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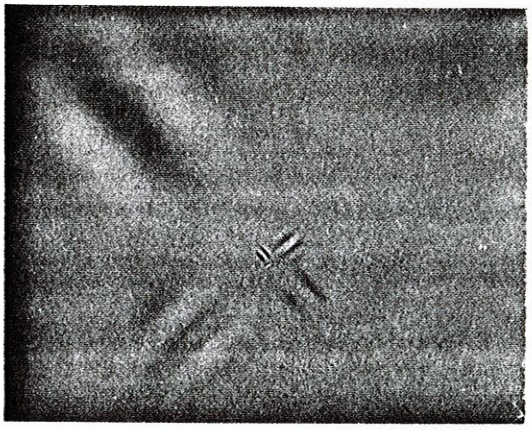
The mind's eye

CAMBRIDGE, England — The researchers wanted to know what kinds of images the eye sees best. They fiddled with the contrast on a television screen here as they showed spots, stripes, rectangles, disks, and blobs of assorted shapes. The winner, defined as the pattern that could be seen with the least intense contrast, turned out to be an odd-looking thing: a round patch with fuzzy bars.

So what? Well, this odd patch may be the basic unit of human sight. Many vision researchers theorize that we make sense of an image only after neurons in the brain have broken it down into simple patterns—roughly comparable to the way a television breaks every picture into dots. Because the "grating patch," as it's known, seems to be the image that's easiest to perceive, NASA psychologist Andrew Watson theorizes that it's the human equivalent of the TV's dot. The case isn't proven—a more readily detectable pattern could still be found, or the underlying theory of how images are processed could be wrong. But Watson suspects that you understood this page only after converting it to an overlapping set of variously sized patches with bars.

"It's a hard concept to understand—after all, when we look at the world, we don't see these little fuzzy bars," says Watson, who did the experiment with physiologists H. B. Barlow and John Robson of Cambridge University. "But there's no reason why we should be sensitive to the workings of our brain. It's got to take patterns of light and transform them into electrical signals—basically, a bunch of numbers—so it knows what it's looking at. The grating patch may be the most efficient way to break down the information."

i.e. relevant to our own discovered CRV signal processes.



NASA

Researchers suspect the brain breaks down any image into simple, overlapping patterns: round patches with blurred stripes of various sizes, as shown here.

machinery can physically interact with this tail (*9–11*). Proteins that bind to this tail have the potential to modulate CFTR gating by stabilizing or disrupting its interaction with the R domain. The NH_2-terminal tail of CFTR could serve as a target for physiologic regulators of CFTR gating or for pharmacologic maneuvers to modulate CFTR activity.

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12. Cystic Fibrosis Genetic Analysis Consortium (www.genet.sickkids.on.ca/cftr).
13. All point mutations were introduced into a 1-kb fragment of the CFTR coding region by the Quickchange site-directed mutagenesis kit (Stratagene). This fragment was sequenced completely to verify each mutation and then ligated into pCDNA3 vector (Invitrogen) containing the rest of the CFTR coding region.
14. A. P. Naren, E. Cormet-Boyaka, K. L. Kirk, unpublished data.
15. A. P. Naren, M. Villain, D. Muccio, K. L. Kirk, unpublished data.
16. The triple and quadruple N-Tail mutants are not completely inactive; for example, cAMP-activated currents can be detected when higher cRNA amounts for these mutants are injected. Indeed, because of the nonlinearity of the oocyte expression system, the currents mediated by these mutants can approach (but not reach) wild-type levels at very high cRNA amounts (such as 50 ng).
17. J. Fu and K. L. Kirk, unpublished data.
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19. M. W. Quick and K. L. Kirk, unpublished data.
20. Recombinant NBD1 (amino acids 433 to 584) bound weakly to GST–N-Tail, but this binding was not inhibited by the N-Tail mutations that inhibit CFTR channel activity.
21. A. P. Naren and K. L. Kirk, unpublished data.
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24. Assays were done as described (*9, 10*). Briefly, soluble GST–N-Tail peptide was added to a lysate of COS-7 cells [0.2% Triton X-100 in phosphate-buffered saline (PBS)] transiently expressing recombinant CFTR (or various R domain constructs) and mixed for 1 hour at room temperature. Bound proteins were precipitated with excess glutathione agarose, washed extensively in 0.2% Triton X-100 in PBS, and analyzed for CFTR or R domain by immunoblotting with monoclonal antibodies to the COOH-terminus or R domain (Genzyme). CFTR immunoprecipitations were done on portions of the same lysates (*9, 10*).
25. Peptides were synthesized on a PerSeptive Biosystems 9050 peptide synthesizer. The biotin was conjugated to the NH_2-terminus with fluorenyl methoxycarbonyl-amino caproic acid as spacer. Peptide binding (biotin P30-63) was assessed by mixing 1.25 μM peptide and 2.5 μM soluble GST-R domain peptide in PBS for 1 hour at 22°C. A 100-fold molar excess of unbiotinylated peptide was used for competition experiments. The complex was precipitated with excess glutathione agarose and washed with PBS. Streptavidin–horseradish peroxidase (HRP, 1 μM) was added in PBS and incubated for 20 min. The beads were washed extensively in PBS plus 0.2% Triton X-100 and assayed for HRP activity with 2,2′-azinobis(3-ethylbenzothiozoline)-6 sulfonic acid (ABTS) according to manufacturer's instructions (Pierce, St. Louis, MO).
26. The single-channel properties of wild-type CFTR and the N-Tail mutants were analyzed in inside-out membrane patches excised from oocytes. The pipette solution contained 140 mM *N*-methyl-D-glucamine (NMDG), 0.2 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 10 mM Hepes (pH to 7.4 with HCl). The bath solution contained 140 mM NMDG, 0.5 mM $MgCl_2$, 1 mM EGTA, and 10 mM Hepes (pH to 7.4 with HCl) supplemented with 1.5 mM Mg-ATP and PKA catalytic subunit (80 U/ml, Promega). Records of multichannel patches (holding potential −80 mV) were digitized using an Axopatch 200B amplifier, filtered at 200 Hz, and analyzed using PCLAMP 6.0 software. The open probabilities of the N-Tail mutants should be considered to be maximal estimates given the very brief open times of these mutants, which leads to underestimating channel number. Open-channel burst durations were estimated with the cycle time method and a minimal interburst duration of 20 ms (*8*). The N-Tail mutants also exhibited reduced open-channel burst durations relative to wild-type CFTR when analyzed at shorter minimal interburst durations such as 10 ms; however, this gave an underestimate of the true open-channel burst duration. The single-channel properties of the ΔR CFTR constructs were analyzed in membrane patches excised from transfected HeLa cells as described (*22*) with a minimal interburst duration of 20 ms. All patch clamp experiments were performed at 20° to 22°C.

27. We thank M. Welsh for providing the original ΔR-S660A CFTR construct. Supported by NIH grants DK51868 and DK50830 (K.L.K.), DA10509 (M.W.Q.), and MH52527 (J.E.B.).

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Neurogenesis in the Neocortex of Adult Primates

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In primates, prefrontal, inferior temporal, and posterior parietal cortex are important for cognitive function. It is shown that in adult macaques, new neurons are added to these three neocortical association areas, but not to a primary sensory area (striate cortex). The new neurons appeared to originate in the subventricular zone and to migrate through the white matter to the neocortex, where they extended axons. These new neurons, which are continually added in adulthood, may play a role in the functions of association neocortex.

