Changes in grey matter induced by training

Newly honed juggling skills show up as a transient feature on a brain-imaging scan.

Does the structure of an adult human brain alter in response to environmental demands[1,2]? Here we use whole-brain magnetic-resonance imaging to visualize learning-induced plasticity in the brains of volunteers who have learned to juggle. We find that these individuals show a transient and selective structural change in brain areas that are associated with the processing and storage of complex visual motion. This discovery of a stimulus-dependent alteration in the brain's macroscopic structure contradicts the traditionally held view that cortical plasticity is associated with functional rather than anatomical changes.

Animal studies indicate that experience-related changes may occur in mammalian brain structures, but so far there has been no evidence of comparable alterations in the human brain[3–5]. To investigate this possibility, we divided a homogeneous group of volunteers (21 female, 3 male; mean age, 22 yr ± 1.6 s.d.), who were matched for sex and age, into two groups, designated as jugglers and non-jugglers. Both groups were inexperienced in juggling at the time of their first brain scan.

Subjects in the juggler group were given 3 months to learn a classic three-ball cascade juggling routine. A second brain scan was performed when they had become skilled performers (that is, when they could sustain juggling for at least 60 seconds). A third scan was carried out 3 months later; during the intervening period, none of the jugglers practised or attempted to extend their skills — for example, by learning a four-ball or a reverse cascade. In fact, most subjects were no longer fluent in three-ball cascade juggling by the time of the third scan.

We used voxel-based morphometry, a sophisticated objective whole-brain technique, to investigate subtle, region-specific changes in grey and white matter by averaging results across the volunteers. This method is based on high-resolution, three-dimensional magnetic-resonance imaging, registered in a common stereotactic space, and is designed to find significant regional differences by applying voxel-wise statistics in the context of gaussian random fields[6,7].

Group comparison at the beginning (the baseline) showed no significant regional differences in grey matter between jugglers and non-jugglers. In the longitudinal analysis, the juggler group demonstrated a significant (44 d.f., $P<0.05$) transient bilateral expansion in grey matter in the mid-temporal area (hMT/V5) and in the left posterior intraparietal sulcus between the first and the second scans. This expansion decreased in the third scan (Fig. 1). We found a close relationship in these regions between the transient structural grey-matter changes and the juggling performance. These findings were specific to the training stimulus, as the non-jugglers showed no change in grey matter over the same period.

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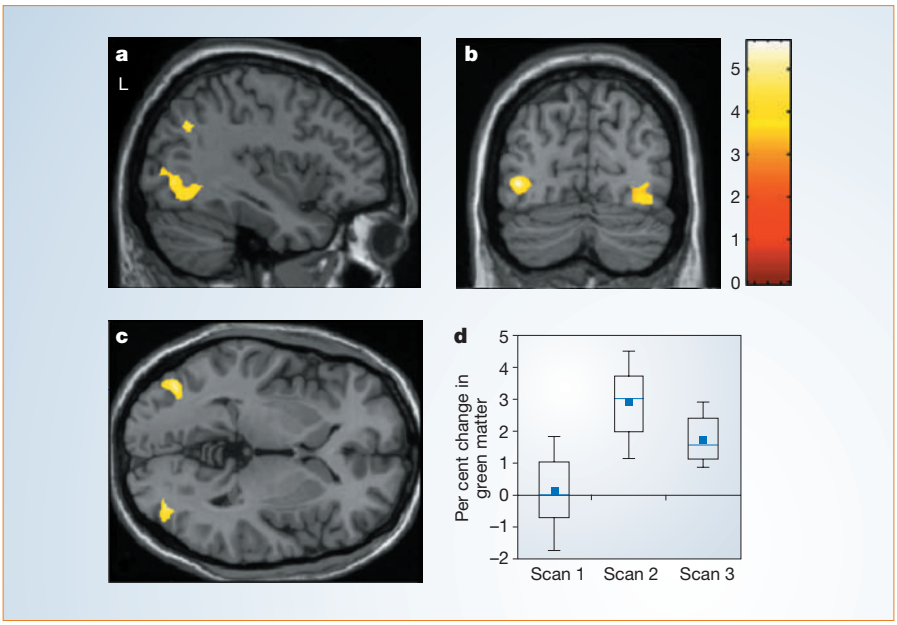

