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Glossary

5-CSRT	5-choice serial reaction time
5-HT	serotonin
5-HTTLPR	serotonin transporter-linked polymorphic region
ACC	anterior cingulate cortex
ADHD	attention-deficit/hyperactivity disorder
ATD	acute tryptophan depletion
BGTC	basal ganglia-thalamic-cortical
BOLD	blood oxygen level dependent
BZP	benzylpiperazine
CFFT	critical flicker fusion threshold
DA	dopamine
DEX	dexamphetamine
DLPFC	dorsolateral prefrontal cortex
i.v.	intravenous
fMRI	functional magnetic resonance imaging
FWE	family wise error
GABA	gamma-Aminobutyric acid
IFG	inferior frontal gyrus
LSD	lysergic acid diethylamide
MA	methamphetamine
mCPP	meta-chlorophenylpiperazine
MDMA	3, 4-methylenedioxymethamphetamine
mPFC	medial prefrontal cortex
MPH	methylphenidate
NA	noradrenaline
NAcc	nucleus accumbens
OFC	orbitofrontal cortex
PD	Parkinson's disease
PE	prediction error
PET	positron emission tomography
PFC	prefrontal cortex
SSRI	selective serotonin reuptake inhibitor
SSRT	stop-signal reaction task
TFMPP	trifluoromethylphenylpiperazine
vmPFC	ventromedial prefrontal cortex
WCST	Wisconsin card sorting task
VTA	ventral tegmental area

Problems and Aims

Recreational drugs have been used historically to elicit pleasure. Recreational drugs are those drugs which are used for personal enjoyment rather than for medical purposes. They can be categorised into depressants (such as heroin) and stimulants (such as amphetamine). Society tolerates the use of those drugs that are legal, such as alcohol and caffeine versus those that are illegal, for example, 3,4-methlenedioxymethamphetamine (MDMA; ecstasy) and methamphetamine (MA).

Recently, there has been a group of synthetic drugs introduced and sold legally worldwide. These drugs are marketed with provocative names such as “XTC” and “Charge” and sold in shops and via the internet, and have been known as “party pills.” These party pills have been sold and used for much of the past decade. The major constituents of party pills were benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP), sold both alone and in combination, and marketed as safe alternatives to illicit recreational drugs such as MDMA and MA. However, their safety has not been clinically evaluated.

BZP is used for its stimulant-like effects and the combination of BZP and TFMPP has been promoted to mimic the effects of MDMA in environments such as “raves”. Concern has been raised following comparisons with MDMA and MA because the use of these agents has been linked with impairments in memory, cognitive function and neurological abnormalities. Despite recent legislative change in many countries, including New Zealand (NZ), Australia, the United Kingdom (UK) and the United States (US), the use of BZP and/or TFMPP is expected to continue (1).

Functional magnetic resonance imaging (fMRI) is a validated non-invasive functional brain mapping technique that can be used to study the effects of drugs, due to its high spatial resolution. The research reported in this thesis used this technique in an exploratory manner to demonstrate the regions of the brain affected by BZP, TFMPP and the combination of BZP+TFMPP when completing specific cognitive tasks. For comparison purposes, an acute dose of placebo was also given. The subjective and physiological effects of BZP have been compared to dexamphetamine (DEX) in previous studies (2), therefore an acute dose of DEX was administered to allow a direct comparison between the two drug states.

Specifically, this thesis will present the investigation of the effect of these drugs on brain circuitry involved in the reward pathways and executive function. In addition, a finger

tapping validation task was completed by participants to determine whether fMRI is a technique that can be used to investigate the effects of BZP, TFMPP and BZP+TFMPP, despite their direct and indirect effects on blood flow via vasculature.

Aims

The overall aim of this thesis was to investigate the effects of an acute dose of BZP and TFMPP, as individual constituents and in combination with placebo. In addition, we compared BZP with DEX.

Specifically we aim:

1. To determine differences in regional activation elicited by a gambling (guessing) task that requires participants to guess the colour of a presented card to obtain a monetary reward. Completing this task will allow this research to focus on:

    a. The anticipatory phase of processing by comparing responses to the anticipation of a uncertain reward;

    b. The reward outcome phase and whether magnitude (i.e. small (50c) versus large ($4) monetary amounts) and valence (wins versus losses) effect the results.

2. To understand the effects of these drugs on executive function. Specifically by assessing the behavioural and imaging data collected whilst subjects complete an event-related colour-word Stroop task to identify changes in selective attention and inhibition.

3. To investigate whether fMRI is a technique that can be used to study the effects of psychoactive drugs on the brain, using a finger tapping task that aims to compare responses in the motor cortex.

Thesis Outline

This section will give an overview of the papers included in each Chapter, describing the experiments and techniques used. All imaging data was collected using a Siemens 1.5T Magnetom Avanto scanner, at the Centre for Advanced MRI (CAMRI) located at the University of Auckland.

Chapter 1: Introduction

Since the research presented in this thesis encompasses a variety of diverse topics ranging from pharmacology to imaging techniques, after introducing the party pill constituents BZP and TFMPP, the introduction will give a brief summary of information about these topics as background, to allow the results of this thesis to be read in context. These summaries are intended to be informative rather than critical. More critically focussed material is presented in association with the chapters reporting the different analyses and in the discussion.

Chapter 1 introduces and provides an insight into what is known about the major constituents of party pills, BZP and TFMPP. It also discusses similar dopaminergic recreational drugs, specifically cocaine, DEX and MA and the amphetamine derivatives that are more serotonergic in nature, such as MDMA, meta-chlorophenylpiperazine (mCPP) and fenfluramine. Reward processing and executive function will be discussed with special consideration of the effects that dopaminergic and serotonergic modulation has on these processes. Finally, fMRI will be described in detail, including the theory of fMRI, and the specific analysis used in this research.

Chapter 2: Reward Processing

Chapter 2 will describe the gambling (guessing) task conducted by our participants. Thirteen participants were recruited in a double-blind cross-over study. An oral dose of BZP (200 mg) and TFMPP (50 mg for participants weighing < 60 kg or 60 mg if weighing > 60 kg) alone, and a combination of the two were given at lower doses (100 mg + 30 mg respectively). Additionally, for comparison, placebo and DEX (20mg) were administered on separate trial days. Sixty-five imaging sessions were conducted (13 participants returning five times). Ninety minutes after administration, participants completed a custom designed gambling (guessing) task, whilst undergoing fMRI. The gambling (guessing) task was designed to assess distinct phases of reward processing, that is, selection, anticipation and outcome, with the additional facet of high and low magnitude wins and losses. This section will present three papers prepared for publication: one reporting the effect of BZP, TFMPP,

and the combination of BZP+TFMPP on the anticipatory stage of processing relative to placebo; a further paper describing the effects of BZP, TFMPP, and BZP+TFMPP on the outcome stage of reward; and a third that compares the effects of BZP relative to DEX on both anticipation and the outcome of reward.

Chapter 3: Executive Function

Chapter 3 describes the investigation of executive function using an event-related colour-word Stroop paradigm, whilst under the influence of BZP, TFMPP or a combination of BZP+TFMPP. The first section of this Chapter presents behavioural and imaging data, where BZP and TFMPP alone and in combination were compared to placebo. The second section and paper contrasts BZP with DEX. The first paper is currently under review, and the second is also intended for publication.

Chapter 4: A Validation Task

Chapter 4 describes a validation task, based on a previous study by Murphy and colleagues (3). The task involves a simple finger tapping task to compare the effects of each drug on the motor cortex. It is based on the assumption that there should be no change in the motor cortex while under the influence of each drug or placebo. If this is the case, one could extend the finding to say that there are no changes in the brain related to the hemodynamic response/neurovascular coupling induced by the drugs and thus, the results that we find in other cognitive tasks are based on specific changes in neural recruitment, not just effects of the drugs on blood flow.

Chapter 5: Discussion and Conclusion

The final Chapter presents an overall discussion, linking the imaging and behavioural data. This describes the limitations of this study, and suggests future analyses that can be completed using the current data and subsequent studies that should be conducted to further our knowledge about the effects of BZP, TFMPP and the combination of BZP+TFMPP.

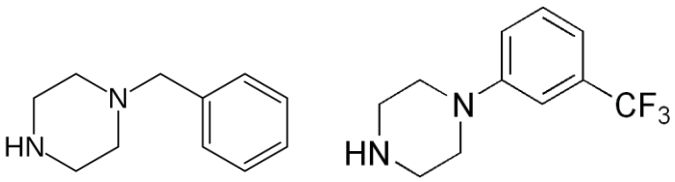
Chapter 1: Introduction

Benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP) are two constituents (Figure 1) most often used in a group of synthetic drugs that, since the late 1990s, have been marketed worldwide as safe and legal alternatives to illicit recreational drugs, such as MDMA or MA. This group of relatively new synthetic drugs was sold in the so-called party pills. Despite their popularity, there is a distinct lack of research describing their effects on the human brain.

1.1. BZP and TFMPP

1.1.1. What, Where and Who?

BZP and TFMPP are two constituents (Figure 1) most often used in a group of synthetic drugs that, since the late 1990s, have been marketed worldwide as safe and legal alternatives to illicit recreational drugs, such as MDMA or MA. This group of relatively new synthetic drugs was sold in the so-called party pills. Despite their popularity, there is a distinct lack of research describing their effects on the human brain.



(a) (b)

Figure 1: Chemical structure of (a) BZP and (b) TFMPP

The majority of BZP and/or TFMPP users have typically been in their late teens and early twenties. These drugs are used to enhance confidence, extend hours of socialising, induce euphoria and increase energy (4). The most common route of administration of BZP and TFMPP is by tablet or capsule (4, 5), with rare reports of intravenous use (6, 7). Party pills containing BZP and TFMPP first emerged in California in 1996 and since this time have been found in similar recreational settings to MDMA. In recent years, legislative change has rendered them illegal in the majority of countries, albeit their use is expected to continue. A press release by the Drug Enforcement Administration (DEA) of the US released figures demonstrating that the number of drug seizures of BZP is rising; in 2004, 48 items identified as BZP were seized, 437 by 2007, 6,088 in 2008 and by 2009, 13,822 (1). BZP was reclassified under Schedule 1 during 2004 in the US (1), and TFMPP was

emergency scheduled but controversially is the only drug to be removed from this schedule and not renewed.

Reports in the *Microgram Bulletin*, an online publication from the DEA, US Department of Justice, describes tablets thought to be MDMA, but after examination were found to contain combinations of BZP, TFMPP and other drugs. For example, tablets that mimic MDMA, have been found to contain combinations of BZP and caffeine; BZP, TFMPP and dextromethorphan; and also BZP, TFMPP caffeine and dextromethorphan, in varying ratios (8).

It is possible that because BZP and TFMPP were legal for a substantial period of time this widened the population of their users, and increased their acceptance. Cohen and Butler (9) propose that in the social context BZP may perform in two ways: as a gateway drug to illicit substances but also to reduce harm, that is, the compounds may be used preferentially due to their perceived safety. In a recent drug use survey, 13.5% of respondents answered that they had started off using party pills, but now mostly use other illegal drugs (4).

This study also highlighted the usage patterns within NZ. One in five people who were surveyed (aged 13-45 years) had tried party pills containing BZP and TFMPP, and of those people, 86.5% said that they had combined party pills with other drug use. The most common being alcohol (91%), followed by tobacco (39.5%) and cannabis (22.4%).

1.1.2. Benzylpiperazine (BZP): What We Know So Far

BZP was originally marketed as a “herbal high”, and promoted as a natural product despite being a synthetic compound (7, 9, 10). BZP was found to reverse the sedative effects of tetrabenazine, a dopamine (DA)-depleting agent that depresses vesicular monoamine accumulation in rats and mice (11). In addition, BZP was also investigated as a potential anti-depressant (12, 13), however the trial was discontinued due to effects similar to amphetamine, and concerns about the subsequent possibility of abuse (9, 13). The stimulant effects have also been reported in preclinical research, with Oberlander and colleagues (14) reporting that BZP, amphetamine and MA all induced contralateral turning behaviour, which was affected by α-methylparatyrosine, an inhibitor of tyrosine hydroxylase – the enzyme involved in the synthesis of DA, by converting tyrosine to the precursor of DA. This illustrated that BZP, amphetamine and MA all affected DA release. In a separate study, rats generalised to BZP, cocaine and methylphenidate (MPH) when trained to recognise a bupropion cue (15).

In humans, BZP exhibits similarities to other psychostimulants, with both physiological and subjective data being comparable to MDMA and DEX (2, 12, 13).

BZP's central mechanism is mainly dopaminergic. It has been shown to inhibit dopaminergic uptake in a manner similar to cocaine (16, 17), release DA from nerve terminals in a similar fashion to amphetamine (18, 19) and act as a direct agonist on postsynaptic dopaminergic receptors (14). In addition, BZP has lesser activity on both noradrenaline (NA) and serotonin (5-HT) release.

BZP intravenously administered to rats (3 mg/kg and 10 mg/kg) induced a dose-dependent elevation in extracellular DA and 5-HT in the nucleus accumbens (NAcc), while 5-HT release was only affected with the higher dose (19). BZP has also been shown to cause peripheral release of NA by blocking synaptic reuptake in an in vitro preparation (20). The actions of BZP on alpha-2 adrenoreceptors are reported to modulate reflex tachycardia and hypertension (7).

1.1.3. Trifluoromethylphenylpiperazine (TFMPP)

TFMPP is another often used component of this group of drugs; however, it is rarely used alone and commonly combined with BZP. When TFMPP has been given alone to healthy participants (60 mg, oral) its subjective effects included increased ratings of "dexamphetamine-like effects", "tension/anxiety", "stimulated" and "high". However it also induced effects of "dysphoria" and "confusion/bewilderment" which is similar to subjective effects of fenfluramine and mCPP (21). mCPP and fenfluramine are predominantly agents that affect 5-HT, with mCPP found to induce feelings of anxiety and confusion in control subjects, and after the administration of fenfluramine subjects have reported an unpleasant experience, sedation and in some participants hallucinations (22). Research using electroencephalography (EEG) in human males, has shown that TFMPP speeds the inter-hemispheric transfer of information across the corpus callosum. This effect was thought to be mediated by its indirect effects on a range of neural pathways including glutamatergic, serotonergic, gamma-Aminobutyric acid (GABA) -ergic and dopaminergic pathways (23).

