cooling and recovery. The receptive fields of recorded neurons were located in the central 4° of visual field. Electrolytic lesions were used to aid reconstruction of electrode tracts on histological sections stained for Nissl substance, cytochrome oxidase and Cat301. We relied principally on Cat301 labelling[22] to identify V3 and MT/V5.

Cooling. The general procedure was similar to that described earlier[23]. Several months before the experiment, two probes, consisting of a 7 mm × 3 mm loop of hypodermic tubing, were placed side-by-side in the depth of the STS at the level of area V5, ipsilateral to the recordings. The proper placement of the probes was verified post-mortem on histological sections stained as above. Cooled methanol was circulated simultaneously through both probes. Probe temperatures (mean value 7.2 °C, range 2.5–12 °C) were monitored by thermocouples at the base of the cooling loops.

Runs usually lasted for 3.5 min. Two control runs were done before cooling was applied. Recording during the cooling run was started once the probes reached constant temperature, a few minutes after the chilled methanol began circulating. After a pause of 15–30 min after the end of the cooling run, one or several recovery runs were done.

It is important to determine to what extent the tissue is affected by the cold. It was shown earlier that the average temperature gradient created by the probe is 10 deg mm^{-1} and that neuropil beyond 3–3.5 mm from the cooling probe is at physiological temperature (Fig. 1 of Lomber *et al.*[23]). This was confirmed by 2DG mapping showing that the metabolic activity of cortex located 3 mm away from the cooling probe was totally unaffected by the cooling[7]. From earlier anatomical publications[2], we measured the following shortest distances between retinotopically corresponding regions in STS and V1, V2, V3 and V4: 7, 5.7, 7 and 3.4 mm. Thus it is highly unlikely that these areas are affected by the cold. This was confirmed by measuring in two instances the temperature from the surface of area 17 down to a depth of 8 mm (corresponding to our recordings in V3). In one experiment there was no change in temperature throughout the cortical depth and in the other case the temperature dropped by less than 2 °C when the probes were cooled.

Furthermore, because fibres of the optic radiations travel 1–2 mm below the STS grey matter[24], even for the lowest temperature of our probes (2.5 °C), these fibres did not reach temperatures lower than 27 °C. Because fibres have a blocking temperature at least 10 deg lower than cell bodies that are inactivated below 20 °C[25,26], it seems very unlikely that direct blocking of LGN fibres could explain our results.

Visual stimuli. Visual stimuli were presented on a computer monitor driven by a Truevision Vista Board under the control of a Matlab program. Stimuli were presented twenty times in an interleaved fashion. The bar was approximately optimized in size and velocity for each neuron site studied. Orientation was optimized to within 15° by measurement of an orientation tuning curve. The background covered a field that was 12.7° wide and 8.4° high and that comprised randomly distributed checks of a size equal to the bar width. The background had the appearance of a set of bars of variable length and similar orientation as the central bar, which was only visible against the background if it differed in contrast or relative movement. One set of luminance values was used at each site, with mean background luminance, L_0, being in the range 9–24 Cd m^{-2}. The contrast of the bar relative to this luminance, $C_{bar} = (L_{bar} - L_0)/L_0$, was in the range 0.72–12.6. The contrast of the light background checks relative to L_0, $C_{check} = (L_{check} - L_0)/L_0$ (equal to the Michelson contrast of the light–dark checks) was in the range 0.06–0.98. The salience is equal to C_{bar}/C_{check}. Selection of different luminance combinations for the bar and background was not systematic.

Data processing. To limit the presence of false positive results due to poor stationarity of the cortex, data were analysed only when the responses to the first two control runs were not significantly different ($P > 0.01$). Responses were measured as the mean number of spikes over the stimulation period for 20 repetitions of the stimulus. Because of the non-gaussian distribution of the data and occasional changes in variance between conditions, we used a bias-corrected and accelerated-bootstrap Student-*t* procedure[27] to assess the statistical significance of differences in the mean number of spikes across the different runs.