The traditional view of the adult primate neocortex is that it is structurally stable and that neurogenesis and synapse formation occur only during development (*1, 2*). Yet structural plasticity in adult brains is found both among lower vertebrates (*3*) and in phylogenetically older mammalian structures such as the olfactory bulb and hippocampus (*4, 5*), even in primates (*6, 7*). Furthermore, neurogenesis is widespread in the adult avian brain including in the hyperstriatum (*8, 9*), a structure homologous to the mammalian cerebral cortex (*10*). Thus, it may seem paradoxical that there is no compelling evidence for neurogenesis in the neocortex of adult mammals (*11*) and there are even strong claims against it for primates (*1*). Using bromodeoxyuridine (BrdU) labeling, which marks proliferating cells and their progeny (*12*), combined with retrograde tract tracing and immunohistochemistry for neuronal markers, we attempted to resolve this paradox. We report that in adult macaques, new neurons are indeed added to several regions of association cortex where they extend axons. The presence of new neurons in brain areas involved in learning and memory (*13*) supports earlier suggestions that adult-generated neurons may play a role in these functions (*9, 14, 15*).

We injected 12 adult *Macaca fascicularis* with BrdU and used immunohistochemistry for cell-specific markers to examine BrdU-labeled cells in prefrontal, inferior temporal, posterior parietal, and striate cortex (*16*). The following markers were used: for mature neurons, (i) NeuN (neuronal nuclei), (ii) NSE (neuron-specific enolase), or (iii) MAP-2 (microtubule-associated protein-2); for immature neurons, TOAD-64 (turned-on-after-division 64-kD protein); for astroglia, GFAP (glial fibrillary acidic protein) (*17, 18*).

In animals perfused 1 week or more after the last BrdU injection, we observed BrdU-labeled cells in prefrontal, inferior temporal, and parietal cortex (Figs. 1 and 2; Table 1). In the region of the principal sulcus in prefrontal cortex, the majority (62% to 84%) of BrdU-labeled cells had round or oval nuclei, morphological characteristics of mature neurons (nuclear diameter = 10 to 25 μm). Confocal laser scanning microscopic analysis of immunostained tissue (*19*) indicated that a subset of these cells expressed markers of mature neurons (Figs. 1 through 3; Table 1). BrdU-

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labeled cells with neuronal characteristics were found in layers 1 through 5, but not 6. Those that were immunoreactive for MAP-2 exhibited dendritic processes (Fig. 3).

Similar proportions of BrdU-labeled cells were co-labeled with the neuronal marker NeuN in inferior temporal cortex (animals #4 and #5), posterior parietal cortex (#10, #11, #12), and in the vicinity of the anterior cingulate sulcus (#5). In contrast, very few BrdU-labeled cells in these regions co-labeled with a marker of astroglia, GFAP (Fig. 3; Table 1). Unlike the association cortex areas examined, in striate cortex, the few BrdU-labeled cells found never co-labeled with the neuronal markers, although some were positive for GFAP (#3, #4, #10, #11, #12). The absence of new neurons in striate cortex was probably not the result of either their rapid death or a longer migration time to reach striate cortex, because they were absent in animals perfused from 1 to 7 weeks after the first BrdU injection.

In the animals (#1, #2) that were perfused 2 hours after a single BrdU injection, labeled cells were observed in the subventricular zone (svz) lining the wall of the lateral ventricles (Fig. 4). In these animals, very few BrdU-labeled cells were observed in the neocortical areas examined; all had small, irregularly shaped nuclei and none expressed neuronal markers. In animals that received several BrdU injections with survival times ranging from 1 to 3 weeks, we not only found BrdU-labeled cells in the svz but also observed evidence of migrating BrdU-labeled cells in the white matter of frontal and temporal sections (Figs. 4 and 5). The BrdU-labeled cells in the white matter were elongated or fusiform in shape, and those that were co-labeled with TOAD-64 had leading and trailing processes characteristic of migrating cells (Fig. 5) (*20*). In animals #3, #4, #5, and #7, these putative migrating cells were arrayed in a stream from their likely site of origin in the wall of the lateral ventricle, through the white matter, to their probable destination in frontal neocortex (Figs. 4 and 5). Elongated BrdU-labeled cells in the white matter did not co-label with markers of mature neurons or astroglia. This putative migratory route for newly generated cells was also observed in two additional animals that received a single BrdU injection and were perfused either 1 week (#8) or 2 weeks (#9) later (Table 1, Fig. 4).

These results suggest that in the adult macaque brain, new cells originate in the svz and migrate through the white matter to certain neocortical regions where they differentiate into mature neurons. At a short survival time (2 hours), BrdU-labeled cells were observed in the svz. At longer survival times (1 to 3 weeks), BrdU-labeled cells that appeared to be migrating were observed in the white matter, and

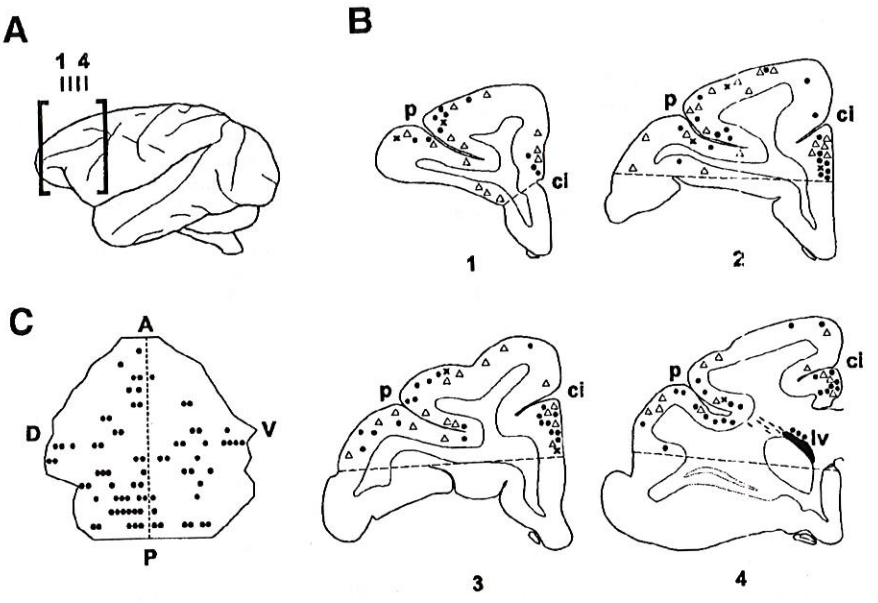

Fig. 1. The distribution of BrdU-labeled cells in prefrontal cortex in adult macaques. **(A)** Lateral view showing levels (1 through 4) of the coronal sections shown in (B) and the principal sulcus region (boxed area) from which the flattened map shown in (C) was made. **(B)** Coronal sections [adapted from (*30*)] showing the distribution of BrdU-labeled cells in the region of the principal sulcus and the anterior cingulate sulcus from animal #5. Solid dots represent BrdU-labeled cells that were not immunoreactive for NeuN or GFAP. Open triangles represent BrdU-labeled cells that were immunoreactive for the neuronal marker NeuN. X represents BrdU-labeled cells that were immunoreactive for the astroglial marker GFAP. Dashes (−) represent BrdU-labeled cells with elongated nuclei in the white matter. Regions below the dashed lines were not examined. ci, cingulate sulcus; lv, lateral ventricle; p, principal sulcus. **(C)** Flattened map of the principal sulcus region (#3) showing the distribution of BrdU-labeled cells (in coronal sections 1 mm apart). The dashed line represents the floor of the sulcus. Each dot represents one BrdU-labeled cell. There were no obvious dorsal-ventral or anterior-posterior gradients in BrdU-labeled cells in this area. A, anterior; V, ventral; D, dorsal; P, posterior.

