

## Using [<sup>11</sup>C]Ro15 4513 PET to characterise GABA-benzodiazepine receptors in opiate addiction: Similarities and differences with alcoholism



Anne Lingford-Hughes<sup>a,b,c,d,\*</sup>, James Myers<sup>a,b,c</sup>, Ben Watson<sup>b,c,d</sup>, Alastair G. Reid<sup>b,c,d</sup>, Nicola Kalk<sup>a,b,c</sup>, Adrian Feeney<sup>b,c</sup>, Alexander Hammers<sup>c,1</sup>, Daniela A. Riaño-Barros<sup>c</sup>, Colm J. McGinney<sup>c,1</sup>, Lindsay G. Taylor<sup>b</sup>, Lula Rosso<sup>c</sup>, David J. Brooks<sup>c,d</sup>, Federico Turkheimer<sup>c,1</sup>, David J. Nutt<sup>a,b,c</sup>

<sup>a</sup> Centre for Neuropsychopharmacology, Imperial College London, Du Cane Rd., London W12 0NN, United Kingdom

<sup>b</sup> Psychopharmacology Unit, University of Bristol, Whitson Street, Bristol BS1 3NY, United Kingdom

<sup>c</sup> MRC Clinical Sciences Centre, Faculty of Medicine, Imperial College, London, United Kingdom

<sup>d</sup> Hammersmith Imanet Ltd., Hammersmith Hospital, Du Cane Rd., London W12 0NN, United Kingdom

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### ABSTRACT

The importance of the GABA-benzodiazepine receptor complex and its subtypes are increasingly recognised in addiction. Using the  $\alpha 1/\alpha 5$  benzodiazepine receptor PET radioligand [<sup>11</sup>C]Ro15 4513, we previously showed reduced binding in the nucleus accumbens and hippocampus in abstinent alcohol dependence. We proposed that reduced [<sup>11</sup>C]Ro15 4513 binding in the nucleus accumbens was a marker of addiction whilst the reduction in hippocampus and positive relationship with memory was a consequence of chronic alcohol abuse. To examine this further we assessed [<sup>11</sup>C]Ro15 4513 binding in another addiction, opiate dependence, and used spectral analysis to estimate contributions of  $\alpha 1$  and  $\alpha 5$  subtypes to [<sup>11</sup>C]Ro15 4513 binding in opiate and previously acquired alcohol-dependent groups. Opiate substitute maintained opiate-dependent men ( $n = 12$ ) underwent an [<sup>11</sup>C]Ro15 4513 PET scan and compared with matched healthy controls ( $n = 13$ ). We found a significant reduction in [<sup>11</sup>C]Ro15 4513 binding in the nucleus accumbens in the opiate-dependent compared with the healthy control group. There was no relationship between [<sup>11</sup>C]Ro15 4513 binding in the hippocampus with memory. We found that reduced [<sup>11</sup>C]Ro15 4513 binding was associated with reduced  $\alpha 5$  but not  $\alpha 1$  subtypes in the opiate-dependent group. This was also seen in an alcohol-dependent group where an association between memory performance and [<sup>11</sup>C]Ro15 4513 binding was primarily driven by  $\alpha 5$  and not  $\alpha 1$  subtype. We suggest that reduced  $\alpha 5$  levels in the nucleus accumbens are associated with addiction since we have now shown this in dependence to two pharmacologically different substances, alcohol and opiates.

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### Introduction

Opiate addiction and its treatment is a global health issue. In the US, 1.8% of individuals over the age of 12 have tried heroin in their lifetime, with 0.1% in the past month (National Survey of Drug Use and Health, 2012). In England, there are an estimated ~300,000 opiate and/or crack users (8.67 per 1000 of 15 to 64 year olds; Statistics on Drug Misuse: England, 2013). Many addicts relapse or find it hard to achieve abstinence, with only about 50% leaving treatment drug-free (Statistics on Drug Misuse: England, 2013). To improve understanding and

treatment of opiate addiction, characterising its underlying neurobiology is critical. Although heroin and other opioid agonists act on opioid receptors, preclinical evidence supports involvement of the GABA system in their effects (Zetterström and Fillenz, 1990; Finlay et al., 1992 and Creed et al., 2014). Endorphins or mu opiate agonists inhibit GABA-ergic tonic inhibition of dopamine neuronal activity in the ventral tegmental area (VTA) resulting in increased dopamine neuronal firing and may thus contribute to the desire or compulsion to use opiates (Johnson and North, 1992 and Ting-A-Kee and van der Kooy, 2012).

There are multiple subtypes of the GABA-benzodiazepine receptor with the type of  $\alpha$  subunit determining benzodiazepine and alcohol sensitivity (Rudolph and Mohler, 2004). There is evidence that the  $\alpha 1$ ,  $\alpha 2$  and  $\alpha 5$  subtypes contribute to drug-liking and/or drug-seeking in a variety of preclinical models with a range of substances of abuse (Rudolph and Knoflach, 2011; Engin et al., 2012; Dixon et al., 2010; June et al., 2001). Associations have also been reported between

\* Corresponding author at: Centre for Neuropsychopharmacology, Imperial College London, Du Cane Rd., London W12 0NN, United Kingdom.

E-mail address: [anne.lingford-hughes@ic.ac.uk](mailto:anne.lingford-hughes@ic.ac.uk) (A. Lingford-Hughes).

<sup>1</sup> Present address: Division of Imaging Sciences and Biomedical Engineering, King's College London, SE1 7EH.

**Table 1**  
Demographic and clinical data.

Clinical variable	Control (opiate)	Opiate dependent
Number	13	12
Age	40 ± 5	36.2 ± 7.6
Beck's depression inventory	n/d	9.8 ± 7.9
Spielberger–State anxiety	n/d	33.64 ± 7.9
Spielberger–Trait anxiety	n/d	37.1 ± 9.8
Severity of Alcohol Dependence Questionnaire	n/a	n/a
Wechsler delayed verbal memory score	n/d	10.2 ± 3.4
Length of abstinence (months)	n/a	n/a
Years of opiate use or alcohol dependence	n/a	16 ± 6.8
Adjective checklist (opiate) withdrawal	n/a	4 ± 3.8
Adjective checklist (opiate) agonist-like	n/a	19.8 ± 3.9

n/a: not applicable; n/d: not done in whole sample.

variants of the GABA-benzodiazepine receptor gene and alcohol and drug use disorders (Engin et al., 2012; Li et al., 2014). In addition, the  $\alpha 5$  subtype plays a role in learning and memory and its impairment under the influence of alcohol (Nutt et al., 2007; Atack, 2011).

