Single Neurons in the Monkey Hippocampus and Learning of New Associations

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The medial temporal lobe is crucial for the ability to learn and retain new declarative memories. This form of memory includes the ability to quickly establish novel associations between unrelated items. To better understand the patterns of neural activity during associative memory formation, we recorded the activity of hippocampal neurons of macaque monkeys as they learned new associations. Hippocampal neurons signaled learning by changing their stimulus-selective response properties. This change in the pattern of selective neural activity occurred before, at the same time as, or after learning, which suggests that these neurons are involved in the initial formation of new associative memories.

Findings from human and animal studies support the idea that the hippocampus is important for the successful acquisition of new declarative memories, including associative memories (*1–3*). Consistent with this idea, neurophysiological studies report changes in hippocampal activity during acquisition of simple classical (*4*) or trace (*5*) conditioning tasks as well as during tasks of spatial learning (*6*). In monkeys, cells in the medial temporal lobe signal retrieval of well-learned associative memories (*7–9*). Only a handful of studies, in contrast, have examined neural activity during the acquisition of new associative memories in the medial temporal lobe (*10–12*). Because learning in these studies is either near floor (*10*) or approaching ceiling (*11*) levels of performance, the dynamics of neural activity associated with learning could not be analyzed in detail.

To examine the patterns of neural activity observed during the acquisition of new associative memories, we trained two macaque monkeys to perform a location-scene association task. The activity of individual hippocampal neurons was recorded as monkeys learned which one of four identical targets superimposed on a complex visual scene was associated with reward (Fig. 1A). Each day, animals were presented with a random mix of two to four novel location-scene associations together with two to four highly familiar "reference scenes" (*13*). Over the course of 18 months, animals saw a total of 378 new scenes, of which they learned 290 to criterion (*13*). During each session, animals learned an average of three new scenes and completed an average of 33 ± 1 trials for each new scene. The mean number of completed trials (i.e., correctly or incorrectly executed trials, not including break fixation or no-response trials) required to learn a new scene to criterion was 12 ± 1.

We recorded the activity of 145 cells throughout the hippocampal region in two macaque monkeys (82 cells in monkey 1 and 63 cells in monkey 2; Fig. 1B). An analysis of the average firing rate during the baseline fixation period revealed a bimodal distribution of firing rates (Fig. 1C). The average firing rate of the population of cells with low baseline rates (i.e., <20 spikes/s) was 8.1 ± 0.4 spikes/s (n = 87) and the average firing rate of the population of cells with high baseline rates was 45.8 ± 3.6 spikes/s (n = 58). This pattern is similar to the distribution seen in the rat hippocampus, where putative principle cells are associated with low firing rates and putative interneurons are associated with higher firing rates (*14*). However, because we did not collect spike waveforms for all the cells, we cannot make strong conclusions about the identity of specific cell types in the monkey hippocampus. Here, we refer to the two populations as high firing rate and low firing rate cells. An analysis of interspike interval distributions showed that 16 ± 13% and 11 ± 11% of spikes were associated with bursts for the population of high and low firing rate cells, respectively (Fig. 1C). These proportions are substantially lower than what is typically found in the rat hippocampus (*14*).

Using an analysis of variance ($P < 0.01$) with scene identity as the main factor, we found that 89 of 145 hippocampal cells (61%) responded in a scene-selective fashion during the scene period only, delay period only, or both periods of the task (Table 1). Of these 89 selective cells, 51 were low firing rate cells and 38 were high firing rate cells. We next hypothesized that cells signaling learning would change their activity in close association with the animal's behavioral learning curve. From the 89 cells with scene-selective neural activity obtained for a total of 241 learned scenes, we identified 69 cells (108 individual scenes) that exhibited significant activity during the scene or delay periods of the task relative to baseline (*15*). For these 108 individual scenes (69 neurons), we calculated the correlation between the behavioral learning curve (expressed as five-trial moving averages) and the raw trial-by-trial neural activity during the scene period, delay period, or both periods of the task. A total of 25 cells (32 scenes) showed a significant correlation between behavioral performance and neural activity during one or both task periods (*16*). We refer to the 25 cells with significant correlations as "changing" cells (Fig. 2, A and C; Fig. 3, A and B; and Table 1). From these 25 changing cells, we identified a total of 37 individual task periods that exhibited significant correlations between neural activity and behavior ($P < 0.01$, 21 positive correlations with r values ranging from 0.3 to 0.84; $P < 0.01$, 16 negative correlations with r values ranging from −0.42 to −0.71). The significance of each r value was confirmed by computing the correlation on shuffled data 1000 times and evaluating the probability that each r value was obtained by chance ($P < 0.01$). We next compared the r values estimated from the 108 scenes for which there was a significant and selective response during the scene period, delay period, or both periods of the task to the distribution of the r values obtained with shuffled data. The distribution of r values from the actual data was significantly different from what would be expected by chance (F =

