Review

Journal of Psychopharmacology
25(9) 1192–1198

DOI: 10.1177/0269881111408455
jop.sagepub.com

Validating the inhalation of 7.5% CO_2 in healthy volunteers as a human experimental medicine: a model of generalized anxiety disorder (GAD)

Jayne E Bailey[1], Gerard R Dawson[2], Colin T Dourish[2] and David J Nutt[3]

Abstract

Anxiety is a complex phenomenon that can represent contextually different experiences to individuals. The experimental modelling in healthy volunteers of clinical anxiety experienced by patients is challenging. Furthermore, defining when and why anxiety (which is adaptive) becomes an anxiety disorder (and hence maladaptive) is the subject of much of the published literature. Observations from animal studies can be helpful in deriving mechanistic models, but gathering evidence from patients and reverse translating this to healthy volunteers and thence back to laboratory models is a more powerful approach and is likely to more closely model the clinical disorder. Thus the development and validation of a robust healthy volunteer model of anxiety may help to bridge the gap between the laboratory and the clinic and provide 'proof of concept' in screening for novel drug treatments. This review considers these concepts and outlines evidence from a validated healthy volunteer model of generalized anxiety disorder (GAD) following the inhalation of 7.5% CO_2.

Keywords

7.5% CO_2, anxiety, experimental medicine, generalized anxiety disorder, translational medicine

Introduction

Anxiety is a complex phenomenon that can represent contextually different experiences to individuals. The experimental modelling in healthy volunteers of clinical anxiety experienced by patients is challenging. Furthermore, defining when and why anxiety (which is adaptive) becomes an anxiety disorder (and hence maladaptive) is the subject of much of the published literature. Anxiety disorders induce a maladaptive level of arousal that impacts on all levels of performance, they can alter behaviour and have a huge economic burden. It has been reported that within the European Union approximately 27% of the population have experienced at least one mental health disorder within a 12-month period (Wittchen and Jacobi, 2005). Of these, among the most frequent were the anxiety disorders (panic disorder, agoraphobia, social anxiety disorder, generalized anxiety disorder, specific phobias, post-traumatic stress disorder and obsessive compulsive disorder). Anxiety disorders were estimated to be responsible for more days off work than the combined total of days lost through diabetes, lung disease and heart disease (Wittchen and Jacobi, 2005).

The existing drug treatments for anxiety disorders such as benzodiazepines (which are particularly effective for acute anxiety) and monoamine reuptake inhibitors, have many limitations including sub-optimal efficacy, delayed onset of action, side effects and tolerance or withdrawal issues (Lader 2008; Lader et al., 2009). Therefore, there is a need to develop new drug therapies that can treat specific symptoms of anxiety and/or subtypes of anxiety disorders. Recent advances in neuroimaging techniques and in our understanding of brain neurotransmitter systems, together with the ability to conduct large scale genotyping studies (e.g. Wray et al., 2009), has led to the identification of many new potential targets for the treatment of anxiety disorders. However, although the development of novel specific targeted agents has been underway for some time, few drugs with novel mechanisms of action have emerged. This inability to bring new treatments to market is partly due to the selection of compounds that had efficacy in animal models, but subsequently failed in clinical trials (Conn and Roth, 2008). This failure to translate the promising preclinical efficacy of novel compounds to successful clinical results is an ongoing challenge for the pharmaceutical industry and represents an increasingly costly problem when compounds fail after expensive Phase II and III clinical trials in patients. Thus the

[1]Severnside Alliance for Translational Research, University of Bristol, Bristol, UK.
[2]P1Vital Department of Psychiatry, University of Oxford, Warneford Hospital, Oxford, UK.
[3]Neuropsychopharmacology Unit, Division of Experimental Medicine, Imperial College London, London, UK.

Corresponding author:
Jayne E Bailey, Severnside Alliance for Translational Research, School of Medical Sciences, Bristol, BS8 1TD, UK
Email: jayne.bailey@bristol.ac.uk

development and validation of a robust healthy volunteer model of anxiety may help bridge the gap and manage the risk between the laboratory and the clinic and provide proof of concept (PoC) in screening for novel treatments.

