NEURODEVELOPMENT

Early life experience drives structural variation of neural genomes in mice

Tracy A. Bedrosian,* Carolina Quayle, Nicole Novaresi, Fred. H. Gage*

The brain is a genomic mosaic owing to somatic mutations that arise throughout development. Mobile genetic elements, including retrotransposons, are one source of somatic mosaicism in the brain. Retrotransposition may represent a form of plasticity in response to experience. Here, we use droplet digital polymerase chain reaction to show that natural variations in maternal care mediate the mobilization of long interspersed nuclear element–1 (LINE-1 or L1) retrotransposons in the hippocampus of the mouse brain. Increasing the amount of maternal care blocks the accumulation of L1. Maternal care also alters DNA methylation at YY1 binding sites implicated in L1 activation and affects expression of the de novo methyltransferase DNMT3a. Our observations indicate that early life experience drives somatic variation in the genome via L1 retrotransposons.

The brain exhibits plasticity in response to environmental experience, particularly during the first weeks of life. A portion of this plasticity can be attributed to modification of DNA through epigenetic changes such as methylation or chromatin remodeling. However, dynamic neuronal DNA sequences suggest a role for mobilization of retrotransposons or induction of other structural variants in experience-driven brain plasticity (*1*, *2*). We developed Taqman assays for droplet digital polymerase chain reaction (ddPCR) to probe the number of long interspersed nuclear element–1 (L1) retrotransposon copies present in mouse genomic DNA (fig. S1). Although L1 is the most abundant class of retrotransposons, comprising about 17% of human and mouse genomes, most copies are truncated or otherwise mutated such that mobilization is no longer possible. The average human genome retains 80 to 100 active L1 copies; the mouse genome retains more than 3000. We designed our assays to enrich for the currently active families of L1 in the mouse genome [L1MdT, L1MdGf, and L1MdA (*3*)] (fig. S1A). Full-length L1 retrotransposons are composed of a 5′ untranslated region (5′UTR) with an internal Pol II promoter, two open reading frames (ORFs), and a 3′UTR followed by a Poly(A) tail. L1 elements mobilize through a "copy and paste" mechanism, in which full-length L1 mRNA is reverse transcribed beginning at the 3′ end and inserted into a new genomic location. In many cases, either reverse transcription stops early or the intermediate single-stranded DNA (ssDNA) is degraded before the insertion is resolved, resulting in 5′ truncated insertions. To account for these multiple forms, we designed four assays spanning different regions of the element to gain insight into the length of L1 copies detected by ddPCR. As expected, assays for the 5′ end of L1 had fewer sequence matches in the mouse reference genome and were more likely to belong to a full-length element than assays for the 3′ end of L1 (fig. S1, B and C). In addition, we developed assays for mouse 5*s* ribosomal DNA and mouse minor satellite DNA for use as stable, multicopy endogenous reference genes (fig. S1, D and E).

Rodents exhibit natural variations in maternal care that influence the neurodevelopment and adult behavior of their offspring (*4*). Some of the lasting effects of maternal care have been linked to epigenetic changes precipitated by the amount of licking/grooming and arched-back nursing that a pup receives from its mother (*5*). Different gene expression patterns, stress responses, and DNA methylation profiles are activated depending on the quality and quantity of maternal care. We leveraged this range of maternal care to examine the effects of neonatal care on L1 copy number in mice. We monitored the behavior of dams with their pups during the first 2 weeks after parturition. Individual variations were observed in maternal style, as previously reported (Fig. 1A) (*6*). We divided mice into two groups based on median total maternal behavior, which revealed two distinctive maternal styles, high maternal care and low maternal care, across the 2-week observation period (Fig. 1B and fig. S2A). Variations in maternal care did not affect body mass gain in the pups (fig. S2B). At weaning on postnatal day (PND) 21, the total percent time dams spent on maternal care was significantly correlated with L1 copy number measured in the hippocampus of their offspring (Fig. 1C and fig. S3) but not in the frontal cortex or heart (fig. S4, A and B). Because this effect was not identified in all tissues, it is unlikely to be a result of inherited differences in L1 copy number. Furthermore, we studied genetically homogeneous inbred mice, in which the L1 copy number was similar among the dams and sires (fig. S4C). To investigate cell-type specificity and to rule out differences in cytoplasmic L1 DNA, we sorted hippocampal nuclei by NeuN expression using fluorescence-activated cell sorting (FACS). By single-nuclei quantitative PCR, we detected a higher hippocampal L1 copy number in $NeuN^+$ nuclei from PND 21 pups reared with low maternal care (Fig. 1, D and E).