[<sup>11</sup>C]Ro15 4513 has a tenfold higher affinity at  $\alpha 5$  than  $\alpha 1$  receptors (Lingford-Hughes et al., 2002). We have used [<sup>11</sup>C]Ro15 4513 to characterise benzodiazepine receptor binding in man, particularly the  $\alpha 5$  subtype, but due to the ubiquity of the  $\alpha 1$  subtype, [<sup>11</sup>C]Ro15 4513 PET also can detect this subtype (Myers et al., 2012). We previously reported lower levels of [<sup>11</sup>C]Ro15 4513 binding in the nucleus accumbens of abstinent alcoholics compared with controls (Lingford-Hughes et al., 2012). We proposed that since the extrasynaptic  $\alpha 5$  subtype provides tonic inhibition, this lower level of receptors would contribute to instability of this critical dopaminergic brain region which in turn could lead to enhanced drug consumption or seeking. We also reported that in alcoholics, although not healthy controls,  $\alpha 5$  receptor subtype availability in the hippocampus positively correlated with verbal memory performance.

This paper complements our [<sup>11</sup>C]Ro15 4513 PET study in alcoholism by studying opiate dependence to assess whether lower levels of [<sup>11</sup>C]Ro15 4513 binding are a marker of addiction. We hypothesised that [<sup>11</sup>C]Ro15 4513 binding in opiate dependence would be lower in the nucleus accumbens compared with controls. Since we suggested the lower [<sup>11</sup>C]Ro15 4513 binding in the hippocampus was a consequence of chronic alcohol abuse, we did not expect to see such a reduction in opiate addiction. Subsequent to our study in alcoholism (Lingford-Hughes et al., 2012), we have developed the use of spectral analysis to assess the contribution of  $\alpha 1$  vs  $\alpha 5$  subtypes to the [<sup>11</sup>C]Ro15 4513 image (Myers et al., 2012). We report here for the first time levels of these subtype in opiate addiction and in alcoholism.

## Materials and methods

### Participants

Opiate-dependent males (DSM-IV) receiving substitute medication (either methadone ( $n = 9$ ; range 10–75 mg daily; mean daily dose 46.7 mg ± 19.7 mg) or buprenorphine ( $n = 3$ ; 2.8 mg, 10 mg, 24 mg daily; mean 12.3 mg ± 10.8 mg)) were recruited from local addiction outpatient treatment services (see Table 1). The control group consisted of 13 men recruited for this study or other studies (Myers et al., 2012; Stokes et al., 2014) to match the opiate-dependent group. They had never met criteria for abuse or dependence (DSM-IV) on alcohol or opiates, although recreational non-dependent use of other illicit drugs (eg cannabis, ecstasy) was permitted.

In the opiate-dependent group, none were or had been dependent on any other drug including alcohol, cannabis, benzodiazepines or stimulants, although occasionally cocaine, crack or amphetamine or cannabis had been used, mostly when they were younger. All were abstinent from alcohol and illicit drugs at the time of the study confirmed

with negative urinalysis and breathalyser reading. Duration of opiate use ranged from 9 to 30 years (mean ± SD, 16 ± 6.8) with most smoking and injecting although 4 had never injected. The opiate-dependent subjects were all current tobacco smokers except for one who had stopped 6 weeks previously; in the control group, 2 were smokers.

Individuals with clinical evidence of hepatic, cognitive or neurological impairment or medical disorder were excluded. No individual had a previous or current history of psychosis. None were taking psychotropic drugs, other than opiate substitute medication, except for one opiate-dependent individual on mirtazapine.

### Assessment: clinical

The opiate-dependent group was assessed using a semi-structured questionnaire for all drugs of abuse, including alcohol and with Adjective Checklist (Jasinski, 1997) for their state at the time of the scan. They underwent a similar assessment as in the alcohol-dependent study which is described elsewhere (Lingford-Hughes et al., 2012) and included Beck Depression Inventory (Beck et al., 1961), Spielberger State-Trait Anxiety Inventory (Spielberger et al., 1970) and the Wechsler Memory Scale and logical memory with immediate and 30-min delayed recall of two stories. The controls recruited for other studies did not complete all these tests ( $n = 9$ ; Stokes et al., 2014).

After complete description of the study to the subjects, written informed consent was obtained. The study was approved by the local Ethics Committees and by the UK Administration of Radioactive Substances Advisory Committee.

### [<sup>11</sup>C]Ro15 4513 PET

We followed the same scanning protocol as described in Lingford-Hughes et al. (2002). [<sup>11</sup>C]Ro15 4513 was synthesised by *N*-methylation of the corresponding *N*-desmethyl derivative with [<sup>11</sup>C]iodomethane. The product was purified by reverse phase HPLC and the specific radioactivity was ~14,000 MBq/μmol at end of synthesis.

[<sup>11</sup>C]Ro15 4513 (mean ~475 MBq) in ~2 ml scans of opiate-dependent individuals and all corresponding control subjects were performed on a Siemens ECAT EXACT HR+ (CTI/Siemens, model 962; Knoxville, TN, USA) scanner.

Blood sampling was carried out to produce a calibrated, metabolite corrected plasma input function for quantification of distribution volume with spectral analysis (Lingford-Hughes et al., 2012).

### Image processing

PET emission data were corrected for attenuation and scatter using a 10-min transmission scan and reconstructed using Fourier rebinning and 2D filtered backprojection with a 2.0 mm kernel Ramp filter, into 24 dynamic frames (1 × 30, 4 × 15, 4 × 60, 2 × 150, 10 × 300 and 3 × 600 s). The final reconstructed volume had voxel dimensions of 2.094 × 2.094 × 2.42 mm. All subjects also underwent T1-weighted magnetic resonance imaging (MRI) with a Philips 1.5 T Gyroscan Intera scanner (Philips, Best, The Netherlands), to provide anatomical images to aid in region definition. A T1-weighted protocol was used to acquire images viewed by a clinical Consultant Radiologist, and none were reported as having significant atrophy or abnormalities.

Reconstructed [<sup>11</sup>C]Ro15 4513 images were analysed on Sun workstations (Sun Microsystems, Mountain View, CA, USA) using Analyze AVW version 8.1 (Biomedical Imaging Resource, Mayo Clinic, Rochester, MN; Robb and Hanson, 1991), Matlab 6.5 (The MathWorks, Inc., Cambridge, UK) and SPM5 (available via <http://www.fil.ion.ucl.ac.uk/spm/>).