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Stress and glucocorticoids impair retrieval of long-term spatial memory

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Extensive evidence from animal and human studies indicates that stress and glucocorticoids influence cognitive function[1–11]. Previous studies have focused exclusively on glucocorticoid effects on acquisition and long-term storage of newly acquired information. Here we report that stress and glucocorticoids also affect memory retrieval. We show that rats have impaired performance in a water-maze spatial task after being given footshock 30 min before retention testing but are not impaired when footshock is given 2 min or 4 h before testing. These time-dependent effects on retention performance correspond to the circulating corticoster-

one levels at the time of testing, which suggests that the retention impairment is directly related to increased adrenocortical function. In support of this idea, we find that suppression of corticosterone synthesis with metyrapone blocks the stress-induced retention impairment. In addition, systemic corticosterone administered to non-stressed rats 30 min before retention testing induces dose-dependent retention impairment. The impairing effects of stress and glucocorticoids on retention are not due to disruption of spatial navigation *per se*. Our results indicate that besides the well described effects of stress and glucocorticoids on acquisition and consolidation processes, glucocorticoids also affect memory retrieval mechanisms.

Young adult male Sprague–Dawley rats (aged 10–12 weeks) from Charles River Laboratories were trained in a single session of eight trials in a water maze to find a submerged platform located in a fixed position[12]. Memory for the location of the platform was tested 24 h later using a 60-s free-swim probe trial. In the first experiment, rats received three footshocks (0.8 mA for 1 s with a 5-s intershock interval) either 2 min, 30 min or 4 h before the retention probe trial. Non-stressed controls spent significantly more time in the target quadrant than in the opposite quadrant ($P < 0.01$), indicating memory for the location of the platform (Fig. 1a). Retention was impaired when the animals were tested 30 min ($P < 0.01$), but not 2 min or 4 h, after the footshock stimulation. Figure 1b shows representative examples of the swimming pattern of a non-stressed control and that of a rat given footshock stimulation 30 min before testing. Control rats displayed focused searching for the platform in the correct location, whereas stressed rats (30-min group) showed random swimming patterns. The four groups did not differ in total pathlengths during the probe trial ($F_{3,36} = 0.04$, $P > 0.98$). These results indicate that the stress impaired specific aspects of spatial performance and that the retention performance on the probe trial was not affected by any gross disturbances in motor performance.

These results suggest that stress induces time-dependent impairment of memory retrieval. Stress initiates a cascade of physiological events, including high circulating levels of catecholamines and

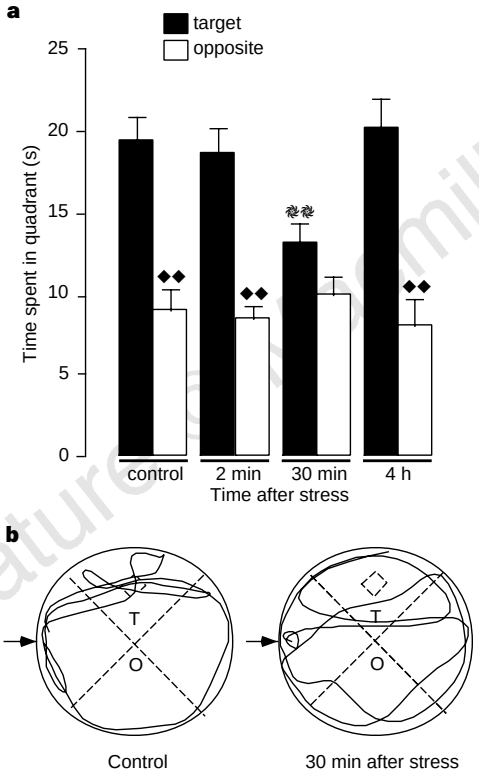

Figure 1 Effects of pre-testing exposure to footshock on retention performance. **a**, Time (mean ± s.e.m.) in seconds spent in target quadrant (T; filled bars) and opposite quadrant (O; open bars) for the four experimental groups. A two-way ANOVA showed significant treatment ($F_{3,36} = 3.68$, $P < 0.05$) and quadrant effects ($F_{1,36} = 55.86$, $P < 0.0001$). **, $P < 0.01$ compared with non-stressed controls; ◆◆, $P < 0.01$ compared with time in target quadrant. Sample size is ten individuals per group. **b**, Representative swimming paths of a non-stressed control and an animal given footshock stress 30 min before testing. Arrow indicates starting placement.

