BHV-1 *LR* and the HSV-1 *LAT* have greatly different sizes and little or no sequence similarity. Although the BHV-1 *LR* antiapoptosis function correlates with expression of an LR protein (*33*), the *LAT* antiapoptosis function may be mediated by the *LAT* RNA, because *LAT* does not appear to encode a protein (*34*).

Our results suggest that *LAT* promotes neuronal survival after HSV-1 infection by reducing apoptosis. This hypothesis is supported by studies indicating that LAT^- mutants establish latency less efficiently than does the wild type (*5, 6, 35, 36*) and that a mutant expressing an altered *LAT* has increased neurovirulence (*12*). In general, stress is associated with reactivation and increased corticosteroid levels. Because corticosteroids induce apoptosis (*37, 38*), stress and viral gene expression during reactivation may induce apoptosis. Because *LAT* facilitates reactivation (*3, 39, 40*), inhibition of apoptosis by *LAT* would increase the probability that productive infection (i.e., productive reactivation from latency) succeeds. Whether reactivation was successful or not, LAT^+ neurons may have a better chance to survive and resume latency. Thus, a *LAT* antiapoptosis function could allow *LAT* to enhance reactivation by (i) enhancing the establishment and maintenance of latency, thereby providing more latently infected neurons in which future reactivations could occur; (ii) facilitating productive reactivation by protecting against apoptosis in neurons in which reactivation occurs; and (iii) facilitating the resumption of latency after a reactivation insult by protecting neurons against apoptosis.

It is unlikely that *LAT* is the only factor that promotes neuronal survival, because terminally differentiated neurons must have a well-devised mechanism to prevent programmed cell death. Furthermore, *LAT* may have additional mechanisms by which it enhances reactivation. Nonetheless, our results strongly suggest that suppressing apoptosis is an important mechanism by which *LAT* enhances HSV-1 reactivation. In addition, the ability of *LAT* to prevent HSV-1–induced apoptosis may be important in preventing the virus from causing extensive neuronal damage and subsequent neuronal disorders.

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Porphyrin and Phthalocyanine Antiscrapie Compounds

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The transmissible spongiform encephalopathies (TSEs) are fatal, neurodegenerative diseases for which no effective treatments are available. The likelihood that a bovine form of TSE has crossed species barriers and infected humans underscores the urgent need to identify anti-TSE drugs. Certain cyclic tetrapyrroles (porphyrins and phthalocyanines) have recently been shown to inhibit the in vitro formation of PrP-res, a protease-resistant protein critical for TSE pathogenesis. We now report that treatment of TSE-infected animals with three such compounds increased survival time from 50 to 300%. The significant inhibition of TSE disease by structurally dissimilar tetrapyrroles identifies these compounds as anti-TSE drugs.

The TSEs are a group of rare, fatal neurodegenerative diseases that include scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease in deer and elk, and Creutzfeldt-Jakob disease (CJD) in humans. The onset of the BSE epidemic in cattle in Great Britain raised concerns that humans could be at risk through exposure to contaminated cattle by-products. In 1996, a previously unknown form of CJD (variant CJD or vCJD) was identified in young people in Great Britain (*1*). The hypothesis that the most likely cause of vCJD was exposure to BSE-contaminated materials has since been supported by several different studies (*2*). At present, over 40 cases of vCJD have been confirmed, and there is some concern that the number of cases could be on the rise (*3*). Thus, with the potential exposure of millions of people to BSE and the onset of vCJD, the need for effective anti-TSE drugs has become acute.

A critical event in TSE pathogenesis is the conversion of the normal protease-sensitive host prion protein, PrP-sen, to a partially protease-resistant form (PrP-res) that is closely associated with disease pathogenesis. Studies have shown that there is a close correlation between compounds that inhibit PrP-res formation in vitro and compounds that inhibit TSE disease (*4*). Therefore, PrP-res is an obvious target for therapeutic intervention. The conversion of PrP-sen to PrP-res involves changes in

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protein conformation (*5*). Certain cyclic tetrapyrroles, a class of compounds that includes biologically important hemes and chlorophylls (*6*), bind strongly and selectively to proteins and effect changes in protein conformation. Thus, these compounds were good candidates for inhibitors of PrP-res formation and TSE disease.