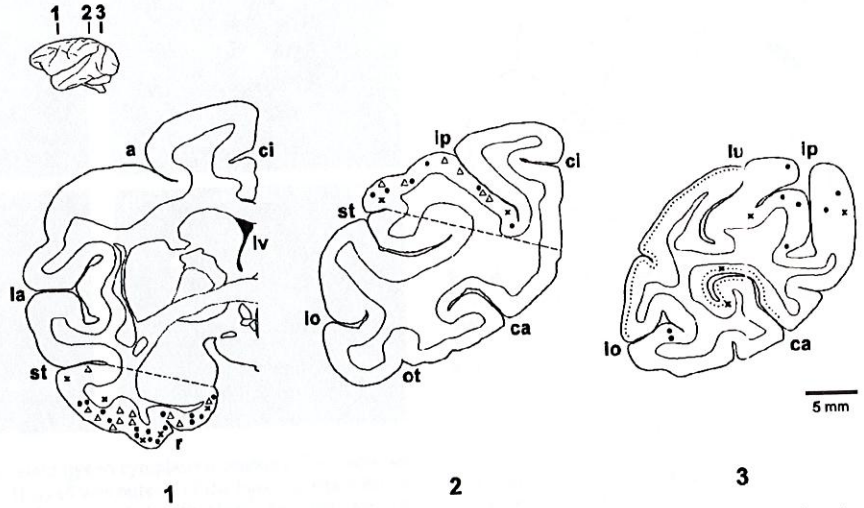

Fig. 2. The distribution of BrdU-labeled cells in coronal sections through inferior temporal cortex (section 1), posterior parietal cortex (section 2), and occipital cortex (section 3) (from animal #10). Symbols are as in Fig. 1. BrdU-labeled cells co-labeled with NeuN were not observed in striate cortex (shown with dotted lines in section 3). a, arcuate sulcus; ca, calcarine sulcus; io, inferior occipital sulcus; ip, intraparietal sulcus; la, lateral sulcus; lu, lunate sulcus; ot, occipito-temporal sulcus; r, rhinal sulcus; st, superior temporal sulcus. The area above the dashed line in 1 and below the dashed line in 2 was not analyzed.

those with mature neuronal characteristics were found in the neocortex. In the adult rodent, the svz produces new cells that migrate in the rostral migratory stream to the olfactory bulb, where they differentiate into neurons (*5*). Our results suggest that in the adult macaque, the svz is the source of an additional population of new neurons that migrate through fiber tracts to neocortical regions.

To establish further the neuronal identity of the new cells and explore their cortical connections, we carried out a combined BrdU labeling and fluorescent retrograde tracing study in animals #10, #11, and #12 (*21*). Several weeks after BrdU administration, these animals were

Table 1. Characteristics of experimental animals and BrdU-labeled cells in the principal sulcus region. All animals, except #7, were given one to five i.p. injections (injs.) daily of 75 to 100 mg/kg BrdU. For all animals but #7, survival time represents the time after the last BrdU injection when the monkey was perfused. Animal #7 received four i.v. injections of 100 mg/kg BrdU, each separated by 1 week with the last injection 24 hours before perfusion. Animals #1 to #6 were also used in a different study of neurogenesis in the dentate gyrus (*7*). The stereological optical dissector method was used to estimate the number of BrdU-labeled cells/mm^3 (*19*). The percentages of BrdU-labeled cells that expressed specific markers were each obtained from a sample of 100 BrdU-labeled cells per marker per animal. As mentioned in the text and in (*22*), three additional male animals (#10, #11, #12) were used for a retrograde tracer study and for identifying new neurons.

No. (sex)	Age (years)	BrdU injs.	Survival time	Percent BrdU-labeled cells				Number of BrdU-labeled cells/mm^3
				NeuN	NSE	MAP-2	GFAP	
1 (m)	5	1	2 hours	0	0	0	20	0.4
2 (m)	16	1	2 hours	0	0	0	31	0.6
3 (m)	5	5	2 weeks	52	43	37	5	26.5
4 (f)	7	5	2 weeks	47	39	33	8	13.2
5 (m)	10	3	1 week	38	32	26	4	17.6
6 (f)	15	5	2 weeks	–	28	–	2	13.7
7 (m)	15	4	24 hours	53	48	29	3	15.6
8 (m)	5	1	1 week	–	–	–	–	7.2
9 (m)	5	1	2 weeks	–	–	–	–	14.4