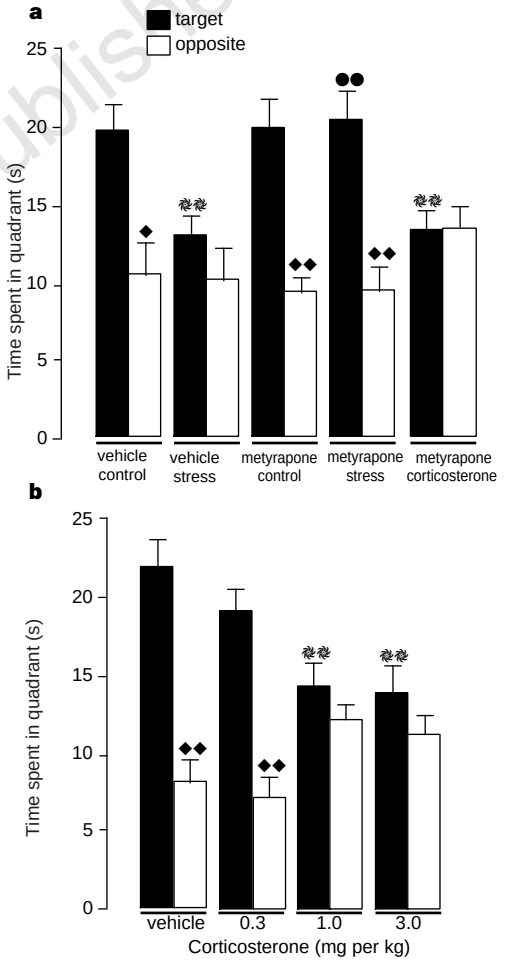

Figure 2 Effects of pre-testing manipulation of glucocorticoid levels on retention performance. Time (mean ± s.e.m.) in seconds spent in target quadrant (filled bars) and opposite quadrant (open bars). **a**, Effect of metyrapone on stress-induced impairment in retention performance. A two-way ANOVA showed significant treatment ($F_{4,42} = 4.94$, $P < 0.005$) and quadrant effects ($F_{1,42} = 22.01$, $P < 0.0001$). **, $P < 0.01$ compared with vehicle-control group; ●●, $P < 0.01$ compared with vehicle-stress group; ◆, $P < 0.05$; ◆◆, $P < 0.01$ compared with time in target quadrant. **b**, Effects of pre-testing corticosterone administration on retention performance. A two-way ANOVA showed significant quadrant effects ($F_{1,36} = 35.47$, $P < 0.0001$) and treatment × quadrant interaction effects ($F_{3,36} = 5.63$, $P < 0.01$). **, $P < 0.01$ compared with vehicle group; ◆◆, $P < 0.01$ compared with time in target quadrant. Sample size is between nine and ten individuals per group.

glucocorticoids. Catecholamines are released rapidly and typically return to baseline levels within 10 min, but the glucocorticoid response is much slower[13]. The finding that plasma levels of corticosterone, from blood collected immediately after the probe trial, were elevated in the impaired 30-min group ($P < 0.01$), but not in any of the other stress groups (Table 1a), suggests that increased adrenocortical function induced by the stressor may have disrupted memory retrieval. To examine this possibility, rats were given metyrapone, a drug that reduces the synthesis of corticosterone by inhibiting the 11β-hydroxylation reaction in the adrenal glands. Metyrapone (50 mg per kg, subcutaneous (s.c.) injection; Sigma) was dissolved in a vehicle containing 40% polyethylene glycol and 60% saline. Consistent with previous findings[14], metyrapone injected 40 min before footshock had no effect on baseline corticosterone levels, but blocked the stress-induced increase in plasma corticosterone ($P < 0.01$; Table 1b). Metyrapone also blocked the stress-induced retention impairment ($P < 0.01$, Fig. 2a). Other possible actions of metyrapone were excluded as metyrapone did not block the retention impairment in rats given corticosterone (3.0 mg per kg, s.c.) 30 min before testing. Neither of the treatments altered swimming pathlengths ($F_{4,42} = 0.61$, $P > 0.64$).

