# Cholinergic Modulation of Disorder-Relevant Neural Circuits in Generalised Anxiety Disorder (GAD) by BNC210, a negative allosteric modulator of α7 nAChR

Toby Wise*[1,2], Fiona Patrick[1], Nicholas Meyer[3], Ndaba Mazibuko[4], Alice E Oates[5], Anna M H van der Bijl[6], Philippe Danjou[7], Susan M O'Connor[8], Elizabeth Doolin[9], Caroline Wooldridge[4], Christine Macare[1], Steven CR Williams[2,4], Adam Perkins[1] & Allan H Young[1]

[1] Centre for Affective Disorders, Department of Psychological Medicine, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK.
[2]National Institute for Health Research Biomedical Research Centre, South London and Maudsley NHS Foundation Trust, London, UK.
[3]Department of Psychosis Studies, Institute of Psychiatry, Psychology & Neuroscience, King's College London, UK.
[4]Department of Neuroimaging, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK.
[5]South London and Maudsley NHS Foundation Trust, London, UK.
[6] Faculty of Social and Behavioural Sciences, University of Leiden, Leiden, Netherlands.
[7]Senior Director R&D, Biotrial, Paris, France
[8] Neuroscience Research, Bionomics, Australia.
[9] Clinical Development, Bionomics, Australia.

This study was funded by Bionomics Limited, Australia. BNC210 is their proprietary drug, which is currently being evaluated in a Phase II clinical trial in PTSD in Australia and the USA.

## Introduction

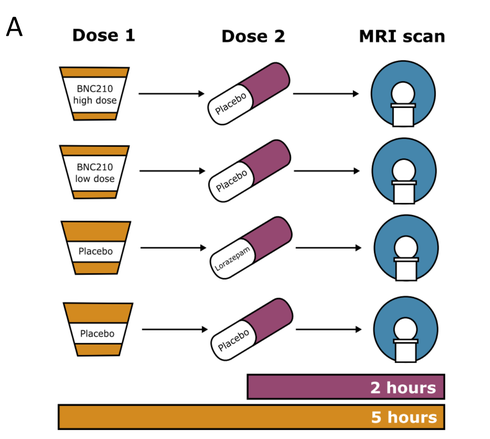
Amygdala hyperactivity has been associated with GAD[1]. Anxiolytic drugs diminish this hyper-reactivity[2], suggesting that normalisation of amygdala response is critical to the successful treatment of symptoms. Amygdala activity in response to aversive stimuli is regulated through functional interactions with the anterior cingulate cortex[3,4], while functional connectivity in these circuits is elevated in clinical anxiety[5].

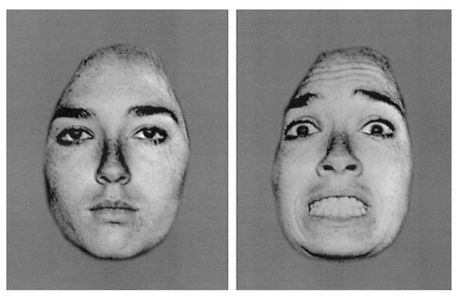
Existing treatments typically target GABAergic and serotonergic neurotransmission, but increasing evidence suggests that cholinergic systems are important in fear-related behaviours, and may play a role in clinical anxiety. In particular, alpha-7 nicotinic acetylcholine receptor (nAChR) antagonist infusion has been linked to anxiolytic behaviour[6]. However, there is no research investigating the role of cholinergic neurotransmission in the function of networks associated with anxiety disorders in humans. We tested this by administering a novel α7 nAChR negative allosteric modulator, BNC210, to individuals with GAD.

## Method

Twenty-four volunteers with GAD participated in the study; GAD diagnosis was established in accordance with DSM-IV, using the Mini International Neuropsychiatric Interview[7] (MINI).

This study constituted a four-way crossover, double blind, double dummy, randomised controlled trial (BNC210 at 300mg and 2000mg, lorazepam at 1.5mg and also placebo (to lorazepam and BNC210). As the absorption rates of BNC210 and lorazepam differ, with Cmax at 5 hours and 2 hours respectively, the dosing schedule incorporated two dose administrations per visit in accordance with these properties, (Figure 1A).



