



# Anxiolytic activity and enhancement of fear extinction demonstrated by BNC210 in rodent models translates directly to the clinic in a CCK-challenge model of panic attack in healthy volunteers



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## INTRODUCTION

BNC210 is a negative allosteric modulator of the  $\alpha 7$  nicotinic acetylcholine receptor in development for the treatment of anxiety disorders.

Deficits in fear extinction are thought to contribute to the development of anxiety disorders. Fear conditioning is a behavioural paradigm used to assess memory associated with fear and emotion with the pairing of an environmental cue or conditioned stimulus (CS - tone) and an aversive unconditioned stimulus (US) such as a mild foot shock (2). This model was used in mice to explore whether BNC210 could enhance fear extinction and thus improve recovery from panic attack.

Cholecystokinin (CCK) is a neurotransmitter, hormone and neuromodulator which binds to CCK<sub>A</sub> and CCK<sub>B</sub> receptors. Administration of CCK-4 produces anxiety in rodents and panic attacks in man which provides a model for assessing novel anxiolytic compounds in healthy volunteers. Here it is shown that BNC210 activity in animal models translates to the clinic.

## METHODS

### ELEVATED PLUS MAZE (1)

BNC210 (1-100 mg/kg, p.o.) and Diazepam (0.5-3 mg/kg, p.o.) were administered to rats in single doses with and without CCK-4 (0.2 mg/kg) to assess the effect on CCK-4 induced anxiety. BNC210 and Diazepam were administered 1 hour prior to testing with CCK-4 administration (i.p.) 30 min prior to testing. Data was expressed as Entries (into) and Time (spent on) the open arms of the EPM. Statistical analysis was performed using the Unpaired T-test (Graph Pad PRISM Version 6).

### CONTEXTUAL FEAR CONDITIONING

A conditioned fear response (freezing) was acquired during the CS (tone) and US (shock) pairing session. CS-US pairing session: C56BL6 mice were placed into an enclosure with an electrified grid bottom. A tone cue was played (CS) which was concluded by a mild foot shock (US). After a 120 sec inter-trial interval, animals were presented with a second tone cue-shock pairing (CS-US). Extinction was initiated 24 hours after the acquisition of fear response.

CS only extinction session: mice were dosed daily for 6 days with either vehicle or BNC210 (p.o.) or Diazepam (i.p.) 1 hour prior to the first extinction session each day. On days 1-3 mice were exposed to 10 CS only presentations at intervals of 60 seconds. On days 4-7, 2 CS only presentations occurred. Time spent freezing was measured and used as an endpoint for strength of the CS-US association and the rate of fear extinction. Comparisons of all treatment groups were conducted using two-way ANOVA followed by a Bonferroni's post hoc test for multiple comparisons using Graph Pad PRISM Version 6.

### HUMAN MODEL OF CCK-4 INDUCED PANIC

Symptoms of CCK-4 induced panic attacks were assessed on the Panic Symptoms Scale (PSS) (3, 4). Healthy male volunteers received a single oral dose of BNC210 (2000mg) or vehicle over two consecutive periods one week apart. In each period the subjects received a single i.v. bolus injection of 50µg CCK-4 at T0+7 hours (BNC210 TMAX). An emotional Visual Analogue Scale (eVAS) (5) was used to subjectively assess recovery to emotional stability at 5, 10, 20, 30 and 60 min following CCK-4 injection. Statistical analysis was performed using the Unpaired T-test (Graph Pad PRISM Version 6).

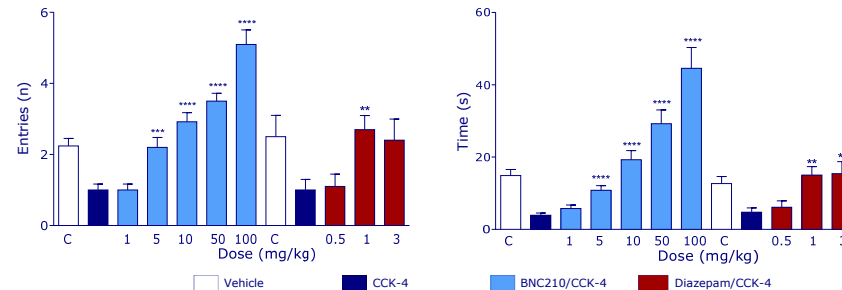
## REFERENCES

1. Pellow et al; (1985) Journal of Neuroscience Methods; 14:149-167.
2. Caldarone et al; (2000) Neuropharmacology; 39:2779-2784.
3. Bradwejn et al; (1991) Journal of Psychiatry & Neuroscience; 16(2):91-5.
4. Eser et al; (2007) Psychopharmacology; 192:479-87.
5. Lees et al; (1999) European Journal of Cancer Care (England); 8(4):220-3.
6. Bouton et al; (1990) State-dependent fear extinction with two benzodiazepine tranquilizers. Behavioural Neuroscience; 104(1):44-55.
7. <http://www.bionomics.com.au/upload/investors/announcements/4736/>
8. Kessler et al; (1995) Posttraumatic stress disorder in the National Comorbidity Survey. Archives of General Psychiatry; 52:1048-1060.
9. Goswami et al; (2013) Animal models of post-traumatic stress disorder: face validity. Frontiers in Neuroscience; 7:89-103.

