(objects or events). No visual pattern matching procedure could, by itself, account for the detection of these correspondences. The infant's enumerative procedure must be more general.

It remains to be determined whether infants' numerical categories are as differentiated as those of older children and whether they are absolute (in the sense of "two" and "three") or relative (in the sense of "more numerous" and "less numerous"). It is also not known how the abilities of infants are related developmentally to those of older children. Answers to these questions may begin to elucidate the psychological foundation of number.

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4. For one development of this view, see D. Klahr and J. G. Wallace [*Cognitive Development, An Information Processing View* (Erlbaum, Hillsdale, N.J., 1976)].
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6. Infants were seated either in an infant seat or on the lap of a parent who wore glasses that occluded his or her view of the displays. The slides were rear-projected onto a 64-cm by 89-cm screen located 60 cm from the infant. The projected displays subtended visual angles of 21.8° (vertical) by 25.2° (horizontal) and were separated by 25.2°. The objects in each slide were presented against a white background, each spatially separated from the others. Room lighting was dim.
7. The experiment was terminated if an infant became fretful or drowsy. Eleven infants completed all 32 trials. One infant failed to complete 16 trials and was replaced. The remaining five infants completed from 17 to 29 trials. All infants were presented with 16 unique pairs of visible displays (Fig. 1). The order of presentation and the lateral position of displays within a pair were counterbalanced across infants. Each infant was presented with two drumbeats on half of the trials and with three drumbeats on the other half. For half of the infants, a particular display pair was accompanied by two drumbeats; for the rest, it was accompanied by three beats. The presentations of the materials on the first 16 trials were repeated on the second 16 trials.
8. Interobserver reliability was greater than 0.9. The observers viewed the infants through peepholes located to the left or right of the projection screen. Partitions blocked their view of the screen and hence the displays. Parents' opaque glasses did not reflect light from the displays. Moreover, two experiments revealed that the observers could neither see reflections of the displays on the infants' corneas nor analyze the infants' patterns of eye scanning to determine the number of objects on each side. Use of corneal reflections was tested in experiment 4, in which four infnats were presented with the materials in Fig. 1. Eight observers (two per infant) who had also served as observers in the main experiments monitored corneal reflections from the displays and judged, as best they could, the lateral position of the two-object display. The observers' proportions of correct judgments did not differ from that expected by chance (proportion, 0.49). Use of scanning patterns was tested in experiment 3 by instructing one of the two observers present at each session to use such patterns to judge the position of the two-object display. Again, judgments were at chance level.
9. Across experiments, a preference for the two-object display when accompanied by two drumbeats was present in the first block of trials (proportion of duration, 0.54, $P < 0.05$), in the second block (proportion, 0.55; $P < 0.01$), and across both blocks (proportion, 0.55, $P < 0.01$); a preference for the three-object display when accompanied by three drumbeats was not present in the first block of trials (proportion, 0.51) but was present in the second block (proportion, 0.58, $P < 0.01$) and across both blocks (proportion, 0.54, $P < 0.01$).
10. Of the 30 infants who had an overall preference for the corresponding display, 11 exhibited one or more long uninterrupted runs as identified by a runs test [S. Siegel, *Nonparametric Statistics* (McGraw-Hill, New York, 1956)] for the presence of significantly few runs of trials in which either the corresponding display was preferred or the noncorresponding display was preferred. An additional five infants, who did not exhibit a long run, nevertheless preferred the corresponding display on a significant number of trials as indicated by a sign test.
11. Supported by NIH postdoctoral fellowship MH 07949 and by a University of Pennsylvania cognitive science fellowship to P.S., by NIH grant HD 13248 to E.S.S., and by NSF grant BNS 80-04881 to R.G. We thank R. G. Cooper, J. E. Hochberg, and the reviewers for their comments, and W. S. Born, S. Mangelsdorf, and C. Norris for assistance in conducting the research. Portions of this work were presented at the meetings of the Psychonomic Society, Philadelphia, 1981, and the International Conference on Infant Studies, Austin, Texas, 1982.