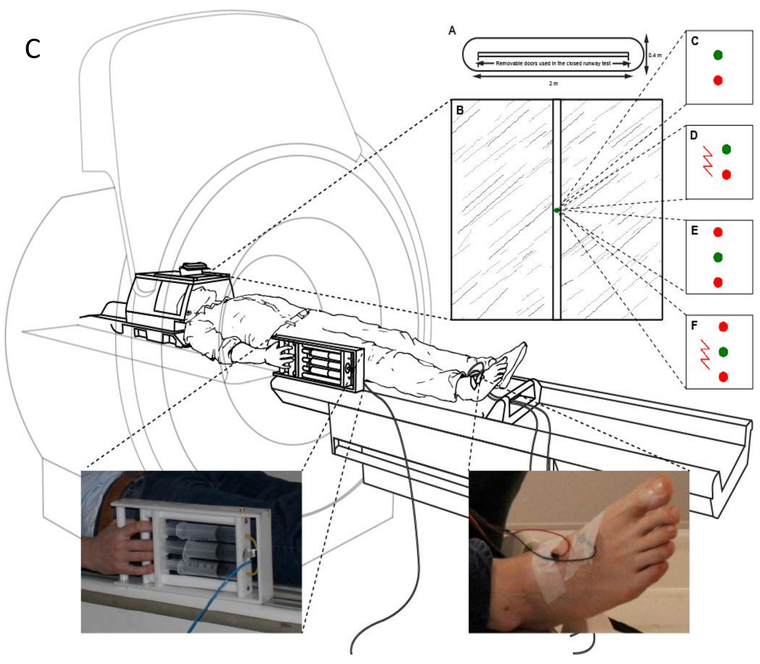




Figure 1: A) Dosing schedule; B) Example of Ekman's emotional faces set, fear; C) JORT paradigm set up

### Emotional Faces Task:

Faces at two intensities (medium and high) displaying fear (Figure 1B) taken from the Ekman emotional faces set[8] were displayed for 2 seconds each, interleaved with neutral faces. Participants were asked to report the sex of the face using a button box with their right hand, ensuring that the emotion of the face was processed implicitly.

### Joystick Operated Runway Task (JORT):

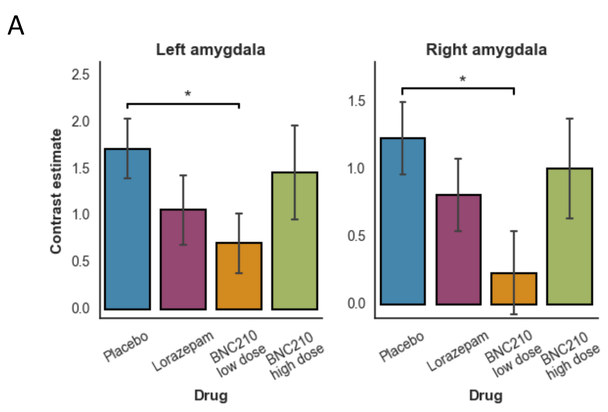
The JORT is a human translation of a rodent defence task, adapted for fMRI use in this study, with the aim being to provide a convergent, behavioural test of the drug effects. The JORT measures the intensity of threat-avoidance behaviour (mild electric shock).

### Data processing & statistical analysis:

Data were pre-processed and analysed with SPM12, testing for drug effects using paired t-tests against placebo in SPM12, with head motion, measured as the total distance travelled, as a covariate. We used a region of interest (ROI) approach with the MarsBAR toolbox (www.marsbar.sourceforge.net) to compare mean activity in our *a priori* amygdala ROI while viewing fearful faces between drug conditions, correcting for multiple comparisons across the two hemispheres using false discovery rate (FDR) correction.

## Results

Relative to placebo, the low dose of BNC210 reduced left ($t(20) = 2.09$, $p = 0.027$, $d = 0.45$) and right ($t(20) = 2.05$, $p = 0.027$, $d = 0.46$) amygdala reactivity (Figure 2A & 2B). In contrast, there was no difference between placebo and BNC210 high dose conditions ($p = 0.33$, both left and right amygdala). Lorazepam reduced left ($t(20) = 2.16$, $p = 0.086$, $d = 0.5$) and right ($t(20) = 1.32$, $p = 0.19$, $d = 0.4$) amygdala reactivity, however this did not reach significance.



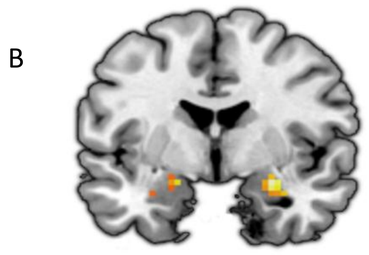
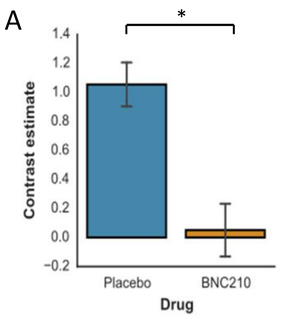




Figure 2: A) Mean (+/- standard error) amygdala response to fearful faces for each drug condition; B) Effect of BNC210 low dose on amygdala activity, thresholded at p < .05 uncorrected for illustration.