Arterial blood input functions calibrated against discrete blood samples using a well counter and further corrected for parent fraction and

plasma: blood ratio. Weighted summed PET images were used for rigid-body co-registration to anatomical MRI data.

Maximum probability maps defined in standard space (73 ROIs for the earlier study with alcohol-dependent individuals, and 83 ROIs for opiate dependence; Hammers et al., 2003; Gousias et al., 2008) were fitted after transformation into native space. Prior to regional sampling, the goodness-of-fit of each object map to the individual brain was checked visually. The ROI sampling was performed in Analyze 8.1 to obtain the mean radioactivity concentration through all frames for all regions in the atlas, and used to generate time-activity curves (TACs).

**Spectral analysis**

Spectral analysis is a basis function technique that uses a set of poly-exponentials convolved with the parent plasma input function to fit tissue TACs with no prior assumption of model order (Cunningham and Jones, 1993; Turkheimer et al., 1994). Although [<sup>11</sup>C]Ro15 4513 is relatively selective for the GABA-benzodiazepine α5 receptor subtype, there is measurable signal from the ubiquitous α1 subtype, which can be partially quantified using band-pass spectral analysis (Myers et al., 2012). [<sup>11</sup>C]Ro15 4513  $V_T$  can therefore be described as comprising α1, α5 and non-displaceable components,  $V_{\alpha1} + V_{\alpha5} + V_{ND}$ . Although it is not possible to directly quantify the contribution of these components to the total signal, spectral analysis has been used to provide estimates by separating  $V_{\alpha5}$  on the basis of slow kinetics. One hundred exponential functions were generated, with the decay parameter logarithmically distributed between 0.00006371 and  $1\text{ s}^{-1}$  (Barros et al., 2010) and convolved with the arterial parent plasma input function ( $C_p$ ) in order to generate a set of kinetic basis functions. A non-negative least-squares algorithm was then used to fit this over-complete basis set to the measured data ( $C_{PET}$ ):

$$C_{PET}(t) = \sum_{i=1}^{100} \phi_i e^{-\theta_i t} \otimes C_p(t)$$

subject to  $\phi_i \geq 0$ . Thus, an integral of the slow kinetic component of the spectrum has been hypothesised to relate to GABA<sub>A</sub>α5 binding, and

$V_{\alpha5}$  is defined by the limits  $a$  ( $0.00006371\text{ s}^{-1}$ ) and  $b$  ( $0.001\text{ s}^{-1}$ ):

$$V_{\alpha5} = \sum_{i=a}^b \frac{\phi_i}{\theta_i}$$

Specific binding of [<sup>11</sup>C]Ro15 4513 to α1 is approximated by defining the limits 0.0010–0.00040  $\text{s}^{-1}$ . Total volume of distribution ( $V_T$ ) is defined as the integral of all peaks in the spectrum.

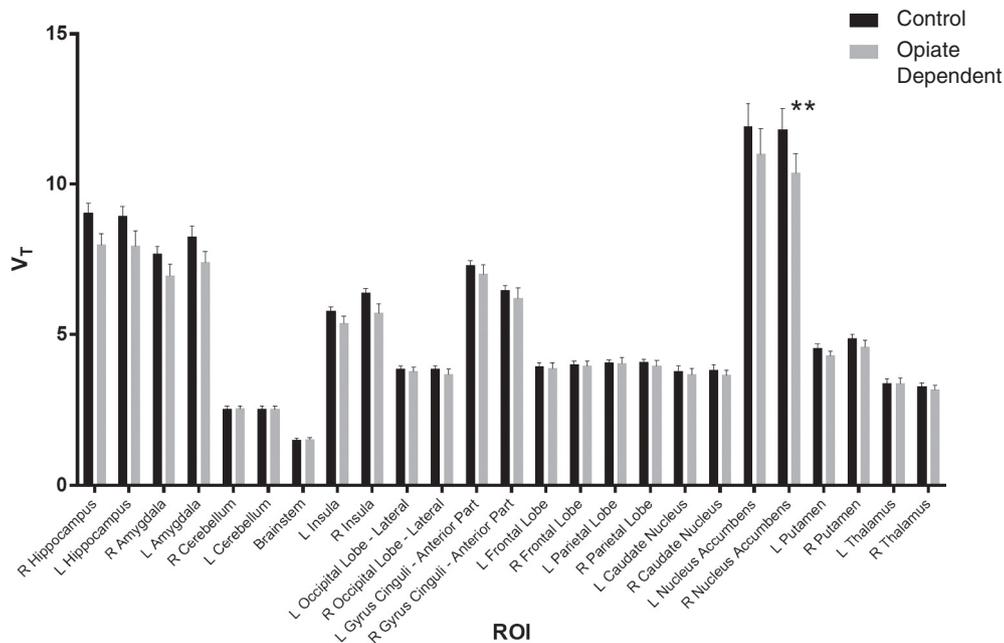
**Statistical analysis**

Statistical analyses were conducted using GraphPad Prism (version 6 for Windows, GraphPad Software, San Diego, CA, USA, www.graphpad.com). Relationships between [<sup>11</sup>C]Ro15 4513 volume of distributions  $V_T$ ,  $V_{\alpha1}$  and  $V_{\alpha5}$  and measures of memory were tested with non-parametric Spearman's ranks correlation coefficients. Repeated-measures two-way ANOVA with ROI and group (alcohol or opiate-dependent vs controls) was used to compare [<sup>11</sup>C]Ro15 4513 distribution volume in dependent individuals with that in controls. Results were corrected using post hoc Šidák correction for multiple comparisons.