The pharmacological effects of TFMPP are well described. TFMPP has been used as a biomarker for serotonin activity due to its effects being almost exclusive to 5-HT (24). TFMPP is relatively selective for the 5-HT_1 and 5-HT_2 receptors with low affinity for 5-HT_3 receptors (25). Specifically, TFMPP has been reported to be a partial agonist at 5-HT_{1A} (26) and 5-HT_{1B} (27) receptors, an agonist at 5-HT_{2C} (28) receptors and a partial agonist at 5-HT_{2A} receptors (29). Its stimulus effects are thought to be mediated by its action on 5-HT_{1B}

and 5-HT_{2C} receptors (27). TFMPP, like MDMA, also stimulates 5-HT transporter-mediated release from neurons (19, 30, 31).

Although TFMPP's effects are mainly serotonergic, it also has indirect effects on DA release via interactions with 5-HT_{2C} and GABA receptor blockade (32-34), and NA release via either 5-HT_{2C} or 5-HT_{1B} receptors (33, 35). Rodent studies have shown TFMPP has abuse potential, with rats trained to discriminate MDMA generalising to a TFMPP cue (36, 37). However, this does not appear to be due to a stimulant-like effect, as other research has reported that TFMPP was not self-administered by rhesus monkeys trained to self-administer cocaine; it did not induce reinforcement of cocaine; and it's discriminative stimulus properties did not generalise to amphetamine (37). This potentially implies that TFMPP displays characteristics of other serotonergic hallucinogens, which are known to be recreationally abused by humans, but fail to show consistent self-administration behaviour in preclinical studies (38, 39).

1.1.4. BZP and TFMPP Combined

The subjective effects of the combination of BZP and TFMPP have been reported to be similar to MDMA, with commercial branding of this combination appearing to promote this, with names such as "XTC" and "Legal X". The ratio of BZP and TFMPP in party pill preparations ranged from 2:1 to 10:1 (40). When the combination of BZP and TFMPP (1:1) was given to rats in low (3 mg/kg) and high (10 mg/kg) doses, Baumann and colleagues (19) reported a parallel increase in dialysate 5-HT and DA, with low dose BZP+TFMPP mimicking the DA and 5-HT release of low dose MDMA (threefold less potent). High doses led to a greater extracellular DA level than BZP or TFMPP alone (19), suggesting a synergistic interaction when the two are co-administered. Fantegrossi and colleagues (37) found that the combination of BZP+TFMPP (1:1) was a less effective reinforcer than BZP alone in adult rhesus monkeys. This may be due to TFMPP being an agonist at 5-HT_{2C} receptors, which are known to reduce the neuronal firing within the dopaminergic mesolimbic system (41, 42). This suggests that the effects of TFMPP on serotonergic circuitry could alter the reinforcing effects of BZP via its indirect effects on the mesolimbic pathway. The results of these studies reflect the similarity between the combination of BZP+TFMPP and psychostimulants such as MDMA (19), and therefore their possible abuse by humans.

1.2. Recreational Drug Use

1.2.1. Introduction to Recreational Drug Use

To characterise BZP and TFMPP, their effects will be viewed in the context of the literature describing the effects and actions of other recreational drugs. Because the effects of BZP and/or TFMPP appear to most closely resemble those of stimulants, this next section will detail the effects of the psychostimulants cocaine, amphetamine and amphetamine derivatives.

Preclinical studies have reported that increased concentrations of DA in the NAcc leads to the reinforcing effects of many recreational drugs, and furthermore, that there is a common pathway for signalling reward and reinforcement. This pathway is the mesolimbic dopaminergic pathway, originating in the ventral tegmental area (VTA) and projects forward to the NAcc and the prefrontal cortex (PFC), amongst other structures (43) (Figure 2). The rewarding effects of recreational drugs have been linked with the subjective reports of feeling “high” or pleasure (44). Subsequent imaging studies have correlated these results with subjective feelings of “high” to investigate regional activation, neurotransmitter release and receptor occupancy associated with reward.

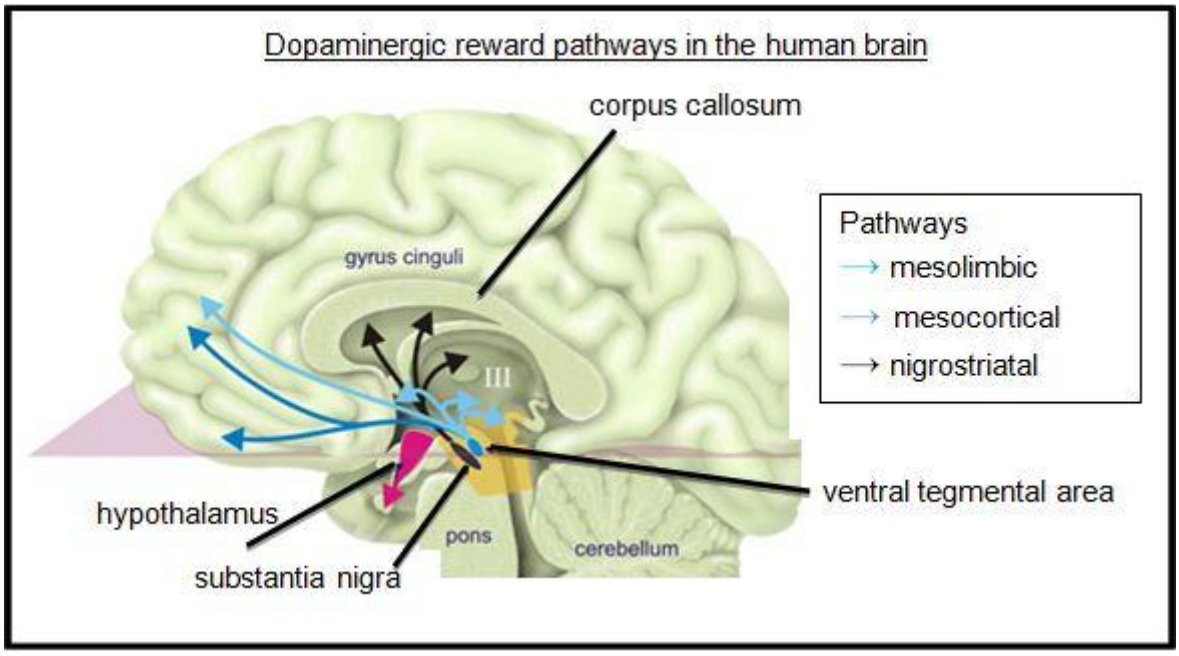


Figure 2: The common reward pathways for reward in the human brain (adapted from (45))

1.2.2. Psychostimulants: Cocaine and Amphetamines

Psychostimulants are a class of drug that evoke characteristic effects, including increased energy, elevated mood, reduced sleep, cardiovascular stimulation and, at higher doses,

psychoses (46). In addition, stimulants can enhance cognitive performance in healthy controls (47) and those with dopaminergic dysfunction (48). Two drugs belonging to this class are cocaine and amphetamine. Cocaine and MA both reach peak uptake in the brain within several minutes after intravenous (i.v) administration; however, MA is cleared at a slower rate, which leads to accumulation of MA in the brain for hours (49). BZP has also been described as having stimulant characteristics, with similarities to the amphetamines (2). Low doses of the combination of BZP and TFMPP have a similar effect to MDMA on DA and 5-HT release (50).

1.2.2.1. Cocaine

Cocaine use is common worldwide. Surveys from the US have reported that 2.4 million Americans over the age of 12 are current users of cocaine and 18% of these users will become problem users (51, 52). Furthermore, it's use has been associated with 40% of drug-related deaths (52). Cocaine is an alkaloid derivative (Figure 3 [d]) extracted from the leaf of the erythroxylon coca. It is commonly administered by intranasal or i.v administration, or the free-base form, known as crack, is smoked (53). The subjective effects of cocaine are due to its ability to increase extracellular DA content in the mesolimbic and mesocortical pathways by blocking the DA transporter, and subsequent binding of DA to postsynaptic receptors. Cocaine also binds to the NA and 5-HT transporters, blocking the presynaptic uptake of NA and 5-HT (46) and enhancing their transmission in the NAcc (54).

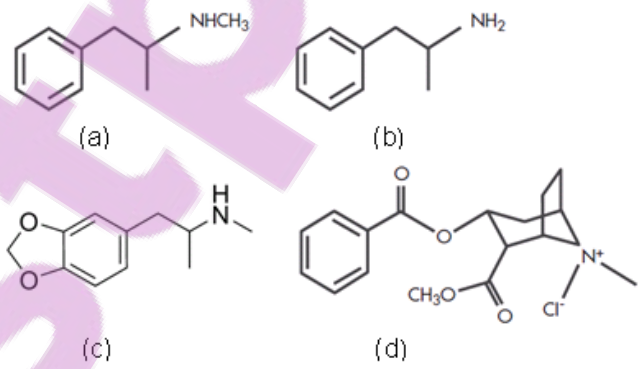


Figure 3: Chemical structure of (a) MA (b) amphetamine, (c) MDMA and (d) cocaine

Volkow and colleagues (55) reported that there is a quantitative relationship between the levels of D_2 receptor occupancy and the degree of rewarding effects, using positron emission tomography (PET). Cocaine was given as a performance-based reward whilst undertaking a task requiring cognitive demand in non-human primates. Specifically, an acute dose was associated with an increase in dorsolateral prefrontal cortex (DLPFC) metabolic activity, a change in task-related firing and a reduction in performance (56).

However, acute doses of cocaine improve selective and sustained attention in rodents (57), which is a characteristic effect of stimulants. The difference between the results of the two studies may lie with the dose of cocaine; as it was the higher dose that was associated with deficits.

1.2.2.2. Amphetamine

Amphetamine (1-methyl-2-phenthylamine) is used for the treatment of a number of medical conditions including attention-deficit/hyperactivity disorder (ADHD) and narcolepsy. A recent survey of drug use in NZ reported that of people aged 16-64 2.1% used amphetamines for recreational purposes in the previous 12 months (58). In addition, MA is currently the most widespread stimulant that is illegally manufactured, distributed, and abused in the US. The 2003 National Survey on Drug Use & Health in the US, reported the lifetime use of methamphetamines at 12.3 million, representing 5.2% of the population of age 12 years and older (59).

Amphetamine and the structural analogues including MA and MDMA share a common component of their structure, that is, a phenyl ring connected to an amino group and a two carbon side chain (60) (Figure 3). Barr and colleagues (61), in a review of MA use and misuse, present a summary of amphetamines actions. The amphetamines are thought to increase extracellular levels of DA by releasing DA from synaptic vesicles and through reverse transport through the plasma membrane via the vesicular monoamine transporter (vMAT). Amphetamines also block reuptake via the DA transporter (DAT) in a similar manner to cocaine and inhibition of the monoamine oxidase (MAO) enzyme that breaks down the neurotransmitters DA, 5-HT and NA. Amphetamine is also thought to decrease the expression of the DA transporters on the cell surface and increase the expression of tyrosine hydroxylase, an enzyme involved in the synthesis of DA, by converting tyrosine to the precursor of DA, dihydroxyphenylalanine (DOPA).

Amphetamine use, in particular MA is increasing. MA is predominantly ingested, smoked, snorted or injected intravenously (62). In healthy naïve participants, low doses of MA has been reported to induce feelings of heightened alertness, attentiveness and energy, with higher doses leading to euphoria, enhanced self-esteem and a sense of wellbeing. An fMRI study reported increased activation of the reward circuitry after MA administration, including the orbitofrontal cortex (OFC), the anterior cingulate cortex (ACC) and the ventral striatum in drug naïve participants (63). MA can also induce negative effects such as, restlessness, insomnia, paranoia and psychoses with chronic use (62). In comparison to amphetamine, MA has a higher lipid solubility profile and passes through the blood-brain barrier into the brain more readily (62). In preclinical studies, albeit to a lesser extent, MA also induces the

release of NA and 5-HT (64, 65). Acute low doses of DEX and MA in humans improve cognitive processing speed, attention and concentration (66-69).

Whilst amphetamine and MA are described as psychostimulants, MDMA has mild hallucinogenic properties, and is referred to as an “enactogen” (70). MDMA use has increased in NZ, with Wilkins and colleagues (71) reporting that the percentage of current users had increased from 1% to 2.3% and those who had used MDMA in the past year had increased from 1.5% to 3.4% between 1998 and 2001. This pattern of use is reflected worldwide with the National Survey on Drug Use and Health (NSDUH), finding 14.2 million lifetime users of ecstasy among people age 12 and older in the US and 2.8 million users of ecstasy in the past year (72).

MDMA is rapidly absorbed following oral administration and its subjective effects include increased energy, emotional warmth and closeness to others and enhanced sensory perception. It is a potent releaser and/or reuptake inhibitor of presynaptic 5-HT and to a lesser extent DA and NA. Its actions are facilitated mainly by inhibiting presynaptic 5-HT reuptake via transporters and releasing 5-HT from intracellular stores (73, 74). MDMA has a lower affinity at 5-HT_{2C} and 5-HT_{2A} receptors (75). The effects on serotonergic transmission have been correlated to the subjective effects of MDMA (76), with activity at 5-HT_{2A} receptors being implicated in causing hallucinogenic effects. Liechti and colleagues (76) found the characteristic acute effects of MDMA in humans, including increased self-confidence, derealisation and intensification of sensory perception were reduced by the administration of citalopram, a selective serotonin reuptake inhibitor (SSRI), strongly implicating a role for the 5-HT transporter in the subjective effects of MDMA. Release of striatal DA has also been shown (77), which has been proposed to be due to the interaction with the DA transporter (78). In addition, MDMA has affinity for the NA reuptake site (79). Actions on dopaminergic and noradrenergic transmission are proposed to underlie its arousing effects (80, 81).