Table 1. Categories of task-related responses.

	Sample period only	Delay period only	Both periods	Total
Selective cells	29	15	45	89
Changing cells*	8	11	6	25
Sustained	4	7	3	14
Baseline-sustained	4	4	3	11
Total hippocampal cells = 145				

*Both sustained and baseline-sustained changing cells (see text) were observed in both animals M1 and M2.

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2.89, $P < 0.001$; Fig. 2E). In no case did the baseline firing rate of the changing cells change during the course of the recording session, according to a rank sum test (*17*). To determine whether changing cells were selective for learned scenes, we examined the neural responses to 27 scenes that were never learned. None of the neural responses showed significant change in activity over time, according to a rank sum test (*17*).

In contrast to the robust changes in firing rate observed in response to particular new scenes (Fig. 2, A and C), changing cells typically gave little or no response to the reference scene with the same rewarded target location (Fig. 2, B and D). This suggests that changing cells do not provide pure motor signals selective for particular eye movements or the locations of particular targets in space. In additional control experiments, we recorded neural activity for five changing cells as animals learned two consecutive sets of novel scenes containing overlapping rewarded target locations. All five of these cells showed changing activity to only one of the two new scenes with the same rewarded target location, which suggests that changing cells do not exhibit response/location-specific activity. Because we did not examine the effect of reversal trials, we cannot rule out the possibility that changing cells exhibit stimulus-specific response properties (i.e., similar changing responses for any new learning involving a particular scene).