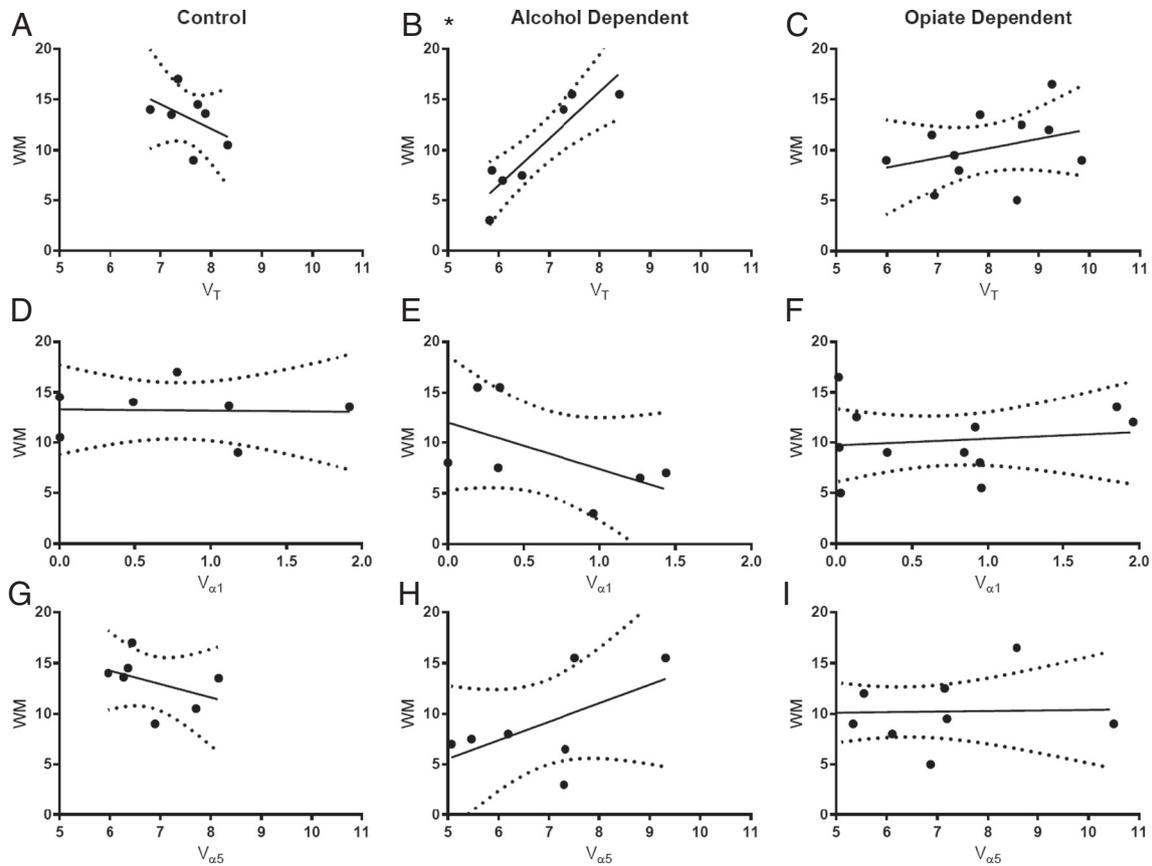
**Results**

*Opiate dependence: [<sup>11</sup>C]Ro15 4513  $V_T$*

There was a significant effect of group in the two-way ANOVA comparing [<sup>11</sup>C]Ro15 4513  $V_T$  in all ROIs between opiate-dependent and healthy control groups, with lower  $V_T$  in the opiate-dependent group ( $F(1, 1743) = 27.85; p < 0.0001$ ). Data from ROIs selected for their relevance to addiction and [<sup>11</sup>C]Ro15 4513  $V_T$  binding are shown in Fig. 1. After correction for multiple comparisons, the right nucleus accumbens [<sup>11</sup>C]Ro15 4513  $V_T$  in the opiate-dependent group was the only ROI where lower  $V_T$  compared with healthy controls remained significant ( $t = 3.971; p < 0.01$ ). No difference in nucleus accumbens volume, as defined by our atlas registration, was found between the groups. There was a trend ( $p = 0.06$ ) towards lower  $V_T$  in the hippocampus of



**Fig. 1.** [<sup>11</sup>C]Ro15 4513  $V_T$  (mean ± SEM) in controls ( $n = 13$ ) and opiate-dependent ( $n = 12$ ) individuals in selected regions of interest (ROIs). There was significantly lower  $V_T$  in the addict group across all ROIs with a two-way ANOVA ( $F(1, 1743) = 27.85; p < 0.0001$ ), the right nucleus accumbens remained significant ( $t = 3.971; p < 0.01$ ) after multiple comparison correction (\*\*).



**Fig. 2.** Correlations of [ $^{11}\text{C}$ ]Ro15 4513  $V_T$ ,  $V_{\alpha 1}$  and  $V_{\alpha 5}$  in the hippocampus with Wechsler Fig. 1 delayed verbal memory score (WM; with 95% confidence intervals) in controls ( $n = 11$ ; A, D, F), alcohol-dependent ( $n = 8$ ; B\*, E, H; see Supplementary information for more details) and opiate-dependent ( $n = 12$ ; C, F, I) individuals. (\* $p < 0.005$ ).

opiate-dependent individuals, in contrast to the significantly lower [ $^{11}\text{C}$ ]Ro15 4513  $V_T$  in the alcohol-dependent group (see below).

No significant correlations were found between delayed verbal memory performance and [ $^{11}\text{C}$ ]Ro15 4513  $V_T$  in opiate-dependent individuals in the hippocampus (see Fig. 2C). There was no significant association of age or any clinical variables with  $V_T$ . We did not see differences in  $V_T$  between methadone and buprenorphine or a relationship between the prescribed dose of either substitute with  $V_T$ , but the study was not sufficiently powered for such analyses.

#### Opiate dependence: $\alpha 1$ and $\alpha 5$

There was no effect of group on the  $\alpha 1$  component ( $F(1, 1743) = 0.1020$ ;  $p = 0.7495$ ), and no individual regions were prominent after correction for multiple comparisons. There was, however, an effect of group on the  $\alpha 5$  component ( $F(1, 1660) = 4.760$ ;  $p = 0.0293$ ) with lower  $\alpha 5$  levels in the opiate-dependent group. The large inter-subject variability resulted in no significant differences being found in any individual ROI after correction for multiple comparisons (see Fig. 3B,D,F). No significant correlations with memory performance were found (see Fig. 2C,F,I).