Other genomic events besides retrotransposition could account for an increase in L1 copy number. For one, mobile elements could be reverse transcribed and exist as extrachromosomal ssDNA or circular DNA without integrating into the genome. To investigate this possibility, we treated samples with single-stranded deoxyribonuclease or size-selected high-molecular-weight DNA, but neither treatment significantly changed the pattern of L1 3′UTR detected (fig. S5, A and B). Alternatively, L1 copy number could be increased as a result of large-scale genomic duplications. However, we observed similar copy numbers of L1MdV, a nonmobile family of L1, between mice reared with high or low maternal care (fig. S5C). Further, early life experience seemed to specifically affect L1 retrotransposons, because copy numbers of other mobile elements—short interspersed nuclear element (SINE) B1, SINE B2, and intracisternal A particle (IAP) elements—did not correlate with maternal care (fig. S5D).

The generation of genomic diversity by L1 elements is likely a dynamic, lifelong process that begins during embryonic development. Retrotransposition activity is increased as neural progenitors differentiate into neurons (*2*); thus, early life (when the brain is undergoing extensive growth and differentiation) represents a prime stage in which to uncover the sensitivity of L1 to experience. L1 retrotransposition rates are higher in the mouse brain compared with other tissues and, among brain subregions, highest in the hippocampus, a region sensitive to environmental stimuli (*7*, *8*). During the first week of life, the hippocampus is one of the few structures in the rodent brain that is still undergoing extensive cell division and differentiation, making it more likely to foster retrotransposition than other brain regions at that time (*9*).

To confirm that low maternal care was eliciting an increase in hippocampal L1 copy number, we examined the timeline of L1 accumulation and the effects of manipulating maternal behavior (Fig. 2A). We used a paradigm of separation-induced maternal care by exposing dams to 3 hours of maternal separation daily. Maternal separation was initially developed as a rat model of neglect, but mice and some strains of rats actually compensate for the separation by increasing their care upon reunion with the litter (*10*–*13*). Maternal separation in our study did not reduce maternal care, in agreement with previous reports in mice (*12*–*14*), but it did reduce the natural variations between individual mothers, such that dams undergoing maternal separation showed a consistently high level of arched-back nursing, licking-grooming, and contact time when reunited with their litters (Fig. 2, B and C). At PND 0, before the pups received any appreciable maternal care, we measured similar hippocampal L1 copy numbers among mice born to high- or low-maternal-care dams; however, by PND 7, we observed more L1 copies in mice reared with low maternal care (Fig. 2D). The accumulation of L1 copies was blocked in mice reared by highly maternal dams

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that compensated for separation (Fig. 2D). In addition, we performed a cross-fostering experiment in which two to four pups from each litter were reared by a foster dam beginning at PND 0. L1 3′UTR copy number was better correlated with the maternal care of the dam that reared the pups and not with the care of the biological dam (Fig. 2E).

To examine the mechanism whereby maternal care affects L1 copy number, we analyzed the rate of neurogenesis. Stimuli that enhance neurogenesis may promote retrotransposition because dividing cells are permissive to the L1 ribonucleoprotein complex entering the nucleus. We injected mice daily, for the first 7 days of life, with EdU, a marker of cell proliferation. Then we collected the hippocampi at PND 21 and used flow cytometry to quantify cells expressing EdU and Prox1, a marker of dentate granule neurons. Mice reared with high or low maternal care had similar numbers of $EdU^+/Prox1^+$ neurons and $EdU^+/Prox1^-$ cells, suggesting no difference in neurogenesis rate (fig. S6, A and B).