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A Microtubule Meshwork Associated with Gametic Pronucleus Transfer Across a Cell-Cell Junction

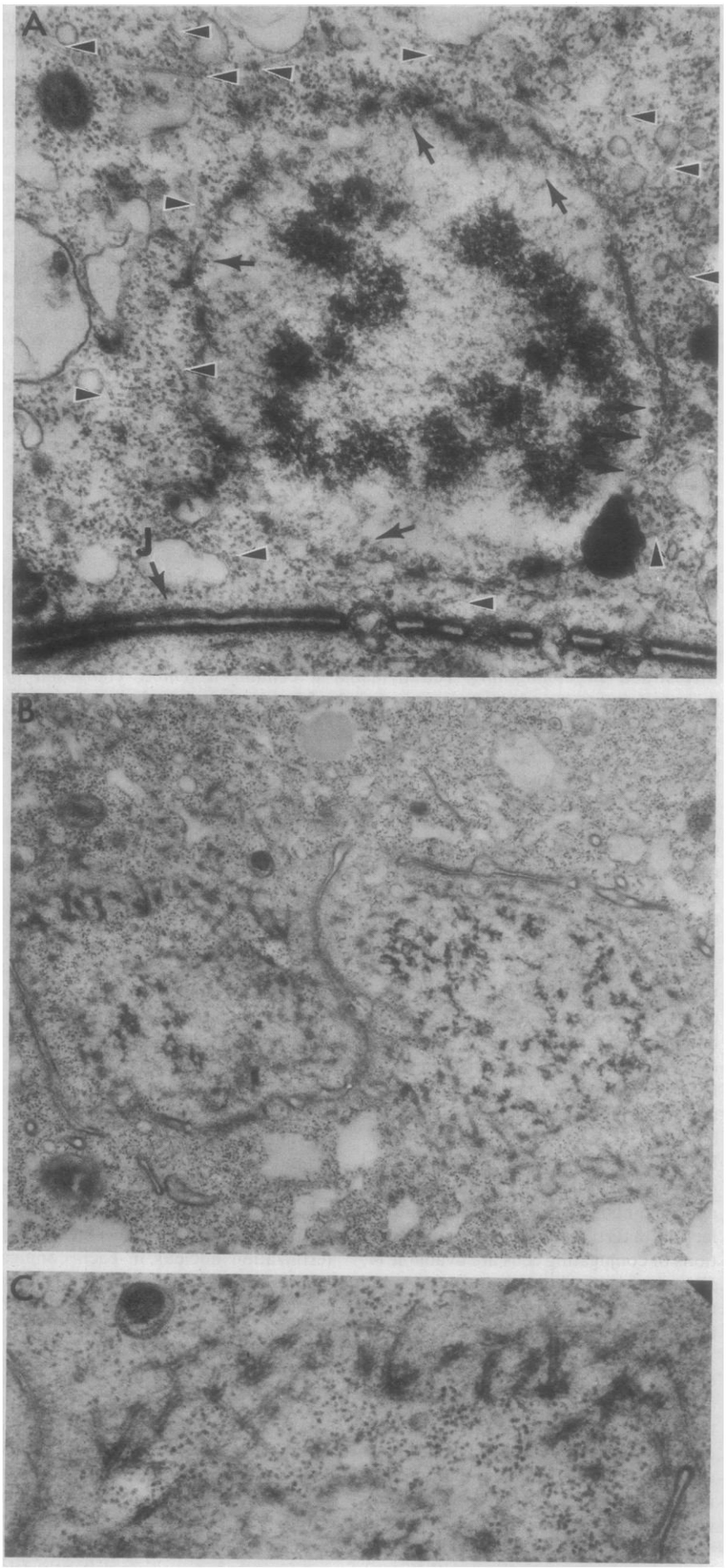
Abstract. *In conjugating* Tetrahymena, *a cellular assembly composed of a microtubule meshwork appears to be required for the transfer of gametic pronuclei across the junction that separates the conjugating cells. This assembly is suggestive of a gametogenic cell division in ancient predecessors of ciliates, with* Tetrahymena *retaining only the associated nuclear division and export.*

Fertilization in the unicellular eukaryote *Tetrahymena thermophila* includes a reciprocal exchange of migratory gametic pronuclei across a temporary junction that separates the two conjugating cells. *Tetrahymena thermophila* is a useful model system for the study of fertilization. Conjugation can be induced efficiently and synchronously in large populations of *T. thermophila*. The nuclear events occurring during conjugation have been observed by light microscopy and are well characterized (*1*). The exchange of gametic pronuclei can be blocked with hyperosmotic shock (*2*) and microtubule assembly inhibitors (*3*). Genetic methods for the detection and selection of fertilization failures in populations of conjugating cells are available (*4, 5*) and should allow the isolation and characterization of mutants defective in fertilization functions.

The two conjugating cells are separated by a specialized junction (*6*) covering an area close to 100 μm^2. It consists of the plasma membrane of each of the two cells, separated by a very regular gap of around 30 nm and interrupted by channels or pores that provide cytoplasmic connections between the two cells (Fig. 1A). The cytoplasmic side of the membrane is continuously lined with an epiplasmic layer approximately 35 nm thick (*7*).