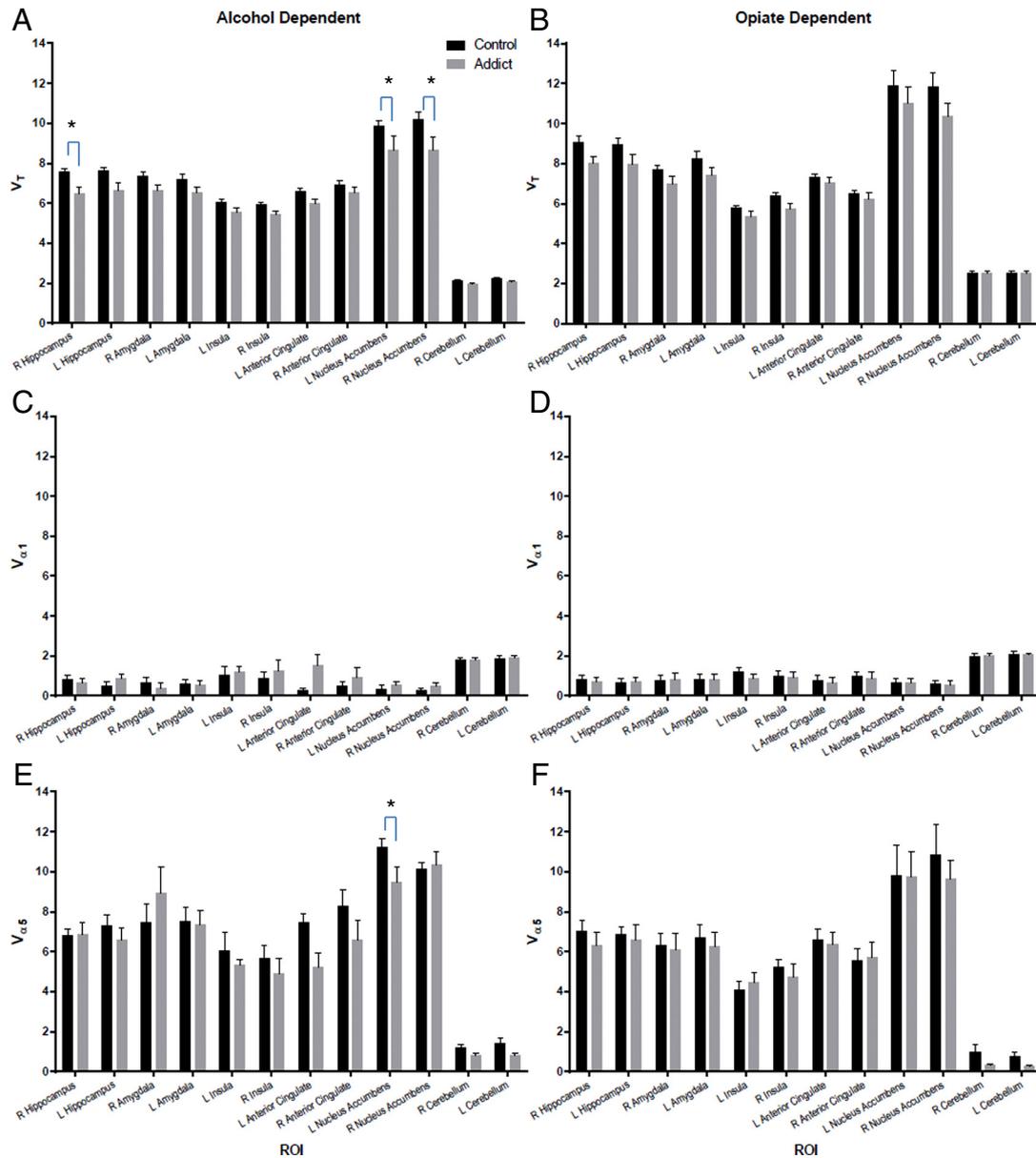
#### Discussion

In this first [ $^{11}\text{C}$ ]Ro15 4513 PET study of opiate dependence, we have shown significantly reduced levels of [ $^{11}\text{C}$ ]Ro15 4513  $V_T$  in individuals on opiate substitute medication compared with controls. A region of interest analysis revealed a significant reduction in the right nucleus accumbens. This complements our study in abstinent alcohol dependence where we also reported reduced [ $^{11}\text{C}$ ]Ro15 4513  $V_T$  in the nucleus

accumbens. Using our spectral analytical approach to determine contribution of  $\alpha 1$  and  $\alpha 5$  binding to [ $^{11}\text{C}$ ]Ro15 4513  $V_T$ , we found lower levels of  $\alpha 5$  subtype in nucleus accumbens in both alcohol and opiate dependence compared with controls no significant contribution from  $\alpha 1$ . Given the different neuropharmacology of opiates and alcohol, our studies support our original proposal that low  $\alpha 5$  levels may be fundamental to addiction rather than only associated with alcoholism.

We are not aware of any other in vivo imaging study of the GABA-benzodiazepine receptor in opiate dependence in man. In alcoholism using non-selective tracers, [ $^{11}\text{C}$ ]flumazenil with PET or [ $^{123}\text{I}$ ]iomazenil with SPET, have consistently shown a reduction in GABA-benzodiazepine receptors particularly in the frontal cortex (Abi-Dargham et al., 1998; Lingford-Hughes et al., 1998) whilst with [ $^{11}\text{C}$ ]Ro15 4513, reductions are seen in limbic areas where binding is high (Lingford-Hughes et al., 2002, 2012). It would be interesting to know if a similar difference in distribution of [ $^{11}\text{C}$ ]flumazenil vs [ $^{11}\text{C}$ ]Ro15 4513 is seen in opiate dependence and the impact of the different opiate substitute medications. Whilst we did not see any differences between methadone and buprenorphine and [ $^{11}\text{C}$ ]Ro15 4513 binding, due to the small numbers we cannot exclude an interaction.

There are limited and inconsistent preclinical receptor binding studies to inform this clinical study. Direct competition between opiate substitute medication and [ $^{11}\text{C}$ ]Ro15 4513 is unlikely since opiate agonists have low affinity ( $\sim \mu\text{M}$ ) for the [ $^3\text{H}$ ]GABA site (Dingledine et al., 1978; Goldinger et al., 1981). Chronic morphine exposure has been reported to increase [ $^3\text{H}$ ]GABA binding (Ticku and Huffman, 1980) and to increase [ $^3\text{H}$ ]muscimol, [ $^3\text{H}$ ]flunitrazepam or [ $^3\text{H}$ ]Ro15 1788 (flumazenil) binding in the cortex, which return to control levels in withdrawal (Sivam et al., 1982; Lopez et al., 1990; Rocha et al., 1993). However, other studies using active and passive or acute and chronic morphine



**Fig. 3.** Comparison of [ $^{11}\text{C}$ ]Ro15 4513  $V_T$ ,  $V_{\alpha 1}$  and  $V_{\alpha 5}$  (mean  $\pm$  SEM) between controls (for alcohol:  $n = 11$  (see Supplementary information for more details); for opiate  $n = 13$ ) and alcohol ( $n = 8$ ; A, C, E) and opiate ( $n = 12$ ; B, D, F)-dependent individuals in selected regions of interest (ROIs). \* $t = 3.713$ ;  $p < 0.01$ .

administration resulted in reduced or no changes in [ $^3\text{H}$ ]flunitrazepam binding in a range of cortical regions and hippocampus, although chronic morphine did reduce sensitivity to GABA-enhancement of binding (Smith et al., 1984, Sivam and Ho, 1982). Given the variability of models, length of opiate exposure and ligands for benzodiazepine receptor or GABA site markers used, it is difficult to extrapolate these findings to our clinical [ $^{11}\text{C}$ ]Ro15 4513 PET study. The clinical importance of the interaction between opiates and the GABA-ergic system suggests further characterisation would be beneficial. In addition, it would provide evidence to understand if the lower level of [ $^{11}\text{C}$ ]Ro15 4513 binding in alcohol and opiate addiction reflect a vulnerability endophenotype to addiction.