We have recently demonstrated that the cyclic tetrapyrroles PcTS (phthalocyanine tetrasulfonate), TMPP-Fe^{3+} [meso-tetra(4- *N*-methylpyridyl)porphine iron(III)], and DPG_2-Fe^{3+} [deuteroporphyrin IX 2,4-bis-(ethylene glycol) iron(III)] (Fig. 1) all strongly inhibited PrP-res formation in vitro (*7*). To determine if they could also inhibit TSE disease in vivo, we infected transgenic mice overexpressing hamster PrP-sen (Tg7) (*8*) intraperitoneally (IP) with a high dose of hamster 263K scrapie. Starting on the day of infection [0 days after infection (dpi)], animals were injected IP three times a week over 4 weeks with one of the three compounds, for a total of 12 treatments (*9*, *10*). Every compound tested significantly delayed disease when compared with untreated controls (Fig. 2A). Treatment with DPG_2-Fe^{3+}, which of the three compounds tested was the least effective inhibitor of PrP-res formation in vitro (*7*), increased mean survival time by 37 days, whereas treatment with the stronger inhibitor TMPP-Fe^{3+} increased mean survival time by 90 days (Table 1). PcTS, the strongest inhibitor of PrP-res in vitro, was also the most effective compound in vivo, with over 50% of the animals surviving an average of 135 days longer than untreated controls (Fig. 2A, Table 2). Similar levels of PrP-res were detected in the brains of terminally ill treated and untreated animals (*11*). The data demonstrated that three tetrapyrroles, each of a structurally different type, strongly inhibited TSE disease, showing that cyclic tetrapyrroles can act as anti-TSE agents.

To determine if PcTS, DPG_2-Fe^{3+}, or TMPP-Fe^{3+} inhibited disease progression during the later stages of infection, we began similar treatments of infected animals at either 28 or 56 dpi (*10*). When treatment was started at 28 dpi, PcTS slightly increased survival times (Fig. 2B, Table 2). DPG_2-Fe^{3+} treatment slightly increased incubation times when compared with the untreated controls (Fig. 2B, Table 1), but this increase was not significantly different from infected animals treated with dimethyl sulfoxide (DMSO) alone ($P = 0.08$), the diluent used to solubilize DPG_2-Fe^{3+} (*10*). If treatment was started at 56 dpi, neither DPG_2-Fe^{3+}– nor TMPP-Fe^{3+}–treated animals were significantly different from untreated controls (Fig. 2C, Table 1). Treatment with PcTS starting at 56 dpi significantly shortened the time to death by about 1 week (Fig. 2C, Table 2), although the reason for this result is unclear. Overall, the data showed that treatment with any of the compounds later during the pathogenic process did not inhibit disease as strongly as treatments administered earlier during pathogenesis. This suggests that PcTS, DPG_2-Fe^{3+}, or TMPP-Fe^{3+} could be useful anti-TSE agents if administered prophylactically but not therapeutically (*12*).

Animals treated with porphyrins or phthalocyanine at 0 dpi generally demonstrated a broader range of disease incubation times when compared with untreated controls (Fig. 2A), suggesting a significant drop in agent titer as a consequence of treatment. To determine if this result was due to inactivation of the infectious agent, we mixed the most potent inhibitor, PcTS, with the infectious inoculum and injected the mixture IP. Mixing PcTS with the agent led to a significant increase in survival time when compared with untreated controls (Fig. 3A, Table 2). Furthermore, there was no significant difference in survival between animals infected with agent plus PcTS or animals treated with PcTS starting at 0 dpi ($P = 0.28$). The ability of PcTS to inhibit disease when mixed with an infectious inoculum suggests that it could be used for inactivation of TSE infectivity in po-