In considering the development of human volunteer experimental models of anxiety that can support PoC, a multidisciplinary approach is necessary. The spectrum of anxiety is not a single concept and contributions from behavioural genetics, cognitive psychology, behavioural pharmacology and functional neurophysiology are all relevant. Observations from animal studies can be helpful in deriving mechanistic models, but gathering evidence from patients and reverse translating this to healthy volunteers and thence back to laboratory models is a more powerful approach and is likely to more closely model the clinical disorder. In addition, drug treatments that are efficacious in anxiety disorders can help to elucidate the neuropharmacological mechanisms involved and to validate new models of anxiety in humans. Current drug treatment options include the benzodiazepines (e.g. lorazepam) serotonin reuptake inhibitors (SSRIs, e.g. citalopram) and the serotonin-noradrenaline reuptake inhibitors (SNRIs, e.g. venlafaxine), which are often prescribed as first-line treatments. Benzodiazepines are effective in patients after acute administration (Lader, 2008) but the reuptake inhibitors are efficacious only after several weeks of treatment and evidence suggests that longer-term treatment will further increase response rates (Baldwin et al., 2005). Benzodiazepines are generally of greater value in the validation of experimental medicine models of anxiety where acute or short term dosing is necessary from both an ethical perspective and to meet the required timeliness for determining the efficacy of novel compounds. The ideal healthy volunteer model of experimental anxiety would incorporate both the psychological and physiological arousal seen in patients with anxiety disorders. The requirements of such an 'ideal' healthy volunteer model are outlined in Table 1.

During the past 20 years, various healthy volunteer models of anxiety have been developed that meet the criteria outlined in Table 1 with varying degrees of success. These models can be broadly divided into three categories which differ in how they induce anxiety experimentally namely, pharmacological, physiological and psychological. Pharmacological challenge tests incorporate the delivery of an anxiogenic agent. Physiological challenge tests may influence the respiratory system to produce dyspnoea or suffocation symptoms, or can be based on pain, such as that induced by electric shock or cold water. Some examples of pharmacological and physiological challenge agents are outlined in Table 2.

Psychological challenge tests comprise behavioural approaches using exposure to phobic stimuli or to specific fearful situations. For example, public speaking is a common cause of fear and in a simulated public speaking model anxiety ratings and autonomic symptoms increase as a result of the public speaking procedure (Guimaraes et al., 1997). One variant of this model is the Trier Social Stress Test or TSST (Kirschbaum et al., 1993) which increases levels of subjective anxiety by asking a volunteer to perform a hastily prepared speech or mental arithmetic in front of an audience, sometimes with the participant observing themselves via video feedback during the performance. The degree to which an individual experiences anxiety in such situations depends upon personal experiences and tolerance of social pressure or embarrassment. Consequently the degree of anxiety induced by these procedures may vary markedly between individuals and in small experimental medicine studies such individual differences could mask any potential effect of the test compound.

Another effective method for inducing fear and/or anxiety is to employ a threat or aversive procedure. Malizia (2001) used such a procedure in a neuroimaging study that examined the areas of brain activity involved in anticipatory anxiety. Participants were told that they might receive a painful electric shock to their hand when the colour of a screen changed from blue to red. When the red screen appeared, the shock could happen at any time and was more likely to be stronger the longer the stimulus remained on the screen. As expected from this simple Pavlovian conditioning procedure heart rate, heart rate variability and subjective anxiety significantly increased during the red screen display compared with the blue screen display.

These procedures are models of conditioned anxiety and preclinical studies in animal models often employ such aversive Pavlovian conditioning paradigms to study the biological and psychopharmacological mechanisms of fear, with good predictive validity (Hijzen et al., 1995). Results from preclinical conditioned anxiety studies can translate into human experimental medicine models, for example the measurement of skin conductance responses to neutral and aversive white noise has shown to be sensitive to anxiolytic drug treatments (Hellewell et al., 1999). Furthermore, the distinction between anticipatory anxiety, contextual anxiety and phasic fear can begin to be unravelled using such procedures (Grillon, 2008). However, some psychological challenges can be time-consuming and difficult to apply consistently from study to study and variability is a significant limitation in the screening of potential anxiolytic treatments. For example, the human fear-potentiated startle test is not sensitive to standard anxiolytics such as the benzodiazepines (Baas et al., 2002).