We have previously reported in our healthy control group that a current or previous history of smoking was associated with higher levels of [ $^{11}\text{C}$ ]Ro15 4513 binding compared with those who had not smoked; current smokers had lowest [ $^{11}\text{C}$ ]Ro15 4513 binding levels and were more similar to controls than ex-smokers (Stokes et al., 2014). Since all the opiate-dependent participants were current smokers, we are

unable to distinguish between the roles of opiate and smoking dependence to [ $^{11}\text{C}$ ]Ro15 4513 binding. In the alcohol-dependent group, smokers had higher levels than the non-smokers, although there was only one ex-smoker and two smokers. An [ $^{123}\text{I}$ ]iomazenil SPET study reported higher binding in a non-smoker alcohol-dependent group compared with a smoker alcohol-dependent group after one week of abstinence but no differences were seen at four weeks (Staley et al., 2005). Given the differences in length of abstinence from alcohol, it is hard to directly compare the studies; however, both suggest that smoking tobacco may alter GABA-benzodiazepine receptor availability. If a reduction in  $\alpha 5$  is critical in addiction, then why is tobacco smoking not associated with reduced [ $^{11}\text{C}$ ]Ro15 4513 binding? Analogous to studying alcohol dependence, where controls drink low amounts of alcohol, comparing non-smokers, non-dependent ‘chippers’ and dependent smokers will be required to determine impact of smoking versus addiction on [ $^{11}\text{C}$ ]Ro15 4513 binding.

Characterising benzodiazepine subtypes in man is important to inform our understanding about its involvement in neuropsychiatric

disorders. The  $\alpha 1$  subtype is ubiquitous (~60% of all benzodiazepine receptors), is found in the synapse and is responsible for fast inhibitory neurotransmission, whereas  $\alpha 5$  is less abundant (~5%), extrasynaptic, and responsible for tonic inhibition (Rudolph and Knoflach, 2011). Each subtype is associated with particular roles such as sedation and anti-convulsant effects for the  $\alpha 1$  subtype and mediation of alcohol reward and memory for  $\alpha 5$ . Using our spectral analytical approach, we have demonstrated that the lower [ $^{11}\text{C}$ ]Ro15 4513  $V_T$  in the nucleus accumbens predominantly reflects lower  $\alpha 5$  levels in alcohol and opiate dependence. There are no preclinical studies of  $\alpha 1$  and  $\alpha 5$  subtypes in opiate exposure. Whilst reduced  $\alpha 1$  subtype protein and mRNA have been reported consistently from chronic alcohol exposure in male rats, by contrast increases or no change in binding of the  $\alpha 1$  selective tracer, [ $^3\text{H}$ ]zolpidem, have been reported (Grobin et al., 2000). Similarly, alcohol-induced increases, decreases or no change have been reported in  $\alpha 5$  subunit protein or mRNA in the cortex (Devaud et al., 1995; Charlton et al., 1997). It is difficult to extrapolate such preclinical studies to alcohol dependence in man with its greater complexity of alcohol exposure and withdrawal.

In alcohol dependence, we previously reported that [ $^{11}\text{C}$ ]Ro15 4513  $V_T$  was significant lower in the hippocampus compared with healthy controls and that there was a positive relationship with delayed verbal memory performance (Lingford-Hughes et al., 2012). We further show here that this relationship between delayed verbal memory performance is associated with [ $^{11}\text{C}$ ]Ro15 4513  $V_{\alpha 5}$  and not  $V_{\alpha 1}$  in the hippocampus in alcohol dependence. No such relationship was found in opiate dependence or either control group. This is consistent with our suggestion that hippocampal  $\alpha 5$  levels here reflect adaptation to excessive alcohol exposure (Lingford-Hughes et al., 2012) since none in opiate group were alcohol dependent or had significant history of alcohol abuse. Therefore, we believe our confirmation of  $\alpha 5$  rather than  $\alpha 1$  being associated with memory adds to the growing evidence of the importance of  $\alpha 5$  in memory in neuropsychiatric disorders in man (Atack, 2011). Concerning the role of the  $\alpha 5$  subtype in reward, whilst there is preclinical evidence of lower  $\alpha 5$  levels associated with reduced alcohol preference and alcohol-seeking (June et al., 2001), there are no comparable studies in opiate reward or dependence.

Whilst this is the first study of [ $^{11}\text{C}$ ]Ro15 4513 in opiate dependence, and we report for the first time delineation of  $\alpha 1$  and  $\alpha 5$  in both alcohol and opiate dependence, a limitation of our studies is that the numbers are small. As a consequence of the loss of the scanner on which the alcohol dependent and their control group were studied, we had to perform the study in opiate-dependent participants on a different scanner and acquire an additional control group. Therefore, any inferences from comparisons between alcohol and opiate dependence are cautiously stated. As with our alcohol dependence study (Lingford-Hughes et al., 2012), we think it unlikely that partial volume effects substantially contribute to the reductions in [ $^{11}\text{C}$ ]Ro15 4513 binding seen in the opiate-dependent group. We did not find any differences in striatal volume between groups and have previously reported no atrophy in the striatum in a similar group of opiate-dependent individuals (Reid et al., 2008).

In summary, we have shown lower [ $^{11}\text{C}$ ]Ro15 4513 binding associated with lower [ $^{11}\text{C}$ ]Ro15 4513  $V_{\alpha 5}$  in opiate dependence, particularly in the nucleus accumbens. We have also shown lower  $\alpha 5$  levels in the nucleus accumbens in alcohol dependence, and suggest that such a reduction is fundamental to addiction and independent of the substance of abuse. We propose that lower levels of  $\alpha 5$  could result in less GABA-ergic inhibitory activity on the mesolimbic dopaminergic system resulting in its greater instability and thus greater response to rewarding stimuli or salient cues. It remains to be elucidated whether these lower levels contribute to a vulnerability endophenotype for addiction more generally or result from substance abuse. Modulating the  $\alpha 5$  subtype presents an innovative target to probe substance abuse and how it can be prevented or treated, particularly now agents selective for this receptor subtype are being developed for human use (Atack, 2011).