Recently, studies investigating the cognitive effects of an acute dose of MDMA found recreational MDMA users have a deficit in spatial memory, but not processing of contextual information (82, 83). In a separate investigation by Lamers et al. (84) users also displayed deficits in their ability to predict object movement during a divided attention task. Many recreational MDMA users are poly-drug users, and previous experience could have influenced their performance. In contrast, Dumont and colleagues (85) gave an acute dose of MDMA to healthy volunteers and found an increased psychomotor speed without affecting accuracy; however, it significantly impaired the delayed recall of words.

To assess the effects of MDMA on impulse control, an acute dose was given to recreational users, and an improvement in performance on a tracking task and a decrease in reaction times were seen (84). Similar findings were reported by Raemakers and Kuypers (86) with increased impulse control using a stop-signal reaction task. However, after sleep deprivation, the stimulant effects of MDMA are not sufficient to improve performance (87), which was worsened in tasks of divided attention and tracking performance (88). Although, the authors report that the MDMA plasma concentrations in participants when tested were approximately 2.5 times lower than earlier studies.

1.2.2.3. Amphetamine derivatives: mCPP and fenfluramine

TFMPP's subjective effects have been likened to mCPP and fenfluramine (Figure 4 (a) and (b)), with increases in feelings of dysphoria and confusion/bewilderment. mCPP and fenfluramine are two amphetamine derivatives that also have predominant effects on 5-HT.

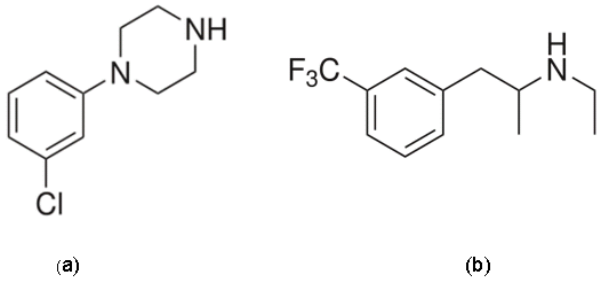


Figure 4: Chemical structure of (a) mCPP and (b) fenfluramine

mCPP is the active metabolite of the antidepressants, trazodone and etoperidone, and it has been suggested that mCPP partially causes their psychoactive properties (89). mCPP has been used in patients groups, as a marker for serotonergic activity to assess receptor sensitivity (90) and has also been found in party pill preparations (21). mCPP has induced anxiety and confusion in control subjects, and in patient groups has tended to worsen symptoms. For example, it induced panic in patients with panic disorder and psychoses in patients with schizophrenia (90). mCPP induces 5-HT, in vivo and in vitro, and its effects are inhibited by the administration of fluoxetine, an SSRI (91). Schoeffter and Hoyer (26) reported that mCPP acts as a partial agonist at 5-HT_{1A}, 5-HT_{1B} and 5-HT_{2C} receptors, with Gommans and colleagues (89) reporting that mCPP's discriminative stimulus properties are largely mediated via 5-HT_{2C} receptors, and to a lesser degree by 5-HT_{1B} receptors. In addition, it acts as an antagonist at both 5-HT_{2A} (28) and 5-HT_3 (25) receptors.

Studies investigating the effects of mCPP on cognition in healthy control subjects have found that it affects specific areas of cognitive functioning, whilst not affecting others. mCPP's effects on inhibition have been investigated in studies using the Go/No-go task, a

task that measures behavioural inhibition. mCPP evoked activation in the mid-cingulate, caudate (92) and OFC (93), which are regions involved in motor behavioural inhibition (94-96). These studies combined suggest that mCPP affects inhibitory responding, leading to recruitment of additional resources to ensure performance in the task, because the behavioural performance of people under the influence of mCPP was the same as controls. In addition, mCPP was reported to slow cognitive but not motor processing in a copying task (97).

Fenfluramine was prescribed as an appetite suppressant prior to being withdrawn from the market due to cardiac side effects, such as pulmonary hypertension (98). It has been shown to increase extracellular 5-HT levels by inducing release from presynaptic storage vesicles and blocking reuptake of 5-HT (99, 100), and is an agonist at 5-HT receptors, in particular 5-HT_2 subtypes (101). When compared to amphetamine, fenfluramine induces an unpleasant experience, sedation and in some participants hallucinations (22). In addition, fenfluramine also reduces aggression in participants with and without a history of conduct disorder (102). The cognitive effects of fenfluramine have been investigated in children with subnormal mental ability, there were improvements in attention, activity level, mood and memory (103). However, impairments in episodic memory have been found (104). Furthermore, fenfluramine administration has caused improvements in impulsive responses in patient groups with conduct disorder, but conflicting results have been reported in healthy controls (102, 105) .

1.3. Reward

1.3.1. Introduction to Reward

Recreational drugs activate reward circuitry and stimulants induce subjective feelings of being “high”. Whilst the subjective effects of BZP and BZP+TFMPP have shown similarities to other stimulants, there have been no studies undertaken to assess regional activation in humans using reward paradigms. To further understand the circuitry involved in reward, the next section will introduce reward and the regions involved in its processing. In addition, BZP and TFMPP both modulate dopaminergic and serotonergic pathways respectively, particular attention will be given to what is already known about drugs and dysfunctions that modulate these circuits.

Reward has an important role in developing and monitoring motivated goal directed behaviour (106), with both humans and animals showing a tendency to seek reward and avoid punishment (107, 108). Reward processing involves a number of specific brain regions, with the cortical-basal ganglia system having a central role. Reward processing

can be divided into distinct phases, and depending upon the task given to participants, each phase of this processing can be evaluated. These stages include selection, anticipation and reward outcome. It has been suggested that the anticipation and outcome stages of reward activate separate regions of the brain (109, 110). Berridge and colleagues (109) presented evidence for the differentiation of “liking” versus “wanting”, proposing that there is a functional and neural dissociation between the two domains. Wanting is reflective of the anticipation of the reward, whereas liking is representative of the receipt of reward. Knutson and colleagues (110, 111) report that *reward anticipation* activates the NAcc, whereas *reward outcome* activates the ventromedial prefrontal (vmPFC) and medial prefrontal cortices (mPFC).

Anticipation has been reported to have a direct effect on learning and decision making. Decision making can be considered a component of goal –directed action where anticipation and feedback have key roles (112). Anticipation has been evaluated in imaging studies using monetary incentive delay tasks. These tasks involve the presentation of a cue, which indicates a potential reward, followed by a delay, and a target. If the participant responds correctly to the target stimulus, they receive the reward (113). Activation has been reported after rewards and losses in the mesial prefrontal regions, dorsal striatum and insula, with additional activation in the thalamus after losses (114).

It has been hypothesised that during reward processing, the ventral striatum acts as the “engine”, providing impetus behind the motivation, whereas the vmPFC directs the processing of reward, in a role similar to that of a “steering wheel” (111, 115, 116). A recent study by Dillon and colleagues (115), reported increased activation in the ACC after anticipation, whereas consumption activated both mPFC and OFC. The prefrontal regions are also involved in anticipation (115, 117) and its involvement has been confirmed in studies reporting activation during anticipation of rewards and losses (118, 119).

Natural rewards, such as food activate similar regions to those induced by recreational drugs and secondary (learned) rewards, for example, money. However, in some studies using natural rewards, for example, food and water there have not been significant differences on reward circuitry, possibly due to baseline hunger or thirst (120). We wanted to ensure that the mesolimbic system was activated during our research, to allow the comparison of BZP, TFMPP and the combination BZP+TFMPP to placebo. Therefore, this research used a secondary reward, that is, money, shown to evoke activation in a more consistent manner in previous studies (121). A recent paper by Knutson and Cooper (116) reviewed imaging papers concerned with reward processing published in the previous year, they found that the most reproducible paradigms used monetary incentives. Money holds a

universal value and is a useful reward in imaging paradigms as both its magnitude and valence can be manipulated.

1.3.2. The Neuroanatomy of the Reward System

Neuroimaging research has identified a number of regions associated with reward processing, including the PFC and the dorsal and ventral striatum.

1.3.2.1. The prefrontal cortex

The PFC is the anterior portion of the frontal lobes and includes the OFC, mPFC and ACC (see Figure 5 below).

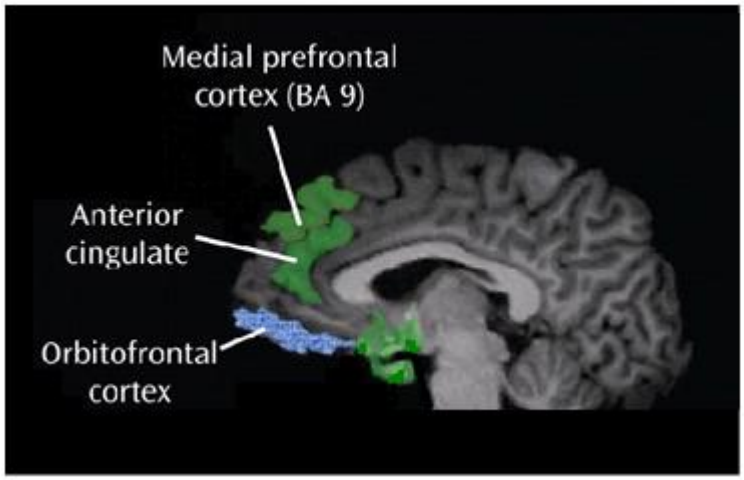


Figure 5: Prefrontal cortex in the human brain showing locations of the mPFC, OFC and the ACC (adapted from (122))

The OFC induces signalling of reward expectation, with an increase in firing of neurons prior to expected rewards in comparison to those that were unexpected (123). The OFC is activated in response to a variety of rewards including natural rewards such as smell, and secondary rewards, such as money. Using a task that presented varying strengths of positive and negative olfactory stimuli, Anderson and colleagues (124) found the OFC was activated in response to the valence of the stimuli, that is, whether it was a pleasant or an unpleasant smell. Similarly, reward processing was investigated using pleasant, painful and neutral touch stimuli with subsequent activation in the OFC, with distinct areas of the OFC coding for pleasant versus painful stimuli (125). Activation of the OFC has also been found in response to secondary rewards, for example, during a gambling (guessing) task where money could be won or lost (118). Kringelbach and Rolls (96), reported two major distinctions in a meta-analysis of OFC function. First, sensory rewards activate more posterior regions of the OFC, whereas abstract rewards, such as money, induced activation in the more anterior regions. Second, when comparing rewards versus

punishments, rewards evoked activation in the medial regions of the OFC and punishments in more lateral regions.

The mPFC is another region implicated in processing reward-related stimuli. As previously described, Knutson and colleagues (110, 111) report that receipt of reward activates the vmPFC and mPFC. The mPFC has been associated with monitoring the specifics of a task response, where it is able to adjust behaviour towards stimuli likely-to-obtain reinforcement. Similarly, the mPFC responds to the probability of rewards. A task that assessed the expected value of rewards found mPFC activity was associated with the probability of a large outcome (126).

Whilst the ACC is generally associated with the resolution of conflict (127), its role can be extended to reward processing. For example, ACC activation has been associated with investing paradigms where conflict occurs (128, 129). In addition, an acute dose of MA induced activation in the ACC and OFC. This activation could indicate that even from the initial administration of MA, it has direct effects on regions involved in decision making (63).

1.3.2.2. The dorsal and ventral striatum

The role of the dorsal (caudate and putamen) and ventral (NAcc) striatum (130) (anatomical locations seen in Figure 6) has been proposed by several authors to include the detection of an affective stimulus, its predictability and its valence (reward versus punishment). Imaging studies reported activation in the striatum in response to a range of rewards including primary rewards, monetary rewards and after the administration of recreational drugs.

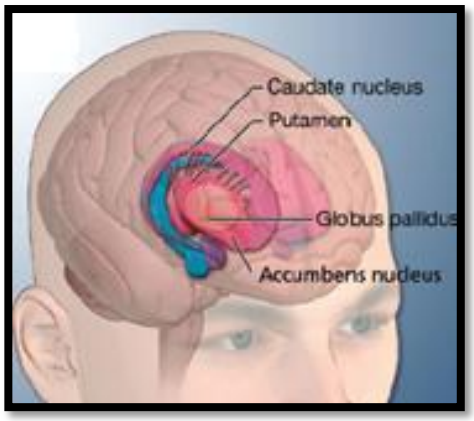


Figure 6: The anatomical location of the dorsal (caudate and putamen) and ventral (NAcc) striatum (adapted from (131))

The dorsal striatum appears to have a distinct role from the ventral striatum. The dorsal striatum is activated in response to both primary and secondary rewards, and is able to differentiate between reward and punishment (132). Dorsal striatal activation has been

found in both PET and fMRI studies investigating reward. For example, in a card selection task, where monetary amounts could be obtained, increases in DA transmission in the left medial caudate were found (133). Similarly, during a fMRI task caudate activation has been reported after administration of cocaine (134) and nicotine (135). It has been suggested by several authors and in a review by Balleine and colleagues (136) that the dorsal striatum is involved in action contingent learning, whereby the caudate and the putamen are associated with learning actions and their reward consequences. Furthermore, it has been proposed that the putamen is involved with stimulus-action coding (137), whereas the caudate acts to code reward prediction errors during goal-directed behaviour (137, 138).