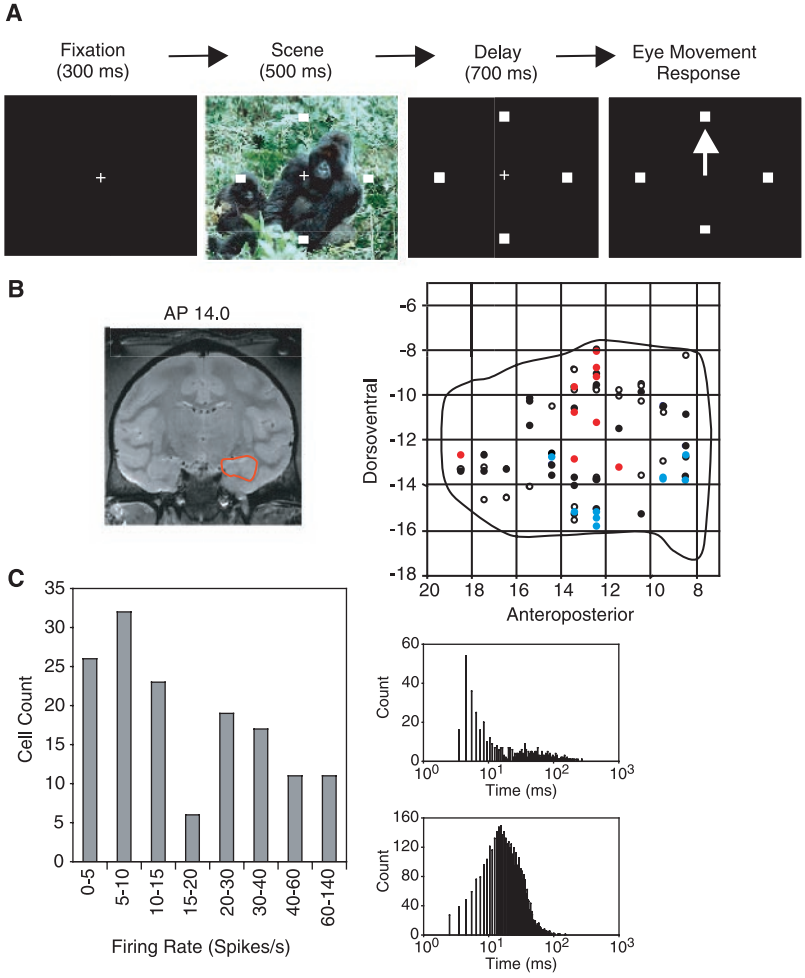

Fig. 1. (**A**) Schematic illustration of the location-scene association task. (**B**) A coronal magnetic resonance imaging (MRI) image showing the center of the recording chamber, with the boundaries of the hippocampal region outlined in red. The graph shows a sagittal view of the anterioposterior (plotted in mm from interaural line) and dorsoventral (plotted in mm from the tip of the guide tube) locations of the nonresponsive/nonselective (open circles), selective (solid circles), sustained (red circles), and baseline-sustained (blue circles) cells in monkey 1. Recording sites appeared to cover all hippocampal subdivisions (i.e., dentate gyrus, CA3, CA1, and subicular complex). However, without histological verification (animals are still participating in ongoing studies), no conclusion can be made on subdivision-specific localization of the different categories of hippocampal cells. The recording sites for animal 2 were the same as for animal 1. (**C**) Illustration of the bimodal distribution of firing rates during the baseline fixation period of the task. The graphs on the right show the distribution of interspike intervals for a bursty (top) and nonbursty (bottom) cell.

Table 2. Properties of changing cells.

	Baseline firing rate (spikes/s)	Response* before learning	Response* after learning
Sustained	16.7 ± 4.0†	6.3 ± 1.1	12.6 ± 1.7
Baseline-sustained	38.9 ± 12.8†	11.2 ± 2.7	3.6 ± 1.6

*Absolute firing rate (spikes/s) relative to baseline in the task period (scene or delay) correlated with learning. †There is a significant difference in the baseline (fixation) firing rate between sustained and baseline-sustained changing cells ($P < 0.05$).

Further analysis showed that all changing cells exhibited sustained activity such that the changes in scene or delay activity were maintained for as long as the cell was isolated (*18*). Two categories of sustained activity were observed. The first category, termed sustained changing cells (14 of 25 changing cells), initially showed little or no response during the scene or delay periods of the task; these cells signaled learning with a significant increase (12 of 14) or decrease (2 of 14) in neural activity that was maintained for the duration of the recording session (Figs. 2C and 3A). The second category, termed baseline-sustained changing cells (11 of 25 changing cells), responded to novel scenes with either increased (3 of 11) or decreased (8 of 11) activity relative to baseline and signaled learning by returning to baseline firing rates (Figs. 2C and 3B). Sustained and baseline-sustained changing cells were found throughout the anterior-posterior extent of the hippocampus (Fig. 1B). Although there was a significant difference between the baseline firing rate of the sustained and baseline-sustained changing cells (t test, $P < 0.05$; Table 2), both populations included both low and high firing rate cells (Fig. 1C; 11 of 14 sustained changing cells and 4 of 11 baseline-sustained changing cells had low firing rates).

Previous studies suggest that stimulus-selective response properties of neurons in the medial temporal lobe change during associative learning (*8, 10*). To address this possibility, we used a selectivity index (*19*) to examine the depth of selectivity before and after learning for the sustained and baseline-sustained changing cells (*13*). We found that the depth of selectivity increased significantly in sustained changing cells (paired t test, $T = -2.68$, $P < 0.01$) and decreased significantly in baseline-sustained changing cells (paired t test, $T = 3.39$, $P < 0.01$) (Fig. 3, C and D). These findings suggest that the striking changes in neural activity exhibited by the changing cells represent changes in the neuron's stimulus-selective response properties.

To relate the changes in neural activity to learning, we examined the temporal relationship between neural activity and behavioral performance by comparing the trial number when learning occurred to the trial number when neural activity changed (*13*). The distribution of the trial number at which neural activity changed relative to learning ranged from 12 trials before to 14 trials after learning, with an average lag/lead time of 6 ± 1 trials (Fig. 2F). Overall, the change preceded learning for 14 task periods, occurred at the same time as learning for 4 task periods, and followed learning for 19 task periods.

