Transposition-Driven Genomic Heterogeneity in the Drosophila Brain
Paola N. Perrant,1 Shamik DasGupta1,2 Jie Wang,3 William Theurkauf,4 Zhiping Weng,3 Michael Rosbash,3 Scott Waddell1,2*

Recent studies in mammals have documented the neural expression and mobility of retrotransposons and have suggested that neural genomes are diverse mosaics. We found that transposition occurs among memory-relevant neurons in the Drosophila brain. Cell type–specific gene expression profiling revealed that transposition expression is more abundant in mushroom body (MB) αβ neurons than in neighboring MB neurons. The piwi-interacting RNA (piRNA) proteins Aubergine and Argonaute 3, known to suppress transposons in the fly germline, are expressed in MB neurons. Gene expression profiling revealed that transposon expression is more abundant in MB neurons than in neighboring MB neurons. Loss of piRNA proteins correlates with an increase in transposon expression in MB neurons. The Piwi-interacting RNA (piRNA) proteins Aubergine and Argonaute 3, known to suppress transposons in the fly germline, are expressed in MB neurons.

Aubergine and Argonaute 3, known to suppress transposons in the fly germline, are expressed in MB neurons. Recent studies in mammals have documented the neural expression and mobility of retrotransposons and have suggested that neural genomes are diverse mosaics. We found that transposition occurs among memory-relevant neurons in the Drosophila brain. Cell type–specific gene expression profiling revealed that transposition expression is more abundant in mushroom body (MB) αβ neurons than in neighboring MB neurons. The piwi-interacting RNA (piRNA) proteins Aubergine and Argonaute 3, known to suppress transposons in the fly germline, are expressed in MB neurons. Gene expression profiling revealed that transposon expression is more abundant in MB neurons than in neighboring MB neurons. Loss of piRNA proteins correlates with an increase in transposon expression in MB neurons. The Piwi-interacting RNA (piRNA) proteins Aubergine and Argonaute 3, known to suppress transposons in the fly germline, are expressed in MB neurons.

Transposons constitute nearly 45% of the human genome and 15 to 20% of the fly genome (1–3). Mobilized transposons can act as insertional mutagens and create lesions where they once resided (2). Recombination between homologous transposons can also delete intervening loci. Specific regions of the mammalian brain, such as the hippocampus, might be particularly predisposed to transposition (4, 5). LINE-1 (L1) retrotransposons mobilized during differentiation appear to insert in the open chromatin of neurally expressed genes (4–6). One such insertion in neural progenitor cells altered the expression of the receiving gene and the subsequent maturation of these cells into neurons (6). The mosaic nature of transposition could therefore provide additional neural diversity that might contribute to behavioral individuality and/or neurological disorders (7–9).

The Drosophila melanogaster mushroom bodies (MBs) are brain structures critical for olfactory memory. The approximately 2000 intrinsic MB neurons are divisible into αβ, γ, and αβ according to their morphology and roles in memory processing (10–13). Here, we used cell type–specific gene expression profiling (14) to gain insight into cellular properties of MB neurons. Intersected geneticities (15) (Fig. 1A) allowed us to exclusively label MB αβ, γ, and αβ neurons in the brain with green fluorescent protein (GFP) (Fig. 1, B to D). For comparison, we also assayed a “no MB” genotype in which GFP labels other neurons (Fig. 1, E to G). While MB neurons can be dissected from the head capsule and dissociated by proteolysis and agglutination; GFP-expressing single cell bodies were then collected by fluorescence-activated cell sorting (FACS). Total RNA was isolated from 10,000 cells per genotype, and polyadenylated RNA was amplified and hybridized to Affymetrix Drosophila 2.0 genome expression arrays. Each genotype was processed in four independent replicates (Fig. 2A).

Routine statistical analysis for differentially expressed genes, including a multiple-testing correction across all 16 data sets, did not reveal significant differences at a false discovery rate (FDR) of <0.05. The only differences we observed were in MB neurons, which had also been observed in αβ neurons by a factor of 22. Of the top 60 transcripts from this list, 29 were significantly different from αβ signals (by raw P value) and represent transposons (Fig. 2A and table S1). Alignment of the corresponding values from the γ and no-MB profiles showed a similar significant bias in transposon expression over these samples. We identified retrotransposons that transpose via a replicative mechanism involving an RNA intermediate and DNA elements that use nonreplicative excision and repair. Retrotransposons can be subdivided into long-terminal repeat (LTR) elements and long interspersed nuclear elements (LINEs). We found 11 LTR elements (Tabor, mdg1, roo, qbert, gypsy, invader3, gypsy2, microcopia, 412, accord, and blood), 11 LINE-like elements (G6, RT1b, HeT-A, Ivk, Crla, Felement, Doc2,
baggins, R2, Doc3, and Doc), and four DNA elements (Bar1, pogo, Tc3, and transib3).