Next, we analyzed methylation of L1 because gene- and brain region–dependent effects of maternal care on DNA methylation have been reported (*5*, *15*). We narrowed our analysis to the Tf family of L1 elements, because this is the most active and evolutionarily recent family of mouse L1 elements (*3*). The L1MdTf promoter consists of a variable number of repeat monomers, with each representing a CpG island and including a YY1 transcription factor binding site required for L1 gene expression (Fig. 3A) (*16*). Using bisulfite sequencing, we assessed a region of 13 individual CpGs containing the YY1 binding site to determine the average methylation level in the hippocampus (Fig. 3B). Mice that experienced low maternal care had less methylation across the L1 promoter (Fig. 3C), particularly at the YY1 binding site (Fig. 3D). This difference corresponded to more L1 mRNA expression in the hippocampus at PND 4 (Fig. 3E) but not in the frontal cortex (fig. S7). To investigate how maternal care,

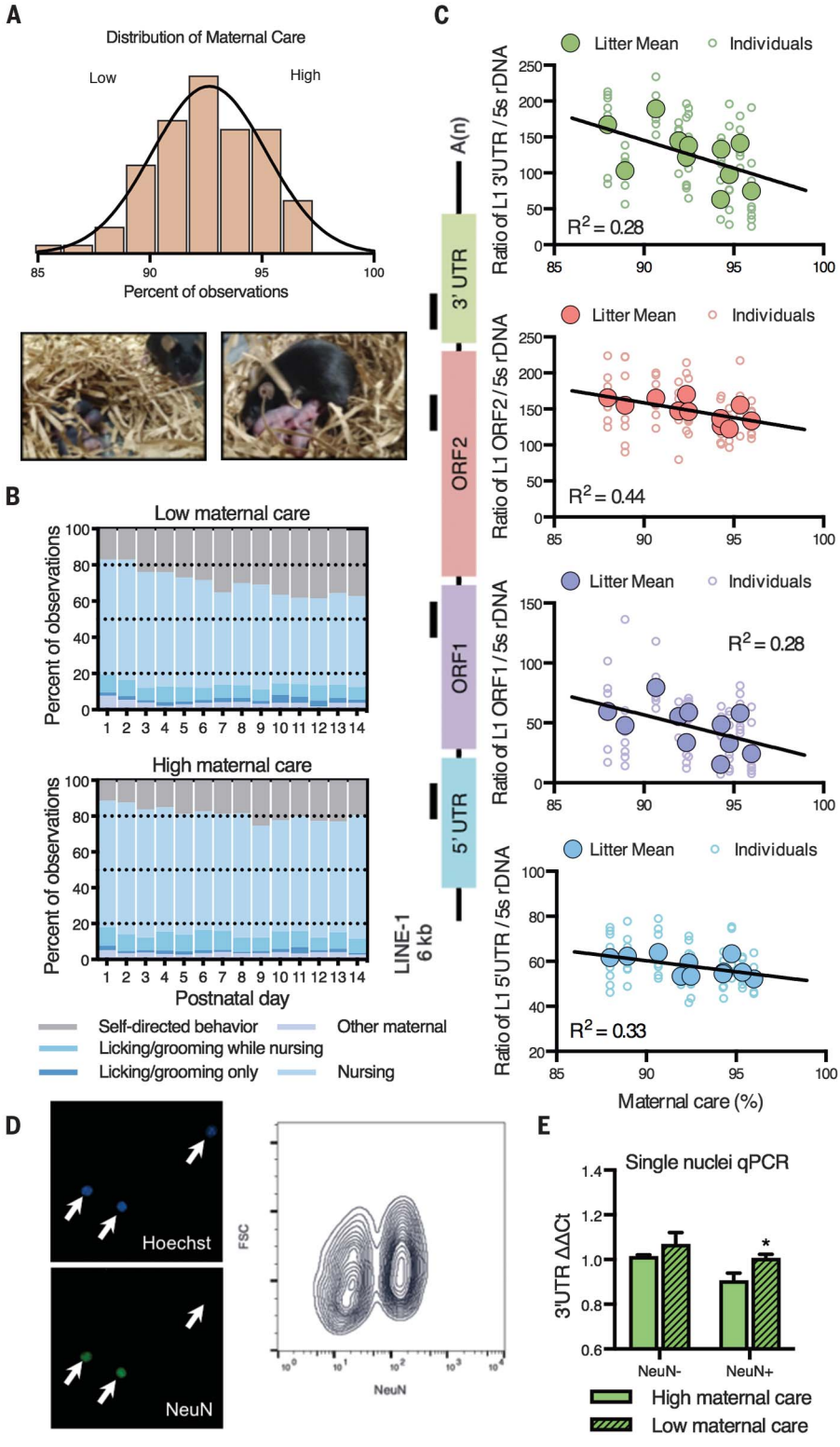
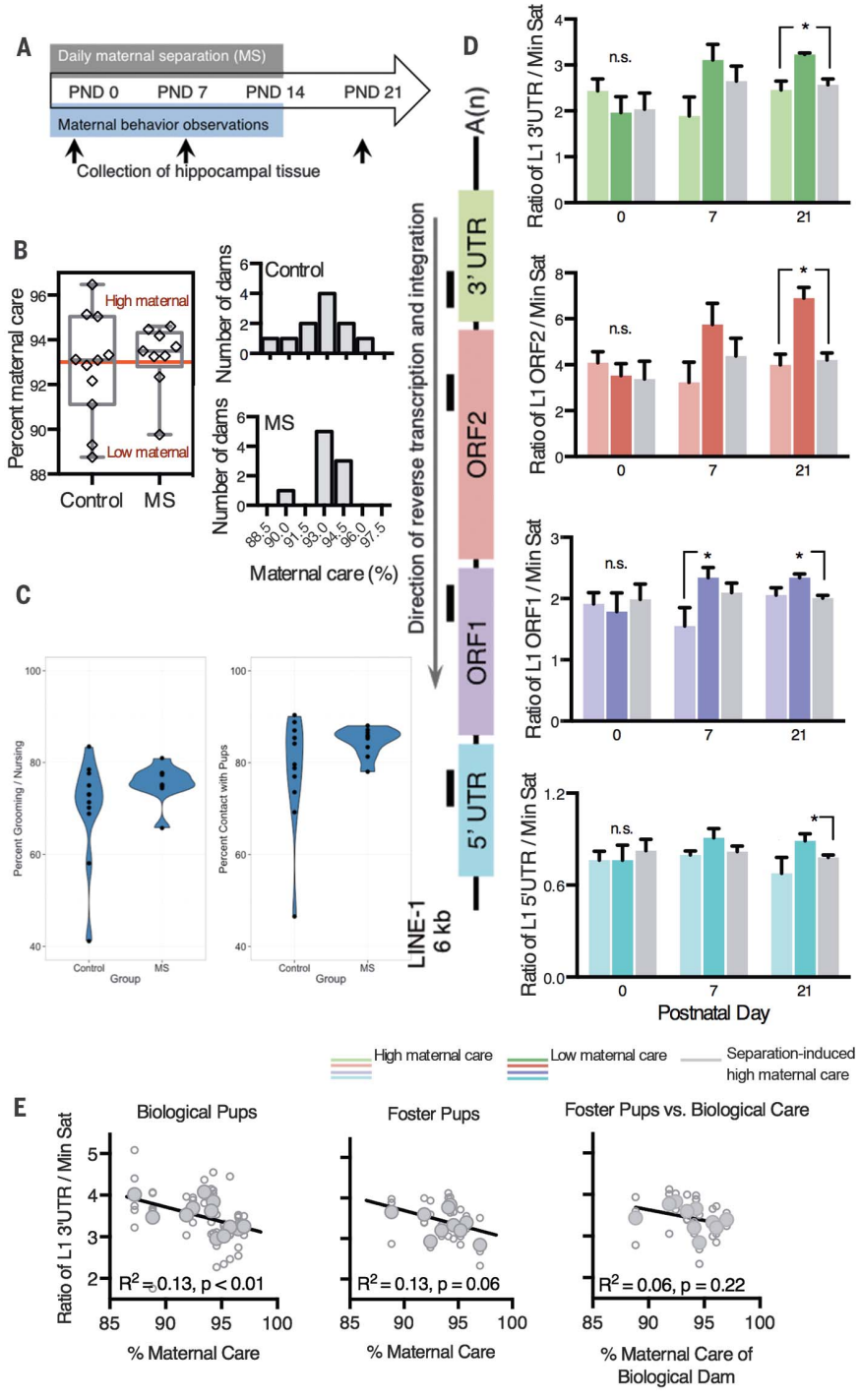