Table 1. Suggested requirements of a human experimental model of anxiety

Requirement	
SAFE	◆ for all participants
	◆ for investigators delivering the challenge
	◆ non-invasive
ACCEPTABLE	◆ to participants receiving challenge
	◆ to ethics committees
	◆ to regulatory bodies
RELIABLE	◆ is there a measurable endpoint?
	◆ can the challenge be performed in different environments by different personnel?
TRANSLATIONAL	◆ from the laboratory to the clinic and back again
	◆ across species
	◆ equivalent doses
	◆ measurable endpoints
EASE	◆ of use
REPEATABLE	◆ can be used on more than one occasion
	◆ no attenuation of response
SUBJECTIVE	◆ measurable psychological effects
OBJECTIVE	◆ measurable physiological effects

Table 2. Pharmacological and physiological models of human anxiety provocation

Challenge	Probable Mechanism
Pharmacological Challenges:	
Benzodiazepine receptor inverse agonists*	Decrease GABA
Caffeine*	Increase NA
Cholecystokinin (CCK), pentagastrin*	Increase CCK
Flumazenil (benzodiazepine antagonist)+	Decrease GABA
m-chlorophenylpiperazine (MCPP)*	Increase 5HT
Pentylenetetrazol*	Decrease GABA
Selective serotonin reuptake inhibitors (SSRIs)	Increase 5HT
Tricyclic antidepressants (TCAs)	Increase 5HT and NA
Yohimbine	Increase NA
Yohimbine/naloxone combination*	Increase NA
Physiological challenges:	
Hypercapnia (inhalation of carbon dioxide)	? Decrease GABA/increase NA
Hyperventilation	Decrease in pCO_2
Sodium lactate+	? Increase noradrenaline/respiration
Sodium bicarbonate+	? Increase noradrenaline/respiration
Cold pressor test	? Increase noradrenaline

*Challenge effective in patients and healthy volunteers; +, challenge effective in panic patients only; GABA, gamma amino butyric acid; 5HT, serotonin; NA, noradrenaline; pCO_2, carbon dioxide partial pressure. SSRIs and TCAs are occasionally anxiogenic in patients in that anxiety is exacerbated early in treatment, healthy volunteers may become anxious. The CO_2 challenge depends on the percentage inhaled.

In addition, a recent report suggests that acute sleep deprivation enhances the effect of a biological challenge on anxiety (Babson et al., 2009). However, it is well known that sleep deprivation can have acute antidepressant effects (Gillin et al., 2001; Bendetti and Smeraldi, 2009) suggesting that sleep deprivation is a non-specific challenge. Such findings underline the difficulty of generating a robust experimental medicine model of anxiety with a stable baseline response that can be used routinely to assess the potential efficacy of novel therapeutic agents.

Models in which anxiety is induced by pharmacological challenges are also not ideal for the screening of new treatments. There is a potential risk of a pharmacological or pharmacodynamic interaction that could lead to a false positive or false negative result. A further risk is that pharmacological agents that induce immediate anxiety generally require intravenous administration which may cause an intrinsic anxiogenic effect in some participants. Pharmacological agents with a delayed response often require repeated oral doses and thereby have limited utility in healthy volunteer studies. For example, the physiological challenge of lactate infusion is only anxiogenic in patients with anxiety and requires intravenous administration. Nevertheless, to study experimental anxiety in human and animal models, it is important to study a clinical population using the same challenge since no disorder can be reproduced in its entirety in healthy volunteer groups. This issue is particularly important in anxiety research, where the disorder can modulate the cognitive processes and belief system of the patient.

