retinal transfer is observed in various kinds of perceptual learning[18,19].

This study does not directly address the question of whether templates are stored as 'eidetic' images[4–7] or as sets of characteristic parameters[2,8–11]. Although the results favour the former alternative they can also be reconciled with the latter if the animals use retinal position as a crucial parameter. By contrast, in many of the experiments that cannot be explained by simple retinotopic matching, perhaps insects extract individual pattern parameters by comparing different consecutively stored templates[8–10].

The pattern position traces in the flight simulator reveal that the retinal image is in continuous motion except during the brief moments (<30 ms) of rotational inversion[12]. Template formation may thus require pattern motion. Feature detection neurons specific for the orientation of moving bars or edges have recently been recorded in the dragonfly optic lobe[20]. If the template is static as Srinivasan *et al.*[21] have argued, it must be blurred unless exposure time is very short. The flight simulator may help to bridge the gap between free-flight behaviour and single unit recording. □

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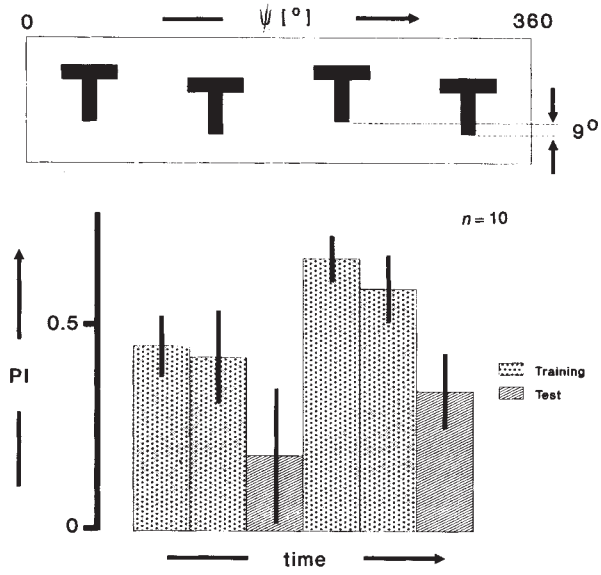


FIG. 4 Flies can discriminate identical figures (T) that are presented at slightly different heights (difference: 9°). Each column represents a 2-min training or test period (striped). Half of the flies are conditioned to avoid the upper figures, the other half the lower ones. Between training and test, the arena is shifted to a random angular position. The performance index (PI) of 0.37 ± 0.10 in the final test is significantly different from zero (*t*-test, $t = 3.44$, $P < 0.01$). Controls confirm that with four identical Ts at the same height no learned pattern preference can be detected after randomizing[13]. Vertical bars, s.e.m.s; *n*, total number of flies.

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# Prefrontal neuronal activity in rhesus monkeys performing a delayed anti-saccade task

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**PATIENTS with damage to the dorsolateral prefrontal cortex are impaired on cognitive tasks such as the Wisconsin Card Sort Test[1], the Stroop Test[2] and an anti-saccade paradigm[3], in which sensory-guided habitual responses must be suppressed in favour of conceptually or memory-guided responses. We report here recordings from prefrontal neurons in rhesus monkeys trained to perform a delayed anti-saccade task based on tests that have been used with humans[3]. Activity in the same prefrontal neurons was recorded across conditions when saccades were made toward a remembered target, and also when this prepotent response was suppressed and a saccade in the opposite direction required. Our findings show that most prefrontal neurons code the location of the visual stimulus in working memory, and that this memory can be engaged to suppress as well as prescribe a response. These results establish, in a subset of prefrontal neurons, the iconic nature of the memory code, and suggest a role for visual memory in response suppression.**

We examined the activity of neurons in the dorsolateral prefrontal cortex (Fig. 1*a*), in two rhesus monkeys trained on a compound oculomotor delayed-response task (Fig. 1*b*), in which on some trials, deferred saccades were directed to the location signalled by the visual cue (standard ODR task), whereas on other trials saccades were made in the opposite direction (anti-saccade oculomotor delayed response, AS-ODR). On anti-saccade trials, the monkeys learned to override the prepotent tendency to look toward the location of the remembered visual stimulus. Impairments on anti-saccade models have been demonstrated both in patients with large frontal lobe lesions[3], and in patients suffering from schizophrenia[4–6].