Figure 1 Transient changes in brain structure induced while learning to juggle. **a–c,** Statistical parametric maps showing the areas with transient structural changes in grey matter for the jugglers group compared with non-juggler controls. **a,** Sagittal view; **b,** coronal view; **c,** axial view. The increase in grey matter is shown superimposed on a normalized T1 image. The left side (L) of the brain is indicated. A significant expansion in grey matter was found between the first and second scans in the mid-temporal area (hMT/V5) bilaterally (left: *x*, –43; *y*, –75; *z*, –2, with $Z = 4.70$; right: *x*, 33; *y*, –82; *z*, –4, with $Z = 4.09$) and in the left posterior intraparietal sulcus (*x*, –40; *y*, –66; *z*, 43 with $Z = 4.57$), which had decreased by the time of the third scan. Colour scale indicates *Z* scores, which correlate with the significance of the change. **d,** Relative grey-matter change in the peak voxel in the left hMT for all jugglers over the three time points. The box plot shows the standard deviation, range and the mean for each time point.

Our results contradict the traditionally held view that the anatomical structure of the adult human brain does not alter, except for changes in morphology caused by ageing or pathological conditions. Our findings indicate that learning-induced cortical plasticity is also reflected at a structural level.

As all of our volunteers have normal fine-motor skills, we conclude that juggling, and consequently the perception and spatial anticipation of moving objects, is a stronger stimulus for structural plasticity in the visual areas (used for the retention of visual-motion information[8,9]) than in the motor areas (involved in the planning and execution of coordinate motion — that is, the supplementary motor area and/or the motor cortex, cerebellum and basal ganglia).

Although the observed transient increase in grey matter takes place in specific motion-selective areas, the microscopic changes underlying these dynamic structural alterations are unclear. Macroscopic alterations may be based on changes at the level of

synaptic bulk and neurites, or they might include increased cell genesis, for example, of glial or even neuronal cells[4]. Imaging results need to be compared with histological data for identification of the structural basis at the microscopic level of temporary, training-dependent structural changes in our brains.

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Competing financial interests: declared none.

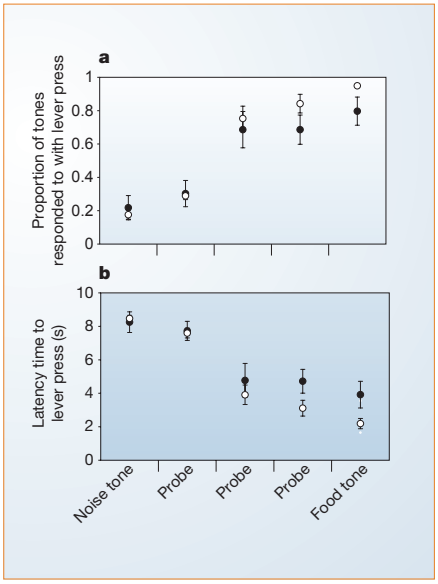

Figure 1 Mean (±1 s.e.) responses to tones during 10 daily 30-min test sessions for male Lister hooded rats housed under 'predictable' (open circles, $n=4$) and 'unpredictable' (filled circles, $n=5$) conditions. **a,** Proportion of tones to which the animals responded to by pressing a lever. **b,** Latent time between sounding of the tone and pressing of the lever. 'Noise' and 'food' tones are the tones used during training (2 and 4 kHz, respectively, for about half of the rats, and 4 and 2 kHz, respectively, for the remaining rats). 'Probe' tones are non-reinforced, intermediate tones (2.5, 3, 3.5 kHz), each randomly interspersed with a probability of 0.085 between the reinforced training tones. Regression equations were calculated for each rat, correcting for nonlinear relationships by using binary logistic regression (for proportions) and logarithmic transformations for linear regression (for latencies). Animals were checked daily and remained healthy throughout the experiments.