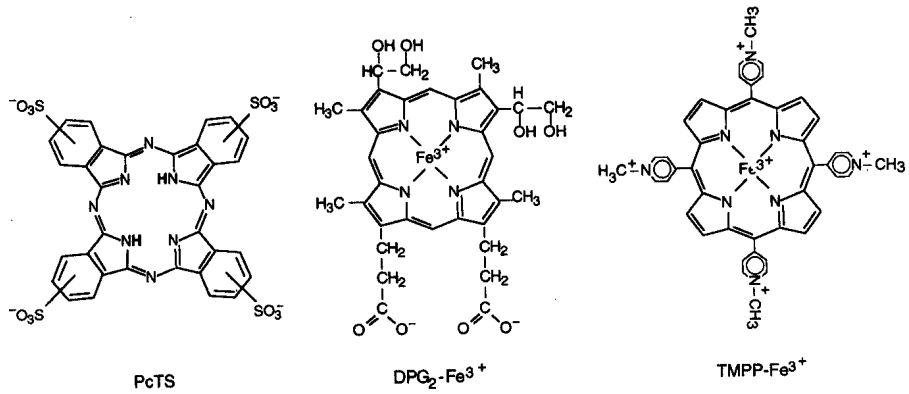

Fig. 1. Structures of phthalocyanine and porphyrins tested. PcTS consists of a mixture of isomers, as reported previously (*7*).

Table 1. Effect of porphyrin treatment on scrapie-infected mice.

Treatment		Time to death (dpi)			Delay of death (days)	
Compound	Treatment*	Dead/total	Mean†	Last death	Mean‡	Significance§
None	NA	18/18	81.6 ± 1.2	93	NA	NA
DPG_2-Fe^{3+}	0	8/8	119 ± 12.5	177	37.4 ± 8.3	$P = <0.0001$
	28	11/11	88.3 ± 2.4	100	6.7 ± 2	$P = 0.01$
	56	14/14	87.4 ± 4.3	113	5.7 ± 3	$P = 0.05$
TMPP-Fe^{3+}	0	11/11	171.5 ± 13.1	226	89.9 ± 10.2	$P = <0.0001$
	56	10/10	84.0 ± 7.6	131	2.4 ± 5.8	$P = 0.68$

*Treatment was started at 0, 28, or 56 dpi and continued three times a week for 4 weeks (12 treatments total). When treatment was started at 56 dpi, the number of treatments ranged from 7 to 12, depending on when the animals were terminal and had to be killed. NA, not applicable. †Time to death in days expressed as the mean ± SEM. ‡Difference in time to death of treated compared with untreated animals expressed as the mean difference ± SEM. §Significance of the survival time of treated compared with untreated animals with the unpaired *t* test.

Table 2. Effect of PcTS treatment on scrapie-infected mice.

Treatment		Dead/total	Time to death (dpi)		Delay of death (days)	
Number*	Initiated (dpi)		Mean†	Last death	Mean‡	Significance§
None	None	18/18	81.6 ± 1.2	93	NA	NA
1	0	9/9	114 ± 8.5	164	32.5 ± 6	$P = <0.0001$
PcTS+263K	0	9/9	183.3 ± 22	341	102 ± 16	$P = <0.0001$
12	0	17/17	216.6 ± 18	389	135 ± 17	$P = <0.0001$
12	28	17/17	86.3 ± 1.8	105	4.7 ± 2.1	$P = 0.03$
4–7	56	12/12	75.1 ± 0.8	79	−6.5 ± 1.6	$P = 0.0003$

*Total number of treatments. The designation "PcTS + 263K" represents animals infected with the inoculum mixed with PcTS and thus equals a single treatment. The range 4–7 reflects the fact that all of the mice died before a full course of 12 treatments could be administered. †Time to death in days expressed as the mean ± SEM. ‡Difference in time to death of treated compared with untreated animals expressed as the mean difference ± SEM. §Significance of the survival time of treated compared with untreated animals with the unpaired *t* test.

tentially contaminated materials of medical importance such as blood.

If the sole mode of action of PcTS was to inactivate the infectious agent in the initial inoculum, then a single treatment with PcTS at the time of infection would have the same effect as multiple treatments over an extended period. To determine whether a single treatment had the same effect as multiple treatments, we first infected Tg7 mice with scrapie and then treated them only once with PcTS. Animals treated once with PcTS survived significantly longer than untreated controls (Fig. 3B, Table 2). However, the single PcTS treatment was significantly less effective at delaying disease than multiple treatments ($P = 0.0006$). The intensely colored PcTS was detected by visual examination of peritoneal tissues months after multiple PcTS treatment had stopped but was not easily detectable a few weeks after a single treatment. This is consistent with the stability of PcTS and the likelihood that it is not rapidly metabolized but rather slowly excreted over time. Altogether, the data suggested that if PcTS was maintained at detectable levels in the periphery, it could act not only to interfere with the infectious agent in the initial inoculum but also to interfere with the infectious agent throughout the course of disease.