Validation of the 7.5% CO_2 healthy volunteer model of anxiety

In an effort to establish a robust, stable and consistent human experimental model of anxiety we have used the inhalation of different concentrations of carbon dioxide (CO_2) to induce anxiety in healthy volunteers and patient groups that was pioneered by Gorman and colleagues (Gorman et al., 1988; Goetz et al., 2001). Inhaling a mixture of air and CO_2 in which the concentration of CO_2 is 7.5% or greater reliably increases the physiological symptoms of anxiety, such as increased heart rate, blood pressure and sweating. These increases in physiological symptoms are accompanied by changes in a participant's subjective state. Using visual analogue scales (VAS) during a 20 minute period of breathing a gas mixture containing 7.5% CO_2, participants report feeling anxious and worried, and less relaxed and happy (Bailey et al., 2007; Bailey and Nutt, 2008). These subjective changes induced by the CO_2 challenge are sensitive to pharmacological manipulation and examples from studies performed in our laboratories are given in Table 3. In addition, CO_2 inhalation can reproduce clinical symptoms in patients with anxiety, e.g. a 20 minute inhalation of 7.5% CO_2 in patients with GAD (Seddon et al., 2011; Hood et al., 2010) and it has been established for many years that a single inhalation of 35% CO_2 produces panic in patients with panic disorder (Woods et al., 1986; Sanderson et al., 1994). Finally, Poma (2005) demonstrated good test-retest reliability with the CO_2 inhalation challenge. They selected 12 healthy volunteers from a group of 21 who 'responded anxiously' during an initial inhalation session with 7% CO_2. When re-tested during a second 7% CO_2 inhalation session the 12 'responders' responded again suggesting that the CO_2 procedure could be used to test putative anti-panic or anxiolytic drugs using a within subject, crossover design.

A key question is how the inhalation of 7.5% CO_2 compares as a model of anxiety with, for example, the public speaking model, CCK-4 infusion, or the fear-potentiated startle paradigm. To date a direct comparison between these models has not been performed, and therefore their relative

Table 3. Effect of drugs on the behavioural response to inhalation of 7.5% CO_2 in healthy volunteers

Study drug	Percentage CO_2	Change from placebo at peak CO_2 effects: VAS fear and worry		Reference
Paroxetine*	7.5%	−10.0	−9.5	Bailey et al., 2007
Lorazepam 0.5 mg	7.5%	−6.5	−1.8	Unpublished
Lorazepam 2 mg	7.5%	−12.9	−12.5	Bailey et al., 2007
Alprazolam 1 mg	7.5%	−16.6	−8.2	Bailey et al., 2009
	35%	−8.7	−5.9	
Zolpidem 5 mg	7.5%	−9.4	−10.9	Bailey et al., 2009
	35%	−0.4	4.1	
Propranolol 40 mg	7.5%	10.0	13.9	Papadopoulos et al., 2010
	35%	−0.9	2.5	
Hydroxyzine 25 mg	7.5%	7.9	13.9	Papadopoulos et al., 2010
	35%	13.3	2.9	
Flupentixol 0.5 mg	7.5%	4.0	12.6	Papadopoulos et al., 2010
	35%	8.5	3.4	
Venlafaxine*	7.5%	−7.0	−12.3	Diaper et al., 2011
	35%	7.5	1.9	P1vital Consortium study
Pregabalin*	7.5%	−7.8	−10.6	Diaper et al., 2011
	35%	−5.9	−2.5	P1vital Consortium study

Changes in VAS (mm) from peak effects of CO_2 inhalation: difference between study drug and placebo. VAS, visual analogue scales.
*please refer to individual papers for dosing regime for these studies.