The nuclear events of conjugation (*1*) begin with meiosis of the diploid (germline) micronucleus. Only one of the four haploid meiotic products remains functional in each cell. A mitotic division of this product generates to gametic pronuclei; one, the migratory pronucleus, lies against the junction that separates the two cells while the other, the stationary pronucleus, lies farther away. Fertilization involves the reciprocal and generally simultaneous exchange of migratory pronuclei across the junction. The incoming migratory pronucleus immediately fuses with the stationary pronucleus of the recipient, thus generating a fertilization nucleus in each conjugant. Within a few minutes after fusion, the fertilization nucleus undergoes the first postzygotic mitotic division.

Our objective was to characterize the ultrastructure of fertilization in *Tetrahymena* in an attempt to understand why inhibitors of microtubule assembly block pronuclear transfer across the junction. Our study revealed a basket-like structure, consisting of a meshwork of microtubules, associated with each migratory pronucleus. This meshwork is similar to one reported in a ciliate distantly related



to *Tetrahymena*, the suctorian *Heliophrya erhardi* (*8*).

Cells were grown and prepared for conjugation as described (*4*). Starved cells were mixed in Dryl's medium (*9*) at 30°C; 5½ hours later, the cells were fixed for electron microscopy with 2 percent glutaraldehyde in 5 m*M* potassium phosphate buffer (*p*H 7.0) containing 1 percent sucrose, followed immediately with 1 percent osmium tetroxide. After 1 hour, the cells were washed twice with 20 m*M* potassium phosphate buffer at *p*H 7.0, block-stained with 1 percent uranyl acetate in 50 percent ethanol, dehydrated through increasing alcohol concentrations and 100 percent propylene oxide, and embedded in Araldite. After sections were cut, the grids were stained in 1 percent uranyl acetate and Reynolds' lead citrate (*10*).

Electron microscopy of more than 30 pairs of conjugating cells (*11*) suggests that pronuclear transfer proceeds through the stages described below. Figure 1A shows a nucleus that has just completed gametogenic mitotic division. In addition to the parallel array of intranuclear microtubules that constitute the mitotic spindle (arrows), there is an abundance of less organized microtubules that crisscross the space surrounding the migratory pronucleus (arrowheads). The migratory pronucleus then acquires the biconvex lens shape observed earlier by light microscopy (*1*). A bowed junction lines one face of the lens, and a conspicuous microtubule meshwork lines the other (*12, 13*) (not shown). The midplane of the lens coincides with the position originally occupied by the junction. Possible connections between

Fig. 1. Electron micrograph of conjugation stages related to fertilization in *Tetrahymena*. (A) Migratory pronucleus that has just completed the gametogenic division before transfer. The chromatin is still condensed. Two microtubule systems are observed: the mitotic spindle (parallel microtubules just inside the nuclear envelope, arrows) and a set of microtubules oriented in diverse directions outside the nuclear envelope (arrowheads). The junction (*J*) is still intact (×27,700). (B) Simultaneous and reciprocal transfer of gamete pronuclei across the junction between conjugating *Tetrahymena* cells. The upper pronucleus is being transferred from the cell on the left to the cell on the right; the lower pronucleus is being transferred in the opposite direction. Each nucleus lies between its fertilization basket (behind) and the deforming junction (ahead) (×27,100). (C) Portion of the fertilization basket behind the lower migrating pronucleus shown in the cover figure. Frequent microtubule contacts, the amorphous material among the microtubules, and the partial exclusion of ribosomes in the area of the basket can be seen (×40,400).

microtubules of this meshwork and the pronuclear envelope are occasionally seen in our preparations. The next recognizable stage consists of a disintegrating junction ahead of a pronucleus that has become more nearly spherical, at least in the forward hemisphere. Figure 1B illustrates a section through a pair of passing pronuclei. The microtubule meshwork of the two pronuclei, taken together, describes an *S*-shaped arc. The two ends and the center of the *S* are still approximately lined up with the original position of the junction. After pronuclear transfer is completed, the meshwork remains aligned with the original position of the junction, filling the breach that still exists in the junction; a complete junction is eventually regenerated (not shown).

If, at 5½ hours after being mixed, the cells are treated for 30 minutes with vinblastine (final concentration, 3 μM) and immediately fixed, the cell pairs do not have a basket; few, if any, microtubules are seen around the pronuclei (not shown). This observation, together with the genetic and autoradiographic evidence that treatment with vinblastine or with other inhibitors of microtubule assembly blocks the exchange of pronuclei (*3*), is consistent with the idea that the basket performs an essential function in pronuclear exchange.

