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Predicting Anxiety-Related Behaviour Using a Spatial Learning Test

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Abstract

Background

Classic experimental behaviour models generally utilise painful stimuli to elicit anxiety-stress responses. This is inconsistent with human anxiety-stress behaviours, which generally result from internal psychological conflict. Here, we use a non-noxious conflict model, predator scent stress (PSS) to model the development of low-level chronic anxiety in a more naturalistic manner.

Previous data revealed that rodents responded to PSS in two ways: avoidance or confrontation. This division could potentially be useful for drug development, allowing targeting of recessive or defensive behaviours, comparable to depressive and manic behaviours observed in many affective disorders, including bipolar. In order to predict performance in the stress test, here we investigated the relationship between spatial learning paradigm and response to stressor exposure.

Methods

Animals were assessed using a modified Barnes maze spatial task. The time taken to locate a food reward using visual spatial cues was recorded. Following maze exposure, rats were subjected to the PSS paradigm.

Animals were positioned in the test box for 15 min daily for 6 days. On habituation days no PSS was present in the box; on test days the hide area contained either PSS (cat scent) or no scent (Control). Behaviour was recorded, analysed and correlated to performance in the maze test.

Results

Presence of PSS evoked behaviour changes in all exposed rats, compared to control groups. Time spent in close proximity to the odour differed significantly, 5/12 rats avoiding the odour and 7/12 remaining in close proximity. These distinct differences in avoidance behaviours correlated to results obtained from the Barnes maze experiments. The time spent on the maze (day 2) directly correlated to a propensity for avoidance behaviour.

Conclusion

The ability to predict anxiety-related behaviour is a significant refinement of the conflict model; enabling targeting of investigations into specific behavioural traits associated with the affective disorders.

Background

Anxiety disorders are characterised by dramatic responses to situations that exceed the normal behavioural response to the presented situation and are commonly linked with affective disorders, which encompass alterations in baseline levels of mood. The spectrum incorporates numerous disorders spanning generalised anxiety disorder, to phobias and post-traumatic stress disorder. One of the most common of the anxiety disorders is generalised anxiety disorders, which can present with or without associated depression. This type of anxiety disorder describes a low-level chronic form of anxiety that lacks a focus or trigger for the behaviours.

Current treatment for the affective disorder spectrum is generally dependent on the utilisation of antidepressants and antipsychotics in combination with non-pharmaceutical treatments such as psychotherapy and cognitive behavioural therapy, yet effects remain unpredictable and treatment is primarily reserved for more severe cases.

With the incidence of anxiety and stress-related disorders increasing the need for treatments selective to symptoms, improving effectiveness, reducing side effects, and problems with compliance, tolerance and dependence is essential. However, there is currently no single animal model for stress/anxiety disorders which mimic the full spectrum of symptoms displayed in humans, thus multiple models are required in order to investigate the effect of test compounds. Furthermore, there is no current way of predicting behavioural responses to low-level triggers of anxiety, outside of exposure to the stressor.

In animals, characteristic behaviours associated with anxiety can be divided into two groups: defensive or recessive. Comparable behavioural responses are also commonly observed in human. We hypothesise that by developing a model which can predict the behavioural response to a stressor it may be possible to target drugs more selectively toward these behavioural subtypes, leading to drug-development aimed at improving effectiveness and compliance.

Here we present a novel model for low level anxiety, utilising a non-noxious conflict model for anxiety (Collins, 2009), expanded to incorporate a method for predicting outcome of stressor exposure, enabling selective targeting of behaviours for research and refinement of the current anxiety model.

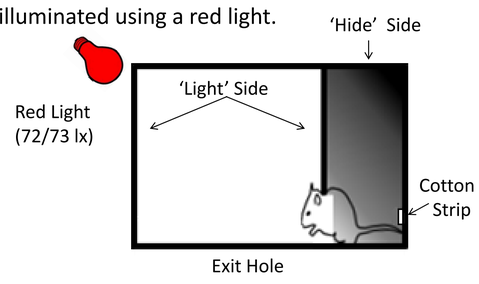
Methods

All procedures were performed in accordance with the UK Animals (Scientific Procedures) Act 1986. Experiments were performed on 32 male Sprague Dawley rats (aged 3 weeks at commencement). All animals were exposed to a Barnes maze spatial learning task for 3 days prior to predator stress model (PSS) exposure.



The Barnes maze consisted of a circular platform at a height of 100 cm with 20 holes along the parameter. A small, dark box containing bedding and a food reward, known as the 'treat box,' was randomly positioned under one of the holes. Animals were positioned in the centre of the maze at the start of the trial and the time taken to locate the 'treat box' using spatial cues was recorded.

PSS Behavioural testing boxes consisted of a dark 'hide' side and a 'light' side separated by a panel incorporating an exit hole. Strips of cotton were positioned on the back wall of the hide side of the box. The light side of the box was illuminated using a red light.