merits are not clearly established. Although many studies have been conducted to determine the effect of these challenges and others (see Table 4) very few experiments were designed specifically to directly compare biological, physiological and psychological responses to different challenges and this is an area that requires further investigation. One well designed study compared the physiological and psychological effects of a mental arithmetic stressor, cold pressor, and a 5% CO_2 inhalation in healthy volunteers and patients with panic disorder (Roth et al., 1992). There were no significant differences in the physiological responses of patients or controls, but the patients reported more tonic anxiety to the inhalation of CO_2. Moreover, participants from both groups that had greater anticipatory anxiety prior to the procedure experienced increased anxiety in response to the tasks. These data suggest that equivalent levels of heightened physiological responses may be a necessary condition for increasing subjective feelings of anxiety, and that an individual's bias at baseline may determine whether a physiological response leads to normal or pathological psychological response. The hypothesis that patients have a 'negative frame' or bias in which the impact of negative stimuli is greater than in controls has proved very useful in furthering our understanding depression (see Harmer et al., 2011) and such an approach may also be useful in understanding GAD. Consequently, a number of studies investigating the emotional and cognitive responses to inhalation of CO_2 in healthy volunteers are underway with the objective of increasing our understanding of how negative framing influences subjective feelings of anxiety. Finally, it is not yet clear how breathing a gas mix of oxygen, nitrogen and 7.5% CO_2 induces anxiety. Clearly enriching breathable gas with CO_2 above its normal induces physiological responses which increase heart-rate and blood pressure which give rise to psychological feelings that healthy volunteers describe subjectively as an increase in their levels of 'worry' and/or 'anxiety'. However, although benzodiazepines, such as lorazepam, reduced subjective feeling of worry and anxiety, they do not reduce blood pressure or heart rate suggesting that their effects are downstream of the physiological effects of CO_2. Whether or not the phenomenological experience of participants breathing CO_2 encompasses all of the symptoms experienced by patients or just a subset of symptoms is not known. However, given that SSRIs and more recently venlafaxine and pregabalin are without effect in participants breathing 7.5% of CO_2 it would suggest that the symptoms induced by chronic conditions such as GAD only partially overlap with those induced by acute challenges.

The assessment of novel compounds in the 7.5% CO_2 model

The work described above suggests that benzodiazepine receptor agonists such as lorazepam have robust anxiolytic-like effects in the 7.5% CO_2 model of anxiety. The effects of benzodiazepines in this model are reproducible indicating that the level of anxiety induced is stable both within and between studies and that the model is sufficiently validated for this class of compound. The action of SSRIs, at least in the case of paroxetine, though significant appears less robust and was only detected after three weeks of daily dosing (Bailey et al., 2007). This may reflect the relatively low efficacy of such drugs in GAD compared with their pronounced effects in panic disorder, or it may be due to the fact that the 7.5% CO_2 inhalation model is more sensitive to drugs that are rapidly effective in anxiety. Similarly, venlafaxine and pregabalin had relatively weak effects in the 7.5% CO_2 inhalation model (Diaper et al., unpublished).

Interestingly, venlafaxine (75 or 150 mg/day) is effective in GAD, but only reduced HAM-A scores after three weeks,

Table 4. Number of publications in a range of human anxiety models identified in a PubMed literature search (performed on 13/08/08)

Challenge/term used	Number of hits	Example
CO_2 inhalation and human anxiety	135	This paper and references
Fear-potentiated startle	75	Grillon et al., 2006
Sodium lactate infusion	56	Facchinetti et al., 1992
Cold pressor test	49	Tavernor et al., 2000
Trier social stress test	36	O'Leary et al., 2007
CCK infusion	14	Gunnarsson et al., 2003

and not after two weeks of dosing (Allgulander et al., 2001). Similarly one week of dosing with 400–600 mg/day of pregabalin is required before a reduction of anxiety scores is observed (Rickels et al., 2005). Thus the profiles of venlafaxine and pregabalin are similar to that of paroxetine in that chronic dosing is required for clinical efficacy. Therefore, it is possible that the 7.5% CO_2 challenge model may differentiate between drugs that have acute effects on anxiety and those that depend on chronic dosing for several weeks for their efficacy. While the clinical effects of benzodiazepines support this observation it is interesting to note that a corticotropin releasing factor (CRF_1) receptor antagonist when given for 7 days had similar efficacy to lorazepam in this model (Bailey et al., 2011). Thus it could be speculated that CRF antagonists might have rapid anxiolytic effects in patients rather than a delayed response as seen with paroxetine, venlafaxine and pregabalin.

To some extent this begs the question of the effects of compounds that target neurotransmitter systems other than GABA or monoamines. The paucity of compounds with alternative mechanisms of actions that are effective in the treatment of acute anxiety has ensured that until recently it has proved difficult to further validate the model.