We suggest that pronuclear transfer takes place by the following mechanism. After (or during) the mitotic division, a large number of microtubules assemble around each migratory pronucleus near the junction (Fig. 2A). The microtubules are usually arranged tangentially around the nucleus, but otherwise are not very regularly organized (like sticks in a bird's nest); collectively they form a cup around each migratory pronucleus. Next, the microtubule meshwork tightens and shrinks; the microtubules become more densely packed and the curvature decreases. This causes the junction to buckle and pushes the pronucleus through the junction into the other conjugant (Fig. 2B). The junction is locally disrupted at this time, perhaps by a different but coordinately timed mechanism. After pronuclear transfer, the condensed meshwork remains at the site of the junction and could serve as a backstop for the nucleus and as a screen to prevent the exchange of other cellular organelles.

The mechanism that provides the motive force for the shrinking of the microtubule meshwork is not clear. The force may be derived from sliding interactions between the microtubules themselves. Indeed, sections through the meshwork invariably show an abundance of microtubule crossings where the microtubules come into contact with one another (Fig. 1C). The microtubules of the meshwork are embedded in a matrix that excludes ribosomes and that seems to be amorphous but may be finely fibrillar (Fig. 1C) (*14*); perhaps contraction of this material provides the motive force for the shrinking of the meshwork (*15*) while the microtubules provide passive structural reinforcement.

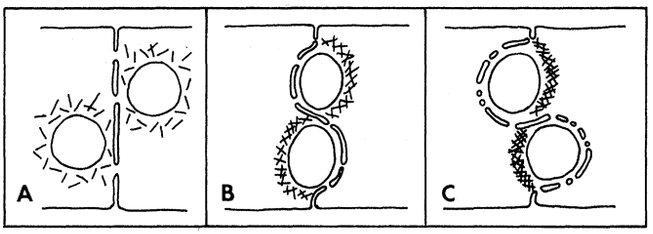


Fig. 2. Proposed mechanism of pronuclear exchange. (A) Formation of a microtubule basket around the pronuclei. (B) The walls of the basket contract (shrink) and flatten, bowing the junction. (C) Further flattening of the basket pushes the pronucleus into the conjugal mate.

The microtubule meshwork surrounding the posterior hemisphere of the migratory pronucleus of *Tetrahymena* is remarkably similar to the microtubule meshwork in *Heliophrya* (*8, 14*), the only other ciliate well characterized in this respect. The characteristic ultrastructure of the microtubules associated with the gamete pronuclei is also similar in the two organisms (*14*). *Tetrahymena* and *Heliophrya* are both ciliates, but very distantly related (*16*), and this mechanism for the transfer of the gametic pronucleus may have arisen before the evolutionary radiation of this phylum (*17*). Associations between microtubules and a migratory pronucleus have also been seen in *Paramecium* (*18*). We suggest that these microtubules are part of a structure functionally similar (if not identical) to the *Tetrahymena* fertilization basket.

The microtubule meshwork, and its association with a mitotic nucleus, is reminiscent of structures found during cell division in other eukaryotes (*19*). Ciliates probably evolved from unicellular eukaryotes in which a postmeiotic mitosis gave rise to female and male gametes capable of fertilization in the surrounding medium (*20*). The fertilization basket may well be an evolutionary descendant of a structure that functioned during this gametogenic division. Evolution of the initimate interactions between gamete mother cells (that is, the conjugal junction) eventually allowed the deposition of the male gamete cell (reduced essentially to a nucleus) directly into the mate's cytoplasm. Pairing of unicells—triggering gametogenic mitoses and the production of free-swimming cellular gametes in an enclosed volume—occurs in some Foraminifera (*21*). A similar (possibly homologous) stage could have been an intermediate in the evolution of internal fertilization in the ciliates.

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The Use of Hypnosis to Enhance Recall

Abstract. *The forensic use of hypnosis is increasing. A hypermnesic procedure was used in an experiment that calls this practice into question. Subjects tried for a week to recall 60 previously presented pictures. They were then either hypnotized or not and encouraged to recall even more pictures. Most of the newly recalled material was incorrect, especially for highly hypnotizable subjects in the hypnosis condition. Such errors in recall can have profound implications for forensic investigations.*

The increased use of hypnosis in forensic investigation has become controversial (*1*). Although numerous case reports attest to the utility of hypnosis in enhancing the recall of the eye witness (*2*), controlled studies have produced conflicting results. Some studies have failed to demonstrate hypnotic hypermnesia, whereas those that have (*3*), have not reported errors in a systematic way nor controlled for the natural hypermnesic effects that can be achieved through repeated testing (*4*). Still others (*5*) have found that hypnotized subjects are susceptible to leading questions. Although scientists are wary of the reliability of forensic hypnosis, police investigators are lobbying to sanction its use in criminal investigation and the judiciary is seeking evidence on which to base legal decisions. The relation between hypnosis and memory enhancement needs to be clarified.