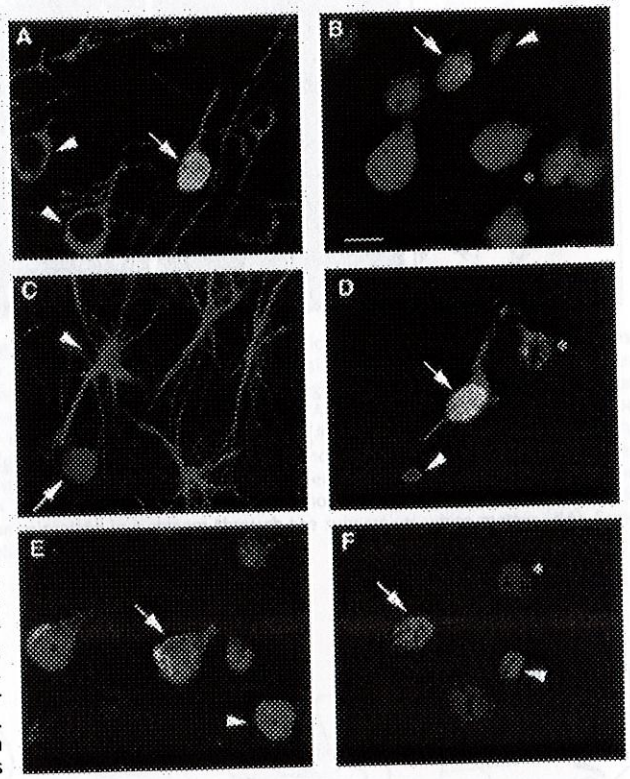

Fig. 3. Confocal laser scanning microscopic images of BrdU-labeled cells in the adult macaque neocortex. (**A**) Arrow: prefrontal cell with neuronal morphology co-labeled for BrdU (blue nuclear stain, cascade blue) and MAP-2 (green cytoplasmic stain, Alexa 488). Arrowheads: BrdU-negative, MAP-2 positive cells (animal #4). (**B**) Arrow: prefrontal cell with neuronal morphology co-labeled for NeuN (green nuclear and cytoplasmic stain, Alexa 488) and BrdU (red nuclear stain, Alexa 568). Arrowhead: cell positive for BrdU, not for NeuN. Asterisk: cells positive for NeuN, negative for BrdU (#3). (**C**) Arrow: prefrontal cell with neuronal nuclear morphology positive for BrdU (red nuclear stain), not stained for the astroglial marker GFAP. Arrowhead: cell with astrocyte morphology negative for BrdU, positive for GFAP (green stain) (#5). (**D**) Arrow: prefrontal cell in ventral principal sulcus with neuronal morphology co-labeled with BrdU (red nuclear stain) and Fluoro-Emerald (green cytoplasmic marker). The tracer was injected into the dorsal bank of principal sulcus (#11). This cell was outside of the injection site diffusion zone. Arrowhead: BrdU-positive cell (red nuclear stain) not labeled with Fluoro-Emerald. Asterisk: Fluoro-Emerald–labeled cell which is BrdU-negative. (**E**) Arrow: posterior parietal cell co-labeled for NeuN (green nuclear and cytoplasmic stain) and BrdU (red nuclear stain). This cell has the morphology of a pyramidal cell (#12). Arrowhead: NeuN-positive, BrdU-negative cell. (**F**) Arrow: posterior parietal cell co-labeled with BrdU (red nuclear stain) and the retrograde tracer Fast Blue (blue cell-body marker). The tracer was injected into area 7A. This cell was outside of the injection site diffusion zone (#12). Arrowhead: BrdU-labeled cell not labeled with Fast Blue. Asterisk: Fast Blue–labeled cell not co-labeled with BrdU. Scale in (B) = 20 μm and applies to all frames.

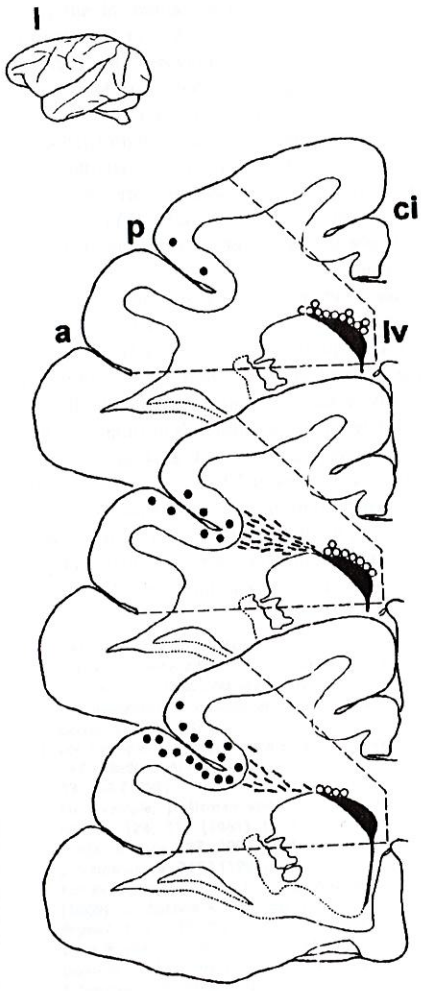

Fig. 4. The distribution of BrdU-labeled cells in animals that were perfused 2 hours (top section, animal #1), 1 week (middle section, #8), and 2 weeks (bottom section, #9) after a single BrdU injection, showing the putative migration of new cells from the svz to the principal sulcus. Area within the dashed lines represents analyzed region. Open circles represent BrdU-labeled cells in the svz. Dashes (–) represent BrdU-labeled cells in the white matter, the majority of which had fusiform nuclei. Solid dots represent BrdU-labeled cells in the cortex surrounding the principal sulcus. Vertical line above lateral view of the brain marks the approximate level of the section shown for each of the three animals.

injected with Fluoro-Emerald in lateral prefrontal cortex (Area 46) and with Fast Blue in posterior parietal cortex (Area 7A) (*22*). These injection sites were chosen because both are known projection targets of neurons in areas in which we had found BrdU-labeled cells, including lateral prefrontal, posterior parietal, and inferior temporal cortex (*23*). We observed cells labeled with BrdU that were retrogradely filled with either Fluoro-Emerald (in frontal cortex) or Fast Blue (in parietal cortex), providing further evidence that some new cells were neurons. Although tracer was transported from the injection sites to non–BrdU-labeled cells in frontal, parietal, and inferior temporal cortex, cells co-labeled with BrdU and tracer were only found within 11 mm of the border of the diffusion zone surrounding the injection sites. Thus, adult-generated cells in prefrontal and posterior parietal cortex extend short axons and may be incorporated into the local circuitry, although the existence of longer connections cannot be ruled out.

Although most neocortical neurons are generated prenatally (*24*), our findings indicate that neurons are added to primate neocortex in adulthood. We observed a considerable number of BrdU-labeled cells with neuronal characteristics, but the numbers generated daily in adulthood are presumably much higher because BrdU is only available for uptake for 2 hours after each injection (*12*). Thus, a single BrdU injection labels a fraction of the cells that divide in 24 hours. Furthermore, it is unlikely that the cells we observed incorporated BrdU during apoptosis, a phenomenon observed in both the developing neocortex and non-neuronal systems (*25*), because many labeled cells were present in neocortex weeks after the last BrdU injection without any signs of degeneration.

Our results are at variance with previous [^{3}H]thymidine autoradiographic studies which claim no neurogenesis in the adult primate neocortex (*1*). This discrepancy may be due to methodological differences. First, since ^{3}H penetrates only 1 to 3 μm into a tissue section, [^{3}H]thymidine autoradiography may underestimate the number of new cells (*26*). Furthermore, the previous study used much longer survival times (3 months to 6 years) for 9 of the 12 animals. New cells that incorporated [^{3}H]thymidine and differentiated into neurons may have died in the interval between injection and perfusion. This is supported by findings that many adult-generated hippocampal neurons die in animals not exposed to complex experiences (*17, 27*). Finally, all of the adult animals in the previous study were pregnant at the time of BrdU injection; pregnancy increases the level of circulating glucocorticoids (*28*), which in turn may inhibit cell proliferation (*29*).

Prefrontal, posterior, parietal, and inferior temporal cortex are areas involved in behavioral plasticity (*13*). Thus, it is conceivable that the new neurons added to these areas in adulthood might play a special role in such functions. Perhaps immature neurons are capable of undergoing structural changes rapidly and therefore may serve as a substrate for learning (*15*). Furthermore, the addition of new neocortical neurons throughout adulthood provides a continuum of neurons of different ages that may form a basis for marking the temporal dimension of memory. The idea that late-generated neurons play an important role in learning and memory was proposed previously by Altman (*14*), and, for the avian forebrain, by Nottebohm (*9*), but direct evidence is still lacking (*15*).