We further analyzed 14 transposons, representing the most abundant in each class. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) of RNA from independently purified cell samples confirmed that transposon expression was significantly higher in αβ neurons than in other MB neurons (Fig. 2B). All transposons, other than R2, were also significantly higher in αβ neurons than in the rest of the brain. R2 is unique, because it exclusively insert in the highly repeated 28S rRNA locus and heterochromatin (17).

Transposition is ordinarily regulated by chromatin structure and posttranscriptional degradation of transposon mRNA guided by complementary RNAs (18–25). The small interfering RNA (siRNA) pathway has been implicated in somatic cells (26). In contrast, the Piwi-interacting RNA (piRNA) pathway has a more established role in the germ-line (19–21). The microarray analysis skewed our attention toward piRNA because the expression level of the translocated Stellate locus, Stellate12D orph (Stell12DOR) mRNA, was higher in αβ than in other MB neurons and the rest of the brain by a factor of >20 (Fig. 2A and table S1). Stellate repeat transcripts are usually curtailed by piRNA, not siRNA (27, 28). Stellate repeats encode a casein kinase II regulatory subunit, and piRNA mutant flies form Stellate protein crystals in testis (29). Immunostaining Stellate in the brain labeled puncta within αβ dendrites in the MB calyx, consistent with high Ste12DOR expression in wild-type αβ neurons (fig. S1).

piRNAs are loaded into the Piwi clade argonauts proteins Piwi, Aubergine (Aub), and Argonaute 3 (Ago3) (21). Piwi and Aub can amplify piRNA pools with Ago3 (30, 31). To investigate piRNA involvement in differential transposon expression, we immunolocalized Piwi proteins and colocalized GFP to assign signals to MB neuron type (Fig. 3). Aub and Ago3 differentially labeled MB subdivisions in addition to structures throughout the brain (Fig. 3 and figs. S2 and S3), but we did not detect Piwi (32). The ellipsoid body of the central complex stained strongly for Aub but not at all for Ago3 (Fig. 3, A and E, and figs. S2D and S3D), which suggests possible functional exclusivity of Piwi proteins in the brain.

Differential Aub and Ago3 labeling was most evident within axon bundles in the peduncle and lobes, where MB neuron types are anatomically discrete (Fig. 3, A to H, and fig. S2). Aub protein colocalized with γ and αβ’ neurons in the peduncle and lobes but was reduced in αβ neurons in both locations (Fig. 3, B to D, and fig. S2). Ago3 did not label MB lobes (fig. S3) but colocalized with γ neurons in the peduncle (Fig. 3H). Ago3 labeled core αβ (αβ`) neurons but did not label outer αβ neurons (Fig. 3F). Therefore, outer αβ neurons do not abundantly express Aub or Ago3, which implies that transposon suppression is relaxed. In contrast, γ neurons express Aub and Ago3, providing potential for piRNA amplification, and αβ’ neurons express Aub. These patterns of Aub and Ago3 in the MB peduncle appear conserved in brains from D. erecta, D. sechellia, and the more distantly related D. pseudoobscura species (fig. S4).

Loss of siRNA function elevates transposon expression in the head (22). We replicated these
findings with ago2αβ and der-2αβi16X mutant flies (fig. S5). In parallel, we used trans-heterozygous aub(aub1011/aub8C42) and ago3 heads (ago32/ ago32) (19, 33) and trans-heterozygous armitage heads (armi/armi72–1) (34) to test whether piRNA suppressed transposon expression (Fig. 3I). Levels of the 14 LTR, LINE-like, and TIR group transposons verified to be expressed in αβ neurons (Fig. 2B) were assayed by qRT-PCR; of these 14 transposons, 13 were significantly elevated in siRNA-abtransitions in embryo DNA that differed from the published Drosophila genome sequence (Fig. 4A). In comparison, αβ neuron DNA revealed 215 additional sites. The remaining brain tissue uncovered 200 new insertions, including 19 that were identical to those in αβ neurons. The sequencing depth for embryos (3.1×) was an order of magnitude greater than for neurons (3.1×) because embryo material could be collected more easily; hence, the αβ and other brain insertions are likely de novo. By randomly sampling reads to yield 1× genome coverage, we calculated 129 new transposon insertions per αβ neuron genome (table S2). Sequencing single neurons (36) would reveal the exact cellular frequency and heterogeneity of transposition events.

