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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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 (30 s of 70 dB 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.

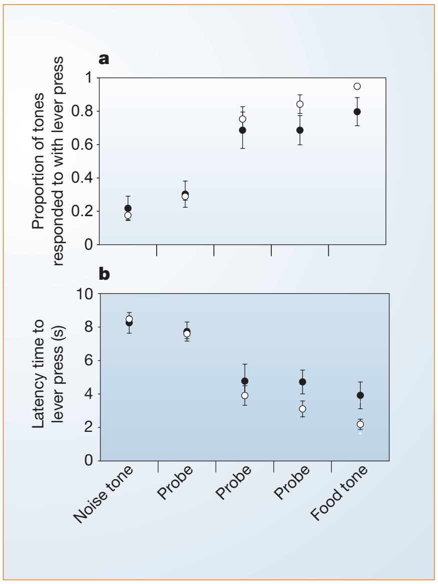

Figure 1 Mean (±1 s.e.) responses to tones during 10 daily 30-min test sessions for male Lister hooded rats housed under ‘predictable’ (open circles, $n=4$) and ‘unpredictable’ (filled circles, $n=5$) conditions. **a,** Proportion of tones to which the animals responded to by pressing a lever. **b,** 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.

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 ($F_{4,28}=2.72$, $P<0.05$) for the latencies.

Regression equations were calculated for each rat. Comparison of the slopes of the response latency regressions indicated that rats in unpredictable housing were slower to press the lever in response to the food tone and the ambiguous tones close to it (Fig. 1b) ($t=-2.44$, d.f. $=7$, $P<0.05$; two-tailed t-tests throughout). These rats also tended to show fewer responses to these tones (Fig. 1a) ($t=1.88$, d.f. $=7$, $P=0.1$). Both findings were still evident when only the slopes of the responses to the ambiguous tones were analysed (latencies: $t=-2.42$, d.f. $=7$, $P<0.05$; proportions, $t=1.92$, d.f. $=7$, $P=0.09$).

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]), body-weight 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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