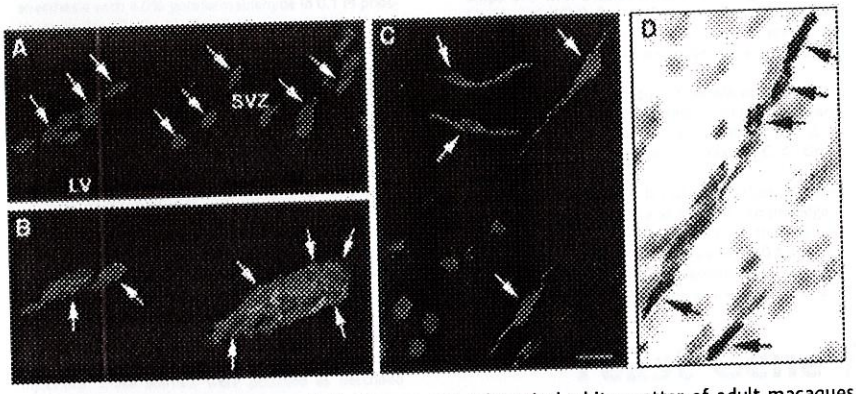

Fig. 5. Immature and migrating cells in the svz and subcortical white matter of adult macaques. (**A**) Arrows: confocal laser scanning microscopic image of chains of BrdU-labeled cells (red nuclear stain Alexa 568) in the svz (#11). (**B**) Arrows: confocal image of BrdU-labeled cells (red nuclear stain) that are TOAD-64–positive (green cytoplasmic stain, Alexa 488). These cells are organized in a chain on the border of the svz and the white matter (#10). (**C**) Arrows: confocal image of TOAD-64–positive cells (green cytoplasmic stain) with the morphology of immature neurons that appear to be migrating from the svz through the white matter to prefrontal cortex (#4). The blue nuclear stain is the DNA dye Hoechst 44323. (**D**) Light photomicrograph of BrdU-labeled cells (arrows) that appear to be migrating in a stream through the subcortical white matter (#4). Scale in (C) = 30 μm and applies to all frames.

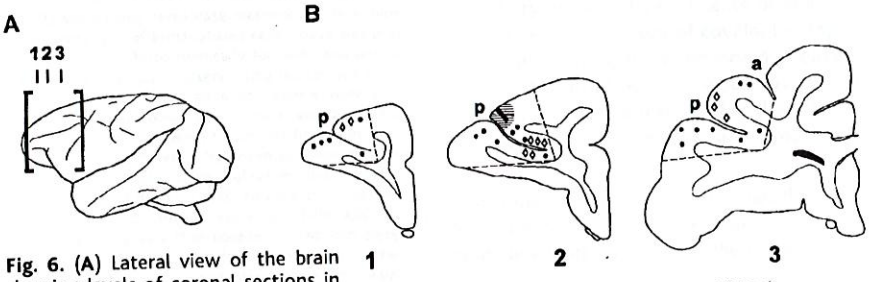

Fig. 6. (**A**) Lateral view of the brain showing levels of coronal sections in (B). (**B**) The distribution of BrdU-labeled cells in the principal sulcus from an animal (#10) that received an injection of the retrograde tracer Fluoro-Emerald into dorsal principal sulcus. The area within the dashed lines represents the analyzed region. Solid dots represent BrdU-labeled cells that were not co-labeled with retrograde tracer. Open diamonds represent BrdU-labeled cells that were retrograde-labeled with Fluoro-Emerald. Section 2 shows the middle of the injection site (solid line) surrounded by the diffusion zone (shaded area).

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19. A modified version of the stereological optical dissector method [M. J. West, L. Slomianka, H. J. Gundersen, *Anat. Rec.* **231**, 482 (1991)] was performed on peroxidase-stained tissue on coded slides. For every 20th section through the principal sulcus, the number of labeled cells in both banks of the sulcus was determined using an Olympus BX-60 OptiPlex computer. Labeled cells were counted excluding those in the outermost focal plane to avoid counting cell caps. The total volume of the principal sulcus area was estimated with Stereoinvestigator (MicroBrightField). The data were expressed as number of BrdU-labeled cells/mm^3. Immunofluorescent tissue was viewed with an Olympus BX-60 fluorescent microscope and with a confocal laser scanning microscope (Zeiss 510 LSM) for verification of double labeling. Z-sectioning was performed at 1-μm intervals, and optical stacks of three to six images were produced for figures.
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22. For the tracer injections, 0.4 μl of 2% Fluoro-Emerald tracer (Molecular Probes) was injected into the edge of the dorsal (Fig. 6) or ventral bank of the posterior portion of the principal sulcus (Area 46) or 0.4 μl of 2% of Fast Blue (Sigma) into the approximate center of exposed Area 7A. The injections were made with a Hamilton syringe over a 20-min period at a depth of 1 mm using a Zeiss binocular microscope. For both injections, the cortex was exposed under strictly aseptic conditions and deep isofluorothane anesthesia. After the injections, the surgical opening was closed and, 1 week later, the animal was perfused under deep Nembutal anesthesia (*16*). The tissue was processed for BrdU immunofluorescence (*18*) with Streptavidin-Alexa 568 (1:2,000, Molecular Probes). Only cells outside of the diffusion zone surrounding the injection site (in which both glia and neurons were labeled with the tracer) were examined for BrdU and retrograde tracer labeling.
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Yeast Gene for a Tyr-DNA Phosphodiesterase that Repairs Topoisomerase I Complexes

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Covalent intermediates between topoisomerase I and DNA can become dead-end complexes that lead to cell death. Here, the isolation of the gene for an enzyme that can hydrolyze the bond between this protein and DNA is described. Enzyme-defective mutants of yeast are hypersensitive to treatments that increase the amount of covalent complexes, indicative of enzyme involvement in repair. The gene is conserved in eukaryotes and identifies a family of enzymes that has not been previously recognized. The presence of this gene in humans may have implications for the effectiveness of topoisomerase I poisons, such as the camptothecins, in chemotherapy.

Topoisomerases are cellular enzymes that are crucial for replication and readout of the genome; they work by breaking the DNA backbone, allowing or encouraging topological change, and resealing the break (*1*). The enzymes are efficient because DNA breakage is accompanied by covalent union between protein and DNA to create an intermediate that is resolved during the resealing step. This mechanism, although elegant, also makes topoisomerases potentially dangerous. If the resealing step fails, a normally transient break

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BRAIN MAY GROW NEW CELLS DAILY

Princeton Study on Monkeys Challenges Long-Held View

By NICHOLAS WADE

In a new challenge to the long-standing belief that adults never generate new brain cells, biologists at Princeton University have found that thousands of freshly born neurons arrive each day in the cerebral cortex, the outer rind of the brain where higher intellectual functions and personality are centered.

Though based on research in monkeys, the finding is likely to prove true of people, too. If so, several experts said, it may overturn ideas about how the human brain works and open new possibilities for treating degenerative brain diseases.

If the new brain cells, or neurons, are involved in memory and learning — perhaps with each day's batch of new cells recording that day's experiences — scientists will have to make major revisions in the long-time view that the adult brain's neurons are static in number and that memory is stored only in the way they interconnect.

In addition, if the brain's cells are in constant turnover, as the new finding suggests, physicians may discover ways to use the brain's natural regeneration system for replacing cells that are lost in diseases of aging.

The discovery, by Dr. Elizabeth Gould and Dr. Charles G. Gross, is reported in today's issue of the journal Science.

The belief that the adult brain does not make new cells rested on careful, well-known studies by Dr. Pasko Rakic of Yale University, who looked

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Brain May Grow New Cells Daily, a Study Finds

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for the formation of new neurons in the monkey brain and found none.