The ventral striatum is activated after the anticipation of reward (110), for example, during a gambling task the ventral striatum was activated in response to financial rewards (139). Anticipation of olfactory rewards also induces activation in the NAcc that increased over time. However, in response to aversive stimuli the opposite was seen, that is, a reduction over time (140). The NAcc has also been linked to more abstract rewarding stimuli such as beauty (141). Amphetamine has shown modulation of the blood oxygen level dependant (BOLD) signal in the NAcc. In a monetary incentive delay task, NAcc activation was found in response to the anticipation of losses, which the authors proposed was indicative of increased positive arousal, which led to neural activations normally seen after rewarding stimuli (142). Ventral striatal activation has also been seen after other recreational drug use, including cocaine (134, 143) and alcohol (144). After the omission of an expected reward, activation in this region has shown to be decreased (145). Subsequently, it has been proposed that the ventral striatum acts as a tracking region for reward prediction error (146); that is, it reacts to the difference between the expected and the actual reward.

1.3.2.3. Thalamus

The thalamus (Figure 7 (a)) is part of a circuit involving the basal ganglia and the PFC known as the basal ganglia thalamo-cortical circuit (BGTC). The thalamic nuclei are known to transmit output from the basal ganglia to the frontal cortex, forming a loop that reportedly drives motivation by communicating with parallel circuits (147). The BGTC circuit is also involved in reward-related behaviour, specifically the thalamo-cortical region is associated with linking reward and specific goal directed behaviours (148). Furthermore, imaging studies have shown that the thalamus is a region activated after rewarding (114, 125, 141) and aversive stimuli (114). In a meta-analysis, investigating the changes in brain activation in response to anticipation, thalamic activation was seen after the *anticipation* of rewarding stimuli in comparison to reward outcome. However, it did not appear to have a role in differentiating between reward and loss anticipation (149). The thalamus has also been reported to be specifically involved in the learning aspect of reward (150). Galvan and

colleagues (150) demonstrated that thalamic activity decreased over time to a conditioned response task and that it's role seems to aid in adjusting behaviour to maximise potential outcomes.

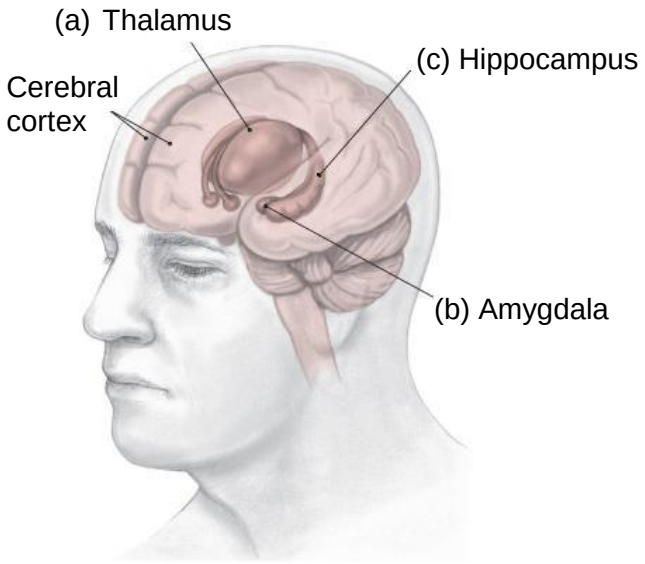


Figure 7: The anatomical location of the thalamus and amygdala (adapted from (151))

1.3.2.4. Amygdala

The amygdala (anatomical location see Figure 7 (b)) is reported to be predominantly activated in response to negative or unpleasant stimuli; however, recent studies have reported activation by positive stimuli. For example, separate studies found amygdala activation in response to both positive and negative emotional stimuli. One study found amygdala activation in response to emotional visual stimuli and in the second study this region was associated with pleasant and unpleasant memories (152, 153). It has been suggested that the amygdala is possibly activated by the intensity of stimuli, rather than whether it has positive or negative valence. This would explain why many studies have associated negatively valenced stimuli with its activity, as negative stimuli tend to be more intense than positive stimuli. This hypothesis was demonstrated in a study by Anderson and colleagues (124) using olfactory stimulation of positive and negative valence at varying intensities. The authors reported that the amygdala was not affected by changes in valence, but instead was activated in response to intensity.

1.3.3. Dopamine and Reward

1.3.3.1. Drugs that affect the dopaminergic system

BZP is mainly dopaminergic in its activity. Dopamine has a critical role in the mediation of reward; and the changes in extracellular levels of DA have been shown to affect the neural responses to the anticipation of reward. To allow the effects of BZP, TFMPP and

BZP+TFMPP to be interpreted, the next section will discuss the results from prior studies, investigating the alterations of dopaminergic transmission on reward processing.

The dopaminergic mesocorticolimbic system consists of the two major pathways associated with reward-related processing: the mesolimbic pathway and the mesocortical pathway (Figure 2, page 9). DA release has been shown after salient stimuli and those that predict reward (154-156). Berridge and colleagues (109) report that the mesolimbic dopaminergic system specifically corresponds to the anticipation of reward, with the NAcc in particular being associated with the anticipation of positive stimuli (157, 158). DA is released prior to the delivery of pharmacological rewards, primary rewards (159-161) and monetary rewards (116).

The initiation of recreational drug use is generally for hedonic effects. As with other forms of rewarding stimuli, recreational drugs induce an increase in DA release in the limbic regions of the brain including the striatum. In human studies, this sudden increase in DA is correlated with increased subjective reports of reward, which have included feelings of "high", pleasure and euphoria (44). Initial administration of drugs of abuse lead to increases in DA release. However, the release of DA is altered after repeated use, and is found in response to the anticipation of the drugs, which leads to a craving. Changes in the motivation for drugs and natural rewards are a key component of addiction (162).

Preclinical studies reported that lesions or blockade of receptors in the mesocorticolimbic system block the reinforcing effects of cocaine and amphetamine (163-165). Further confirmation of DA's involvement is seen by reductions in reward behaviour after administration of DA antagonists (166, 167). Also, DA depletion in the NAcc attenuates the rewarding effects of amphetamine (168) and cocaine (169-171).

Recent imaging studies proposed that the increase in activation of the striatum seen after administration of amphetamine, is thought to be due to increases in phasic firing of dopaminergic neurons (63). Increased DA release, has been linked to subjective effects, including arousal and reward (172). In comparison to placebo, amphetamine increased extracellular levels of DA in the striatum, which correlated with its rewarding effects, such as, euphoria (173, 174). This has also been found following the administration of cocaine (143) and alcohol (144).

Manipulations of the dopaminergic system in humans have been used to explore the effect of DA on anticipation. da Silva Alves and colleagues (175) gave healthy participants α-methylparatyrosine, which depleted DA. They measured activation before and after the acute dose and reported an increase in the caudate and cingulate regions following

placebo, but no activation after DA depletion. Other studies support the involvement of the prefrontal and striatal DA circuits in reward processing. Knutson and colleagues (142) gave amphetamine to healthy volunteers completing a monetary task. They reported activation after anticipation of reward in the ventral striatum (176). Administration of amphetamine has also been shown to amplify wanting (109), suggesting manipulation of the DA system could be leading to the increased motivation and compulsion in drug addiction and seeking.

A relationship has also been identified between dopaminergic firing and prediction error (PE). PE is the difference between the predicted and the actual received reward. Participants learnt associations between visual stimuli and delivery of food flavours, and subsequent activations related to behavioural preferences were found in the ventral midbrain and ventral putamen (177).

1.3.3.2. Dysfunctions of the dopaminergic system

Patients with dysfunctions of the dopaminergic system have provided evidence of dopaminergic involvement in reward processing. One prominent condition is drug addiction. Addiction in characterised by a compulsion to seek and take a recreational drug regardless of its consequences, the loss of control over intake and when the drug is not given a negative emotional state ensues (178). Phasic firing of DA neurons in the NAcc is thought to increase after moderate consumption of recreational drugs; however, after the transition to dependence this phasic firing is reduced. Robinson and colleagues (179) described a shift from “liking” the drug to “wanting” the drug and compulsive use. It has been postulated that it is the excessive DA release after taking recreational drugs, especially cocaine and amphetamine, in comparison to natural rewards that leads to changes within the DA system, which ultimately leads to compulsive drug taking (180). Preclinical research has found increased reward thresholds in measures of reward function during acute abstinence by direct brain stimulation reward (181, 182). This raised threshold is evidence of changes in the underlying reward systems (178). In addition, changes in neurotransmitter systems, for example, depletions of extracellular levels of DA and 5-HT in microdialysis studies during withdrawal (183, 184), are proposed to lead to negative withdrawal states, and produce a vulnerability to relapse (185).

The incentive–sensitization theory of addiction suggests that the dopaminergic systems may be sensitised, whereby there is an increased wanting of the drug even in the absence of liking (186). Imaging studies have demonstrated dopaminergic system dysfunction in addiction. For example, PET studies have reported decreases in metabolic activity in the OFC (187) during withdrawal, and within the ventral striatum and PFC there is a reduction in DA D_2 receptors (178, 188).

Patients with schizophrenia have a dysfunction in DA circuitry. One of the negative symptoms of schizophrenia is affective flattening or anhedonia, thought to stem from dopaminergic dysfunction in the ventral striatum. Imaging studies have found reduced activation in regions of the mesocorticolimbic system, specifically the amygdala (189, 190), hippocampus (190), PFC (191), insula (192) and NAcc (192). However, a confounding factor in these studies is that the patients were medicated with neuroleptics, which block the DA D_2 receptor, and D_2 receptors have been found to affect reward. Jukel and colleagues (193) recruited a non-medicated schizophrenic population and compared these patients to healthy controls. They reported that in response to reward-related stimuli there was a reduction in activation in the ventral striatum, which was associated with reports of negative symptoms.

1.3.4. Serotonin and Reward

TFMPP has been shown to be relatively selective for the serotonergic system. Although it is understood to a lesser degree, modulations of the serotonergic circuitry have proved to affect the processing of reward. The next section will discuss the effects that alterations in extracellular 5-HT levels and serotonergic dysfunctions have on reward.

Serotonergic neurons project from the raphe nuclei to the forebrain, with associated projections to the amygdala and hippocampus (Figure 8). The role of 5-HT in reward processing is not completely understood. 5-HT has been proposed to oppose the role of DA in reward processing (194, 195), with studies reporting evidence of a serotonin-dopamine gradient along the caudal-rostral axis in the striatum (196, 197). In addition, 5-HT has been associated with aversive processing (198, 199).

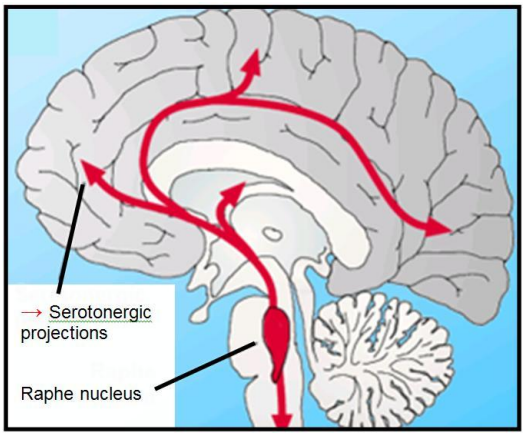


Figure 8: Schematic of the serotonergic system projecting from the raphe nuclei (adapted from (200))

1.3.4.1. Drugs that affect the serotonergic system

Del-Ben and colleagues (201) gave the SSRI citalopram to a group of healthy volunteers and found it decreased activation in the right OFC and right parahippacampal/amygdala region, and increased activation in the bilateral thalamus and fusiform gyri in response to aversive stimuli. In a separate study, citalopram was administered to healthy controls for 7 days, and the authors reported similar findings (202). The effects of paroxetine, another SSRI, were also used to investigate responses to a monetary incentive delay task, with authors reporting diminished activation in regions associated with motivation (203).

Kapur and Remington (204), suggested that the effects of 5-HT oppose DA. There have been reports that 5-HT antagonises the effects of DA in the VTA and the substantia nigra, and this opposition transmits through to the dopaminergic terminals in other regions such as the striatum, leading to a reduction in DA transmission. This proposal was expanded by Daw and colleagues (194), who also suggested that 5-HT and DA have opposing roles, where DA acts in an appetitive manner, and the dorsal raphe 5-HT projections would oppose these actions and encourage avoidance.

1.3.5. Losses and Uncertainty

Under the influence of recreational drugs, people are reported to make sub-optimal decisions. Regional responses to the prospect of aversive stimuli and uncertain or risky choices are a key component in making these decisions. Therefore, understanding these responses to aversive and uncertain stimuli is of particular interest to this research. To determine the effects that BZP, TFMPP and BZP+TFMPP have on the anticipation of uncertain outcomes and their responses to aversive stimuli, an event-related gambling task was designed. This task was designed to allow the evaluation of each of these distinct aspects of reward processing – anticipation and outcome of reward or punishment.

The mesocorticolimbic dopaminergic system is involved in the processing of aversive stimuli with the underlying role of incentive motivation, in the pursuit of safety being hypothesised (157). However, others argue that the mesocorticolimbic system has a role in aversive motivation itself. Matsumoto and Hikosaka (205) propose that DA modulates the processing of aversive stimuli (205, 206) and determines the motivational salience of both types of stimuli—rewarding and aversive. In addition, both rewarding and aversive events are thought to trigger orienting of attention, cognitive processing and increases in motivational salience (108). Similar regions are reportedly activated in response to monetary losses and rewards with additional activation within the ACC and thalamus after losses (114).

After the administration of amphetamine, an increase in activation was reported in response to the anticipation of losses. This may be reflective of increasing motivation regardless of outcome (142). Similarly, a study by Ikemoto and Panksepp (157), hypothesised that in response to aversive events there would be an increase in NAcc activity due to the anticipation of a positive outcome. The effects of amphetamine on PE were researched and showed amphetamine evoked a wider network of activations, including the ventral striatum, globus pallidus, putamen, insula, ACC and VTA/substantia nigra (176). In addition, the DA agonist pramipexole also induced an exaggerated response to reward and a reduction in top-down responses to the control of behaviours (207).

The OFC, a region previously discussed and associated with reward processing, is also implicated in aversive processing and punishment, and often associated with the inhibition of future responses (208). Alternatively, reports have suggested activation of the OFC is reflective of the response to punishments rather than solely motor responses; humans with lesions in the OFC respond to punishments but are less likely to respond to the anticipation of punishment (209).