Our results show that hippocampal neurons signal learning by changing their stimulus-selective response properties. Because these changes could occur before, at the same time as, or after learning, this suggests that there is a gradual recruitment of a network of hippocampal neurons associated with the formation of new associative memories (*20*–*22*). Prefrontal (*23*) and premotor cortex (*20, 24*) exhibit similar patterns of changing neural activity. Further

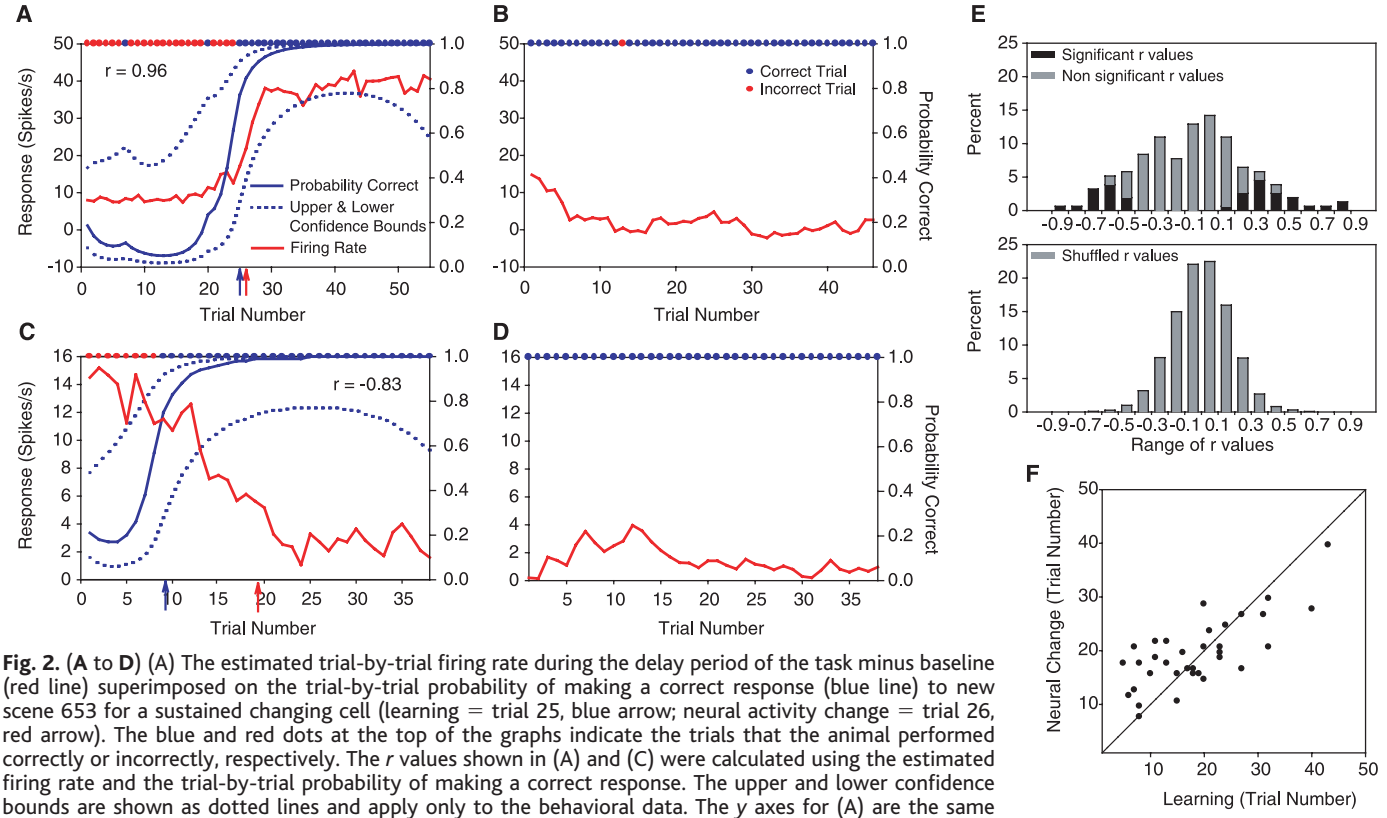

Fig. 2. (**A** to **D**) (A) The estimated trial-by-trial firing rate during the delay period of the task minus baseline (red line) superimposed on the trial-by-trial probability of making a correct response (blue line) to new scene 653 for a sustained changing cell (learning = trial 25, blue arrow; neural activity change = trial 26, red arrow). The blue and red dots at the top of the graphs indicate the trials that the animal performed correctly or incorrectly, respectively. The *r* values shown in (A) and (C) were calculated using the estimated firing rate and the trial-by-trial probability of making a correct response. The upper and lower confidence bounds are shown as dotted lines and apply only to the behavioral data. The *y* axes for (A) are the same as in (B) to (D). (B) The response of the same cell shown in (A) to the reference scene (scene 67) with same rewarded target location as new scene 653. (C) The estimated neural activity during the scene period minus baseline and behavioral learning curve for a baseline-sustained changing cell to new scene 386 (learning = trial 9; neural activity change = trial 19). (D) The response of the same cell shown in (C) to the reference scene (scene 68) with the same rewarded target location as new scene 386. (**E**) The distribution of *r* values obtained by