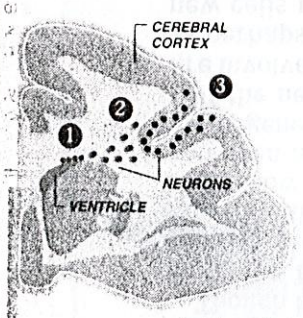
But the Princeton work is likely to be convincing because it builds on previous reports of brain cell turnover, notably by Dr. Fernando Nottebohm of Rockefeller University, who showed that canaries grow new neurons to learn new songs, and recent studies showing that new cells are formed in the hippocampus, a brain region where initial memories of faces and places are formed.

"The scientific community can easily believe something it is 50 percent ready to absorb, but not something that comes out of left field," said Dr. Eric R. Kandel, a leading neuroscientist at Columbia University. "But here, we are prepared for it."

A CLOSER LOOK

More Gray Matter

Researchers have found that the brains of macaque monkeys produce new brain cells that migrate to the cerbral cortex, where higher functioning is centered.



Frontal view of brain

1. Neural stem cells, neurons in their early stage of development, are produced in the central area of the brain.
2. The neurons develop as they migrate.
3. The mature neurons reach the outer cortex, the location of advanced functions in the brain.

Source: Science

The New York Times

Dr. Kandel compared the likely change in view to the paradigm shifts described by the historian of science Thomas Kuhn as occurring when one major scientific theory is replaced by another.

Although the new study was done in macaque monkeys and has yet to be confirmed in humans, as fellow primates monkeys are usually quite predictive of what occurs in people.

Dr. Gould, who has studied new cell formation in the hippocampus, and Dr. Gross, an expert on the cerebral cortex, injected macaques with a chemical that is incorporated in the new DNA formed when a cell divides.

They found that a stream of new neurons were generated in the monkeys' brains in a zone just above the brain's fluid-filled central chambers. This zone was recently identified by other scientists as the home of the brain's stem cells, the source cells from which an organ is replenished.

The new neurons migrated toward the cortex, matured and sent out axons to make connections with other brain cells, the Princeton biologists found.

The researchers looked for new neurons in four areas of the cortex, and found them in three areas where memories are known to be stored: the frontal cortex, used for decision-making, and two areas on the side of the brain used for visual recognition. No new neurons were detected in the fourth area, the striate cortex, a region at the back of the head that simply processes visual information from the eyes and passes it on to other parts of the cortex.

Whatever the new cells are doing in the cortex, they affect regions of the brain that are central to human thought and identity. The Princeton work, said Dr. Ronald D. G. McKay, an expert on brain stem cells at the National Institutes of Health, "places new neurons in the region of the brain involved in the highest level of personality: it's the frontal cortex that is important in determining who you are in a very human way."

Dr. Gould said it was possible that the new neurons arriving in the cortex would be particularly sensitive to recording information for a certain period while they matured.

"They would become integrated in the circuitry and represent the information being learned at that particular time," she said, after which they would not record anything more.

In other words, the conveyor belt of new neurons might record successive days' experiences almost like a moving tape.

"We know the characteristic of memory is that events are tagged with times," Dr. Gross said. "We have no idea how that is done. But since we have now shown there are new cells added every day, which cover a spectrum of ages, these cells could possibly provide the substrate for the temporal dimension of memory."

Dr. Kandel, of Columbia University, said the idea was perfectly possible, given how little was now known about the brain's system for ultimate long-term memory storage.

"How do you distinguish the memory of 20 years ago from the memory of 30 years ago? You would have to mark the birthday of the cell in some way," Dr. Kandel said, suggesting that the train of new neurons offered a plausible mechanism whereby the brain might somehow do this.

The notion that new memories are stored in a train of new nerve cells was advocated in the 1960's by Dr. Joseph Altman, then of the Massachusetts Institute of Technology. But his proposal was not widely accepted. And when Dr. Rakic, an authority on neuron formation in the embryonic monkey brain, reported in 1985 that no new neurons were formed in the adult monkey's brain, this became the accepted view.

Even when Dr. Gould and others showed recently that new cells were formed in the hippocampus, Dr. Rakic argued that this was a primitive area of the brain — even reptiles have a hippocampus — and that brain organs acquired more recently in evolution, like the primates' cerebral cortex, would not be expected to behave the same way.

Dr. Gould said it was this argument that had made her determined to look for new cells being formed in the cerebral cortex, despite the expense of doing work on monkeys and the risk in "redoing an experiment that a very well respected person," Dr. Rakic, had already performed.

Dr. Rakic's office said he was traveling yesterday and unavailable for comment.

If indeed the brain is constantly renewing the cells in its cortex, hippocampus and maybe other areas, the prospects for learning how to repair the aged or damaged brain begin to look much more hopeful.

"Degenerative diseases of the brain are really defined by loss of nerve cells," Dr. Kandel said. Though diseases like Parkinson's affect specific areas of the brain, it might become possible to channel young new neurons into the areas of disease. "This is pie in the sky," he said, "but at least there is now the possibility of thinking about it."

Dr. William T. Greenough, a neuroscientist at the University of Illinois, said the Princeton work created a "whole new ball game" for addressing brain diseases, by harnessing the brain's own restorative potential.

The Princeton biologists plan to follow up their discovery by blocking the formation of new neurons in monkeys' brains and seeing what happens. If the new neurons are essential for memory and learning, then serious deficits should appear in the monkeys' performance. The researchers as yet have no idea whether the loss of brain cells and the generation of new ones are separate events or part of the same cycle.

"Our discovery," Dr. Gross said, "suggests more questions than answers."

October 1999

PSI Research and New Paradigms in Science

by Alexander Imich

Abstract: A look at the history of science is given and the ever changing concepts observed.

We can look at the history of science in two ways. We can view it as an affirmation of the extraordinary feats of mind which the human race can produce when operating at its best. But we can also see the history of science as an ongoing lesson in humility, as a consistent attack on the inflated human ego. Copernicus showed us that we are not the center of the universe. Darwin demonstrated that we were the end result of an almost infinite set of experiments and that we were a product of nature like the other animals. Freud told us that our unconscious functioning determined much of what we thought and felt. Einstein described a universe in which the only constant was the speed of light, and in which no two observers ever had the same experience.

Describing contemporary society, C.P. Snow writes (1993) about two different cultures: the scientific-technical and the humanistic-spiritual. The first is materialistic; it believes that reality is ultimately composed of matter and governed by laws of motion and energy. It believes that there is an objective world which can be viewed by the observers at a distance and studied separately from himself. The scientific-technical view also affirms that only what can be physically measured is real. It holds that the study of component parts will lead inevitably to comprehension of the whole. For those of us with a background in parapsychology and spiritual work, the heaviest burden of the contemporary scientific view is the assumption that we can only acquire information through our five senses.

These seemingly solid paradigms of science, the most respected intellectual authority in Western society, are being undermined by recent advances in theoretical physics and in parapsychology. A new lesson in humility is about to be delivered to the Western mind, even as it perceives a new skill possessed by most of us.

First, let's look at the state of modern science. Its materialistic doctrine was first contradicted by Einstein's discovery that matter is concentrated energy. Subsequently, quantum mechanics demonstrated that micro particles sometimes behave like particles and at other times like waves. Another blow to the belief that there was a hard and fixed reality out there.

Heisenberg took the notion of indeterminacy a step further. His principle declares that the study of the subatomic world disturbs it, thus contradicting the cherished belief that an observer can study a world

independent of himself. Heisenberg's principle of indeterminacy also contradicts the positivistic notion that the real world can be physically measured. Philosophically, the most challenging consequence of Heisenberg's statement is that it undermines the whole idea of causal connection. Something which is not determined cannot be connected to its previous state with certainty. Causal connection requires a perfect one-to-one relationship. Thus, with indeterminacy, a fundamental element of all science, the idea of cause and effect, has been seriously threatened.