Risk taking and uncertainty are other components of decision making, and involve specific regions, including the amygdala, OFC, inferior frontal gyrus (IFG) and insula (210, 211). In addition, in investigations of the neural responses to probability, the ventral striatum has been found to be responsive to uncertainty. For example, in a study by Cooper and Knutson (212), the NAcc was activated after the anticipation of certain rewards, but not *certain* losses. However, the NAcc was also found to evoke activation to stimuli of *uncertain* wins and losses.

1.3.6. Reward prediction error

Electrophysiological studies in monkeys who had undergone classical conditioning reported that after learning a stimulus predicts the availability of reward there is a subsequent burst of firing in DA neurons. Upon receipt of the reward the firing of DA neurons reflects the *difference* between the expected and actual reward. When the reward was greater than expected there was an increase in firing, however, when a reward was less than predicted, firing was inhibited. Furthermore, if the predicted reward was then received then there was little change in firing. This effect is known as the reward prediction error (213, 214).

1.3.7. Magnitude

Neural regions are also responsive to the magnitude of rewards (215). The NAcc has been implicated in the magnitude of reward only (158), while the caudate (215) and the thalamus (150, 158) have been associated with the magnitude of both reward and punishment. A

meta-analysis by Knutson and Greer (149), further verified that the NAcc was associated with magnitude of reward but not loss. Delgado and colleagues (215) proposed that the caudate's role may be to differentiate between not only valence but also magnitude of stimuli, reflecting how valuable the stimuli is. Therefore the caudate's role may be implicated in approach behaviour dependent upon the magnitude. In addition, changes in reward magnitude have also been shown to affect the activation in the PFC (216).

1.3.8. Summary of Reward

The processing of reward and related stimuli activates specific neural structures, with distinct roles. In addition to the processing of reward, characteristic regions are activated in response to aversive and uncertain stimuli. Reward, punishment and uncertainty are all facets that contribute towards decisions made in daily living. However, whilst under the influence of recreational drugs people are reported to make poor judgements and consequently bad decisions. Drugs that modulate dopaminergic and serotonergic circuitry have been shown to affect processing of reward and punishment. BZP and TFMPP have been reported to affect DA and 5-HT respectively. This research aims to identify regions that are activated in comparison to placebo, and compare these results with other well-known drugs. In addition to alterations in reward processing, decision making can also be affected by a dysfunction of executive processing, such as selective attention and inhibition. Whilst this section discussed reward processing, the next section of the introduction will describe executive function, the associated regional circuitry and the effects that modulating the dopaminergic and serotonergic pathways have on this processing.

1.4. Executive Function

1.4.1. Introduction to Executive Function

Executive function controls behaviour in a top-down manner (217). Top-down control is the effortful aspect of self-regulation and is associated with the PFC. Executive function can be further divided into four components: decision making; monitoring and updating information; shifting between one task and another; and inhibition of pre-potent responses (218). Cognitive control allows for flexible goal directed behaviour, so the appropriate action can be taken depending on the task at hand (219) and enables the resolution of conflicting responses.

John Ridley Stroop published an article in 1935, which described a task used to investigate attention and interference. This has become known as the classical colour-word Stroop task and involves the presentation of a stimulus, to which the participant has been

instructed to respond to the colour the word is written in, and not the written word (220). Three conditions are presented: control words that are non-colour words; congruent words, where the word and the colour match, such as RED being presented in red; and incongruent words, where the colour and word do not match, such as RED written in green (Figure 9).

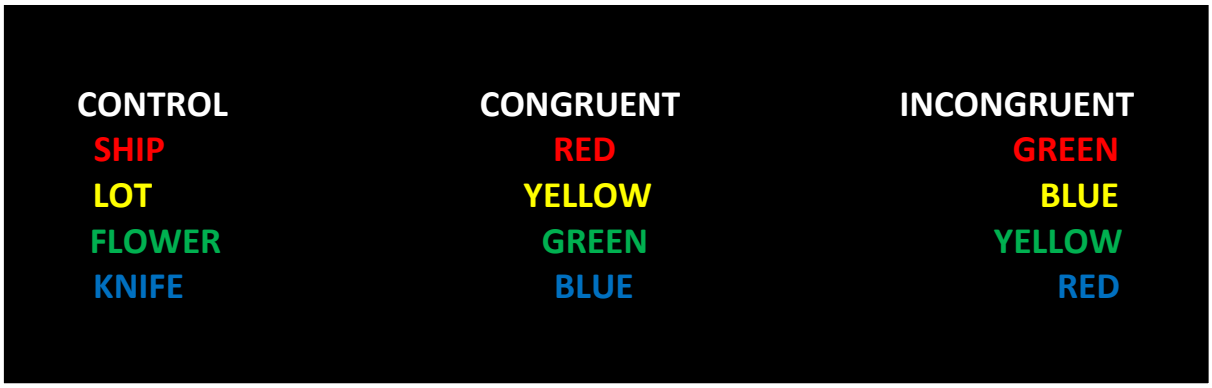


Figure 9: Examples of Stroop control, congruent and incongruent conditions

The Stroop effect measures the interference induced by the incongruent condition relative to the congruent condition for speed, accuracy (220) and in the case of imaging tasks, regional activation. This interference is derived from the difficulty of suppressing the natural or pre-potent response to read and respond to the written word, which is thought to be a more automatic response as it is more practised (221, 222). Since patients with lesions in the frontal lobes were found to have deficits in both speed and word reading on the Stroop task, the task has been increasingly used, as a psychological test to assess selective attention and inhibition (221, 223). It is now one of the most frequently used paradigms used to study cognitive control, when faced with an interference dimension. In subsequent studies, patients including those with ADHD (224), schizophrenia (219, 225), depression and obsessive-compulsive disorder (226) all show deficits in the Stroop effect. In addition, recreational drugs and drug dependence also modulate the Stroop effect (227).

Stroop paradigms can be designed so that participants respond vocally or with a button press. Both types of responding have unwanted effects on imaging data: vocalisation can cause movements of the head, jaw and tongue, whereas the response by button press also recruits the use of motor movements and subsequent motor inhibition. Bernal and colleagues (228) demonstrated cognitive inhibition is lateralised to the left hemisphere, whereas the right hemisphere reflects motor inhibition.

1.4.2. Neuroanatomy of Executive Function

1.4.2.1. Prefrontal cortex

Several areas in the PFC have been linked to executive function, with the ACC and DLPFC being identified as key regions. The ACC has long been implicated in cognitive control, with some studies suggesting a functional dissociation between the caudal and the rostral ACC in regards to their role in responding to conflicting stimuli, where the caudal responds to perceptual conflict and the rostral region responds to response conflict (229). Furthermore, there has been debate over the exact role of the ACC. Botvinick (230) suggests that the dorsal ACC acts as a general monitoring system, whilst others implicate its role in detecting conflict and subsequent recruitment of the DLPFC to resolve the conflict. Additionally, other studies have reported that the ACC, DLPFC and the parietal cortices are involved in resolving the conflict (231, 232). Botnivink (233) observed an increase in activation in the ACC when there is an issue with top-down control. This reflects recent findings by Azizian and colleagues (234) who proposed that an increase in ACC activity in the Stroop effect may demonstrate a compensatory recruitment of neural regions to allow for the support of selective attention processes.

Recently studies have shown a strong association between the OFC and cognitive control. Patients with damage to the OFC performed more poorly on the Stroop and Trail making tasks, suggesting a role for the OFC in response inhibition and attention switching. It has been proposed that the OFC has an integral role in inhibitory control and selection of specific stimulus information that is then processed by the DLPFC (235). Moreover, the OFC was activated in a number of neuroimaging studies that have used the Stroop (236, 237) and the Go/No-go tasks (238), which both require inhibitory responses.

The IFG is reportedly involved in the inhibition of responses (239). Predominantly the right IFG has been found to be associated with inhibitory control, however the left IFG has also been shown a similar role (240). Patients with lesions in the right IFG were found to have impaired inhibitory control (241). Imaging studies have also revealed the involvement of the IFG, using tasks that require the inhibition of pre-potent responses including the Stroop and Go/No-go tasks (242, 243).

1.4.2.2. Dorsal striatum

Healthy participants completed a Stroop and Simon tasks during fMRI to investigate both word and spatial interference respectively. The study found that the head of the left caudate was activated during Stroop interference only, suggesting that the caudate plays a role in the control of word but not spatial interference (231, 244). In addition, Li et al. (245)

demonstrated that during a stop-signal task the caudate plays a role in the inhibitory control of pre-potent responses.

1.4.3. Dopamine and Executive Function

As previously discussed, BZP's pharmacological effects are predominantly dopaminergic, in addition to TFMPP having indirect effects on DA via 5-HT_{2C} receptor agonism. Dopamine has an important role in the circuits involved in executive function and is implicated in the modulation of the Stroop task. This next section will discuss the results from prior studies, investigating the effects of alterations in dopaminergic transmission.

Executive function is mediated to a large extent by the PFC. Dopaminergic neurons project into the PFC from the midbrain, with additional connections within the basal ganglia, which is innervated by the nigrostriatal pathway (Figure 2, page 9).

An inverted U-shaped dose-response curve describes the effect of DA transmission on executive function. It has been suggested that there is an optimum extracellular DA level (246), and DA transmission that is too high or too low within the dopaminergic circuitry results in sub-optimal performance on tasks (246). Hyper or hypodopaminergic levels are a consequence of dopaminergic drugs or dysfunctions in DA circuitry, for example, patients with Parkinson's disease (PD), schizophrenia and ADHD.

1.4.3.1. Drugs that affect the dopaminergic system

Dopaminergic agonists, such as bromocriptine and amphetamine modulate executive function. Administration of amphetamine (0.25 mg/kg body weight) to healthy volunteers showed a typical inverted U-shaped dose-response in the PFC in relation to working memory, as well as an improvement in accuracy in people who have performed poorly prior to drug administration. Moreover, a reduction in performance was found in those participants who showed an optimal performance prior to the drug (247). These findings were corroborated in a recent study where levodopa (100 mg) was given to aging adults and young healthy volunteers to investigate the effect of reductions in DA associated with aging. Subjects were asked to complete a visual–spatial interference task based on a Stroop/Simon-like paradigm. The younger subjects' performance was impaired and associated with increased activity in the ACC, unlike that of the older adults. These results are thought to be due to altered dopaminergic transmission, which aided function of the PFC in older people, whereas in the younger group, there was overstimulation of DA due to baseline DA levels (248).

A number of studies have compared the effects of agonists at different DA receptor subtypes, to elucidate which receptor(s) effect cognition. Roesch-Ely (249) compared the effects of pergolide with bromocriptine and placebo on performance during a Stroop task. Pergolide is a D_1 and D_2 agonist in comparison to bromocriptine, which is a D_2 agonist. Pergolide did not alter the Stroop effect while bromocriptine reduced Stroop interference. This is in line with the cognitive benefits observed in patients with schizophrenia after the giving bromocriptine (250), suggesting a role for D_2 receptors in the processing of executive function that was reversed by the additional stimulation of D_1 receptors.

Acute tyrosine/phenylalanine depletion (ATPD) reduces both DA release and synthesis, and impairs the DA dependent cognitive processing of memory (251, 252). Scholes and colleagues (253) used ATPD to study the effects of DA depletion on the Stroop task in healthy controls, and surprisingly reported improvement in Stroop performance. This is in contrast to other studies, which have described improvement after an *increase* in DA levels; the difference could lie with a global decrease from ATPD, whilst regional changes in DA levels are seen in the other studies.

1.4.3.2. Dysfunctions in the dopaminergic system

Studies have indicated a disruption in the striatal dopaminergic regions in stimulant drug addiction. In a recent study by Nestor and colleagues (227), abstinent MA addicts undertook a Stroop task that resulted in hypoactivation of the right IFG, supplementary motor cortex/ACC and anterior insular cortex during the incongruent condition. The authors propose that this reduction in regional activation reflects the cognitive control deficits associated with MA addiction. This confirmed previous imaging results that also identified a reduction in prefrontal activation of the Stroop effect in MA abusers (254).

PET imaging studies report that MA dependent subjects have a reduction in postsynaptic D_2 receptor levels (255). Subjects dependent on cocaine (256) and alcohol have similar reductions (257). Impulsivity and compulsivity in addiction have been associated with changes in DA circuitry (258). MA and cocaine users have shown a reduction in performance during the Stroop task, indicative of a decreased ability to selectively attend to stimuli or the ability to inhibit pre-potent responses (259-261).

Schizophrenia has been associated with a deficit in performance on tasks that involve cognitive control (262). Weinberger and colleagues (263) first proposed that there was a reduction in the projections of the mesolimbic DA circuitry to the DLPFC, after cerebral blood flow was investigated using the Wisconsin card sorting task (WCST). Whilst some studies have reported an increase in the interference of patients with schizophrenia (264, 265), others have reported no change and an increase in the speed of response to

facilitation (266). The authors from the latter study concluded patients with schizophrenia have a generalised slowing of processes, and that the increase in facilitation could be linked to the reduction in DA activity in the mesocortical projections of the ventral striatum. Greater activation in the right ACC has been reported in patients with schizophrenia during the Stroop effect (267), and reduction in activation in the DLPFC has been reported in first degree relatives (219). Studies that have administered DA agonists to patients with schizophrenia have provided evidence of an involvement of DA with the cognitive deficits seen in this condition. Amphetamine was given to a group of schizophrenic patients completing the WCST, and deficits seen prior to drug administration were improved (268).

1.4.4. Serotonin and Executive Function

TFMPP has shown selectivity for the serotonergic system, and therefore the effect that alterations in serotonergic circuitry have on executive function must be considered. Studies have identified an association between serotonergic circuitry and cognition. It has been proposed that increased extracellular levels of 5-HT impairs learning and memory. The Stroop paradigm recruits a number of functions to maintain performance on cognitive and motor inhibition, including working memory, attention and motivation (228). Serotonin has been found to modulate attention and cognitive flexibility (269).

