support of our hypothesis, PN-FLX-treatment of 5-HTT^{+/+} and 5-HTT^{+/-} mice mimicked the behavior of 5-HTT^{-/-} mice treated with either FLX or vehicle (Fig. 1, A to D). PN-FLX had no effect on 5-HTT^{-/-} mice in these tests, indicating that the effects of FLX were specifically mediated by 5-HTT blockade.

The effect of PN-FLX was specific to exploratory behavior given that we did not detect any differences in measures such as activity in the more aversive portions of these environments (fig. S2). The reduced locomotor activity seen in the open field and the elevated plus-maze was likely related to the novelty of these environments, because no differences in locomotion were seen when mice were assessed in their home cage (Fig. 1E).

To further assess the effect of PN-FLX treatment on adult emotional functioning, we examined their behavior in the novelty-suppressed feeding paradigm. This test is thought to reflect anxiety- and depression-related behaviors because chronic antidepressant administration and anxiolytics reduce the latency to begin feeding (13, 14) and because animal models of depression and anxiety are abnormal in this test (8, 13). Consistent with previous findings (8), 5-HTT^{-/-} mice exhibited longer latencies to begin feeding in this test when compared with vehicle-treated 5-HTT^{+/-} or 5-HTT^{+/+} mice (Fig. 2A). PN-FLX treatment prolonged the latency of 5-HTT^{+/-} mice to the level seen in 5-HTT^{-/-}

mice. Weight loss during food deprivation (Fig. 2B) and food consumption in the home cage (Fig. 2C) were comparable across groups, indicating that the observed differences in latency were not due to motivational factors. The different effect of PN-FLX on the latency of 5-HTT^{+/+} and 5-HTT^{+/-} mice suggests an enhanced sensitivity to pharmacological inhibition in mice with a genetically reduced complement of 5-HTT.

Because the foregoing tests depend on behavior in conflictual situations, we examined the effects of PN-FLX in shock avoidance, a paradigm that assesses behavioral responses to stress. We have previously found that 5-HTT^{-/-} mice exhibit significant impairment in shock avoidance (8), and we replicated that finding here (Fig. 3A). PN-FLX treatment of 5-HTT^{+/-} or 5-HTT^{+/+} mice reproduced the behavioral deficit seen in 5-HTT^{-/-} mice (Fig. 3A). As expected, PN-FLX treatment had no effect on 5-HTT^{-/-} mice, demonstrating that FLX does not produce behavioral effects independent of 5-HTT (Fig. 3A). The shock-escape deficit seen in 5-HTT^{-/-} mice or PN-FLX treated mice was not an artifact of reduced locomotor activity, given that activity during the intershock intervals was comparable across groups (Fig. 3B). This finding suggests that interference with 5-HTT function during early brain development predisposes the organism to maladaptive stress responses.

These findings shed new light on the consequences of early disruption of 5-HTT function on adult emotional behavior. Specifically, the altered behavior of adult mice seen in our study is likely the result of neurodevelopmental perturbations caused by early-

life disruption of 5-HTT function. This conclusion has potentially important implications.

First, these findings support the notion that genetic polymorphisms that reduce 5-HTT expression may exert their effects during early development of the central nervous system (CNS) by altering maturation of circuits that modulate emotional responses to novelty and stress. Such a hypothesis provides a potential explanation for the increased susceptibility of individuals carrying one or two low-expressing 5-HTT alleles to depression in the face of multiple life stressors (6).

Second, our findings may have relevance to the use of SSRI medications during early life. Increasingly, SSRIs are used to treat emotional disorders in children and pregnant women (15). However, the long-term effects of these medications on brain development are largely unknown. The period of development from P4 to P21 in mice corresponds to the events of brain maturation that begin during the third trimester of pregnancy and continue into early childhood. Thus, exposure to SSRI-like antidepressants during this period of development may entail unexpected risks for affective function later in life.

Although animal studies have documented effects of serotonin on craniofacial (16), cardiac (17), and CNS development (18, 19), human studies have shown that prenatal SSRI exposure has no overt teratogenic effects but may increase the risk of premature birth and the occurrence of an SSRI-withdrawal syndrome in the first few days of life (20). Other studies have found that fine-motor skills may be impaired in SSRI-exposed children (21), but no impact on cognitive development has been observed (22, 23). However, these studies have