correlating the raw neural and behavioral data (top) and the distribution of *r* values obtained on shuffled trials (bottom). All data in the distributions are expressed as a percentage of total. (**F**) Temporal relationship between the trial number of learning and the trial number when the neural activity changed for 37 task periods. Because three pairs of data points are superimposed on the plot, the total number of visible data points is 34.

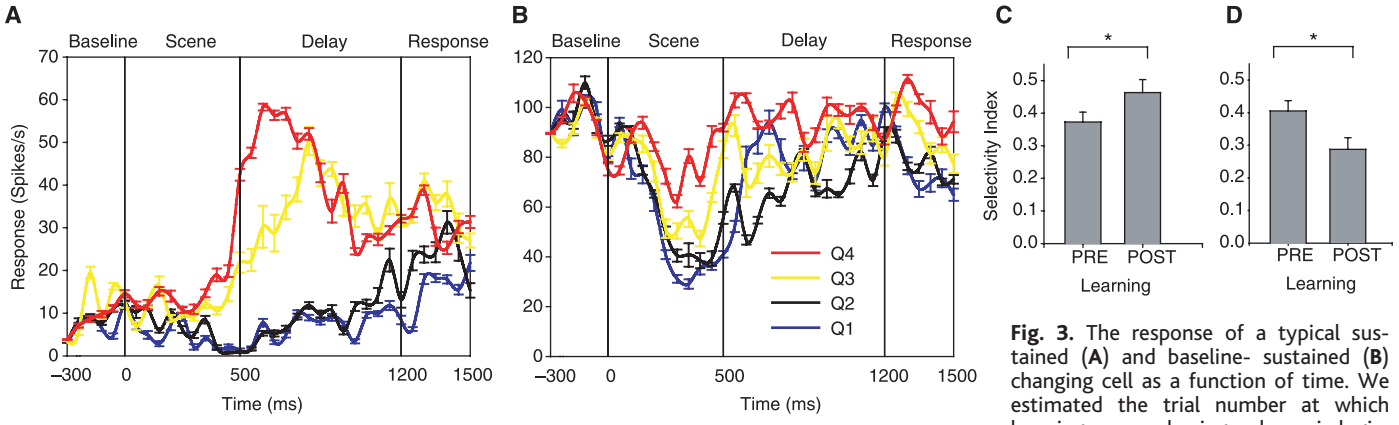

Fig. 3. The response of a typical sustained (**A**) and baseline- sustained (**B**) changing cell as a function of time. We estimated the trial number at which learning occurred using a dynamic logistic regression analysis (*13*) and then subdivided the prelearning and postlearning trials into two equal parts forming four "quartiles" of learning (Q1 to Q4). We then averaged the estimated neural activity during the baseline, scene, delay, and eye movement portions of the trial for each quartile of learning and graphed them separately. Bin width = 20 ms. (**C** and **D**) The difference in the selectivity index calculated on the neural data before and after learning for the sustained changing cells (C) and the baseline-sustained changing cells (D).

studies will be needed to clarify the relationship between the hippocampus and the premotor and prefrontal cortices in associative memory formation. We found that although both sustained and baseline-sustained changing cells signaled when learning occurred, only the sustained changing cells continue to signal selective information after learning. We hypothesize that these sustained changing cells not only participate in the formation of associative memories but also may participate in the neural circuit important for the eventual storage of these associations in long-term memory.