1.4.4.1. Drugs affecting the serotonergic system

Del-ben and colleagues (201) gave an acute dose of citalopram (an SSRI) to participants who completed a Go/No-go task. The authors reported a reduction in the medial OFC after the No-go stimuli. Similarly, in a separate study using the Go/No-go task, acute administration of mCPP resulted in an attenuated response in the lateral OFC (93). However, in contrast healthy subjects in a PET study using a 5-HT transporter (SERT) ligand, found that high SERT binding in fronto-striatal regions is associated with better performance on executive function (270). Madsen and colleagues (270) suggested that 5-HT may influence cognitive function directly or indirectly, by the adverse effect of these drugs (270). For instance, high reductions in 5-HT levels have been shown to affect arousal; and studies have reported memory impairments to be linked with sedation (271).

Focussed attention during a Stroop paradigm is essential to maintain task performance. The flanker and dichotic listening task are two tasks that assess focussed attention. The Stroop, flanker and the dichotic listening tasks are all been affected by a change in the extracellular 5-HT levels. Schmitt and colleagues (272) found that acute tryptophan depletion (ATD) led to a reduction in interference in the Stroop task and an increase in participants' performance in a dichotic listening task. In a separate study, auditory attention was assessed after ATD using EEG and magnetoencephalography (MEG), and the results

suggested that the reduction in 5-HT decreased involuntary attention shifting to task-irrelevant information (273). However, these results have not been replicated in subsequent tasks. Studies reporting the effect of ATD on the Stroop task have also conflicted, with several studies finding an improvement (253, 274), whilst others have not shown any changes (271, 275). This may be due to baseline levels of 5-HT.

The role of 5-HT has also been implicated in decreasing sustained attention. Fluoxetine, an SSRI reduced sustained attention (276), while sertraline, another SSRI, did not. This is thought to be due to the additional effects of sertraline on DA transmission (277), and indicates the involvement of 5-HT. Although the Stroop task does not directly measure sustained attention, there are still ramifications, as participants must maintain attention on the task to ensure adequate performance.

1.4.4.2. Dysfunctions of the serotonergic system

5-HT is associated with depression, bipolar affective disorder, schizophrenia anxiety and impulse control-related disorders (201, 270). Serotonergic modulation in these conditions is proposed to contribute to neural recruitment during cognitive control tasks in these patient groups. Vollm and colleagues (278) investigated behavioural inhibition in patients with a diagnosis of borderline and antisocial personality disorder, a disorder where patients exhibit a disruption in behavioural inhibition. The authors reported a wider network of areas that were activated in relation to healthy controls, including frontal and temporal regions in the Go/No-go task.

5-HT levels are thought to be a contributing factor to depression (279), combined with reductions in DA (280) and NA (281). Cognitive impairments in depression include deficits in attention and executive functions, such as impairments in cognitive flexibility. In a functional imaging study that used ATD in depressed patients an increase in performance was reported in a Stroop test (274).

1.4.5. Summary of Executive Function

Imaging studies have identified key regions in the brain associated with selective attention and inhibition, two components of executive function. Whilst the exact roles of some regions are debated, the effects of DA transmission on regions, particularly on prefrontal regions, have been shown to be critical in executive function. Recreational drugs and other drugs that affect DA have shown an inverted U-shaped dose-response curve in relation to cognition, where too high or too low a dose can lead to sub-optimal performance. The serotonergic system to a lesser extent has also shown the ability to modulate executive function. Whist the previous two sections have been dedicated to exploring the circuitry of

reward and executive function, the next will detail imaging, particularly the background and analysis of fMRI, including the advantages and disadvantages of using this technique to study aspects of human brain function.

1.5. Imaging

1.5.1. Introduction to Imaging

The field of neuroimaging has expanded in the past 20 years and now includes (amongst others) EEG, MEG, fMRI, PET, diffusion tensor imaging (DTI), ^{1}H Magnetic Resonance (MR) spectroscopy and structural analysis, such as voxel-based morphometry (VBM). Whilst EEG and MEG provide excellent temporal resolution (in the range of milliseconds), they provide poor spatial resolution. fMRI and PET on the other hand, have poor temporal resolution (approximately 2 seconds), but high spatial resolution. fMRI has allowed advances in the investigation of specific neural circuits, by providing information about blood flow. Combining fMRI with cognitive tasks has allowed the investigation of components of functional neuroanatomy involving, for example, cognitive control and attention and monetary reward tasks. In addition, a branch of fMRI known as pharmaco fMRI (phMRI) enables research into the effects of specific drugs and their effects on these circuits.

1.5.2. fMRI

fMRI is a relatively new technique that provides non-invasive high spatial resolution and allows the changes in brain activation over time to be examined. fMRI is based on the principle that when there is an increased cognitive demand in a particular region, there will be a subsequent increase in blood flow to that specific region. The increase in blood flow stems from an increase in the metabolic demand for oxygen—this change in blood flow can be measured. Oxygenated and deoxygenated blood have a different magnetic signal: oxygenated blood is diamagnetic and deoxygenated blood in paramagnetic. Specifically, the paramagnetic signal alters the T2* weighted magnetic resonance image signal and thus, in a similar manner to a contrast agent, the deoxygenated blood can be measured (282, 283).

The underlying properties of how fMRI works is based on the physics of the proton. The single unpaired proton spins on its axis (Figure 10) due to the elementary particles known as quarks and their paired antiquarks. These quarks and antiquarks all have orbital motions in relation to each other. The proton exhibits a charge, which combined with the spinning motion induces a magnetic field (284).

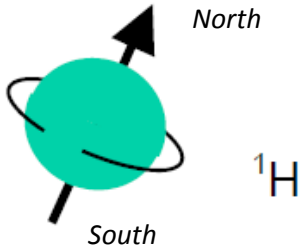


Figure 10: Proton spinning on its axis (adapted from (285))

When the fMRI signal which is derived from the strong magnetic field, is turned on it applies a strong magnetic field to the tissue, which causes these protons to approximately align. Their alignment is not exact, which leads to their axes rotating in an inexact manner, known as precess, and the shape of the spin is cone-shaped (Figure 11). The frequency of time taken for a rotation within this cone shape is known as its resonance, or its Larmor frequency. Each proton has a unique resonance frequency. In the MRI scanner the magnet has a gradient, whereby it is stronger on one end than the other. This allows a slightly different magnetic field to be applied to the tissue, which combined with the proton being specific to each tissue ensures that the Larmor frequency for each proton is unique, and can be distinguished from another (284, 286).

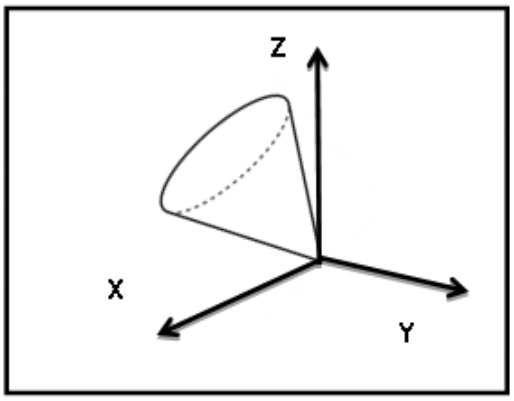


Figure 11: Depiction of the cone-shaped precess

A coil is placed next to the part of the body that is being imaged, and this emits powerful radio frequency pulses. This radio frequency excites the protons, causing them to “tip” or “flip” toward the XY plane and causes them to transition from a state of low to high energy within the magnetic field. The radio frequency in the coil is then turned off and the protons that have absorbed the radio frequency will emit it. This release of energy causes the protons to realign. The coil then detects the time taken for a proton to release that energy. It is this release of energy that allows the development of the images that are seen in fMRI,

of the particular tissue. The signal is then received by the MRI computer and reconstructed using Fourier transforms (284, 285).

The data that are collected contains a series of images that are divided into equally sized portions known as volume elements or voxels. Each voxel's intensity reflects the nuclear spin density in that area. The voxel's intensity at different points in time can be used as a marker of activity. Each fMRI experiment collects hundreds of volumes of images per run and within each image there are approximately 100,000 voxels.

Deoxyhemoglobin is paramagnetic and is able to suppress the MR signal, whilst diamagnetic oxyhemoglobin does not. By collecting a series of images over time whilst the subject is completing a task we can study this change in oxygenation that reflects brain activity. The response in blood flow in a particular region is known as the hemodynamic response function (HRF). There is an increase in signal approximately two seconds post-neuronal activity and this signal peaks after five to eight seconds (287). The signal then decreases, forming an undershoot dip below baseline, as there is a greater concentration of deoxyhemoglobin as blood flow decreases at a faster rate than blood volume (Figure 12).

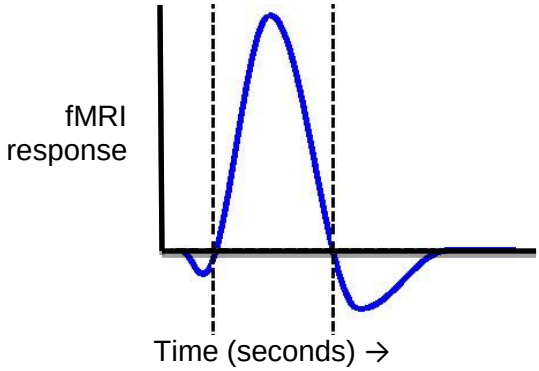


Figure 12: The hemodynamic response function (HRF)

The data collected from these studies are not specifically reflective of a release of a particular neurotransmitter; however, studies have associated the BOLD response to increases in extracellular levels of DA. Knutson and colleagues (288) described the relationship between an increase in BOLD activation and an increase in DA release in the NAcc. They proposed that this increase in activation could be due to agonism at postsynaptic D_1 receptors increasing the NAcc signal. This increase should alter the membrane potential, increasing the BOLD signal through increased energy consumption. Furthermore, that antagonism should lead to the opposite effect.

1.5.2.1. Advantages and disadvantages

The number of studies using fMRI has increased over the past decade due to its many benefits. PET imaging requires the injection of radioactive isotopes, whereas fMRI does not—it is a non-invasive technique. fMRI also provides a manner of imaging that can be collected in a relatively short period of time, allowing both healthy controls and patients to be scanned. However, like every technique there are limitations. Although fMRI provides an excellent tool for spatial resolution, it has poor temporal resolution, which is dependent on time between the acquisitions of scans across the brain, known as the repetition time (TR). The TR can range in studies from half to four seconds depending on the study protocol. This delay in temporal resolution is a downside to fMRI as the neural changes in hemodynamics can occur within milliseconds. However, since the assumption made with fMRI is that the change in the BOLD signal occurs over a time frame of 5–8 seconds after activation, a TR of approximately two seconds is accepted within the field (289).

There are two main experimental designs in fMRI- blocked designs and event-related, again both with their advantages and disadvantages. Blocked designs consist of experimental conditions presented in succession. For example, in the colour word Stroop paradigm there are three conditions, incongruent, congruent and control words. In a blocked design, a run of one particular condition is presented in a group, followed by a break, then a different condition in a group (Figure 13). Blocked designs have the advantage of a high statistical power as the signal is generated from a collective of stimuli presentations rather than just a single presentation (289). However, this technique can lead to fatigue, and if the condition is not changed often enough scanner drift noise can be an influencing factor in the signal. In addition, the differing levels of arousal associated with conditions could be a confounding influence. Event-related designs are another method, where the stimuli conditions are presented in a randomised manner (Figure 13). Event-related fMRI designs are advantageous in many ways, for example, they have estimation efficiency, that is they enable researchers to estimate the HRF to a stimulus of short duration, but they do reduce the detection power of statistical significance of the data (290). This type of design also reduces the amount of boredom of the task (289).

The choice of design protocol may lie with the research being undertaken. For example, in gambling tasks, some studies have used blocked designs to eliminate the effects that are created by unpredictability (291). However, blocked designs do not allow for the investigation of different aspects of reward processing. Event-related designs offer the advantage of a differentiation between the different phases and account for general task-related processing effects, such as a difference in baseline arousal (292, 293). We specifically wanted to assess the effect of BZP, TFMPP and the combination of these drugs

on the different phases of reward—that is, the anticipation and reward outcome phases—so we designed an event-related gambling (guessing) paradigm that would allow this differentiation.