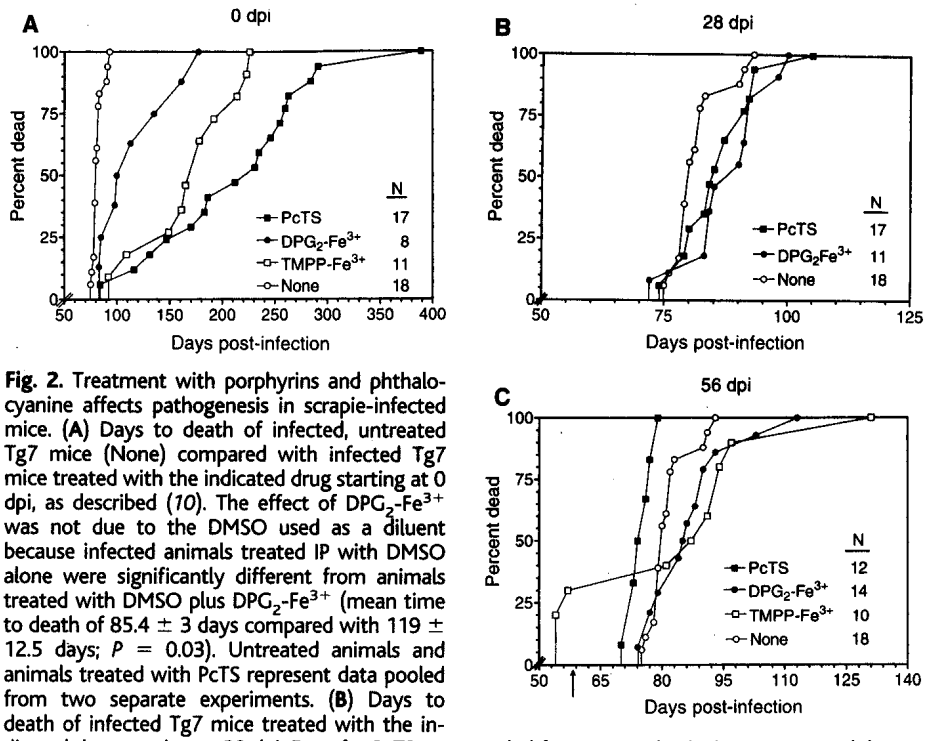

Fig. 2. Treatment with porphyrins and phthalocyanine affects pathogenesis in scrapie-infected mice. **(A)** Days to death of infected, untreated Tg7 mice (None) compared with infected Tg7 mice treated with the indicated drug starting at 0 dpi, as described (*10*). The effect of DPG_2-Fe^{3+} was not due to the DMSO used as a diluent because infected animals treated IP with DMSO alone were significantly different from animals treated with DMSO plus DPG_2-Fe^{3+} (mean time to death of 85.4 ± 3 days compared with 119 ± 12.5 days; $P = 0.03$). Untreated animals and animals treated with PcTS represent data pooled from two separate experiments. **(B)** Days to death of infected Tg7 mice treated with the indicated drug starting at 28 dpi. Data for PcTS were pooled from two individual experiments. **(C)** Days to death of infected Tg7 mice treated with the indicated drug starting at 56 dpi. The arrow indicates the time at which treatment was started. In the TMPP-Fe^{3+} group, two mice died of scrapie before administration of the compound. For all panels, individual data points represent the percentage dead for the total number of mice for that group and may include more than one mouse. The legend is shown on the right, and the number of mice is indicated under "N."