Animals were habituated to the boxes for 3 days prior to testing under 'Control' conditions. On test days; animals were exposed to one of two paradigms: 'No Odour' (n=10), predator (cat) 'Odour' (n=22), where the cotton strip was impregnated with predator scent.

Animals were positioned in the centre of the box at the start of the trial and behaviour was observed for 15 mins and recorded via cameras linked to a computer for off-line analysis. Behaviour was analysed visually and behaviours typically associated with anxiety and stress-related states were measured.

Statistical analysis

Data was analysed using ANOVA (InStat; GraphPad Inc.). *P*<0.05 was considered significant. Data are presented as mean±SEM.

Results

Figure 1. Characteristic anxiety behaviours induced by PSS exposure

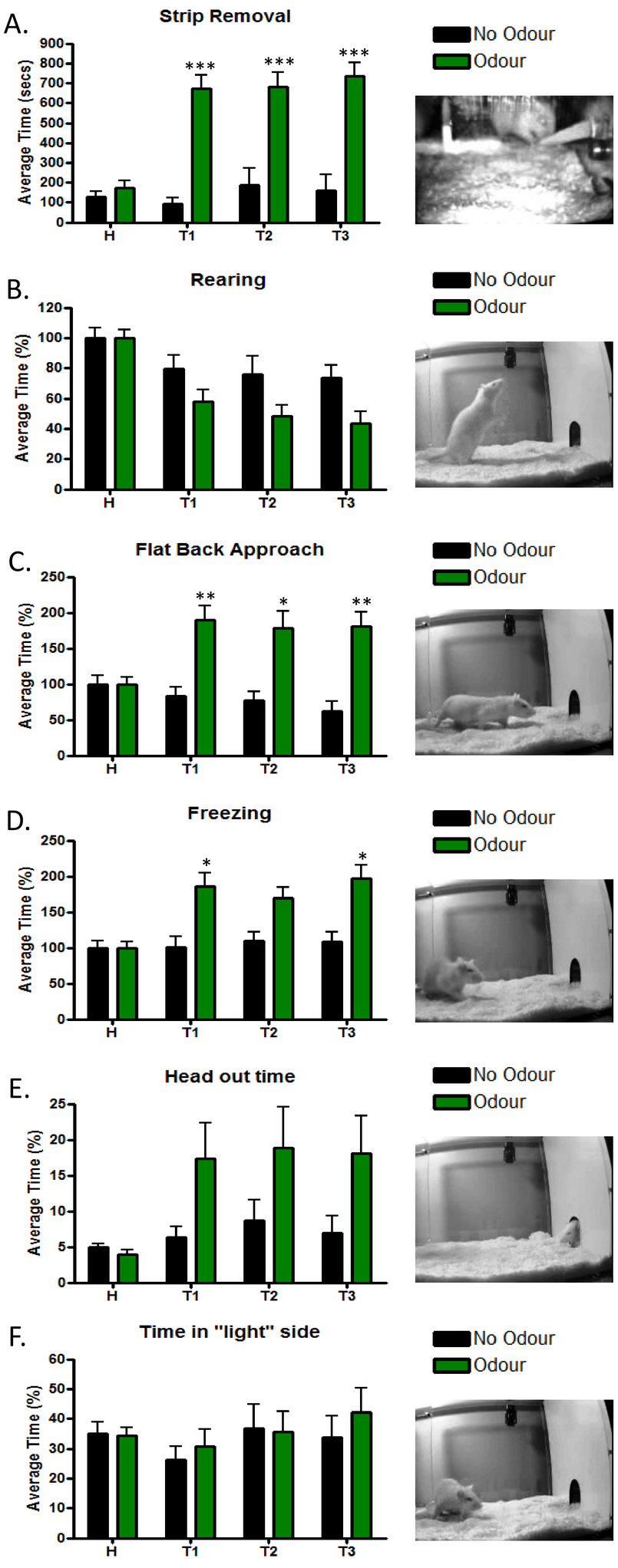


Fig. 1. Anxiety behaviours elicited by PSS model exposure. (A–F) Left panel: Graphs depicting characteristic anxiety-related behavioural changes following exposure to stimuli. Key:* *P*<0.05, ** *P*<0.01, *** *P*<0.001. Right panel: Photographs depicting typical behavioural postures observed.