A reduction in connectivity between the left amygdala and anterior cingulate cortex while viewing fearful faces under low dose BNC210 conditions (peak = 9, 41, 20, $t = 4.18$, $p = 0.04$ FDR corrected, Figure 3A & 3B) was shown. There were no differences in connectivity with the anterior cingulate for placebo and lorazepam conditions.



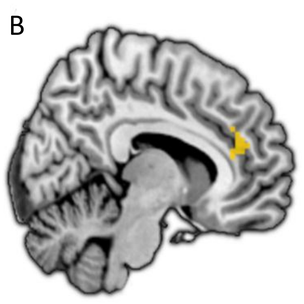
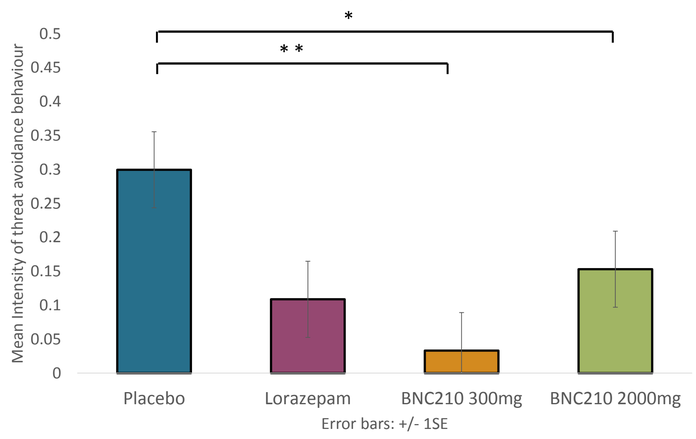




Figure 3. A) Mean functional connectivity strength with left amygdala seed while viewing fearful faces in the anterior cingulate cortex cluster. B) Anterior cingulate cortex cluster showing significant reductions in functional connectivity strength with the amygdala under BNC210 in the PPI analysis.

Echoing these emotional faces data, analysis of JORT results showed both low dose ($f=8.897$, $p =.007$) and high dose ($f = 5.217$, $p =.033$) BNC210 significantly reduced intensity of threat avoidance behaviour, relative to placebo (Figure 4).

Figure 4: Mean intensity of threat avoidance behaviour, across conditions.




## Discussion

Results demonstrated that BNC210 reduced amygdala reactivity to fearful faces relative to placebo. In addition, reduced connectivity was observed between the amygdala and anterior cingulate cortex while viewing fearful faces, after low dose BNC210 administration. Further, in the JORT, both doses of BNC210 reduced the intensity of avoidance behaviour in response to threat, relative to placebo.

These results suggest that the function of anxiety-related neural circuits and anxiety-related behaviours can be altered through negative allosteric modulation of cholinergic neurotransmission by BNC210, highlighting the potential of this molecule as a novel anxiolytic pharmacological approach for GAD.

## References

1. Nitschke, J. B. *et al.* Anticipatory Activation in the Amygdala and Anterior Cingulate in Generalized Anxiety Disorder and Prediction of Treatment Response. *Am. J. Psychiatry* **166,** 302–310 (2009).
2. Paulus, M. P., Feinstein, J. S., Castillo, G., Simmons, A. N. & Stein, M. B. Dose-Dependent Decrease of Activation in Bilateral Amygdala and Insula by Lorazepam During Emotion Processing. *Arch. Gen. Psychiatry* **62,** 282–288 (2005).
3. Etkin, A., Egner, T., Peraza, D. M., Kandel, E. R. & Hirsch, J. Resolving Emotional Conflict: A Role for the Rostral Anterior Cingulate Cortex in Modulating Activity in the Amygdala. *Neuron* **51,** 871–882 (2006).
4. Robinson, O. J., Charney, D. R., Overstreet, C., Vytal, K. & Grillon, C. The adaptive threat bias in anxiety: Amygdala–dorsomedial prefrontal cortex coupling and aversive amplification. *NeuroImage* **60,** 523–529 (2012).
5. Robinson, O. J. *et al.* The dorsal medial prefrontal (anterior cingulate) cortex–amygdala aversive amplification circuit in unmedicated generalised and social anxiety disorders: an observational study. *Lancet Psychiatry* **1,** 294–302 (2014).
6. Mineur, Y. S. *et al.* Multiple Nicotinic Acetylcholine Receptor Subtypes in the Mouse Amygdala Regulate Affective Behaviors and Response to Social Stress. *Neuropsychopharmacology* **41,** 1579–1587 (2016).
7. Lecrubier, Y. *et al.* The Mini International Neuropsychiatric Interview (MINI). A short diagnostic structured interview: reliability and validity according to the CIDI. *Eur. Psychiatry* **12,** 224–231 (1997).
8. Ekman, P. & Friesen, W. V. *Pictures of facial affect*. (Consulting Psychologists Press, 1975).