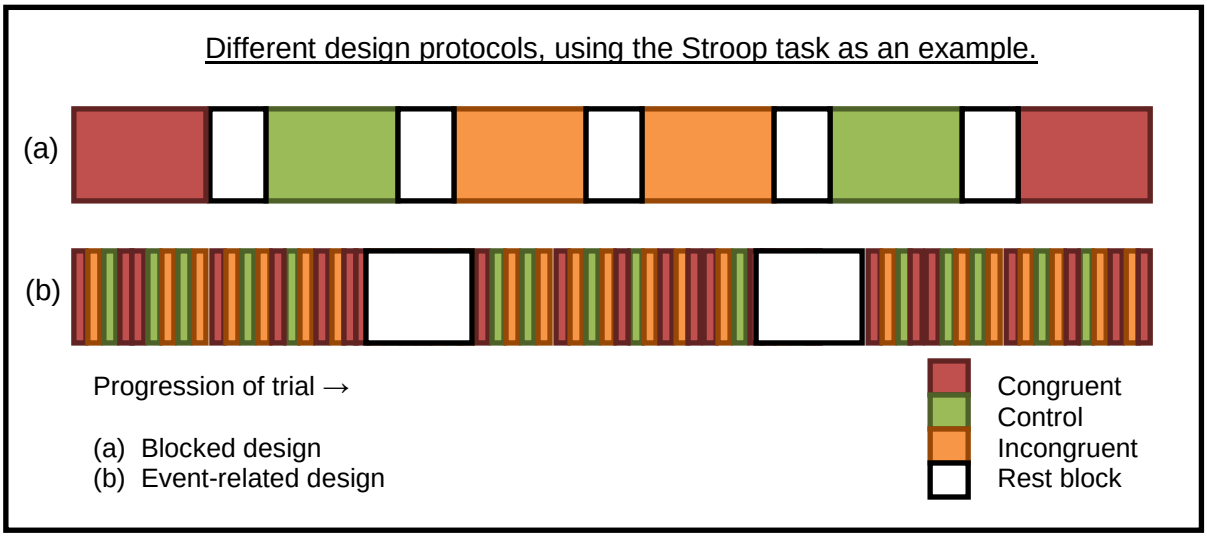


Figure 13: Blocked versus event-related design protocols

The OFC is a region with increasing interest from researchers, it has associations with impulsivity and decision making, amongst other processes; however, this region has a tendency to exhibit artefacts. This artefact stems from the sinuses, which are close by and contain air pockets. In addition, artefact can be generated from the movement of the head during imaging. There are now scanning parameters to target data collection in this area, which combined with the collection field maps, can reduce the amount of signal dropout in this particular area. However, neither of these strategies was used in this data collection. When choosing imaging sequences that are optimised for the OFC there are compromises that must be made. Firstly the angle at which the brain must be acquired must be shifted from the transverse plane to the coronal plane, due to the shape of the brain. As a result, the volume coverage may be compromised, especially for large tilt angles. In addition, although it does improve sensitivity in the OFC, it does not improve sensitivity in all regions affected by susceptibility gradients, indeed it increases signal loss from areas where through-plane gradients are negative rather than positive (294). Since our focus was not solely on imaging the OFC, the imaging sequences were not optimised to this region due to these limitations.

Random noise is another confound that impacts on fMRI data. Noise can come from several different sources, including the scanner drift which cause the slow change over time of voxel intensities, or the subjects themselves. An example of the latter being movement and physiological factors, such as, heartbeat and respiration can generate

noise. These factors can be addressed by using artefact detection and rejection software and by using a high pass filter, that removes this baseline noise (289).

1.5.3. Data Analysis

The previous section described the basis of fMRI. This section will give an insight into what happens after the data is collected, i.e. the analysis. Specialised software has been developed to allow the analysis of the data acquired from MRI sequences. Statistical Parametric Mapping (SPM) is the software package that was used for analysis of this research. The data must firstly be pre-processed, to allow individuals scans to be compared. The data from groups of individuals can then be combined and contrasted with another group. For example, fMRI has been used to image healthy control subjects and those patients with conditions such as schizophrenia, PD and ADHD.

1.5.3.1. Pre-processing

The main steps involved in fMRI pre-processing include slice timing correction, realignment, co-registration of both the structural and functional images, normalisation and smoothing.

First, a slice timing process is completed, accounting for the sequence in which images are collected in the scanner, which is scanner specific. For example, depending on the scanner the slices may be collected sequentially or in an interleaved manner. Slice timing is a critical step, as the slices are collected over the time span of the TR. Thus by instructing SPM of how the data is collected by the scanner, it allows for accurate interpretation of the exact time point of scan acquisition.

The realignment process involves correcting for motion. Motion is an important factor in the collection of imaging data, with minor movements leading to large impacts in the images acquired. A rigid body transformation is conducted which involves the image being moved and rotated in six directions (i.e. x, y, z, pitch, roll, yaw). This image is then resampled using interpolation and a motion corrected file is created (289).

The functional images collected are at relatively low resolution; therefore, a structural image is collected alongside to allow for presentation of anatomical locations. Therefore, the functional and anatomical scans must be aligned using the co-registration process. This process uses either a rigid body or affine registration using either six or twelve parameters respectively.

To allow for subjects to be compared either at the single subject level or at a group level, a normalisation process must take place. This process ensures that subject's brains are warped to a standard template, and allows for consistency in brain region locations. A disadvantage of this step is that it can introduce a source of error, due to the interpolation used and a subsequent reduction in spatial resolution (289).

The final step in pre-processing is to spatially smooth the data, which may aid in the registration between subjects, and reduce the noise which subsequently increases the signal to noise ratio. Smoothing has been described by Lindquist (289) is equivalent to applying a low-pass filter to the data. The smoothing involves convolving the images with a Gaussian kernel, with a full width of the kernel at half its maximal height (FWHM). Typically the FWHM used is between 4 and 12mm, and it is approximately three times that of the voxel size.

1.5.3.2. First and second level analysis

After the pre-processing of data is complete, it can then be analysed and compared. This comparison can be at both the single subject level or combined and compared at a group level. The general linear model (GLM) is applied to the data (Figure 14). The GLM models the time series and conducts a univariate analysis of the varience, creating a t-statistic at each voxel from the data. Where Y is the observed data at each voxel, β are the parameters which define the contribution of each component of the design matrix to the observed data (Y); X is the design matrix, that represents several components to explain the observed data, and ε is the residual error, that is, whatever cannot be explained by the model.

$$Y = \beta x + \varepsilon$$

Figure 14: Equation for the general linear model (GLM)

The *t*-statistic is the beta (β) divided by the standard error of the slope. As the parameter estimates are considered normally distributed, once the data has been fitted to the model, statistical inference can be made as to whether the betas are significantly different from the null hypothesis.

The researcher must then form contrast vectors, for either a single condition or for a difference between two conditions, known as an interaction contrast. Depending on the experimental hypothesis in question, if the aim is to compare the effects at a group level, to

maintain maximum specificity interaction contrasts should only be made at the second (group) level analysis stage.

Second level or group comparisons can be made using the contrast files generated at first level analysis. Whilst the more common practice is to use a full factorial model, this model does not account for individual variance, and thus can cause an unsubstantial inflation of the t-statistic; a flexible factorial model takes this into account (295). The flexible factorial model has a subject factor, which is absent in the full factorial model; this results in an increase in the degrees of freedom. Interaction contrasts can be made at this level to contrast the difference between the groups, albeit the contrasts are more complex.

Although distinct brain regions have been associated with reward and executive function, it was unclear which regions would be affected when the drug state (i.e. BZP, TFMPP or BZP+TFMPP) was compared to placebo. Therefore whole brain fMRI was acquired and analysed to identify regional activation.

Due to corruptions in some of the E-prime data files due to collection error BZP, TFMPP and BZP+TFMPP were compared individually to placebo. This allowed the maximum remaining data sets to be used in the analysis.

1.6. Objectives of this Research

1.6.1. The Concerns: Comparisons with Other Drugs

Despite their frequent use, the effects in humans of BZP and TFMPP, alone and in combination are poorly understood. Studies in animal models have shown that BZP is similar to other stimulants in respect to its release of neurotransmitters DA and 5-HT. However, animal studies are not necessarily predictive of effects in humans (296). TFMPP has been used as a marker for serotonergic activity; however, its effects of specific pathways have not been investigated. This research aims to investigate the effects of BZP and/or TFMPP on reward and executive function.

Studies have reported that BZP has similar effects on mood to psychostimulants, such as MDMA and MA (2, 40, 297). Acute administration of amphetamine and cocaine improve performance on tasks that require attention and memory, but regular or chronic use of these drugs cause deficits. Aron and Paulus (259) reviewed the literature that studied the effects of cocaine, amphetamine and MDMA using fMRI. They reported reductions in activation of the ACC, DLPFC and IFG, regions involved in key aspects of executive function, including inhibition and decision making. However, cocaine was once reported to be a “relatively safe, non-addictive euphoric agent”, despite reports of dependence and

preclinical studies indicating similarities to amphetamine (298). By 1986, the lack of research and claims of safety found up to three million people reporting to be regular users of cocaine in the US, and with this increase, there was a subsequent increase in morbidity and mortality (298).

A recent example of a similar unsubstantiated claim is the emergence of the synthetic drugs, such as BZP and TFMPP, which are marketed as safe alternatives to illicit recreational drugs, despite the lack of knowledge into their effects. Like cocaine and other stimulants, BZP and the combination of BZP+TFMPP have stimulant-like effects, but their effects on human's reward and cognitive function have not been assessed.

Literature has shown that regular use of cocaine, MA and MDMA are associated with impaired cognitive processing. In animal models, chronic cocaine administration revealed impairments in selective and sustained attention (299-301) and increases in impulsivity (302, 303). These impairments have also been seen in humans with cocaine causing both structural (95) and metabolic changes (55). Functional abnormalities in verbal memory and attention correlate with lifetime of cocaine use (304). Recreational cocaine users have also shown deficits in inhibition (305). In a Go/No-go task cocaine users were given an acute dose of cocaine that resulted in improvements in inhibitory control (306). The authors have proposed that cocaine users have hypoactivation in these regions associated with inhibition and the increases seen in this study are "normalising" the activity in this region. This normalisation process is due to the dopaminergic transmission of the drug compensating for the dysfunction in the dopaminergic circuitry induced by chronic use (307). After cessation of cocaine, users display deficits in spatial memory and cognitive flexibility (308).

In addition to cocaine, MA administration has led to long term deficits on cognitive function (309), thought to be due to a modulation of dopaminergic circuitry. Animal studies report degeneration of the nerve terminals and reductions in the DA transporter (DAT) activity (310, 311), in addition repeated administration causes decreases in striatal concentrations of DA and its metabolites (312). In humans there is also evidence of reductions in DAT levels in studies using PET imaging (313). MA abuse leads to impairments in tests of executive function—including the Stroop task (227)— tests of inhibitory control (314, 315), learning (313), memory recall and manipulation of information (261). Less is known regarding the effect on the 5-HT system; however, results of animal studies depict damage to the serotonergic fibres (62).

Neurotoxic damage of the 5-HT neurons has been reported after MDMA use in rodent and primate studies (316-318), with the damage including depletion of 5-HT and its metabolite 5-Hydroxyindoleacetic acid (5-HIAA), reduced density of the 5-HT transporter and reduced

serotonergic axonal density (319). Similarly, clinical studies in MDMA users have shown deficits in memory and mood, albeit these reports have been inconsistent. Moreover, a meta-analysis of cognitive function in MDMA users found deficits in several domains, including attention, verbal and non-verbal learning and memory, and executive function (320). On the other hand, there have been conflicting reports. Several studies were not able to find deficits in impulsivity (321), and others unable to distinguish deficits specifically to MDMA in poly-drug users. The impairments observed in these poly-drug users have been suggested to be due to other drug use (322, 323).

The permanent effects of MDMA have also been disputed, with reports that the 5-HT transporter availability returning to normal after a period of 5 months abstinence. Moreover, although there have been indications of cognitive deficits in MDMA users, this may be due to pre-existing functional abnormalities rather than a direct effect of the drug (319).

Lyvers and colleagues (324) proposed direct drug effects on the circuitry involved in executive function are associated with continued drug addiction. However, the difficulty in assessing cognitive deficits in drug users is the confounding factor of other poly-drug use, which may lead to, or contribute to, the changes reported. Alternatively, abnormalities may have been present prior to beginning drug use and thus may be a predisposing factor to the commencement of drug use in the first place, that is, it caused vulnerability for drug use. For example, increased impulsivity and deficits in behavioural flexibility have been shown to increase the likelihood of a person becoming addicted to a drug. In addition, addiction has been shown impair behavioural inhibition by inducing changes in the brain circuitry (325). A number of studies have tried to compensate for these limitations by correlating their findings with the lifetime of drug use, or matching the control subjects for their use of other drugs.

To be able to assess the effects of drugs abuse whilst minimising confounding issues, one can look at the effects of these drugs after acute administration, in relation to placebo in controlled study designs. This enables the resulting effects of the research to be directly linked to the drug. The effects of BZP and/or TFMPP on reward processing and executive control have never been investigated; subjective comparisons to other psychostimulants such as MDMA and MA have raised concerns regarding their acute and long term effects. This investigation therefore aims to define their acute effects relative to placebo and in addition BZP will be directly compared to DEX.

1.6.1.1. BZP, TFMPP and the combination and reward.

BZP, TFMPP and BZP+TFMPP each modulate DA and 5-HT to differing degrees. These drugs are consumed for their rewarding effects, in particular to mimic the effects of MDMA

and MA. This research aims to compare the effects of these drugs to placebo on reward processing and infer their subsequent effects on motivation and decision making. BZP, as discussed, is mainly dopaminergic in its activity. Based on previous research we hypothesise that alterations of DA transmission by BZP will alter the participant's neural activations during the gambling (guessing) task, in regions known to be modulated by DA, such as the striatum and PFC. Furthermore, we predict to see a distinct patterns of activation in each of the anticipation and outcome phases, with the anticipation phase being predominantly effected by BZP administration.

TFMPP is mainly serotonergic, with indirect activity on DA transmission at 5-HT_{2C} receptors, consequently leading to a reduction in DA in the VTA (41, 42). TFMPP should also influence the effects seen on reward processing, relative to placebo, in a manner similar to other serotonergic agents, or drugs that reduce DA transmission. It is expected that a diminished response in relation to rewarding feedback will be seen, but possibly a heightened response to the aversive stimuli.

When the BZP+TFMPP are combined, it is expected that the effects on regional activation will be diminished due to the opposing effects on DA transmission by BZP and TFMPP.

1.6.1.2. BZP, TFMPP and combination and executive function

Executive function was previously discussed, with a particular consideration for the effects of drugs that affect dopaminergic and serotonergic circuitry. This research presents a direct comparison of BZP, TFMPP and BZP+TFMPP to placebo, for the effects of selective attention and inhibition. The areas predicted to show regional differences to placebo may not solely lie within those mainly associated with the Stroop task, such as, the ACC and DLPFC, but may be found in additional areas involved in both cognitive and motor inhibition, including the OFC and caudate.

We hypothesise that BZP will alter responses to the Stroop effect, by causing a disturbance in the balance of selective attention and inhibition, due to its effects on DA transmission. Specifically, since BZP and DEX have been compared in terms of their effects, we predict that following administration of BZP there will be a reduction in reaction times in the incongruent condition. It is expected that behavioural data and imaging data will demonstrate a reduction in reaction time and a reduction in activation in areas associated with cognitive control, such as the ACC and PFC.