Among 108 prefrontal neurons recorded during both ODR and AS-ODR trials, 51 neurons had directionally specific delay period activity in the ODR task. We concentrate here on the delay period activity of these neurons. Of these 51 neurons, 44 have been histologically localized to the principal sulcus or immediately adjacent cortex. Visual, mnemonic and oculomotor activities of prefrontal neurons during standard ODR task performance, as well as surgical methods, recording procedures and data analysis techniques have been described previously[7–9]. Firing rates in all task-related responses were significantly greater than baseline rates (two-tailed unpaired Student's *t* statistic, alpha level 0.05).

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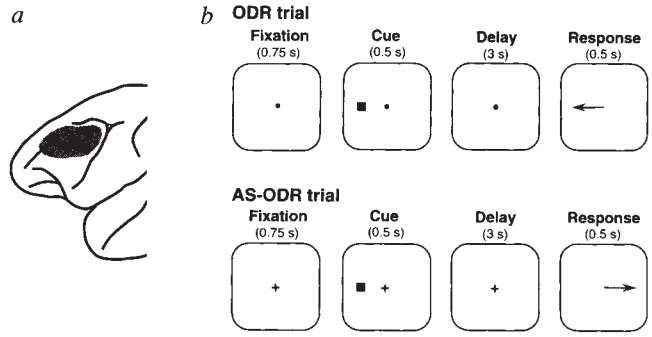


FIG. 1 Schematic drawings of recording location and behavioural tasks. *a*, Region of the prefrontal cortex sampled during unit recording. *b*, The compound ODR/AS-ODR task. Each trial began with fixation of a central target (750 ms), either a spot (ODR trials) or a small plus sign (AS-ODR trials). A visual stimulus was then presented for 500 ms 13 ° to the left or to the right (location at random). Continual fixation was required during the presentation of the peripheral stimulus and throughout the 3-s delay period that followed. At the end of the delay, the fixation target disappeared, signalling the response phase. The monkey had 500 ms to direct a saccade in the dark to an invisible response window (6 ° diameter) centred either on the stimulus (ODR trials), or directly opposite the stimulus (AS-ODR trials). Correct responses were rewarded with a drop of juice. ODR and AS-ODR trials were either randomly interleaved within a session or given in blocks.

Figure 2 illustrates a principal sulcus neuron whose increased firing in the delay is linked to the location of the visual cue, not the direction of the saccade. First, the neuron responded 98 ms after the onset of the visual stimulus when it appeared on the right but not the left (Fig. 2*a*, right; *b*, left; *c*, right). Second, it showed increased activity on ODR trials during the 3-s delay period, again only after the rightward stimulus (Fig. 2*a*, right). Third, and most relevant to the memory code, on anti-saccade trials, activity was again elevated in the delay period following the rightward stimulus (Fig. 2*b*, left), even though the saccade at the end of the delay was directed to the left. Fourth, on AS-ODR error trials, when the monkey mistakenly directed its saccades toward the stimulus, neuronal activity in the delay again reflected the rightward location of the stimulus (Fig. 2*c*, right). Thus, the response of this neuron in the delay was keyed to the direction of a preceding stimulus, and not to that of the impending saccade, whether that saccade was correct or incorrect, and whether it was toward or away from the original stimulus. A similar pattern of stimulus dependence was found in the delay period activity of 30 out of 51 (or 59%) of the prefrontal neurons studied. If the 44 histologically verified principal sulcus neurons are considered alone, this percentage rises to 68%. The preponderance of stimulus-dependent delay period activity in this area is strong evidence of a prefrontal specialization for ideational processing that does not rely on a motor code and is not mediated by motor signals.