New αβ insertions occurred across all chromosomes, without obvious regional bias (Fig. 4B). In addition, insertions resulted from 49 different transposons representing LTR, LINE-like, TIR, and Foldback (FB) classes (Fig. 4C). They included 11 of the 29 transposons in the oβ transcriptome (Fig. 2A), and the number of insertions per class was consistent with their prevalence in the genome (Fig. 4D). Therefore, many transposons mobilize in αβ neurons.

Of the 215 de novo αβ insertions, 108 mapped close to identified genes (Fig. 4E and fig. S6). Of these, 35 disrupted exons, 68 disrupted introns, and 5 fell in promoter regions (<1 kb from transcription start site). The remaining 107 insertions mapped to piRNA clusters or intergenic regions and were not assigned to a particular gene. A similar distribution was observed for the 200 new insertions in the rest of the brain (Fig. 4E and fig. S6). The reference fly genome has 258 transposon insertions in exonic regions, 11,110 insertions in intronic regions, 502 insertions in promoter regions, and 33,008 insertions in intergenic regions. Therefore, both groups of brain cells had a significantly larger fraction of insertions within exons, and fewer in intergenic regions, than the transposons that are annotated in the genome (Fig. 4E and table S8). To test whether such a distribution was unique to neurons, we analyzed de novo insertions in ovary DNA, again using embryo sequence as the comparison. New insertions in ovary DNA revealed a similar skew toward exons (Fig. 4E and table S8).

In mammals, active L1 elements appear to disrupt neurally expressed genes (4–6). New αβ neuron insertions, but not those in other tissue

Fig. 3. Aub and Ago3 are not abundant in αβ neurons. (A) Aub immunostaining (magenta) labels the ellipsoid body (EB), MB subdivision in the peduncle (ped), and cell body layer (rind). A single confocal section is shown at the level of the MB peduncle. Dashed box denotes area shown in (B) to (D). (B) to (D) Aub labels αβ neurons (green) but overlaps with αβ’ neurons (C) and γ neurons (D). (E) Ago3 immunostaining (magenta) labels neurons throughout the brain and MB subdivision in the peduncle. A single confocal section is shown at the level of the MB peduncle. Dashed box denotes area shown in (F) to (H). (F) to (H) Ago3 staining is prominent in the αβ core [(F), solid triangle] and does not overlap with outer αβ-labeled αβ neurons [(G), open triangle], nor with αβ’ neurons (G), but overlaps with γ neurons (H). Scale bars, 40 μm [(A) and (E)], 10 μm [(B) to (D), (F) to (H)]. (I) qRT-PCR analysis from wild-type, aub1011/aub8C42, ago32/ago32, and armi/armi72–1 mutant heads. Several transposon transcripts are elevated in ago3, aub, and armi mutant fly brains. Values are normalized to wild-type heads. *Significant increase, P < 0.05 (t test).
(tables S7 and S9), were significantly enriched in 12 Gene Ontology (GO) terms (Benjamini FDR <5%; tables S4 and S5), all of which are related to neural functions. Moreover, promoter regions from 18 of 20 of the targeted genes drive expression in αβ neurons (table S10). We found exonic insertions in *gilgamesh*, derailed, and mushroom body defect and intronic insertions in *dance* and *rutabaga* (table S3), all of which have established roles in MB development and function (37–40). In addition, MB neurons are principally driven by cholinergic olfactory projection neurons (41) and receive broad GABA-ergic inhibition (42) and dopaminergic modulation through G protein–coupled receptors (43). We identified intronic insertions in *nicotinic Acetylcholine Receptor α 80B*, *G* protein-coupled receptor kinase 1, and cyclic nucleotide gated channel-like and an exonic insertion in *GABA-B-receptor subtype 1* (table S3). Transposon-induced mosaicism could therefore alter integrative and plastic properties of individual MB αβ neurons.

Our data establish that transposon-mediated genomic heterogeneity is a feature of the fly brain and possibly other tissues. Together with prior work in rodents and humans (4–6), our results suggest that genetic mosaicism may be a conserved characteristic of certain neurons. Work in mammals indicates that L1 expression occurs because the L1 promoter is released during neurogenesis (6, 21). Our data are consistent with such a model and also support the idea that transposons avoid posttranscriptional piRNA silencing in adult αβ neurons.