Fig. 1. Natural variations in maternal care predict hippocampal L1 copy number. (**A**) Distribution of time spent on maternal care. Dams above the median were called "high maternal," and dams below the median were called "low maternal." $N = 84$. Photos show representative low maternal (left) and high maternal (right) dams. (**B**) Breakdown of mean time spent performing maternal versus self-directed behaviors. Low: $N = 41$; High: $N = 43$. (**C**) Hippocampal L1 copy number in PND 21 offspring is inversely correlated with maternal care received during the first two postnatal weeks. Linear mixed model (LMM), with maternal care and sex as fixed factors and a random intercept for litter; $N = 75$ pups from 11 litters. Fixed-effect coefficients for care: 3′UTR, −7.66, $P = 0.049$; ORF2, −4.15, $P = 0.01$; ORF1, −3.60, $P = 0.06$; 5′UTR, −1.05, $P = 0.03$. (**D**) Hippocampal tissue collected at PND 21 was sorted based on NeuN staining. Micropictographs show sorted $NeuN^+$ and $NeuN^-$ single nuclei (arrows). (**E**) L1 copy number was higher in single neuronal nuclei from pups with low maternal care. ddCt (ΔΔCt) values for nuclei were averaged to get one value per mouse used for statistical analysis. Two-tailed *t* test, High $NeuN^-$: $N = 3$ mice (83 nuclei), Low $NeuN^-$: 4 mice (83 nuclei), $t = 0.41$, $P = 0.40$; High $NeuN^+$: 4 mice (125 nuclei); Low $NeuN^+$: 4 mice (131 nuclei), $t = 2.85$, $P = 0.03$. Data represented as mean ± SEM. *$P < 0.05$.

Fig. 2. Manipulating maternal care affects accumulation of L1 copy number.
(**A**) Timeline of experimental design. Dams were either undisturbed or subjected to daily 3-hour separation from their pups during the first 2 weeks postpartum. Pups were collected at PND 0, PND 7, and PND 21. (**B**) Dams exposed to separation were more likely to be highly maternal than control dams. Control: $N = 11$; MS (maternal separation): $N = 9$. (**C**) Separated dams showed less variability in time grooming and nursing their pups and time contacting pups. F test for equality of variances (Grooming/Nursing: $F = 7.58$, $P = 0.0085$; Contact: $F = 15.12$, $P = 0.0008$). Control: $N = 11$; MS: $N = 9$. (**D**) Low maternal care was associated with increased hippocampal L1 copy number beginning at PND 7, but separation (which caused compensatory increases in care) blocked the increase in copy number. Two-tailed t test, P0: High $N = 6$, Low $N = 7$, MS $N = 4$; P7: High $N = 4$, Low $N = 5$, MS $N = 5$; P21: High $N = 5$, Low $N = 4$, MS $N = 9$. 3′UTR high versus low, $t = 2.91$, $P = 0.03$; MS versus low, $t = 2.82$, $P = 0.02$. ORF2 high versus low, $t = 4.14$, $P = 0.01$; MS versus low, $t = 4.64$, $P < 0.01$. ORF1 P7 high versus low, $t = 1.94$, $P = 0.04$, P21 MS versus low, $t = 4.04$, $P < 0.01$. 5′UTR MS versus low, $t = 2.70$, $P = 0.02$. (**E**) In another cohort of mice, two to four pups from each litter were fostered to another dam on PND 0. L1 3′UTR copy number in the hippocampus at PND 21 correlated better with the care the pups received than with the care of the biological mother. Pearson correlation, Biological pups: $N = 64$ pups, Foster pups: $N = 28$ pups. L1 3′UTR copy number in foster pups did not correlate with the care of the biological dam. Data in bar graphs represented as mean ± SEM. *$P < 0.05$.



in general, influences methylation of the L1MdTf promoter, we examined gene expression of DNA methyltransferases in hippocampal tissue from our time point cohort. DNMT1 and DNMT3B expression decreased from PND 0 to PND 21 but did not differ between maternal care groups. In contrast, DNMT3A expression peaked at PND 7, at which point expression was reduced in mice that received low maternal care (Fig. 3F). Methylation of IAP elements, which showed no change in copy number with maternal care, was similar for all mice (fig. S8). These observations suggest that methylation of L1 occurs during normal postnatal development but to varying extents depending on the environment to which the developing pup is exposed. Differential methylation and expression are likely only some of the mechanisms that contribute to changes in L1 copy number; another possibility is differential susceptibility of the DNA to accept new L1 insertions.