The modernist attack on the common sense positivism of 19th century science continued. Bell's theorem of non-locality invalidated Einstein's statement that the speed of light is the highest possible speed and introduced the revolutionary notion of infinite speed, or instantaneity. The logical consequence of this concept is the mystical idea of universal interconnection, the cosmic unity of all things.

It is worth mentioning that mystical issues were not unfamiliar to the greatest modern theoreticians of physics Eddington, Einstein, De Broglie, Heisenberg, Jeans, Planck, Pauli, Bohr, Schroedinger. They have each expressed their thoughts on the subject in writing. The developments in theoretical physics which I have discussed weakened the rational foundations of the so called hard sciences, shortening somewhat the distance between the scientific-technological and the humanistic-mystical. It is our belief that future scientific discoveries will continue reducing the differences between these two worlds. We also believe that their final rapprochement will come through the discoveries of parapsychology. For this and other reasons, we believe that parapsychological research is among the most important scientific efforts of our time. The perennial wisdom, as Aldous Huxley calls it, of all religions, states that the goal of human life is to reestablish contact and ultimately to merge with the Absolute. The science of parapsychology is helping us on that path. In the modern world, human beings orient themselves by means of specific organs — the senses. But for survival on the jungle floor or in the tree tops we very likely developed extrasensory capacities. Their perpetual disuse in technological cultures might be the reason why they have become "rusty" or even atrophied in human beings. There are exceptions to this state of things. Rare individuals, like Home, Palladino, Maribelli or, in our times, Gellet, Kulagina, Thomaz—do repeatedly demonstrate paranormal powers.

Do psychics get that way due to genetic mutation? Are these powers related to mishaps in their lives? Accidents with electromagnetic forces, or mechanical traumas? The fact is that many people have PSI abilities, not just the supremely gifted. Some experience them only very sporadically, sometimes only once in a lifetime. Others accept PSI as a normal part of living. We should also note that certain individuals, medicine men, voodoo priests, shamans, yogis, Zen masters and others acquire these powers through deliberate practice. Avatars, messengers of the

Divine, are of course masters of the paranormal.

In spite of the evidence for the paranormal, most science is still governed by the dictum, "Nihil est in intellectu quod not erat in sensu"—"Nothing is in the intellect what has not been in the senses." It was first spelled out by the great wisdom lover, the foundation builder of exact science, Aristotle, who wanted to make sense out of accumulated anecdotal information. His basic statement is still regarded as self evident. It is still the tacitly assumed scientific assumption of the Western World. One could wonder why no one from the scientific community has ever challenged such a basic paradigm and decided to test it experimentally.

I can report on one such experiment and its revolutionary, paradigm bursting implications.

But first, let's return to one of the cornerstones of modern physics, Heisenberg's uncertainty principle.

A young German physicist, Werner Heisenberg, thinking through what Einstein had called "Gedanken-Experiment" realized that it was impossible to determine both the location and the momentum of a moving subatomic particle, for instance an electron. To measure a particle, one has to illuminate it with light, the wave length of which has to be smaller than the particle. In the case of an electron, would have to be gamma rays. Light we know has a dual nature; sometimes it behaves like a wave, sometimes as a particle— a light quantum. Light quantum striking an electron will influence its position. Thus, we are unable and never will be able to determine both parameters with absolute precision. Some uncertainty will always remain.

Uncertainty, a term describing a state of mind, has been transferred to a state of matter. An epistemological hypothesis about the limitation of our cognitive possibilities in regard to the subatomic world has become an oncological statement of physics.

Given the dominant position of contemporary theoretical physics, a position reinforced by the triumph of technology, the idea of uncertainty has become fashionable, spreading beyond the limits of physical science to a view of our cultures. The uncertainty of the political, social and economic situation of the world has undoubtedly contributed to this phenomenon.

But Heisenberg has been challenged. It has been said that, "Heisenberg tries to give a causal explanation about why causal explanations are not possible." Another philosopher, Jaki (1966), wrote about the indeterminacy principle: "It was recognized that the principle itself cannot disprove that have definite positions and velocities. The principle has such bearing only when taken jointly with the methodological assumption that only what can be observed in the laboratory is endowed with reality."

Entering a territory full of metaphysical precipices, physicists, in order not to lose their balance have been forced to adopt many notions and hypotheses more befitting mystics than the aristocrats of the scien-

tific world.

Since everything in the universe is composed of basic physical units and the universe is nothing else but their play, reductionists are convinced that all knowledge is potentially their proper field of study. It's their belief that if every science is not yet a branch of physics, it's only because of the technical difficulty of dealing with the extreme complexity of living beings. Life forms are composed of trillions of macromolecular complexes, each in turn involving hundreds, thousands or millions of atoms. The reductionists argue that with the improvement of our technical means, particularly computational machines, it will become feasible to explain life in terms of physics Some physicists even hope to be able to explain psychological phenomena, and even unsolved philosophical problems.

This hubris is challenged by parapsychology. Parapsychology - the science which studies the interaction of mind and the universe — has established that besides the five senses, there exists an additional mode of information acquisition. This mode, called psi, is not connected to our senses. As a matter of fact, neither how it operates nor its connection to any part of the organism is known.

One thing is certain: The modes of psi operation are much different from those known to science. It is possible that using ESP, the direct knowledge of things or events, we may not disturb microevents, and may be able to correctly measure complementary parameters of a particle, removing the indeterminacy of the operation. Hawking (1988), a sincere disbeliever in the paranormal, yet an honest and powerful thinker, writes, "We could imagine that there is a set of laws that determine events completely for some supernatural being who could observe the present state of the universe without disturbing it." There is no convincing proof of the existence of supernatural beings. There is, instead, a probability that information about physical reality, obtained without the intermediary of senses may not disturb the observed state. In addition to ESP, a second mode of psi operation is also known. Colloquially, it is the effect of mind on matter. Rigorously, it is a multivariate nonlocal action without discernible material connection. Such action is called psychokinesis, PK in abbreviation. When PK is used to influence a micro physical event quantitatively, it could permit defining the parameters of the event, making them known without uncertainty. Thus, when either ESP or PK are used in the study of a micro event, either one may invalidate the indeterminacy principle.

I arrived at this conclusion in the mid-1950s. J.B. Rhine considered publishing it in his Journal of Parapsychology, but eventually decided not to. He had enough public controversy and did not want more.

The empirical confirmation of my hypothesis came in 1969. That year, the prestigious and conservative "New Scientist" published Helmuth Schmidt's experiments. In this research, various subjects were able to predict the time of arrival of an electron emitted spontaneously and

...ally unpredictably by the atoms of radioactive Strontium-90.

Schmidt, is a physicist, turned parapsychologist; few have his background. For his experiments, Schmidt constructed a sophisticated electronic instrument containing radioactive material, a Geiger counter, an electronic four position switch rotating at the rate of one million steps per second, and four colored lamps. The lamps were wired to light up when electrons arrived at specific steps of the counter. The subject's task was to guess which lamp would light up next. With one subject, the probability of obtaining a large number of successful trials by chance alone was less than one in 100,000.