Response-coding neurons were also found, in smaller numbers (25%, 13/51), intermixed among stimulus-coding neurons within the principal sulcus. An example of response-coding delay period activity in a principalis neuron is shown in Fig. 3. Activity was

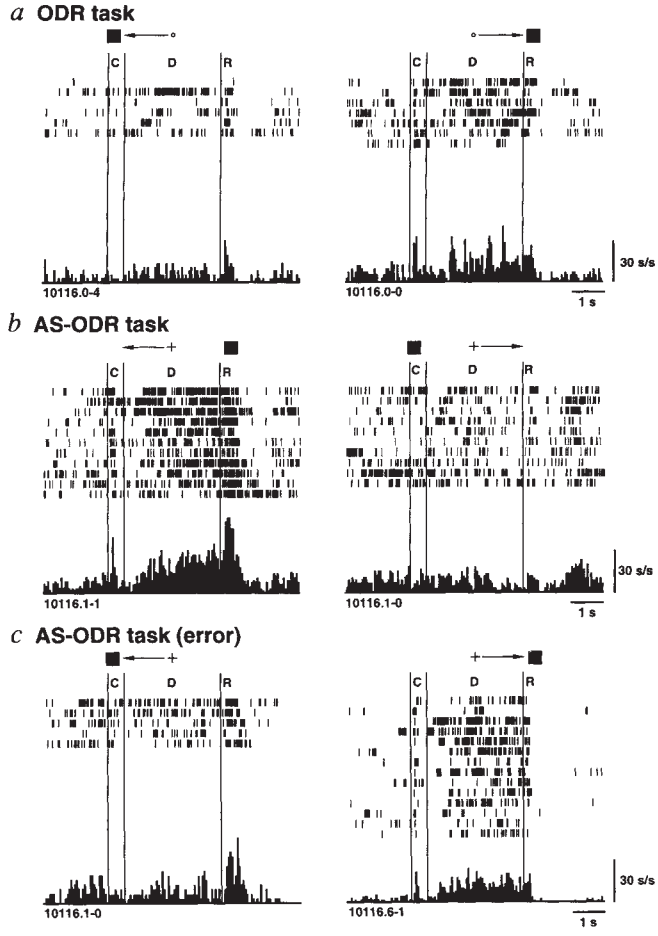


FIG. 2 Stimulus-dependent delay-period activity of a principal sulcus neuron (10116, left hemisphere). *a*, Delay period activity is elevated on ODR trials after rightward stimuli and before rightward saccades (right) *b*, Delay period activity is elevated on AS-ODR trials after the rightward stimulus, before leftward saccades (left). Because the response persists across trials with identical stimuli but opposite saccades, it codes the location of the stimulus. *c*, The response still occurs after rightward stimuli on AS-ODR error trials when the monkey mistakenly makes a saccade to the target (right). The neuron also responded phasically 98 ms after the onset of the visual stimulus on the right (cue (C) period: *a*, right; *b*, left; *c*, right), and 53 ms before saccades to the left (response (R) period: *a*, *b* and *c*, left). The independence of the preferred directions of visual and mnemonic activity on the one hand, and oculomotor activity on the other, is brought out by the inverted target-saccade relationship imposed by the anti-saccade task. In a two-way ANOVA (stimulus location by saccade direction), delay period activity following the rightward stimulus was significantly greater than that following the leftward stimulus ($F_{(\text{stimulus location})} = 19.56$, d.f. = 1, $P \leq 0.000$). Neither saccade direction ($F_{(\text{saccade direction})} = 0.78$, d.f. = 1, $P = 0.385$) nor the interaction term were significant ($F_{(\text{sl/sd})} = 2.70$, d.f. = 1, $P = 0.111$). The filled squares and arrows above each raster indicate the location of visual cue, and directions of saccadic eye movements, respectively. The abbreviations C, D, and R indicate cue, delay and response periods. Vertical lines through rasters indicate, left to right, the onset of the visual stimulus, the offset of the visual stimulus (beginning of the delay period), and the offset of the fixation spot (end of delay, beginning of response period). Rasters are aligned to the beginning of the delay. Vertical and horizontal bars at lower right corner of each pair of histograms indicate 30 spikes per s and 1 s, respectively.