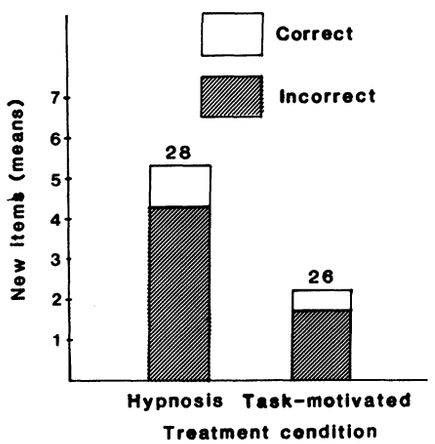

Fig. 1. New items presented as memories by subjects after hypnotic or task-motivating suggestions to enhance recall. All items were designated by subjects as true memories. The number of subjects in each group is shown above each bar.

We now report that any pressure to enhance recall beyond the initial attempt may increase the number of items recalled but increase the number of errors as well. The use of hypnosis exaggerates this process, particularly for those with hypnotic ability. When hypnotized, the highly hypnotizable subjects recalled twice as many new items as controls but made three times as many new errors.

Fifty-four subjects were selected on the basis of their hypnotic ability as measured by a group adaptation of the Stanford C Scale of Hypnotic Susceptibility (SHSS:C) (*6*). Subjects with low susceptibility had SHSS:C scores from 1 to 6, and those with high susceptibility, from 7 to 12. All subjects were presented with a series of 60 slides of simple black-and-white line drawings of common objects (*7*), presented at a rate of 3½ seconds per slide. They were then given a recall sheet and requested to write the name of a line drawing in each of the 60 blank spaces provided for this purpose, indicating as well which items represented memories and which were just guesses. This forced recall procedure is standard in hypermnesia studies (*8*). Subjects were initially given three trials in the laboratory with 3-minute rest periods between trials.

Subjects were then instructed that during the next week they were to recall as many of the line drawings as they could once each day, and to write their recollections on the take-home recall sheets provided. They were asked to deposit each recall sheet in a convenient drop-box daily for 6 days. Altogether, subjects completed nine trials over a period of 7 days before their second laboratory session.

The mean number of items recalled on the first trial was 30. By trial 9 the cumulative mean had risen to 38 items—an increase of 27 percent. The number of errors increased as well, from an average of less than one error on the first trial to an average of four errors by the ninth. Most subjects approached asymptotic levels of output by about trial 7, 4 days after a single viewing of the stimuli.

The next step was to see whether hypnotic suggestions for increased recall would enable subjects to retrieve more information after asymptotic recall had been reached. During this second laboratory session, subjects were told to relax and focus all their attention on the slides they had seen the week before. They did so either while hypnotized (hypnosis condition) or without hypnosis (task-motivated condition). Before this session subjects did not know which condition they would be in, and the experimenters were unaware of subjects' hypnotic ability. Consistent with these precautions, independent sample t-tests indicated no difference between high and low susceptible subjects in the cumulative number of correct items retrieved over the week before treatment [$t(26) = 0.49$] or for the cumulative errors retrieved prior to treatment [$t(26) = 0.14$].

Figure 1 illustrates the number of items reported on the treatment trials that had never been reported as memories before. Subjects in the hypnosis group reported over twice as many new items (both correct and incorrect) as subjects in the task-motivating condition did. The correct information retrieved by subjects in both conditions remained proportional to this shift in total output. Those higher in hypnotic ability in the

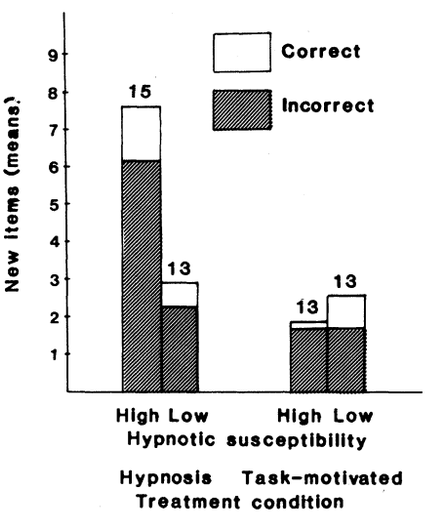

Fig. 2. New items presented as memories by subjects of high and low susceptibility to hypnosis after hypnotic or task-motivating suggestions to enhance recall. The number of subjects in each group is shown above each bar.