These results suggest that activation of the hypothalamic–pituitary–adrenal cortex (HPA) axis mediates the impairing effects of footshock stress on retention for spatial memory and that a selective blockade of the HPA axis is sufficient to block these impairing effects. To investigate further the putative role of glucocorticoids on memory retrieval, non-stressed rats were given corticosterone (0.3, 1.0 or 3.0 mg per kg, s.c.; Sigma) 30 min before the probe trial (Fig. 2b). Corticosterone was dissolved in a vehicle containing 5% ethanol in saline. As expected, the systemic corticosterone injections induced dose-dependent increases in plasma corticosterone levels (Table 1c). The two higher doses of corticosterone induced retention impairment comparable to that seen after footshock ($P < 0.01$ for both doses). Again, the groups did not differ in total pathlengths ($F_{3,36} = 1.70$, $P > 0.18$). Results of a previous study indicated that intracerebroventricular administration of the specific antagonists for adrenal steroid receptors to non-stressed rats shortly before retention testing did not affect retention performance in a spatial water-maze task[15]. Together, these findings suggest that stress levels of glucocorticoids may impair memory retrieval, whereas basal levels do not exert tonic inhibitory effects on memory retrieval. Moreover, because the water-maze training procedure is very stressful and induces high levels of corticosterone, the present effects cannot be explained by state dependency.

Many nonspecific factors affecting sensory, attentional and motor processes influence spatial navigation. As indicated above, motor performance was not affected by any of the experimental manipulations. To examine further whether the effects were due selectively to influences on specific aspects of memory retrieval, footshock, corticosterone (3.0 mg per kg, s.c.) or a vehicle injection were given to naive animals 30 min before a water-maze training session. A two-way analysis of variance (ANOVA), with trials as repeated measures, showed that all three groups learned the task and that they did not differ in escape latencies ($F_{2,189} = 0.08$, $P > 0.92$). In addition, the group did not differ in performance on a probe trial given 20 s after the last training trial ($F_{2,27} = 0.08$, $P > 0.93$). These findings indicate that the impairing effects of stress and glucocorticoids on retention performance are not due to nonspecific effects on spatial navigation. These data further indicated that immediate recall is not affected by the treatments.