However, in a recent study we provided preliminary evidence of efficacy of a selective CRF_1 receptor antagonist, R317573 in the 7.5% CO_2 model of anxiety (Bailey et al., 2011). Preclinical evidence suggests that drugs acting as receptor antagonists on the CRF system may be useful for the treatment of depression, anxiety, and other stress-related disorders (Valdez, 2006). In a double-blind, placebo-controlled, randomized study in 32 healthy participants we examined the effects of 7 days treatment with 40 mg R317573 a dose that is comparable to those effective in preclinical models. On Day 8, eight of the placebo-treated group received the benzodiazepine receptor agonist, lorazepam 2 mg as a positive control. All participants then inhaled 7.5% CO_2 for 20 minutes. Subjective reports of peak gas effects were assessed using VAS and questionnaires.

In this study when compared with placebo, both lorazepam and R317573 decreased: (i) all subjective symptoms; (ii) the total score on the Panic Symptom Inventory; and (iii) the GAD symptom scale. These data suggest that drugs that are antagonists at the CRF_1 receptor have efficacy in the 7.5% CO_2 model of anxiety in healthy participants. These data are the first to show that CRF antagonists are active in a human volunteer model of experimentally-induced anxiety and suggest that CRF_1 antagonists may be useful in the treatment of acute anxiety in patients with anxiety disorders. Moreover they demonstrate that the 7.5% CO_2 inhalation model is sensitive to potential non-benzodiazepine anxiolytics. However, a recent trial with the CRF antagonist, pexacerfont 100 mg/day (after a 1 week loading dose of 300 mg/day), was without effect in patients with GAD (Coric et al., 2010). It would be interesting to test pexacerfont in the CO_2 model to determine whether it was effective at the doses given to patients in the pexacerfont study. If it were then it would suggest an alternative patient population, perhaps those with panic disorder, may be a more appropriate population to study. If pexacerfont was without effect it would further characterize the 7.5% CO_2 inhalation model.

In conclusion, the 7.5% CO_2 inhalation model has proved to be a reliable experimental medicine method for inducing acute anxiety-like symptoms in healthy participants. It has been shown to be sensitive to pharmacological manipulation of a range of drugs used to treat anxiety disorders and emerging new treatment with different mechanisms of action. Consequently, it may be useful in the future development of new drug therapies for anxiety disorders.

Funding

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

The studies in this review from this research team were funded by Novartis, GSK, AstraZeneca, P1Vital Ltd and Johnson & Johnson PRD.

Conflict of interest

JE Bailey has no conflict of interest to declare. Dawson and Dourish are both employees, directors and shareholders of P1vital LtD. David Nutt has provided consultancy services to and/or received honoraria, grants or clinical trial payments from Pfizer, GSK, Novartis, Cypress, Lilly, Janssen, Lundbeck, BMS, Astra Zeneca, Servier, Hythiam, Sepracor, Neurosearch, Reckitt Benkiser, Schering-Plough, Wyeth, Cephalon, and MSD, and holds shares in P1vital Ltd. He is an advisor to British National Formulary, MRC, GMC, Dept of Health, president of the European College of Neuropsychopharmacology, member of the European Brain Council, president-elect of the British Neuroscience Association, and chair of the Independent Scientific Committee on Drugs (UK).

References

Allgulander C, Hackett D and Salinas E (2001) Venlafaxine extended release (ER) in the treatment of generalized anxiety disorder: twenty-four-week placebo-controlled dose-ranging study. *Br J Psychiatry* 179: 15–22.

Baas JM, Grillon C, Böcker KB, Brack AA, Morgan III CA, Kenemans JL, et al. (2002) Benzodiazepines have no effect on fear potentiated startle in humans. *Psychopharmacol* 161: 233–247.

Babson KA, Feldner MT, Trainor CD and Smith RC (2009) An experimental investigation of the effects of acute sleep deprivation on panic-relevant biological challenge responding. *Behav Ther* 40: 239–250.

Bailey JE and Nutt DJ (2008) GABA-A Receptors and the Response to CO_2 inhalation – a translational trans-species model of anxiety? *Pharmacol Biochem Behav* 90: 51–57.

Bailey JE, Kendrick A, Diaper A, Potokar JP and Nutt DJ (2007) A validation of the 7.5% CO_2 model of GAD using paroxetine and lorazepam in healthy volunteers. *J Psychopharmacol* 21: 42–49.