A recent study described a role for piRNA in epigenetic control of memory-related gene expression in *Aplysia* neurons (44). It is therefore possible that MB neurons differentially use piRNA to control memory-relevant gene expression and that transposon mobilization is an associated cost. Because we found transposon expression in αβ neurons of adult flies, it is conceivable that disruptive insertions accumulate throughout life, leading to neural decline and cognitive dysfunction. Alternatively, permitting transposition may confer unique properties across the 1000 neurons in a model and also support the idea that transposon insertions in αβ neurons. (C) Distribution of new insertions from 18 of 20 of the targeted genes drive expression in αβ neurons. Bar height indicates number of insertions in each location. piRNA clusters are shown. (D) The proportion of new insertions found for each transposon class in αβ neurons. (E) Distribution of transposons in the annotated genome significantly differs from distribution of de novo insertions in the brain, and ovary DNA with respect to neighboring genes (P < 2.2 × 10−16 for αβ neurons, the rest of the brain, and ovary; χ2 test).

### References and Notes

19. C. Li et al., Cell 137, 509 (2009).
34. Y. Tomari et al., Cell 116, 831 (2004).
44. P. Rajasekharapathy et al., Cell 149, 693 (2012).

Acknowledgments: Data are available through ArrayExpress (www.ebi.ac.uk/arrayexpress) and NCBI Short Read (www.ncbi.

5 APRIL 2013 VOL 340 SCIENCE www.sciencemag.org
Rats and Humans Can Optimally Accumulate Evidence for Decision-Making

Bingni W. Brunton,1,2* Matthew M. Botvinick,1,3 Carlos D. Brody1,2,4†

The gradual and noisy accumulation of evidence is a fundamental component of decision-making, with noise playing a key role as the source of variability and errors. However, the origins of this noise have never been determined. We developed decision-making tasks in which sensory evidence is delivered in randomly timed pulses, and analyzed the resulting data with models that use the richly detailed information of each trial’s pulse timing to distinguish between different decision-making mechanisms. This analysis allowed measurement of the magnitude of noise in the accumulator’s memory, separately from noise associated with incoming sensory evidence. In our tasks, the accumulator’s memory was noiseless, for both rats and humans. In contrast, the addition of new sensory evidence was the primary source of variability. We suggest our task and modeling approach as a powerful method for revealing internal properties of decision-making processes.

D ecisions in real life often need to be made based on noisy or unreliable evidence. Accumulating evidence from a set of noisy observations made over time makes it possible to average over different noise samples, thus improving estimates of the underlying signal. This principle is the basis for the influential class of “drift-diffusion” models (1–5), which have been broadly applied to explain a variety of phenomena in biology (6–8). Accumulation involves both maintaining a memory of evidence accrued so far and adding new evidence to the memory. Yet no test to date has distinguished between noise associated with each of these two components.

Fig. 1. Psychophysical tasks and summary of behavior. (A) Sequence of events in each trial of the rat auditory task. After light onset from a light-emitting diode (LED) in a center port, trained rats placed their nose into the port and “fixated” their nose there for a fixed amount of time until the light was turned off (1 to 2 s). Trains of randomly timed clicks were played concurrently from left and right free-field speakers during the last portion of the fixation time. After nose fixation and sounds ended, the rat made a choice, poking in the left or the right port to indicate which side played more clicks. Humans performed an analogous version of the task on a computer while wearing headphones. (B) Schematic diagram of a stimulus in the visual pulses version of the task, performed by humans on a computer. (C) Psychometric curves (fits to a four-parameter logistic function for each subject; see methods) for rat subjects. (D) Psychometric curves, as in (C), for human subjects. (E) Chronometric curves for an example rat. Difficulty is labeled by the ratio of click rates played on the two sides. For each difficulty, performance improves with longer stimulus durations. Dashed lines show the best-fit model predictions for this rat, as described in the text. The vertical axis shows mean accuracy and 95% confidence interval (CI).

We developed tasks in which subjects (humans and rats) were concurrently presented with two trains of pulses, one train representing “left”-labeled pulses and the other, “right”-labeled pulses. At the end of each trial, the subjects had to report which of the two trains had the greater total number of pulses. The timing of pulses was random and varied widely, both within and across individual trials (9, 10). We reasoned that the precisely known pulse timing would enable detailed modeling of the subjects’ choices on each individual trial, whereas its variability would allow exploration of the stimulus space and would thus provide statistical power.

In an auditory version of the task, performed by three humans and 19 rats, left pulse trains were clicks presented on a speaker to the left of the subject, and right pulse trains were clicks presented on a speaker to the right of the sub-

1Princeton Neuroscience Institute, Princeton University, Princeton, NJ 08544, USA. 2Department of Molecular Biology, Princeton University, Princeton, NJ 08544, USA. 3Department of Psychology, Princeton University, Princeton, NJ 08544, USA. 4Howard Hughes Medical Institute.

*Present address: Department of Biology and Department of Applied Mathematics, University of Washington, Seattle, WA 98195, USA.
†Corresponding author. E-mail: brody@princeton.edu

www.sciencemag.org SCIENCE VOL 340 5 APRIL 2013 95