Evidence summarized by Lupien and McEwen indicates that glucocorticoids have multiple and often conflicting effects on memory function[6]. These authors stressed that in order to interpret correctly the effects of glucocorticoids on cognition, it is critical to dissociate the effects on the different memory phases. The time-limited effects of stress and glucocorticoids on memory consolidation follow an inverted-U-shape dose–response relationship: extreme low and high levels may impair consolidation[1–4,6–8], but intermediate doses enhance memory[5,6,9–11], and activation of glucocorticoid receptors seems to be a prerequisite for the long-term storage of information. To our knowledge, our report is the first to demonstrate that once memories are consolidated, the efficacy or accuracy of the information retrieved remains vulnerable to glucocorticoids at the time of recall. As the retrieval impairment was found as soon as 30 min after drug injections, which is too fast to consider the classic genomic action via intracellular receptors[16], it is likely that the effects may involve an interaction with neurotransmitter mechanisms[17–19]. Alternatively, other rapid, non-genomic steroid actions have been described via surface receptors linked to GABA receptors[20]. Whatever the exact mechanism, glucocorticoid influences on retrieval mechanisms seem to be exclusively adverse. In fact, the same treatments that enhance memory consolidation when administered immediately post training[9–11], impaired memory retrieval in the present study when given shortly before testing. Although our experiments examined the role of stress and glucocorticoids on retrieval in only a relatively stressful task, the findings reinforce the conclusion that the nature of the effects of glucocorticoids on cognitive performance depends on the specific mnemonic processes examined. Further research is needed to determine whether comparable effects are found with less stressful tasks and with other species. Our results are important in relation to observations in human subjects that indicate that diurnal fluctuations in cognitive performance are negatively correlated with diurnal variations in plasma cortisol levels[21]. Our findings may be of even greater importance in considering experiments that examine the effects, on memory function, of chronic stress or sustained elevated levels of glucocorticoids, as well as in clinical conditions of human patients with hypercortisolism. In contrast to acute treatments, chronic exposure to stress or glucocorticoids seems to induce only cognitive deficits. Traditionally, the impairments seen under those conditions have been interpreted (either implicitly or explicitly) as being due solely to a disruption of memory consolidation. On the basis of the present findings, however, it seems likely that at least some of the effects may be due to impaired memory retrieval. □

Table 1 Effects on plasma corticosterone levels

Treatment	Plasma corticosterone (μg dl^{-1})
(**a**) Footshock stress	
Control	2.3 ± 0.2
2 min	6.1 ± 1.7
30 min	11.2 ± 4.0*
4 h	4.3 ± 0.8
(**b**) Metyrapone	
Vehicle control	4.1 ± 1.3
Vehicle stress	13.8 ± 4.9†
Metyrapone control	4.8 ± 1.1
Metyrapone stress	4.7 ± 0.5‡
Metyrapone corticosterone	29.1 ± 5.8†
(**c**) Corticosterone	
Vehicle	3.3 ± 0.7
0.3 mg kg^{-1}	6.7 ± 1.5
1.0 mg kg^{-1}	12.9 ± 2.6§
3.0 mg kg^{-1}	31.0 ± 6.5‖

a, Plasma corticosterone levels (mean ± s.e.m.) of groups in Fig. 1a. A one-way ANOVA revealed a significant treatment effect ($F_{3,36} = 3.00$, $P < 0.05$). *$P < 0.01$ compared with non-stressed controls. **b**, Plasma corticosterone levels of groups in Fig. 2a. A one-way ANOVA revealed a significant group effect ($F_{4,36} = 9.52$, $P < 0.0001$). †$P < 0.01$ compared with vehicle-control group; ‡$P < 0.01$ compared with vehicle-stress groups. **c**, Plasma corticosterone levels of groups in Fig. 2b. A one-way ANOVA showed a significant group effect ($F_{3,36} = 13.00$, $P < 0.0001$). §$P < 0.05$; ‖$P < 0.01$ compared with vehicle group. Sample size is between 8 and 10 individuals per group.

Methods

The water maze was a circular galvanized steel tank, 1.83 m in diameter, 0.58 m in height, filled with water (27 °C) to a depth of 20 cm. For the training, a submerged Perspex platform (20 cm × 25 cm) was placed 25 cm away from the edge in a fixed location. The single training session consisted of eight trials with four different starting positions that were equally distributed around the perimeter of the maze. After mounting the platform, the animals were allowed

to remain there for 20 s, and were then placed in a holding cage for 30 s until the start of the next trial. After completion of training, the animals returned to their home cages until retention testing 24 h later. On the day of testing, the animals were retained in a waiting room for 1 h before exposure to footshock or systemic injection of corticosterone. Footshock stress was administered in an inhibitory avoidance apparatus (as described elsewhere[9]) in an adjacent, sound-attenuated room. The rats were placed in the starting compartment and, after they stepped completely into the shock compartment, the door between the compartments was closed and a series of three footshocks (0.8 mA for 1 s with a 5-s intershock interval) was administered. After the last footshock, the rats were retained in the shock compartment for 15 s and then returned to their home cages. The animals were assigned randomly to one of the experimental groups. The probe trial consisted of a 60-s free-swim period without a platform and was recorded on video tape for later analysis. The rat was placed in the tank at the arrow (Fig. 1b), a position that was equal in distance to the imaginary target quadrant (T) and opposite quadrant (O). Training and testing was conducted between 10:00 and 15:00 h.