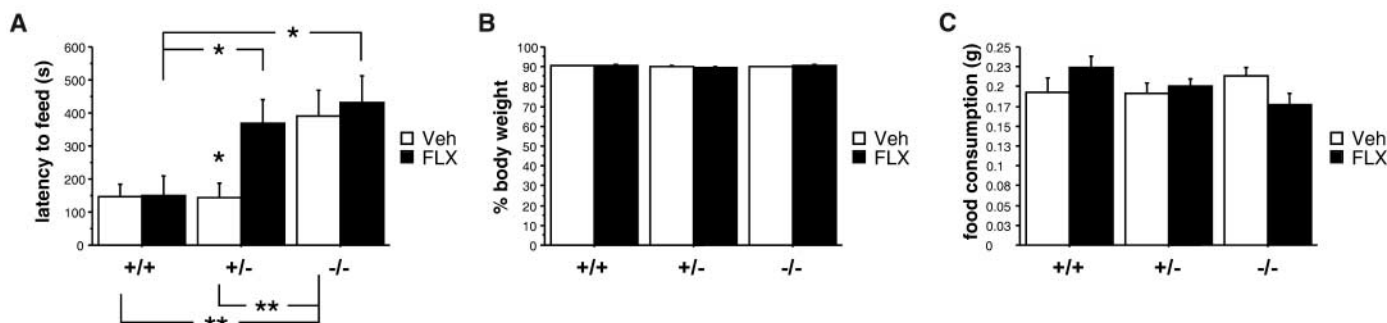


Fig. 2. Novelty-suppressed feeding test. (A) The latency to begin feeding is shown in seconds. (B) Weight loss is expressed as a percentage of free-feeding body weight. (C) Post-test food consumption is shown in grams. *, $P < 0.05$; **, $P < 0.01$ compared to their respective controls; mean \pm SEM; $n = 13$ to 27 per group. Veh, vehicle.

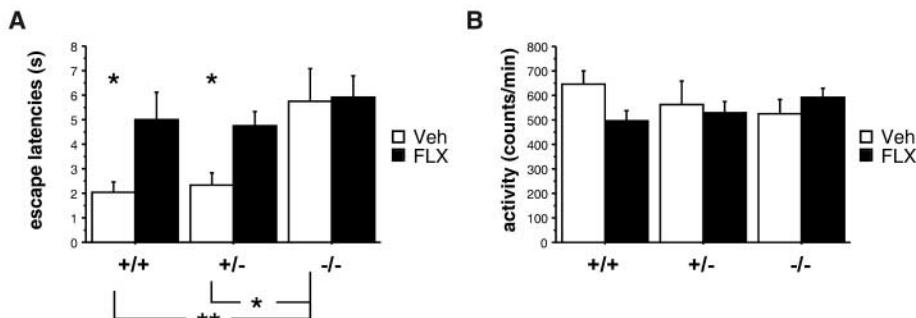


Fig. 3. Shock-escape paradigm. (A) Average latency to escape a foot shock shown in seconds. (B) Total locomotor activity between shocks. *, $P < 0.05$; **, $P < 0.01$ compared with their respective controls; mean \pm SEM; $n = 13$ to 27 per group. Veh, vehicle.

not followed children long enough to determine whether SSRI exposure in utero has the potential to alter emotional function later in life.

Animal studies have also failed to demonstrate teratogenic effects of early SSRI exposure (24). However, inhibition of 5-HTT function has been found to alter brain development in more subtle ways. Genetic inactivation of 5-HTT affects barrel formation in the somatosensory cortex and alters segregation of retinal axons (25, 26). The period between P0 and P4 has been identified as the critical time window during which increased serotonergic tone disrupts barrel-field formation (27). Because the period used in our study started on P4, it is unlikely that such changes in cortical development underlie the behavioral effects of PN-FLX observed in this study.

Mice lacking the 5-HTT gene have reduced dorsal raphé firing rates (8, 28) and fewer serotonergic neurons (8, 29). Similar to 5-HTT^{-/-} mice, rats treated with clomipramine from P8 to P21 were found to exhibit altered emotional behavior as adults (30) and reduced firing rate of raphé neurons (31). Clomipramine is one of the few tricyclic antidepressants that shows a moderate selectivity for 5-HTT over norepinephrine reuptake sites (32), suggesting that these effects may also result from 5-HTT blockade. Thus, alterations in the structure and function of serotonergic nuclei likely contribute to the altered behavioral responses observed in this

study. Other work has shown that developmental or genetic factors affecting anxiety- or depression-related behaviors can alter hippocampal structure (33–35), amygdala function (36, 37), and receptor expression in the prefrontal cortex (38). These structures all receive significant serotonergic innervation and therefore may be affected by presynaptic alterations in serotonergic function.

The present findings demonstrate that 5-HTT function modulates the development of brain systems involved in emotional and stress-related responses. Low-expressing 5-HTT variants may act during development to modify brain circuits or gene expression that predisposes carriers of these alleles to emotional disorders. Likewise, the use of SSRI medications in pregnant mothers and young children may pose unsuspected risks of emotional disorders later in life. Ultimately, careful clinical studies will be required to determine whether our findings have applicability to the risks for psychiatric morbidity in human subjects. Our results may help guide the choice of outcome measures in clinical studies and aid in the identification of molecular and developmental mechanisms that may confer vulnerability to affective and anxiety disorders.

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39. We acknowledge the generous gift of fluoxetine from E. Lilly, the analytical work of T. Cooper, and the helpful comments of F. Menzaghi, J. Gordon, M. Myers, and C. Gross.

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 Materials and Methods
 Figs. S1 and S2
 References

18 June 2004; accepted 26 August 2004