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15. Although all 89 selective cells exhibited a significant difference between baseline and the average response to all scenes during either the scene or delay periods, not all 89 cells exhibited a significant difference between baseline and their responses to individual scenes.
16. Of the 25 changing cells, 19 cells changed for one scene, 5 cells changed for two scenes, and 1 cell changed for three scenes.
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Supporting Online Material

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Materials and Methods
Fig. S1
References

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Programmed DNA Deletion As an RNA-Guided System of Genome Defense

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Genomewide DNA rearrangements occur in many eukaryotes during development, but their functions and mechanisms are poorly understood. Previous studies have implicated a sequence-recognition mechanism based on RNA-mediated interactions between nuclei in ciliated protozoa. In this study, we found that the process recognized and deleted a foreign gene integrated in a *Tetrahymena* chromosome, suggesting an unusual mechanism of genome surveillance. We further found that injection of double-stranded RNA into the cell at specific developmental stages triggers efficient deletion of the targeted genomic regions. Together the results indicate an RNA-based mechanism that directs genomewide DNA rearrangements and serves to disable invading genetic agents.

The ciliated protozoan *Tetrahymena thermophila* contains a germinal nucleus (micronucleus) and a somatic nucleus (macronucleus) in each cell. During sexual conjugation, the micronucleus goes through a series of events to produce a zygotic nucleus, which divides and differentiates to form the new macro- and micronucleus of the progeny cell. The old macronucleus is destroyed. Formation of the new macronucleus involves extensive genomewide DNA rearrangements. Thousands of specific DNA segments, comprising ~15% of the genome, are deleted, and the remaining DNA is fragmented and endoduplicated about 23-fold to form the somatic genome, which is responsible for all transcriptional activities during growth. These deleted segments (referred to here as deletion elements) range from several hundred base pairs (bp) to more than 20 kb in size and are composed of single-copy and moderately repetitive sequences. Some of them are deleted with precise boundaries, whereas for others the boundaries are somewhat variable (*1*). The mechanism, biological role, and evolutionary origin of this deletion process remain largely unknown.

Although programmed deletion of micronucleus-specific sequences is a tightly regulated process, several notable exceptions have been observed to occur in *Tetrahymena* (*2*) and a related ciliate, *Paramecium* (*3*, *4*). Through genetic manipulations, a cell can be modified to contain a particular micronucleus-specific sequence in the macronucleus. This produces an unusual phenomenon: In subsequent matings of this cell, the presence of the anomalous sequence in the old macronucleus prevents this sequence from being deleted in the newly formed macronucleus. These data indicate that, through some type of internuclear communication, a ciliate cell can distinguish sequences that are present only in the germ line from those also present in the somatic genome. Through this same process, the cell could potentially identify the sequences destined for deletion. It suggests a possible mechanism for deleting micronucleus-specific sequences without the need for a specific sequence signal. If true, foreign sequences that are inserted only into the germline genome will also be deleted in the daughter macronucleus through this process.

To test this idea directly, we inserted a bacterial sequence into the germline genome of *Tetrahymena* with the use of homology-directed gene replacement (*5*). The inserted sequence is a 1.5-kb knockout cassette containing the neomycin-resistant gene (*neo*) of *Escherichia coli* flanked by *Tetrahymena* regulatory sequences (*6*). We inserted this sequence downstream of the single ribosomal RNA gene, replacing a 4.2-kb region that includes a normal deletion element with variable boundaries (*7*, *8*) (Fig. 1A). Strains of compatible mating types were generated that were homozygous for this cassette in their micronuclei but lacked the transgene in their macronuclei (*5*). They were crossed to one another to produce progeny for DNA analysis. The macronucleus of the progeny was expected to contain the transgene because it is descended from the parental micronuclei. However, smaller DNA fragments were detected (Fig. 1B) that apparently lost the transgene cassette through simple deletions (Fig. 1A). The deleted regions are slightly heterogeneous, with their right deletion boundaries falling just inside the cassette and the left boundaries just outside of it (fig. S1). We tested the same transgene at two additional

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