For plasma corticosterone determination, the animals were decapitated immediately after the probe trial and trunk blood was collected in heparinized (500 IU ml^{-1}) tubes and stored on ice. After centrifugation at 5000 r.p.m. for 10 min, the supernatant was stored at −50 °C until assay. Corticosterone plasma concentrations were determined by radioimmunoassay using a highly specific antibody (B3-163, Endocrine Sciences, Tarzana, California) and ^{3}H-corticosterone tracer. Coefficients of variation within and between assays were less than 10%.

Retention performance data were analysed with a two-way ANOVA with treatment as between-subject variable and quadrant as within-subject variable. The ANOVAs were followed by either Fisher's tests for between-subject comparisons or paired *t*-tests for within-subject comparisons. Plasma corticosterone levels were analysed with a one-way ANOVA followed by Fisher's tests. A probability of less than 0.05 was considered significant.

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The stomach is a source of leptin

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The circulating peptide leptin, which is the product of the *ob* gene[1], provides feedback information on the size of fat stores to central Ob receptors[2,3] that control food intake and body-weight homeostasis[4–6]. Leptin has so far been reported to be secreted only by adipocytes[1] and the placenta[7]. Here we show that leptin messenger RNA and leptin protein are present in rat gastric epithelium, and that cells in the glands of the gastric fundic mucosa are immunoreactive for leptin. The physiological function of this previously unsuspected source of leptin is unknown. However, both feeding and administration of CCK-8 (the biologically active carboxy-terminal end of cholecystokinin) result in a rapid and large decrease in both leptin cell immunoreactivity and the leptin content of the fundic epithelium, with a concomitant increase in the concentration of leptin in the plasma. These results indicate that gastric leptin may be involved in early CCK-mediated effects activated by food intake, possibly including satiety.

Oligonucleotides deduced from the cloned mouse *ob* gene[1] were used for screening of total RNA extracted from fundic

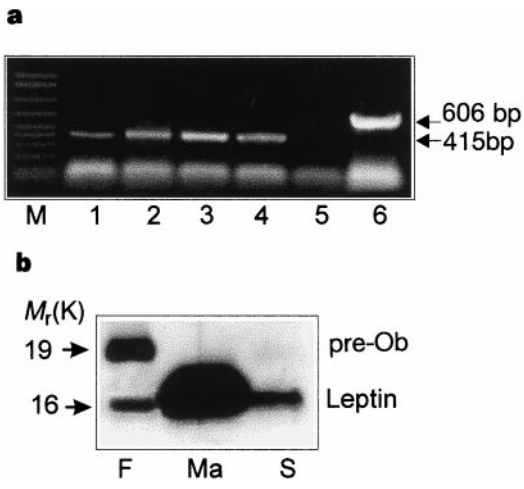

Figure 1 Leptin expression in Wistar rats. **a**, RT-PCR analysis of leptin mRNA: fundic epithelium (lane 1), mesenteric (lane 2), epididymal (lane 3) and perirenal (lane 4) adipose tissues; liver (lane 5); β-actin (lane 6); markers (lane M). Arrows indicate the expected size of the PCR products: 415 bp for *ob* and 606 bp for β-actin. **b**, Western blot of leptin protein: homogenate from fundic epithelium scrapings of Wistar rats (50 μg proteins, lane F), mesenteric adipose tissue (20 μg proteins, lane Ma) and serum (5 μg proteins, lane S). Two leptin immunoreactive bands are seen in the fundic epithelium sample: 16K (leptin) and 19K (leptin precursor, pre-Ob). Note that the 19K band is absent from the adipose tissue and serum.