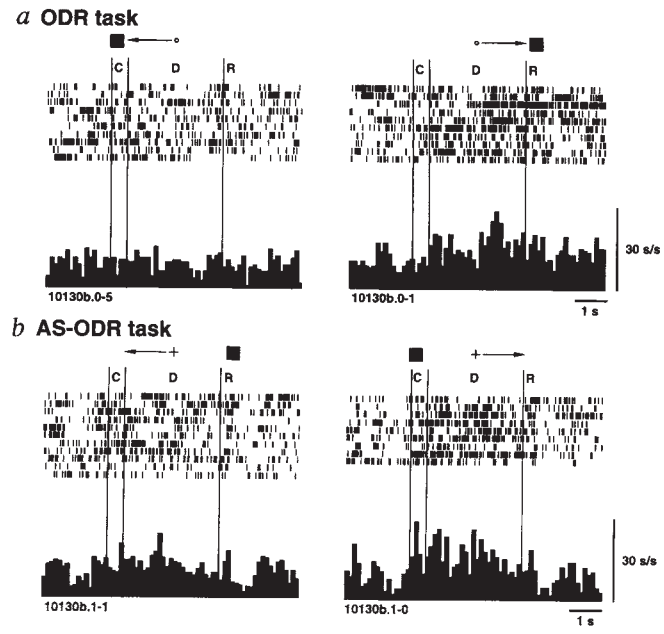


FIG. 3 Saccade-dependent delay-period activity of a principal sulcus neuron (10130b, right hemisphere). *a*, Delay period activity is elevated on ODR trials after rightward stimuli before rightward saccades (right) *b*, Delay period activity is elevated on anti-saccade trials after leftward stimuli before rightward saccades (right). Because the response persists across trials with identical saccades but opposite stimuli, it codes the direction of the saccade. In a two-way ANOVA (stimulus location by saccade direction) delay period activity preceding rightward saccades was significantly greater than that preceding leftward saccades ($F_{(\text{saccade direction})} = 12.44$, d.f. = 1, $P = 0.001$). Neither stimulus location ($F_{(\text{stimulus location})} = 1.74$, d.f. = 1, $P = 0.195$) nor the interaction term ($F_{(\text{sl/sd})} = 0.80$, d.f. = 1, $P = 0.376$) were significant. Conventions are the same as in Fig. 2.

greater in the delay before rightward saccades, irrespective of whether the stimulus signalling the saccade had appeared on the right (Fig. 3*a*, right) or the left (Fig. 3*b*, right). Similar motor-planning activity has been recorded from neurons in the frontal eyefields[10], the posterior parietal cortex[11], the supplementary motor cortex and neostriatum[12], and in the superior colliculus[13]. The prefrontal region from which our recordings were made has connections to all of these structures[14–20], and could through these connections participate in the timing and/or direction of motor output. Eight of the 51 neurons (16%) failed to respond on AS-ODR trials and are not described further.

Evidence that stimulus-coding neurons may have a role in response suppression is provided by the neuron illustrated in Fig. 4, whose complex firing pattern is affected both by the location of the stimulus and the direction of the saccade. Delay period activity is increased before leftward saccades (Fig. 4*a*, *b*, left), but is suppressed after rightward stimuli, strongly on ODR trials (Fig. 4*a*, right), and transiently on AS-ODR trials (Fig. 4*b*, right). Given that the suppression is associated with the rightward location of the stimulus and not the rightward saccade, it may be driven by input from other stimulus coding prefrontal neurons similar to the one illustrated in Fig. 2. Independent response-coding and stimulus-coding mechanisms in prefrontal cortex might thus combine to mediate complex cognitive functions.

Stimulus-coding and response-coding delay period activities have been recorded previously from neurons in the principal sulcus on delayed-response tasks involving manual rather than oculomotor responses[21]. The agreement between those results and our own, obtained with tasks recruiting different motor systems, suggests that the concurrent representation of past stimuli and future actions is a general principle of prefrontal operation. This segregation may enable the flexible re-association of stimulus representations and motor plans on a moment to moment basis on tasks in which stimulus–response relationships are not fixed. Stimulus and response-coding prefrontal neurons may directly or indirectly contribute to activity changes observed in motor areas, such as the supplementary motor area, the motor cortex, and the putamen, where target-dependent and limb-dependent preparatory activity have also been recorded during a delayed-response task[12].