Our results suggest that there is plasticity at the level of the DNA sequence in response to environmental perturbations. It will be necessary to confirm these findings in the future using additional methods like single-cell genome sequencing. PCR-based copy number assays have the advantage of detecting relative changes across a large number of L1 elements but lack specificity for targeting somatic insertions or for distinguishing insertions that arise through bona fide retrotransposition versus another mechanism. Because of this lack of specificity, it is difficult to estimate the number of insertions per cell. Nonetheless, de novo insertions may have a variety of consequences depending on the site of insertion, such as altering expression of nearby genes, affecting splicing of transcripts, or shuffling DNA through L1-mediated transduction (*17*, *18*). Somatic retrotransposition could affect neuronal diversity and function, particularly in light of the highly networked state of the brain. As previously reported (*4*), we observed more anxiety-like behavior in adult mice that were reared with low maternal care (fig. S9), although it remains unknown whether somatic mosaicism contributes to these behaviors. Single-cell sequencing has estimated the rate of L1 retrotransposition in the human

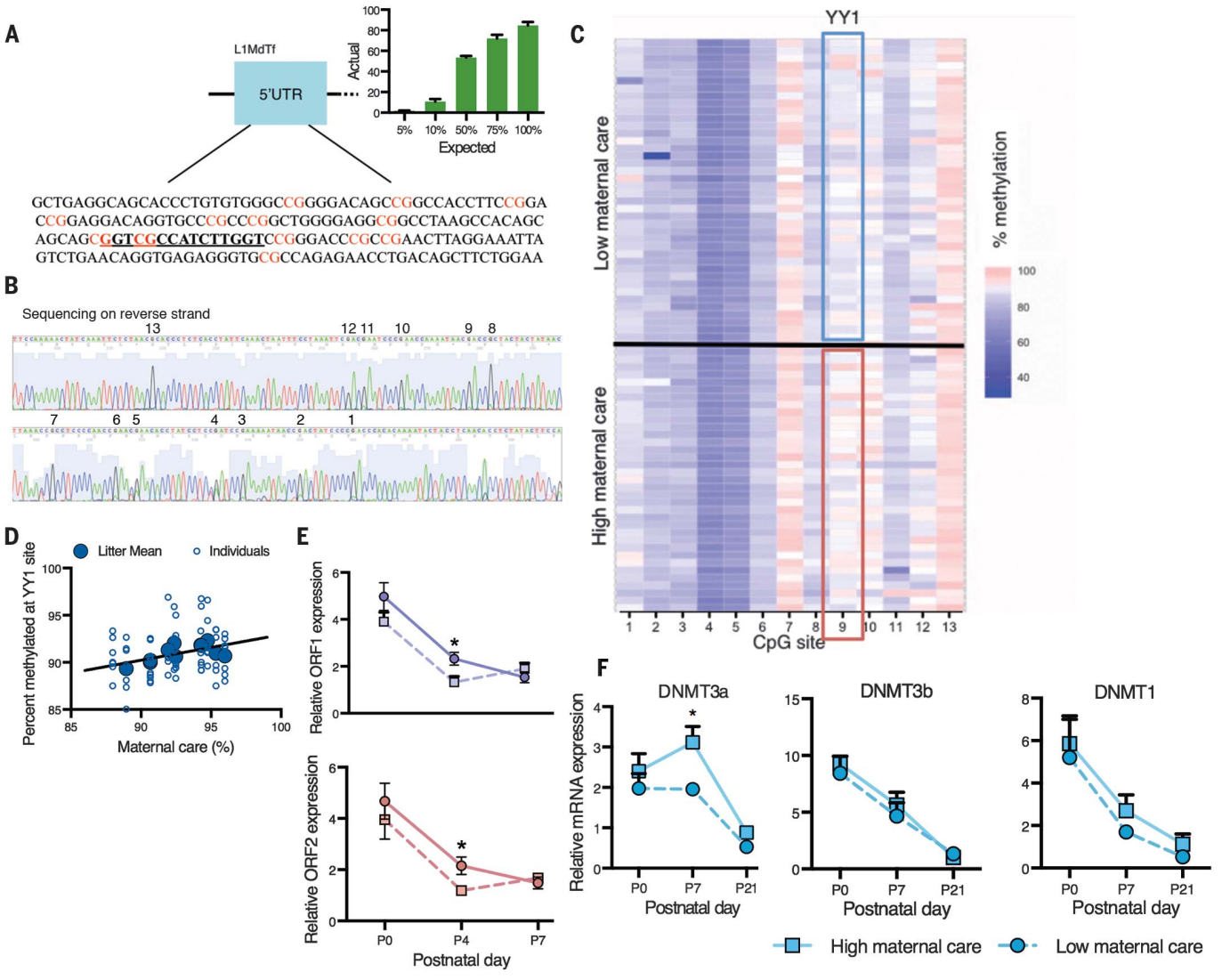


Fig. 3. Maternal care alters methylation of YY1 binding site in the L1 promoter. (**A**) The L1MdTf promoter contains a CpG island and YY1 binding site (underlined and bold) that is important for transcription of the element. Sequencing the bisulfite-converted L1 promoter using methylated DNA standards produced accurate results (inset). (**B**) Methylation was analyzed at 13 individual CpG sites in the L1MdTf promoter of hippocampal DNA from PND 21 mice. (**C**) Low maternal care was associated with less methylation, particularly at the YY1 binding site at position 9. Each row represents an individual mouse. LMM, with maternal care and CpG site as fixed factors and a random intercept for litter. Fixed factor coefficient for care: 0.18, $P = 0.01$. High: $N = 32$; Low: $N = 44$. (**D**) Mice reared with less maternal care had reduced methylation of the YY1 site. LMM, with