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.neuroimage.2016.02.005>.

## References

- Abi-Dargham, A., Krystal, J.H., Anjilvel, S., Scanley, B.E., Zoghbi, S., Baldwin, R.M., Rajeevan, N., Ellis, S., Petrakis, I.L., Seibyl, J.P., Charney, D.S., Laruelle, M., Innis, R.B., 1998. Alterations of benzodiazepine receptors in type II alcoholic subjects measured with SPECT and [ $^{123}\text{I}$ ]iomazenil. *Am. J. Psychiatry* 155, 1550–1555.
- Atack, J.R., 2011. GABAA receptor subtype-selective modulators. II.  $\alpha 5$ -selective inverse agonists for cognition enhancement. *Curr. Top. Med. Chem.* 11, 1203–1214.
- Barros, D.A.R., Heckemann, R.A., Rosso, L., McGinnity, C.J., Keihaninejad, S., Gousias, I.S., Brooks, D.J., Duncan, J.S., Koepp, M.J., Turkheimer, F.E., Hammers, A., 2010. Investigating the reproducibility of the novel alpha-5 GABAA receptor PET ligand [ $^{11}\text{C}$ ]Ro15 4513. *NeuroImage* 52, S112.
- Beck, A.T., Ward, C.H., Mendelson, M., Mock, J.E., Erbaugh, J., 1961. An inventory for measuring depression. *Arch. Gen. Psychiatry* 4, 561–571.
- Charlton, M.E., Sweetnam, P.M., Fitzgerald, L.W., Terwilliger, R.Z., Nestler, E.J., Duman, R.S., 1997. Chronic ethanol administration regulates the expression of GABAA receptor alpha 1 and alpha 5 subunits in the ventral tegmental area and hippocampus. *J. Neurochem.* 68, 121–127.
- Creed, M.C., Ntamati, N.R., Tan, K.R., 2014. VTA GABA neurons modulate specific learning behaviors through the control of dopamine and cholinergic systems. *Front. Behav. Neurosci.* 8, 8.
- Cunningham, V.J., Jones, T., 1993. Spectral analysis of dynamic PET studies. *J. Cereb. Blood Flow Metab.* 13, 15–23.
- Devaud, L.L., Smith, F.D., Grayson, D.R., Morrow, A.L., 1995. Chronic ethanol consumption differentially alters the expression of gamma-aminobutyric acidA receptor subunit mRNAs in rat cerebral cortex: competitive, quantitative reverse transcriptase-polymerase chain reaction analysis. *Mol. Pharmacol.* 48, 861–868.
- Dingledine, R., Iversen, L.L., Breuker, E., 1978. Naloxone as a GABA antagonist: evidence from iontophoretic, receptor binding and convulsant studies. *Eur. J. Pharmacol.* 47, 19–27.
- Dixon, C.I., Morris, H.V., Breen, G., Desrivieres, S., Jugurnauth, S., Steiner, R.C., Vallada, H., Guindalini, C., Laranjeira, R., Messas, G., Rosahl, T.W., Atack, J.R., Peden, D.R., Bellelli, D., Lambert, J.J., King, S.L., Schumann, G., Stephens, D.N., 2010. Cocaine effects on mouse incentive-learning and human addiction are linked to alpha2 subunit-containing GABAA receptors. *Proc. Natl. Acad. Sci. U. S. A.* 107, 2289–2294.
- Engin, E., Liu, J., Rudolph, U., 2012.  $\alpha 2$ -containing GABA(A) receptors: a target for the development of novel treatment strategies for CNS disorders. *Pharmacol. Ther.* 136, 142–152.
- Finlay, J.M., Damsa, G., Fibiger, H.C., 1992. Benzodiazepine-induced decreases in extracellular concentrations of dopamine in the nucleus accumbens after acute and repeated administration. *Psychopharmacology* 106, 202–208.
- Goldinger, A., Müller, W.E., Wollert, U., 1981. Inhibition of glycine and GABA receptor binding by several opiate agonists and antagonists. *Gen. Pharmacol.* 12, 477–479.
- Gousias, I.S., Rueckert, D., Heckemann, R.A., Dyet, L.E., Boardman, J.P., Edwards, A.D., Hammers, A., 2008. Automatic segmentation of brain MRIs of 2-year-olds into 83 regions of interest. *NeuroImage* 40, 672–684.
- Grobin, A.C., Papadeas, S.T., Morrow, A.L., 2000. Regional variations in the effects of chronic ethanol administration on GABA(A) receptor expression: potential mechanisms. *Neurochem. Int.* 37, 453–461.
- Hammers, A., Allom, R., Koepp, M.J., Free, S.L., Myers, R., Lemieux, L., Mitchell, T.N., Brooks, D.J., Duncan, J.S., 2003. Three-dimensional maximum probability atlas of the human brain, with particular reference to the temporal lobe. *Hum. Brain Mapp.* 19, 224–247.
- Jasinski, D.R., 1997. Assessment of the abuse potential of morphine-like drugs (methods used in man). In *Drug Addiction I: Morphine, Sedative/Hypnotic and Alcohol Dependence*. *Handb. Exp. Pharmacol.* 45, 197–258.
- Johnson, S.W., North, R.A., 1992. Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J. Neurosci.* 12, 483–488.
- June, H.L., Harvey, S.C., Foster, K.L., McKay, P.F., Cummings, R., Garcia, M., Mason, D., Grey, C., McCane, S., Williams, L.S., Johnson, T.B., He, X., Rock, S., Cook, J.M., 2001. GABA(A) receptors containing (alpha)5 subunits in the CA1 and CA3 hippocampal fields regulate ethanol-motivated behaviors: an extended ethanol reward circuitry. *J. Neurosci.* 21, 2166–2177.
- Li, D., Sulovari, A., Cheng, C., Zhao, H., Kranzler, H.R., Gelernter, J., 2014. Association of gamma-aminobutyric acid A receptor  $\alpha 2$  gene (GABRA2) with alcohol use disorder. *Neuropsychopharmacology* 39 (4), 907–918.
- Lingford-Hughes, A.R., Acton, P.D., Gacinovic, S., Suckling, J., Busatto, G.F., Boddington, S.J.A., Bullmore, E., Woodruff, P.W., Costa, D.C., Pilowsky, L.S., Ell, P.J., Marshall, E.J., Kerwin, R.W., 1998. Reduced levels of the GABA-benzodiazepine receptor in alcohol dependency in the absence of grey matter atrophy. *Br. J. Psychiatry* 173, 116–122.