Working memory as characterized in studies of human cognition is more than a passive storage device but also a workspace for manipulation of symbolic representations[22]. Such a workspace may be needed to perform the anti-saccade task in monkeys, because this task requires a mental inversion analogous to

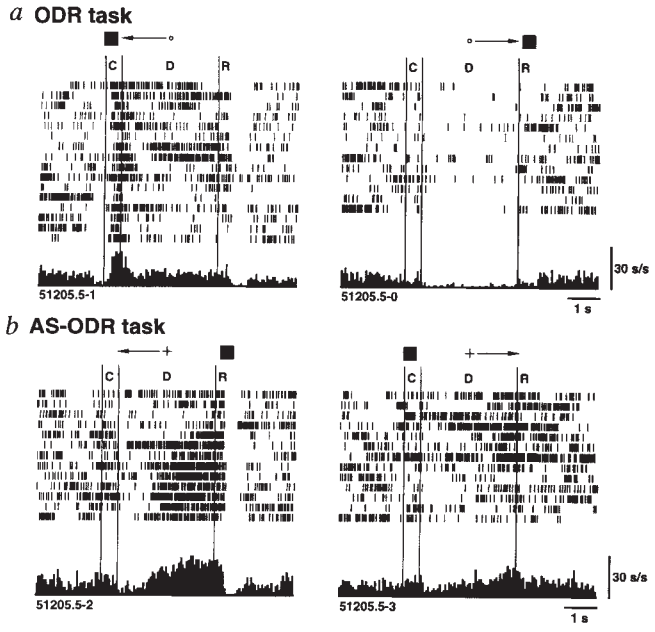


FIG. 4 Saccade-dependent delay period activity of a prefrontal neuron (51205, right hemisphere). *a*, Delay period activity on ODR trials is elevated before leftward saccades (left), and is strongly inhibited before rightward saccades (right). *b*, Delay period activity is again elevated before leftward anti-saccades (left), but inhibition before rightward anti-saccades is absent (right). Because the suppression seen on ODR trials did not occur before saccades of similar direction and amplitude on anti-saccade ODR trials, it cannot be attributed to the metrics of the impending eye movement. Conversely, a transient suppression is seen in the beginning of the delay on anti-saccade trials after the stimulus appears on the right. This transient suppression, and the more lasting suppression seen on ODR trials, have the rightward stimulus location in common. Thus the suppression is more closely associated with the rightward location of the stimulus than the rightward direction of the saccade. The suppression on anti-saccade trials following the rightward stimulus is overridden by an excitatory drive on this unit anticipating the leftward direction of the upcoming saccade. A visual response is evident after the stimulus appears on the left, if a saccade is made to the stimulus (*a*, left). The visual response is not present when the saccade is made away from the stimulus (*b*, right). This pattern of activity may reflect an enhancement of visual responsiveness to stimuli serving as saccade targets similar to that previously reported in the FEF[10] and principal sulcus[23]. A two-way ANOVA (stimulus location by saccade direction) confirms that delay period activity preceding leftward saccades was significantly greater than that preceding rightward saccades ($F_{(\text{saccade direction})} = 18.78$. d.f. = 1, $P = 0.000$). Stimulus location alone was not a significant factor ($F_{(\text{stimulus location})} = 0.64$, d.f. = 1, $P = 0.429$), but the interaction between stimulus location and saccade direction was significant ($F_{(\text{sl/sd})} = 19.79$, d.f. = 1, $P = 0.000$). The significant interaction term reveals that this neuron's pattern of activity in the delay is a function of both stimulus location and saccade direction.

processes engaged in humans performing an anti-saccade task[3], the Stroop Test[2] or Wisconsin Card Sort Test[1]. In these tasks, as in AS-ODR, habitual, generally sensory-driven, responses must be inhibited and less potent, generally instruction-guided, alternative responses selected. Loss of prefrontal neurons that hold instructional information 'on line' may explain why damage to prefrontal cortex commonly results not only in the absence of a correct response but distraction by sensory cues and disinhibition and preservation of competing habitual responses. As mentioned, schizophrenic patients are impaired on a variety of anti-saccade tasks[4–6]. If these deficits reflect the dysfunction of working memory mechanisms residing in prefrontal cortex, as seems reasonable, understanding the neural mechanism subserving delayed-response function in non-human primates could hold clues to cognitive dysfunction in mental illness as well. □

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# MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia

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**CYTOKINES are critical in the often fatal cascade of events that cause septic shock[1–3]. One regulatory system that is likely to be important in controlling inflammatory responses is the neuroendocrine axis. The pituitary, for example, is ideally situated to integrate central and peripheral stimuli[4], and initiates the increase in systemic glucocorticoids that accompanies host stress responses[6–8]. To assess further the contribution of the pituitary to systemic inflammatory processes, we examined the secretory profile of cultured pituitary cells and whole pituitaries *in vivo* after stimulation with bacterial lipopolysaccharide (LPS). Here we identify macrophage migration inhibitory factor (MIF)[9–11] as a major secreted protein released by anterior pituitary cells in response to LPS stimulation. Serum analysis of control, hypophysectomized and T-cell-deficient (nude) mice suggests that pituitary-derived MIF contributes to circulating MIF present in the post-acute phase of endotoxaemia. Recombinant murine MIF greatly enhances lethality when co-injected with LPS and anti-MIF antibody confers full protection against lethal endotoxaemia. We conclude that MIF plays a central role in the toxic response to endotoxaemia and possibly septic shock.**

The anterior pituitary cell line AtT-20 was cultured serum-free in the presence of increasing amounts of bacterial endotoxin *Escherichia coli* 0111 : B4 LPS). Conditioned media were analysed at different times for secreted proteins by SDS–polyacrylamide gel electrophoresis. Endotoxin stimulation resulted in a specific time- and concentration-dependent appearance of a 12.5K protein in the medium (Fig. 1*a*). This protein was isolated and its N-terminal sequence identified it as the murine homologue of human MIF[9] (96% identity over 27 amino acids; Fig. 1*b*). Murine MIF was then cloned from complementary DNA prepared from stimulated pituitary cells, expressed in *E. coli* and used as immunogen for antibody production.

Under serum-free conditions, relatively high concentrations of LPS were necessary to demonstrate MIF release by silver staining. When analysed by western blotting with anti-MIF antibody, however, as little as 100 pg $ml^{-1}$ of LPS induced pituitary cell secretion of MIF (Fig. 1*c*). Supplementation of culture medium with 1% serum markedly increased secretion of MIF, consistent with studies indicating that LPS responses are potentiated by serum factors such as LPS-binding protein[12,13]. In contrast, MIF was not released by incubation of pituitary cells with the inflammatory mediators tumour necrosis factor-$\alpha$, interleukin-1$\beta$, interleukin-6, or interferon-$\gamma$ (data not shown). Western blotting also showed that resting, non-stimulated cells contained significant amounts of pre-formed MIF. Immunocytochemistry confirmed these results and showed that immunoreactive intracellular MIF is released within 16 h of LPS stimulation (Fig. 1*d*).

Human macrophage migration inhibition was one of the first cytokine activities to be described and results from product(s) elaborated by activated T cells[10,11]. Cloned human MIF has an $M_r$ of 12.5K and exerts a broad range of pro-inflammatory activities on monocytes/macrophages[9,14–17]. We used reverse transcription–polymerase chain reaction (RT-PCR) analysis to investigate the expression of pituitary MIF *in vivo* in mice injected with sublethal amounts of LPS (2.25 mg $kg^{-1}$). Pituitary MIF messenger RNA levels increased with time and reached a plateau 16–24 h after LPS challenge (Fig. 2*a*). These findings were confirmed by competitive PCR analysis, which showed a 3-fold increase in pituitary MIF mRNA after LPS administration (Fig. 2*b*). Although infiltrating mononuclear cells were not evident in stained pituitary sections after LPS treatment, we wished to exclude a potential T-cell contribution of pituitary MIF mRNA. Pituitary cDNA analysis for the T-cell gene product CD2 was uniformly negative, ruling out infiltrating T-cells as a possible source of pituitary MIF mRNA (Fig. 2*a*). Also, LPS did not induce pituitary MIF mRNA in the genetically endotoxin-resistant mouse strain C3H/HeJ (Fig. 2*c*).

The pituitary content of MIF protein *in vivo* was analysed by western blotting of pituitary lysates. As predicted from studies of cultured pituitary cells, pituitaries from normal, non-stimulated mice showed substantial smounts of pre-formed MIF protein (Fig. 3*a*). There was a significant decrease in pituitary MIF con-

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