maternal care as fixed factor and a random intercept for litter. Fixed factor coefficient: 0.21, $P = 0.04$. High: $N = 32$; Low: $N = 44$. (**E**) L1 mRNA expression was elevated in pups raised with low maternal care. Each time point, two-tailed t test, P0: $N = 6$ per group; P4: High: $N = 5$; Low: $N = 3$; P7: High: $N = 4$; Low: $N = 5$. ORF1 P4, $t = 2.5$, $P = 0.04$; ORF2 P4, $t = 3.4$, $P = 0.01$. (**F**) The reduced methylation coincided with reduced expression of DNMT3a, a de novo methyltransferase enzyme, at PND 7. Expression of DNMT3b and DNMT1 did not differ between mice with different maternal care. Each time point, two-tailed t test, P0: $N = 6$ per group; P7: High: $N = 4$; Low: $N = 5$; P21: High: $N = 5$; Low: $N = 4$. DNMT3a P7, $t = 2.97$, $P = 0.02$. Data represented as mean ± SEM. *$P < 0.05$.

brain to be <0.6 to 13 insertions per neuron, depending on the brain region and detection method (*19*, *20*). Mice have thousands more active copies of L1 per cell than humans, but whether species differences in L1 copy number result in a proportionally greater L1 insertion rate is unknown. Even a few insertions could have substantial functional effects in the brain. Moreover, it was recently reported that childhood stress and adversity result in hypomethylation of retrotransposons in humans (*21*, *22*). Our results demonstrate that early life experience can drive structural variation of the genome via L1 retrotransposons.

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SUPPLEMENTARY MATERIALS

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Materials and Methods
Figs. S1 to S9
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Genomic plasticity during brain development

Mice genomes contain many mobile retrotransposons. Bedrosian *et al.* analyzed DNA from the mouse hippocampus during development (see the Perspective by Song and Gleeson). They found that the amount of maternal care in the first few weeks of a mouse pup's life affected the number of copies of the L1 retrotransposon. The experience of maternal care was thus "recorded" in the DNA of these mice pups during a time when the brain was still actively developing.

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