Animal behaviour

Cognitive bias and affective state

Information processing by humans can be biased by their emotions — for example, anxious and depressed people tend to make negative judgements about events and to interpret ambiguous stimuli unfavourably[1–4]. Here we show that such a 'pessimistic' response bias can also be measured in rats that are housed in unpredictable conditions[5,6]. Our findings indicate that cognitive bias can be used as an indicator of affective state in animals, which should facilitate progress in animal-welfare studies.

We trained rats to respond by pressing a lever when they heard a tone associated with a positive event (delivery of a 45-mg food pellet) and to refrain from pressing the lever as a way to avoid a negative event (an unpleasant burst of white noise) when they heard another tone. Once the animals were able to score a correct response to each tone more than 50% of the time (binomial testing for three consecutive daily 30-min sessions), they were allocated to either 'unpredictable' housing, which induces symptoms of a mild depression-like state[5,6], or to 'predictable' housing.

In 'unpredictable' housing, between zero and two negative interventions were made at random times on any one day — for example, the cage might be unfamiliar or tilted, or it could contain a stranger of the same species; sometimes the light/dark cycle would be temporarily reversed or bedding left damp. These changes were never imposed simultaneously, and they were made at least two hours before or after test sessions. 'Predictable' housing, in contrast, was maintained as during training, with none of these interventions.

After nine days, during which training was continued, the rats were exposed to non-reinforced tones that had frequencies intermediate between those of the two food-delivery and noise-avoidance tones. Ten test sessions were held to investigate the animals' anticipation of these positive or negative events, as judged by their lever-press response to these ambiguous tones.

The proportion of tones responded to by lever pressing (Fig. 1a) and the time taken to respond to the tones (mean response latencies; Fig. 1b) were calculated for each tone for each rat on each of the test days. Analysis of variance with repeated-measures (tone, test day) and a between-subjects factor (housing) revealed a housing × tone interaction ($F_{4,28}=2.72, P<0.05$).

The proportion of tones responded to with a lever press by individuals kept in unpredictable housing indicated that fewer lever presses were made in response to tones of frequency close to that of the food tone (Fig. 1a) (in two-tailed t-tests, $t=1.88$, d.f. $=7$, $P=0.1$). These rats were also slower to press the lever in response to the food tone and to the ambiguous tones that were close to it in frequency (Fig. 1b) ($t=-2.44$, d.f. $=7$, $P<0.05$). Both findings were still valid when only the responses by the rats to the ambiguous tones were analysed (proportions: $t=1.92$, d.f. $=7$, $P=0.09$; latencies: $t=-2.42$, d.f. $=7$, $P<0.05$).

Overall, rats in unpredictable housing were slower to respond and tended to show fewer responses to ambiguous tones close to the positive tone and to this tone itself. The treatment groups did not differ ($P>0.2$) in tests of feeding motivation (consumption speed of freely available food pellets[7]), anhedonia (amount of sucrose solution consumed[5,6]), activity (hole–board test[8]), bodyweight change across the test period, and response accuracy to training tones before and after the imposition of housing changes, indicating that none of these factors was likely to account for our findings.

By using ambiguous stimuli to probe animals' relative anticipation of positive and negative events, we have shown that rats in unpredictable housing show behaviour indicating reduced anticipation of a positive event. This compares with findings for depressed or anxious humans, who also have reduced expectation of positive events[1,4] and interpret ambiguous stimuli negatively[3].

Our results call for further investigation of the underlying processes involved[9,10]. We find no evidence of enhanced anticipation of the negative event. This may be due to a floor effect and could be revealed using, for example, lever-pressing and nose-poking as counterbalanced positive and negative responses. It is possible that our technique could be adapted to detect an enhanced expectation of positive events — a correlate of happy mood in humans[4]. Being able to assess positive as well as negative affect in animals is an important objective for animal welfare[11].

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Competing financial interests: declared none.