Table 1. Summary of behavioural results

Behaviour	H		T1		T2		T3	
	No odur	Odour	No Odour	Odour	No Odour	Odour	No Odour	Odour
Strip removal (secs)	130.8 ± 26.5	174.9 ± 33.9	94.6 ± 30.7	674.3 ± 66.7***	187.6 ± 83.9	682.2 ± 73.3***	159.8 ± 83.7	737.7 ± 66***
Rearing (%)	100.0 ± 6.4	100.0 ± 5.1	79.7 ± 8.6	58 ± 8.1	76.1 ± 11.6	48.2 ± 7.2	73.8 ± 8.0	43.4 ± 7.8
Flat back (%)	100.0 ± 12.7	100.0 ± 10.0	82.8 ± 13.2	190.1 ± 19.2**	77.5 ± 12.2	178.6 ± 23.9*	62.3 ± 13.5	180.6 ± 20.5**
Freezing (%)	100.0 ± 9.3	100.0 ± 8.5	100.6 ± 15.2	185.7 ± 19.2*	109.2 ± 13.2	169.0 ± 16.1	108.4 ± 14.0	197.0 ± 19.0*
Head out (%)	5.0 ± 0.5	4.0 ± 0.6	6.3 ± 1.5	17.4 ± 4.9	8.7 ± 2.9	18.9 ± 5.7	6.9 ± 2.5	18.1 ± 5.2
"Light" side (%)	35.1 ± 3.8	34.3 ± 2.9	26.4 ± 4.5	30.7 ± 5.8	36.9 ± 7.9	35.5 ± 7.0	33.9 ± 7.0	42.1 ± 8.1

Table 1. Full details of behavioural and statistical analysis of data obtained from the PSS model. Key:* *P*<0.05, ** *P*<0.01, *** *P*<0.001.

Figure 2. Differential behavioural responses to PSS

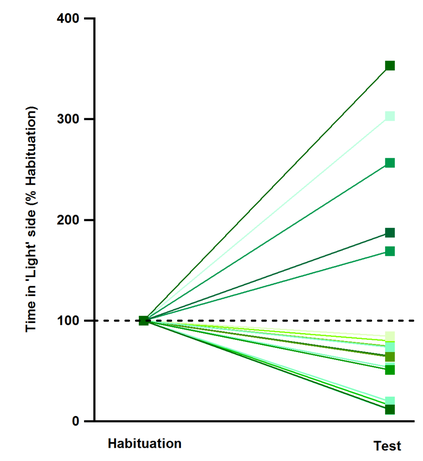


Fig. 2. Data showing split in behaviour in the time spent in the 'light' side (n=18, each line represents one animal). Animals consistently displayed either: avoidance of the odour (more time in the light) or more time in close proximity to the odour (more time in hide), comparable to recessive or defensive behaviours

Figure 3. Behavioural phenotype correlates to maze performance

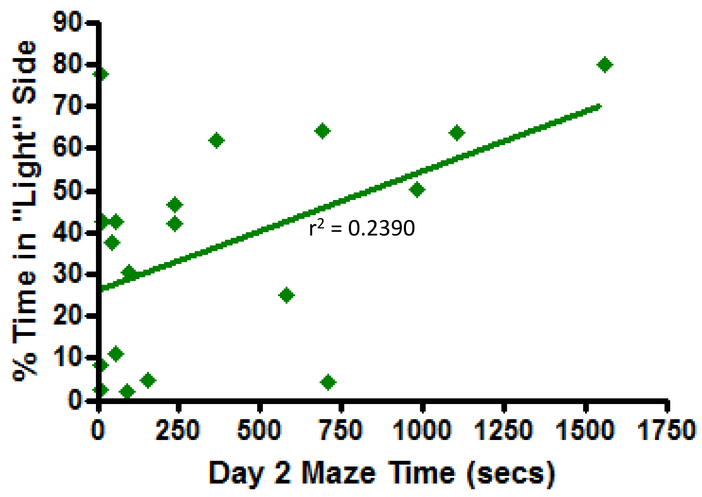


Fig. 3. Data showing correlation between the time spent in the 'light' side (PSS Odour, average of test days) and the time spent to locate the "treat box" on day 2 of the Barnes Maze (n=22, one point per animal). Animals which took longer to remember and locate the "treat box" on day 2 of the Barnes maze spent significantly more time in the 'light' side of the PSS box (*P*=0.037)

Conclusion

In PSS experiments, presence of cat odour evoked characteristic anxiety related behaviours in all animals, compared to the control groups. Furthermore the time spent in close proximity to the odour differed between rats, with rats either avoiding the odour or remaining in close proximity. These distinct differences in avoidance behaviours directly correlated to the results obtained from the Barnes maze experiments, whereby the time taken to locate the 'Treat box' on the maze (Day 2) indicated propensity for avoidance behaviour in PSS experiments.

The ability to predict anxiety-related behaviour is a significant refinement of the conflict model; enabling targeting of investigations into specific behavioural traits associated with the affective disorders.

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References

D.R. Collins (2009) Differential modulation of Hippocampal plasticity in a non-noxious conflict model for anxiety. *Neuroscience* 162, 863–869.