In Schmidt's experiments the unpredictable quantum process was predicted and the idea of uncertainty in the quantum process was invalidated. Quantum theory assumes that there is no causal mechanism and that randomness is the basic attribute of nature. Random events cannot be predicted.

In Schmidt's experiments, if PK and not ESP were the operating mode, then it is possible that PK, and not the electron arriving from the Strontium 90 was instrumental in lighting the lamps of the testing equipment. However, as I learned from Schmidt, the lamps did not light when the radiative material was removed from the equipment. It can therefore be assumed that ESP and not PK was the operating mode in these experiments. Foreknowledge was at work rather than mind influencing nature.

Parapsychology is unique among the sciences. The major theoretical breakthroughs have yet to be made. But while this orphan science struggles to decipher the mysteries of psi, there are people on this globe who already know the secrets we parapsychologists would like to decipher. They are the yogis, practitioners of Eastern esoteric wisdom, and the shamans, the medicine men and women of traditional cultures. It is difficult to understand why, with few exceptions, no vigorous effort is being made by parapsychology researchers to study this knowledge from those who already possess it, or to examine the wisdom presented in written texts that have been translated by scholars into European languages. I, know only one case where such Eastern knowledge has been used by two Westerners.

Taimni (1965), citing aphorism 3.26 of Patanjali's sutras, writes that the yogi can acquire, "knowledge of the small, the hidden, or distant by directing the light faculty. Annie Besant and C.W. Leadbeater did follow this teaching. Almost 100 years ago, they decided to develop the siddhi which would permit them to observe infinitesimally small objects, to enter the atomic and subatomic world.

These two theosophists undertook a series of investigations of micro particles — atoms and molecules of elements and of some chemical compounds. They conducted this 'mental' investigation for 38 years! In 1907 they examined 59 elements and detected variations in the atoms of argon, krypton, neon and xenon. The variations represented isotopes of

these noble gases, isotopes that were discovered by science only five years later. The theosophists turned chemical researchers made several other discoveries that were later confirmed by science.

The results of their work were examined by the particle physicist Steven M. Phillips in 1980. He came to the conclusion that the observations made by the two Western sidhi masters describe, among other things, quarks — particles that were proposed by Murrey Gell-Mann only 31 years later.

Mystics tell us that true knowledge of reality cannot be achieved through sensory perception and that the only way to such perfect knowledge is via a state of immediate awareness. William James in 1902 wrote, "It must always remain an open question whether mystical states may possibly be of much superior point of view, windows through which the mind looks upon a more extensive and inclusive world."

Ken Wilber reviewed the work of a number of important philosophers and psychologists who emphasized the superior value of intuitive knowledge: With summary phrases he cites:

Henri Bergson — intellect versus intuition

Abraham Maslow — intellectual versus fusion knowledge Trigant Burrow — detention versus co-tention

Norman O. Brown — dualistic versus carnal knowledge—"carnal" because subject and object become one in the art of knowing

Andrew Weil — straight versus stoned

Krishnamurti — thought versus awareness

Wei Wu Wei— outseeing versus inseeing

Spinoza — intellect versus intuition

There are, of course, many other humanistic thinkers who have elaborated the supremacy of non-dualistic, intuitive awareness. Strangely, after more than a century of parapshychological study, we still know almost nothing about the very nature of psi or about its mode of operation There are many puzzles.

For example, if the mastery of psi is a sign of high spiritual development, we have difficulty in understanding why psi capacities appear in people after they have experienced trauma. People like Geller, Morton and others acquired psychic abilities after electrical jolts. The famous clairvoyant Croisset fell from a ladder. We are puzzled because these are people who seem to be far from spiritual perfection.

But the mysteries will unravel, The very methods of science are gradually invalidating our present scientific paradigms. A new era in research will open with the recognition and use of extrasensory capacities. Hopefully, the spiritual development of humanity and its acquisition of paranormal capacities will lead to an epoch which fulfills humanity's great dream of a time of peace and plenty, brotherhood and bounty.

Psi research will one day open many doors for the human race. In the tradition of the great scientific breakthroughs, it will expand our abilities as it deflates our egos. With the cultivation of Psi by all people,

we will discover that we have a role in creating and shaping reality, and that at the same time we are part of a long process of evolution which is beyond any single person's conscious control. We will all know at some point what the mystics have always known, that the secret of life is in the moment of intuitive awareness, and that that moment is neither rational nor irrational, formless or structured, empty or full. When we are in that moment we will have achieved the wisdom of the other side.

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Holmes and Watson

Sherlock Holmes and Dr. Watson went on a camping trip. After a good meal and a bottle of wine, they lay down for the night and went to sleep. Some hours later, Holmes awoke and nudged his faithful friend. "Watson, look up at the sky and tell me what you see." "Watson replied, "I see millions and millions of stars." "What does that tell you?" asked Holmes. Watson pondered for a minute and said, "Astronomically, I observe that Saturn is in Leo. Horologically, I deduce that the time is approximately a quarter past three. Theologically, I can see that God is all powerful and that we are small and insignificant. Meteorologically, I suspect that we will have a beautiful day tomorrow. "What does it tell you, Holmes," he asked. "Watson, you idiot. Someone has stolen our tent."

— Submitted by Phyllis and Lenny Stern

Case Report

The Crashing of Swissair Flight 111 on September 2, 1998

by Fred Gurzi

Abstract: The author describes a psychical experience in which he received a message that an aircraft will be shot down. Six days later, Swissair Flight 111 crashed into the ocean killing all on board. This report describes the relationship between the paranormal message and subsequent events.

On 27 August 1998, I e-mailed John Beloff, editor of the Society of Psychical Research in London, and described a psychical experience I had that day. It was an "interruptive" experience, meaning that nomal thought processes were interrupted for paranormal messaging. The message was: "Indications are that an aircraft will be shot down." Six days later Swissair Flight 111 crashed into the ocean off Nova Scotia, killing all 227 persons on board. The cause of the tragedy is still under investigation.

It appears that the prediction of Swissair Flight 111 was affirmed by three actual events in which the number six linked each event to the prediction. In my mind the flight was shot down, as stated in the prediction, although this has yet to be confirmed by Canadian investigators.

The first appearance of the number six (6) occurred psychically to me four days before the crash in the form of six (6) letters. These were B R A I T S or B R A I T T. As it turns out, the latter fits within the name of Lt. Col. Brian Akitt, in charge of Canadian Rescue Operations. Six alphabetic characters share sequentially but not altogether contiguously in the first and last names, as illustrated below. Defying "Intervention Paradox," a more elaborate communications network, worldwide, might have been instrumental in averting the tragic outcome of Swissair on the basis that "Six warns!"

1	2	3	4	5		6	7	8	9	10
B	R	I	A	N		A	K	I	T	T
B	R		A					I	T	T

The second appearance of six (6) occurred during the actual event. As reported by the news media, the aircraft's power system failed six (6) minutes before the crash. This rendered the jet aircraft helpless and subjected passengers and crew to the terror of darkness.

The third appearance of six (6) occurred after the event. Wreckage was spewed over a six (6) mile radius after the crash, as reported by the Canadian news media. Three distinct and related events had occurred before, during and after in the demise of Flight 111. All shared in the commonality of a number, coinciding with the exact number of days