Bailey JE, Papadopoulos A, Diaper A, Phillips S, Schmidt M, van der Ark P, et al. (2011) Preliminary evidence of anxiolytic effects of the CRF_1 receptor antagonist R317573 in the 7.5% CO_2 proof-of-concept experimental model of human anxiety. *J Psychopharmacol* 25: 1199–1206.

Bailey JE, Papadopoulos A, Seddon K and Nutt DJ (2009) A comparison of the effects of a subtype selective and non-selective benzodiazepine receptor agonist in two CO2 models of experimental human anxiety. *J Psychopharmacol* 23: 117–122.

Baldwin DS, Anderson IM, Nutt DJ, Bandelow B, Bond A, Davidson JRT, et al. (2005) Evidence-based guidelines for the pharmacological treatment of anxiety disorders: recommendations from the British Association for Psychopharmacology. *J Psychopharmacol* 19: 567–596.

Benedetti F and Smeraldi E (2009) Neuroimaging and genetics of antidepressant response to sleep deprivation: implications for drug development. *Curr Pharm Des* 15: 2637–2649.

Conn PJ and Roth BL (2008) Opportunities and challenges of psychiatric drug discovery: roles for scientists in academic, industry and government settings. *Neuropsychopharmacology* 33: 2048–2060.

Coric V, Feldman HH, Oren DA, Shekhar A, Pultz J, Dockens RC, et al. (2010) Multicenter, randomized, double-blind, active comparator and placebo-controlled trial of a corticotropin-releasing factor receptor-1 antagonist in generalized anxiety disorder. *Depress Anxiety* 27: 417–425.

Diaper A, Bailey JE, Rich A, Craig KJ, Dawson GR, Dourish CT, Nutt DJ (2011) Venlafaxine and pregabalin reduce CO_2-induced symptoms of anxiety in an experimental human model. *Presented in British Association for Psychopharmacology Summer Meeting, 24-27 July 2011, Harrogate, Poster: TE02 (Group E – Anxiety)*.

Facchinetti F, Romano G, Fava M and Genazzani AR (1992) Lactate infusion induces panic attacks in patients with premenstrual syndrome. *Psychosom Med* 54: 288–296.

Gillin JC, Buchsbaum M, Wu J, Clark C and Bunney W Jr (2001) Sleep deprivation as a model experimental antidepressant treatment: findings from functional brain imaging. *Depress Anxiety* 14: 37–49.

Goetz RR, Klein DF, Papp LA, Martinez JM and Gorman JM (2001) Acute panic inventory symptoms during CO_2 inhalation and room-air hyperventilation among panic disorder patients and normal controls. *Depress Anxiety* 14: 123–136.

Gorman JM, Fyer MR, Goetz R, Askanazi J, Liebowitz MR, Fyer AJ, et al. (1988) Archives of General Psychiatry, Ventilatory physiology of patients with panic disorder. *45* 31–39.

Grillon C (2008) Greater sustained anxiety but not phasic fear in women compared to men. *Emotion* 8: 410–413.

Grillon C, Baas JM, Pine DS, Lissek S, Lawley M, Ellis V, et al. (2006) The benzodiazepine alprazolam dissociates contextual fear from cued fear in humans as assessed by fear-potentiated startle. *Biol Psychiatry* 60: 760–766.

Guimaraes FS, Mbaya PS and Deakin JFW (1997) Ritanserin facilitates anxiety in a simulated public-speaking paradigm. *J Psychopharmacol* 11: 225–231.

Gunnarsson T, Mahoney C, Shlik J, Bradwejn J and Knott V (2003) Acute effects of cholecystokinin tetrapeptide on brain stem auditory evoked potentials in healthy volunteers. *Pharmacopsychiatry* 36: 181–186.

Harmer CJ, de Bodinat C, Dawson GR, Dourish CT, Waldenmaier L, Adams S, Cowen PJ, Goodwin GM (2011) Agomelatine facilitates positive versus negative affective processing in healthy volunteer models. *JPsychopharmacology* 25: 1148–1158.