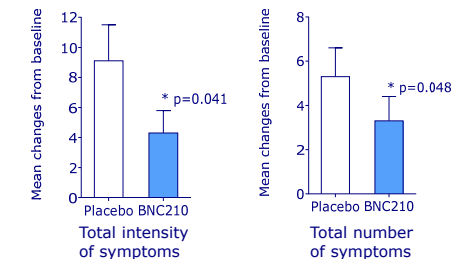
## RESULTS

### EFFECT OF BNC210 ON CCK-4 INDUCED ANXIETY IN RODENT AND HUMAN MODELS

#### 1. THE ANXIOGENIC EFFECT OF CCK-4 WAS REVERSED BY BNC210 IN RAT EPM



#### 2. BNC210 SIGNIFICANTLY REDUCED PSS SCORES IN CCK-4 CHALLENGE IN MAN

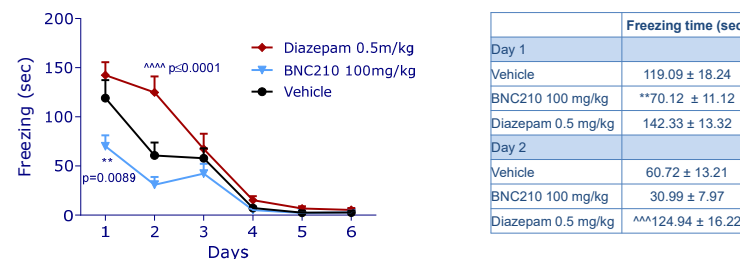


Translation

The ability of BNC210 to reverse CCK-4 (0.2 mg/kg) induced anxiety was studied in the rat EPM. BNC210 (1-100 mg/kg) fully reversed the anxiety induced by CCK-4 from  $\geq 5$  mg/kg as shown by increased number of Entries into, and Time spent in, the open arms. Doses from 10 to 100 mg/kg produced significant anxiolytic responses demonstrating a broad effective dose range. Diazepam also fully reversed the CCK-4-induced anxiety at 1 and 3 mg/kg for both Time and Entries however the 3 mg/kg dose was sedating (data not shown) thus limiting the effect dose range to 1 mg/kg. \*\* $p \leq 0.01$ ; \*\*\* $p \leq 0.001$ ; \*\*\*\* $p \leq 0.0001$  denotes significant differences to CCK-4 treated animals. (Mean  $\pm$  SEM;  $n=10-25$  rats).

### EFFECT OF BNC210 IN MODELS OF FEAR EXTINCTION IN RODENT AND MAN

#### 3. BNC210 DECREASED THE TIME SPENT FREEZING IN A MOUSE MODEL OF CONTEXTUAL FEAR CONDITIONING EXTINCTION

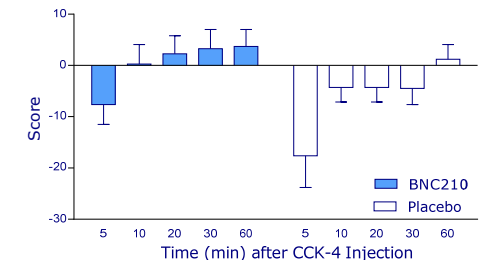


	Freezing time (sec)
Day 1	
Vehicle	119.09 $\pm$ 18.24
BNC210 100 mg/kg	**70.12 $\pm$ 11.12
Diazepam 0.5 mg/kg	142.33 $\pm$ 13.32
Day 2	
Vehicle	60.72 $\pm$ 13.21
BNC210 100 mg/kg	30.99 $\pm$ 7.97
Diazepam 0.5 mg/kg	^^124.94 $\pm$ 16.22

Translation

BNC210 enhanced fear extinction in a contextual fear conditioning extinction paradigm in C56BL6 mice. On Day 1 of extinction, mice treated with 100 mg/kg BNC210 froze significantly less compared to vehicle-treated mice. On Day 2 of extinction, there was a trend for mice treated with BNC210 to spend less time freezing versus vehicle-treated mice. Conversely, Diazepam treated mice (0.5 mg/kg) spent more time freezing on Day 1 compared to control and BNC210 treated mice. This increase in freezing behaviour became highly significant for Diazepam on Day 2 showing that it has an inhibitory effect on fear extinction. On Day 3, time spent freezing was similar for all groups and by Day 4 the conditioned response (freezing) was extinguished. (Mean  $\pm$  SEM,  $n=12$ ).

#### 4. BNC210 ENHANCED RECOVERY OF EMOTIONAL STABILITY FOLLOWING CCK-4 INDUCED PANIC ATTACK IN MAN



An emotional Visual Analogue Scale (eVAS) was used to subjectively assess recovery to emotional stability at 5, 10, 20, 30 and 60 minutes following CCK-4 injection. BNC210 (2000 mg; p.o.) showed a trend towards improved emotional stability in eVAS scores at 5 minutes post CCK-4 injection and a return to baseline at 10 minutes. Placebo treated subjects did not return to baseline emotional stability until 60 minutes post CCK-4 injection. (Mean  $\pm$  SEM,  $n=15$ ).

## CONCLUSIONS

- ❖ The effects of BNC210 on panic symptoms following a CCK-induced panic attack in healthy volunteers, represent direct translation of pre-clinical EPM data to the clinic.
- ❖ The effects of BNC210 on enhanced recovery of emotional stability following a CCK-induced panic attack in healthy volunteers, represent direct translation of rodent fear extinction data to the clinic.
- ❖ The performance of Diazepam in the rodent CCK-4 and fear extinction models has also translated to man. Benzodiazepines are an effective therapy for Panic Disorder with a limited dose range, and are contra-indicated for PTSD due to impairment of fear extinction (6).
- ❖ Recent clinical data in GAD patients (7) have demonstrated that BNC210 significantly dampens down amygdala activation in an emotional faces task and produces significant reduction of simple avoidance responses.
- ❖ Together these data support the evaluation of BNC210 in PTSD patients where high co-morbid anxiety (15-31%) (8) and deficits in fear extinction (9) are major symptoms.
- ❖ Bionomics is currently enrolling PTSD patients in a Phase II trial.