- Lingford-Hughes, A., Hume, S.P., Feeney, A., Hirani, E., Osman, S., Cunningham, V.J., Pike, V.W., Brooks, D.J., Nutt, D.J., 2002. Imaging the GABA-benzodiazepine receptor subtype containing the alpha5-subunit in vivo with [<sup>11</sup>C]Ro15 4513 positron emission tomography. *J. Cereb. Blood Flow Metab.* 22, 878–889.
- Lingford-Hughes, A., Reid, A.G., Myers, J., Feeney, A., Hammers, A., Taylor, L.G., Rosso, L., Turkheimer, F., Brooks, D.J., Grasby, P., Nutt, D.J., 2012. A [<sup>11</sup>C]Ro15 4513 PET study suggests that alcohol dependence in man is associated with reduced  $\alpha 5$  benzodiazepine receptors in limbic regions. *J. Psychopharmacol.* 26, 273–281.
- Lopez, F., Miller, L.G., Thompson, M.L., Schatzki, A., Chesley, S., Greenblatt, D.J., Shader, R.I., 1990. Chronic morphine administration augments benzodiazepine binding and GABA<sub>A</sub> receptor function. *Psychopharmacology* 101, 545–549.
- Myers, J.F., Rosso, L., Watson, B.J., Wilson, S.J., Kalk, N.J., Clementi, N., Brooks, D.J., Nutt, D.J., Turkheimer, F.E., Lingford-Hughes, A.R., 2012. Characterisation of the contribution of the GABA-benzodiazepine  $\alpha 1$  receptor subtype to [(11)C]Ro15-4513 PET images. *J. Cereb. Blood Flow Metab.* 32, 731–744.
- National Survey of Drug Use and Health, 2012. <http://www.drugabuse.gov/national-survey-drug-use-health>.
- Nutt, D.J., Besson, M., Wilson, S.J., Dawson, G.R., Lingford-Hughes, A.R., 2007. Selective blockade of alcohol's amnesic actions by an  $\alpha 5$  subtype- benzodiazepine receptor inverse agonist. *Neuropharmacology* 53, 810–820.
- Reid, A.G., Daghli, M.R.C., Kempton, M., Williams, T.M., Watson, B., Nutt, D.J., Lingford-Hughes, A., 2008. Reduced grey matter volume in opiate dependence may be explained by degree of alcohol use: a voxel-based morphometry study. *J. Psychopharmacol.* 22 (1), 7–10.
- Robb, R.A., Hanson, D.P., 1991. A software system for interactive and quantitative visualization of multidimensional biomedical images. *Australas. Phys. Eng. Sci. Med.* 14, 9–30.
- Rocha, L., Tatsukawa, K., Chugani, H.T., Engel Jr., J., 1993. Benzodiazepine receptor binding following chronic treatment with naloxone, morphine and met-enkephalin in normal rats. *Brain Res.* 612, 247–252.
- Rudolph, U., Knoflach, F., 2011. Beyond classical benzodiazepines: novel therapeutic potential of GABA<sub>A</sub> receptor subtypes. *Nat. Rev. Drug Discov.* 10, 685–697.
- Rudolph, U., Mohler, H., 2004. Analysis of GABA<sub>A</sub> receptor function and dissection of the pharmacology of benzodiazepines and general anesthetics through mouse genetics. *Annu. Rev. Pharmacol. Toxicol.* 44, 475–498.
- Sivam, S.P., Ho, I.K., 1982. Influence of morphine dependence on GABA-stimulated benzodiazepine binding to mouse brain synaptic membranes. *Eur. J. Pharmacol.* 79, 335–336.
- Sivam, S.P., Nabeshima, T., Ho, I.K., 1982. An analysis of GABA receptor changes in the discrete regions of mouse brain after acute and chronic treatments with morphine. *J. Neurochem.* 39, 933–939.
- Smith, J.E., Co, C., Lane, J.D., 1984. Limbic muscarinic cholinergic and benzodiazepine receptor changes with chronic intravenous morphine and self-administration. *Pharmacol. Biochem. Behav.* 20, 443–450.
- Spielberger, C., Gorsuch, R.L., Lushene, R.E., 1970. *Manual for the State-Trait Anxiety Inventory*. Consulting Psychologist Press, Palo Alto.
- Staley, J.K., Gottschalk, C., Petrakis, I.L., Gueorguieva, R., O'Malley, S., Baldwin, R., Jatlow, P., Verhoeff, N.P., Perry, E., Weinzimmer, D., Frohlich, E., Ruff, E., van Dyck, C.H., Seibyl, J.P., Innis, R.B., Krystal, J.H., 2005. Cortical gamma-aminobutyric acid type A-benzodiazepine receptors in recovery from alcohol dependence: relationship to features of alcohol dependence and cigarette smoking. *Arch. Gen. Psychiatry* 62, 877–888.
- Statistics on Drug Misuse: England, 2013. <http://www.hscic.gov.uk/catalogue/PUB12994/drug-misu-eng-2013-rep.pdf>.
- Stokes, P.R., Myers, J.F., Kalk, N.J., Watson, B.J., Erritzoe, D., Wilson, S.J., Cunningham, V.J., Barros, D.R., Hammers, A., Turkheimer, F.E., Nutt, D.J., Lingford-Hughes, A.R., 2014. Acute increases in synaptic GABA detectable in the living human brain: a [<sup>11</sup>C]Ro15-4513 PET study. *NeuroImage* 99, 158–165.
- Ticku, M.K., Huffman, R.D., 1980. The effects of acute and chronic morphine administration on GABA receptor binding. *Eur. J. Pharmacol.* 68, 97–106.
- Ting-A-Kee, R., van der Kooy, D., 2012. The neurobiology of opiate motivation. *Cold Spring Harb. Perspect. Med.* 2 (10), a012096.
- Turkheimer, F., Moresco, R.M., Lucignani, G., Sokoloff, L., Fazio, F., Schmidt, K., 1994. The use of spectral analysis to determine regional cerebral glucose utilization with positron emission tomography and [<sup>18</sup>F]fluorodeoxyglucose: theory, implementation, and optimization procedures. *J. Cereb. Blood Flow Metab.* 14 (3), 406–422.
- Zetterström, T., Fillenz, M., 1990. Local administration of flurazepam has different effects on dopamine release in striatum and nucleus accumbens: a microdialysis study. *Neuropharmacology* 29, 129–134.