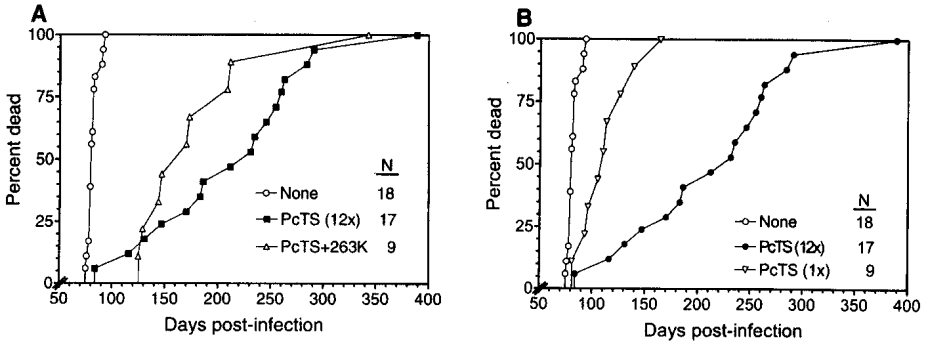

Fig. 3. PcTS mixed with hamster scrapie or given separately as a single treatment at the time of infection increases survival in scrapie-infected mice. **(A)** Days to death of infected, untreated Tg7 mice (None) and infected Tg7 mice treated starting at the time of infection, for a total of 12 treatments [PcTS (12x)] compared with Tg7 mice infected with 263K plus PcTS (PcTS+263K) or **(B)** treated once with PcTS at the time of infection [PcTS (1x)]. The data for the none and PcTS (*12*) treatment groups are the same as in Fig. 2. Individual data points represent the percentage dead of the total number of mice for that group and may include more than one mouse. The legend is shown on the right, and the number of mice is shown under "N."

The anti-TSE effect of PcTS, DPG_2-Fe^{3+}, and TMPP-Fe^{3+} is most likely occurring in peripheral tissues such as the spleen. All were less effective when treatment was started later during disease when replication of the agent in the periphery is no longer essential for disease progression (*13*). None of these compounds are known to cross the blood-brain barrier, and thus it appears unlikely that disease inhibition is occurring through the central nervous system. Mechanistically, the data obtained from the different PcTS treatments of scrapie-infected animals (Table 2) are consistent with the hypothesis that PcTS, and by extension possibly DPG_2-Fe^{3+} and TMPP-Fe^{3+}, interacts directly with the infectious agent in peripheral tissues to slow disease onset. Any of these compounds could bind to PrP-sen, PrP-res, and/or an intermediate complex of PrP-sen/PrP-res. Because PrP-sen is the precursor to PrP-res (*14*) and PrP-sen/PrP-res interactions are known to be crucial in PrP-res formation (*15*), binding to any of these targets could significantly impair PrP-res formation and thus disease. Furthermore, because many of the cyclic tetrapyrroles strongly inhibited both hamster and mouse PrP-res formation in vitro, it is likely that these compounds could be effective against different strains of TSE agents in different species (*7*).

As a common feature of the compounds tested, the planar, polarizable, and hydrophobic central aromatic macrocyclic structure appears to be a critical factor in their inhibition of TSE disease (Fig. 1). Other compounds with some anti-TSE activity, such as congo red, amphotericin B and its derivatives, and the anthracycline 4′-iodo-4′deoxy-doxorubicin (*4*, *16*), also contain polarizable, hydrophobic aromatic rings or extended conjugated unsaturated structures. It is well known that the central ring structure is important in tetrapyrrole interactions with protein surfaces that can induce changes in protein structure (*7*). Thus, the anti-TSE activity of tetrapyrroles represents a use for these compounds that appears to rely on one of their basic biological roles: to effect changes in protein conformation. This in turn suggests that cyclic tetrapyrroles may provide a basis for therapeutic approaches for noninfectious diseases of protein folding and aggregation such as Alzheimer's disease or type 2 diabetes. They have already been successfully used for different medical applications (*6*, *17*) and have been shown to have a low toxicity in humans. A large number of porphyrins and phthalocyanines are available, and there is an extensive literature on methods for the preparation of new structures. Furthermore, because the structures of protein/tetrapyrrole complexes are unusually well understood, optimization of tetrapyrrole structure for pro-

phylactic or even therapeutic effectiveness among this class of anti-TSE agents is especially promising.