Hellewell JSE, Guimaraes FS, Wang M and Deakin JFW (1999) Comparison of buspirone with diazepam and fluovoxamine on aversive classical conditioning in humans. *J Psychopharmacol* 13: 122–127.

Hijzen TH, Houtzager SW, Joordens RJ, Olivier B and Slangen JL (1995) Predictive validity of the potentiated startle response as a behavioral model for anxiolytic drugs. *Psychopharmacol* 118: 150–154.

Hood SD, Hince DA, Davies SJ, Argyropoulos S, Robinson H, Potokar J, et al. (2010) Effects of acute tryptophan depletion in serotonin reuptake inhibitor-remitted patients with generalized anxiety disorder. *Psychopharmacol* 208: 223–232.

Kirschbaum C, Pirke KM and Hellhammer DH (1993) The 'Trier Social Stress Test' – a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 28: 76–81.

Lader M (2008) Effectiveness of benzodiazepines: do they work or not? *Expert Rev Neurother* 8: 1189–1191.

Lader M, Tylee A and Donoghue J (2009) Withdrawing benzodiazepines in primary care. *CNS Drugs* 23: 19–34.

Malizia AL (2001) Positron emitting ligands in the study of the clinical psychopharmacology of anxiety and anxiety disorders. MD Thesis, University of Bristol, UK.

O'Leary MM, Loney BR and Eckel LA (2007) Gender differences in the association between psychopathic personality traits and cortisol response to induced stress. *Psychoneuroendocrinology* 32: 183–191.

Papadopoulos A, Rich A, Nutt DJ and Bailey JE (2010) The effects of single dose anxiolytic medication on the CO_2 models of anxiety: differentiation of subjective and objective measures. *J Psychopharmacol* 24: 649–656.

Poma SZ, Milleri S, Squassante L, Nucci G, Bani M, Perini GI, et al. (2005) Characterization of a 7% carbon dioxide (CO_2) inhalation paradigm to evoke anxiety symptoms in healthy subjects19: 494–503.

Rickels K, Pollack MH, Feltner DE, Lydiard RB, Zimbroff DL, Bielski RJ, et al. (2005) Pregabalin for treatment of generalized anxiety disorder: a 4-week, multicenter, double-blind, placebo-controlled trial of pregabalin and alprazolam. *Arch Gen Psychiatry* 62: 1022–1030.

Roth WT, Margraf J, Ehlers A, Taylor CB, Maddock RJ, Davies S, et al. (1992) Stress test reactivity in panic disorder. *Arch Gen Psychiatry* 49: 301–310.

Sanderson WC, Wetzler S and Asnis GM (1994) Alprazolam blockade of CO_2-provoked panic in patients with panic disorder. *Am J Psychiatry* 151: 1220–1222.

Seddon K, Morris K, Bailey J, Potokar J, Rich A, Wilson S, et al. (2011) Effects of 7.5% CO_2 challenge in Generalized Anxiety Disorder. *J Psychopharmacol* 25: 43–51.

Tavernor SJ, Abduljawad KA, Langley RW, Bradshaw CM and Szabadi E (2000) Effects of pentagastrin and the cold pressor test on the acoustic startle response and pupillary function in man. *J Psychopharmacol* 14: 387–394.

Valdez GR (2006) Development of CRF1 receptor antagonists as antidepressants and anxiolytics: progress to date. *CNS Drugs* 20: 887–896.

Wittchen H-U and Jacobi F (2005) Size and burden of mental disorders in Europe: a critical review and appraisal of 27 studies. *Eur Neuropsychopharmacol* 15: 357–376.

Woods SW, Charney OS, Loke J, Goodman WK, Redmond DE and Heninger GR (1986) Carbon dioxide sensitivity in panic anxiety. Ventilatory and anxiogenic response to carbon dioxide in healthy subjects and patients with panic anxiety before and after alprazolam treatment. *Arch Gen Psychiatry* 43: 900–909.

Wray NR, James MR, Gordon SD, Dumenil T, Ryan L, Coventry WL, et al. (2009) Accurate large scale genotyping of 5HTTLPR and flanking single nucleotide polymorphisms in an association study of depression, anxiety and personality measures. *Biol Psychiatry* 66: 468–476.