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8. Transgenic mice (Tg7) were derived with a hamster PrP-sen gene under the control of the native PrP promoter as described previously for the transgenic mouse line Tg10 (*18*) and were crossed onto a mouse $PrP^{0/0}$ background (*19*). Thus, Tg7 mice do not express endogenous mouse PrP-sen but express high levels of hamster PrP-sen in a wide variety of tissues including brain. Because Tg7 mice have substantially shorter disease incubation times than Syrian hamsters when infected with hamster 263K scrapie either IP (about 82 days compared with 120 days) or intracranially (about 45 days compared with 75 days), they were used to test the therapeutic potential of the porphyrins and phthalocyanines.
9. The compounds tested were used as received from Porphyrin Products (Logan, UT) and dissolved in sterile water at the indicated concentrations: PcTS at 5 mg/ml and TMPP-Fe^{3+} at 10 mg/ml. DPG_2-Fe^{3+} was dissolved at a concentration of 30 mg/ml in 100% DMSO and stored as a stock solution at room temperature.
10. Animals were infected IP with 0.05 ml of a 1:10 dilution of a stock 10% brain homogenate of hamster 263K scrapie. The stock had an intracranial median lethal dose of 1×10^{10}/ml. Animals were injected IP with 0.05 ml of the drug solution three times a week for 4 weeks starting at 0 or 28 dpi. Treatments started at 56 dpi were continued three times a week until the animal was near terminal and killed. For each treatment, the doses given were as follows: PcTS, 5 mg/kg; DPG_2-Fe^{3+}, 30 mg/kg; and TMPP-Fe^{3+}, 10 mg/kg. Uninfected animals treated with the compound alone showed no ill effects (*11*). Because DPG_2-Fe^{3+} was dissolved in 100% DMSO, a group of infected animals was treated with 100% DMSO IP with the same treatment regimen to control for any effect of DMSO on disease progression. All results were analyzed with the unpaired student's *t* test. Animals were monitored for clinical signs and killed when in the terminal phases of disease. To confirm the diagnosis of scrapie, we removed the brain and spleen and analyzed them by Western blot with the hamster PrP-specific antibody 3F4 for the presence of PrP-res as described previously (*18*).
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Mirror-Image Confusion in Single Neurons of the Macaque Inferotemporal Cortex

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Humans and animals confuse lateral mirror images, such as the letters "b" and "d," more often than vertical mirror images, such as the letters "b" and "p." Experiments were performed to find a neural correlate of this phenomenon. Visually responsive pattern-selective neurons in the inferotemporal cortex of macaque monkeys responded more similarly to members of a lateral mirror-image pair than to members of a vertical mirror-image pair. The phenomenon developed within 20 milliseconds of the onset of the visual response and persisted to its end. It occurred during presentation of stimuli both at the fovea and in the periphery.

Behavioral tests in many species (including octopus, pigeon, monkey, and human, both child and adult) have demonstrated that confusion between lateral mirror images is more common than confusion between vertical mirror images (*1–5*). Speculation about why this is so has centered on two general ideas (*6*). The first idea is based on the fact that lateral reversals usually result from a change of viewpoint and thus convey little information about the object viewed (a tiger is equally threatening when seen in right or left profile), whereas vertical reversals usually do not result from a change of viewpoint and thus do convey information about the object (a tiger is less of a threat upside down than right side up). If lateral reversals convey little information, then brain resources dedicated to representing them may have become relatively limited, through an adaptive phylogenetic or ontogenetic process. The second idea is that confusion between lateral mirror images is an accidental consequence of the bilateral symmetry of the nervous system. To the degree that the hemispheres are mirror images of each other and interhemispheric pathways link corresponding points, neurons in the left hemisphere activated by a "b" must be linked to neurons in the right hemisphere activated by a "d," with the consequence that either stimulus will activate both populations, giving rise to confusion. Whichever account is correct, the question remains as to where in the brain the neural correlate of mirror-image confusion resides. One candidate is the inferotemporal cortex (IT), an area critical for visual object recognition in both monkeys and humans (*7*). Visually responsive neurons in IT are selective for particular shapes and for the orientations at which those shapes are presented (*8*). We hypothesized that individual IT neurons would manifest lateral mirror-image confusion by responding more similarly to members of lateral mirror-image pairs than to members of vertical mirror-image pairs.

We prepared two monkeys for microelectrode recording of single-neuron activity in IT (*9*) (Fig. 1). During each recording session, the monkey fixated on the center of a monitor while a series of stimuli was presented at the fovea. Initially, we presented images from a library of 28 white chiral shapes, each ~3° in height and width, in order to find a shape that elicited a strong response from the neuron. Having found such a shape, we then carried out testing with eight variants: the shape itself at orientations in the viewing plane of 0°, 90°, 180°, and 270° and its mirror image at the same four orientations. A

Fig. 1. Parasagittal magnetic resonance image of the right hemisphere of monkey 1. Arrow indicates the center of the recording zone. Also visible are guide-tube tracks in overlying tissue and a dark artifact from a titanium skull screw above the parieto-occipital cortex.

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