TFMPP has been compared to both mCPP and fenfluramine in terms of its subjective effects. In a manner similar to mCPP, it is expected that the effects of TFMPP may also influence executive function. There could be a modulation in activation in the OFC, and

also an additional neural recruitment in a compensatory manner to ensure focussed attention and response inhibition is maintained.

When BZP and TFMPP are combined, executive function may be modulated due to the effect of changes in dopaminergic transmission induced by the individual drugs.

The research reported in this thesis aims to further understand the impact that BZP, TFMPP and BZP+TFMPP combined have on these processes. We have chosen to use the technique of fMRI to conduct these exploratory investigations, as it gives an insight into changes in neural activations in comparison to placebo; and BZP relative to DEX.

Specifically, the following research allows the investigation of reward via a custom gambling (guessing) task which was designed to allow the provocation of reward circuitry, and allowed the distinction between different stages of this processing. In addition, executive function was evaluated by using a classic colour-word Stroop task, a task that has been repeatedly used to investigate the effects of selective attention and inhibition in healthy controls, patient groups and after the administration of selected drugs. The next three chapters will present the results from this research in the form of papers, which are prepared to be published.

1.6.1.3. Trial procedure

The series of investigations that are presented in this thesis were gathered using a cross-over design. Thirteen participants were recruited to take part in this trial, and returned on five separate occasions with at least seven days between trial days. This separation of trial days allowed time for the washout of each drug, ensuring that there was no influence of the preceding drug(s) on behavioural or imaging data collected.

Each trial day was carried out in a standardised manner (Figure 15). Participants were asked to arrive for trials fasted, that is, they were asked not to eat anything or drink any caffeine containing beverages from 9pm of the preceding day. This ensured that the absorption of the drugs would not be affected by food.

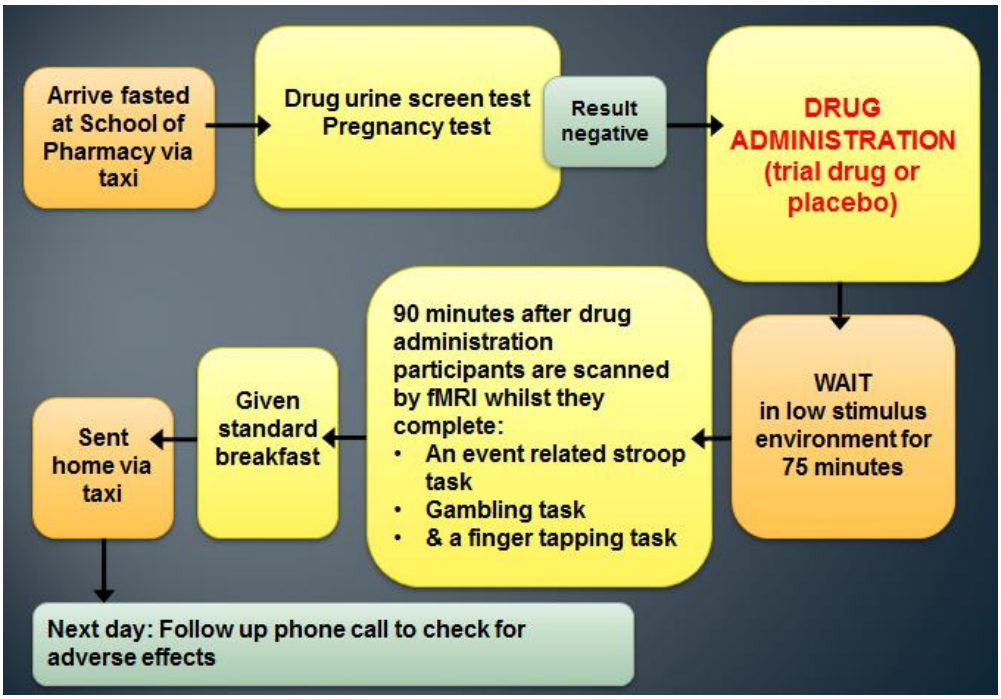


Figure 15: Trial day procedure

Prior to drug administration all participants were required to take a urine-drug test to ensure that they had not taken any other drug prior to coming to the study. In addition, all female participants were required to take a pregnancy test. If either test was positive the participant was excluded from the trial.

The trial drugs (BZP, TFMPP, BZP+TFMPP, DEX and placebo) were manufactured by the School of Pharmacy using good manufacturing practice and capsules were identical in appearance. The drugs were given in a randomised schedule to each subject under double-blinded conditions to ensure minimal bias.

Ninety minutes after drug administration, the participants were scanned using fMRI. A recent pharmacokinetic study reported that the elimination half-life of BZP is 5.5 hours and that TFMPP has two disposition phases with the half-lives being 2 and 6 hours. Ninety minutes was chosen as a suitable time for scanning as the peak plasma concentration of BZP and TFMPP has been reported to be 75 and 90 minutes respectively. Three tasks were completed by participants whilst in the scanner, a standard colour-word Stroop task, a custom-designed gambling (guessing) task and finger tapping task. The sequence (Figure 16) in which the participants completed each of the tasks on each trial day was randomised to ensure that appropriate counterbalancing occurred in respect to drug effect, that is we randomised the tasks to ensure the time after the drug's peak level should not impact results on a specific task, due to the length time in the scanner post drug administration (total scan time approximately 45 minutes).

After completing the trial day procedures the participant was given a standard breakfast (toast and a de-caffeinated beverage) and then sent home to a supervising adult via taxi.

Figure 16: Example of progression of tasks whilst in scanner with duration of activities.

1.6.1.4. Subjective and physiological data

During each trial three subjective rating scales were also collected in addition to monitoring of the physiological effects of blood pressure, heart rate and temperature. The effects of BZP, TFMPP and the combination BZP+TFMPP were evaluated using Profile of Mood States (POMS) prior to drug administration (time zero) and after scanning was completed (140 minutes after drug administration), and via the Addiction Research Centre Inventory (ARCI) and Visual Analogue Scale (VAS) at time zero and every fifteen minutes for an hour after drug administration and after scanning.

Chapter 2: BZP, TFMPP and Reward—Is it Worth the Gamble?

2.1. Preamble

The previous chapter introduced the party pills drugs BZP and TFMPP so they could be placed the context with other recreational drugs. This Chapter will report the effects that BZP, TFMPP and the combination have on the reward circuitry involved in completing a gambling (guessing) task whilst undergoing fMRI.

The gambling (guessing) task used in this series of investigations was designed to specifically look at activation induced in the two stages of reward processing, that is anticipation of an uncertain outcome and the outcome of reward or punishment (loss). To our knowledge there is no task in the current literature that investigates anticipation and reward/punishment outcomes without the added complexity of learning, risk or a manipulation of probability. Unlike other reward tasks, such as the monetary incentive delay task, the probability of a win or loss was held at a fixed probability of 50%. This allowed the neural activations in response to punishment to be investigated with no modifications in baseline anticipation of a reward. The task was named the gambling task, as participants were instructed that they were able to win and lose monetary amounts. Behavioural responses in the form of reaction time could also be measured in this task.

Participants completed this custom designed gambling (guessing) task 90 minutes after the administration of BZP, TFMPP, BZP+TFMPP, DEX or placebo. The reverse side of a playing card was presented to participants and they were asked to guess the colour of the suit, if a question mark (“?”) appeared, by selecting red or black via a button press. The stimulus then progressed to the anticipation stage; the monetary amount that they were playing for—$0, 50 cents or $4 appeared on the back of the card. The card was then flipped to reveal the colour of the suit and the amount that was either won or lost. The stages of this process were named selection, anticipation and reward outcome phases. To investigate the effects that these drugs have on the specific regions involved in reward processing, we wanted to eradicate any direct or indirect effects that these drugs were having on regional activation. A neutral condition was therefore presented to participants; during this stimulus an “X” appeared on the reverse of the playing card, this indicated that the participant was not to actively play this game, that is, they were not to guess the colour of the suit. The game would proceed as in the active task, and in the reward outcome stage a “no-change” would be presented. This allowed a baseline condition for the contrast of interest to be compared with.

Each drug state, BZP, TFMPP, combination of BZP+TFMPP and DEX was compared with placebo. To evaluate the similarities of BZP with DEX an additional comparison was also made.

Reward anticipation and outcome have been shown to affect distinct circuitry; we wanted to evaluate both stages of processing. In addition magnitude is also known to affect results, so we differentiated the results by large and small losses. Therefore the results will be presented as three papers.

All the data were collected on a 1.5T Magnetom Avanto Siemens scanner at the Centre for Advanced MRI, and all data were pre-processed and analysed using SPM8. The group level analysis was conducted using a flexible factorial model.

The main focus of this thesis was analysis of the imaging and behavioural data from the three trial tasks whilst undergoing fMRI. A preliminary analysis was also conducted on the physiological data that was collected at time zero in comparison to 60 minutes after drug administration.

Previous studies in our group have shown that BZP and BZP+TFMPP increased both systolic and diastolic blood pressure, however there was no change after the administration of TFMPP.

Data from this study showed similar trends as BZP and DEX increased both systolic ($p < 0.039$) and diastolic blood ($p < 0.036$) pressure in comparison to placebo. Furthermore, BZP+TFMPP increased heart rate ($p < 0.013$). TFMPP did not affect blood pressure but appeared to cause changes in body temperature ($p < 0.024$).

The subjective effects of the drugs were assessed qualitatively in previous studies using the same doses as this study. Subjectively, BZP had stimulant effects, increased ratings of euphoria and dysphoria and increased sociability and drug liking ($p<0.05$) (2), whilst TFMPP induced increases in dysphoria, dexamphetamine-like effects, tension/anxiety and confusion/bewilderment and increased drug liking, high and stimulation in comparison to placebo (21). When the two were combined BZP+TFMPP had significant dexamphetamine-like effects, increased dysphoria and feelings of self-confidence (40).

We are confident therefore that the drug doses used in this study are pharmacologically active.

It is the intention to complete full quantitative data analysis on the subjective and physiological effects that have been recorded in this trial. Data was collected at 15 minute

intervals throughout the 5 trials for each participant, therefore it may be necessary to condense data into 30 or 60 minute bins in order to compare the pre and post-drug administration to that of placebo.

2.2. Gambling Task: Comparing BZP, TFMPP and Combination BZP+TFMPP to Placebo to Investigate the Anticipatory Stage of Reward Processing

Differential responses to anticipation of reward after an acute dose of the synthetic drugs benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP) alone and in combination using functional magnetic resonance imaging (fMRI)

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2.2.1. Introduction

A relatively new group of designer drugs, which includes BZP and TFMPP, has been regularly used for the past decade as alternatives to illicit drugs, such as MDMA and MA. Despite ongoing use, there is a surprising paucity of research about their effects on the reward circuitry of humans. Studies is animal models have ascertained that BZP mainly affects DA (14, 16-19), whereas TFMPP is serotonergic in its activity (326), with its stimulus properties negated by effects at 5-HT_{1B} and 5-HT_{2C} receptors (27). Recently BZP was reported to induce subjective effects similar to MDMA and amphetamine (2). In contrast, TFMPP's subjective effects have been compared to fenfluramine and mCPP (21).

BZP and TFMPP have been sold both alone and in combination. When combined and administered to rodents (1:1) in low doses (3 mg/kg, i.v.) the effects are reported to mimic those of low dose MDMA (three fold less potent), while higher doses (10 mg/kg, i.v.) produce a synergistic effect on DA release and induced seizures (19). Furthermore, in adult rhesus monkeys, the administration of BZP and TFMPP in combination was reported to be a less effective reinforcer than BZP alone, which was been proposed to be due to TFMPP's effect on 5-HT_{2C} receptors and subsequent actions of DA transmission (37).

Reward and punishment are behavioural stimuli associated with motivation in both animals and humans (107, 108). The anticipation phase of reward processing is reported to activate different regions of the brain in comparison to the receipt of the reward (109, 110). Evidence of neural distinction between the anticipation or "wanting" and the receipt of reward or "liking" has been proposed, with a strong association between the mesolimbic dopaminergic system implicated in anticipation or wanting (109). This hypothesis has been demonstrated in many studies, including the pharmacological manipulation of dopaminergic neurons. For example, amphetamine causes an increase in extracellular DA levels and that subsequently amplifies wanting. In addition, amphetamine has been reported to cause a reduction in the amplitude of the BOLD signal (142). Manipulation of the dopaminergic

system by recreational drugs has been suggested to lead to the increase in motivation and compulsive drug seeking associated with addiction (186, 327, 328).

Preclinical research has identified the neural network that responds to rewarding stimuli which includes the dorsal striatum (the caudate and putamen), ventral striatum (NAcc), OFC, mPFC and ACC (115, 329). These regions all receive dopaminergic input from neurons originating in the VTA (329).

fMRI research has used cue-response outcome contingencies, in the form of monetary incentive delay tasks, to investigate reward anticipation. An initial cue indicates the potential reward to be obtained and after a short delay a target appears, and if the participant responds correctly to the target they receive the reward (113, 126, 132, 158, 330). A distinct network of regions has been found to be responsive to anticipation. For example, a using monetary incentive delay task, anticipation of rewards and losses activated the mesial prefrontal regions, dorsal striatum and insula, with additional activation in the thalamus after losses (114).

Research has distinguished regions associated with anticipation, from those that are activated during the outcome of reward; whereby the ventral striatum is associated with anticipation, and the receipt of reward with the vmPFC and mPFC (110, 111). Furthermore, the ventral striatum has been reported to act in a manner such as the “engine” whereas the vmPFC has the role of the “steering wheel” (111, 115, 116). However, Dillon and colleagues (115) recently reported that there was increased activation in the ACC after anticipation, whereas consumption activated both mPFC and OFC, and the ventral striatum was activated during both phases. Risk taking and uncertainty are other facets of reward processing known to activate specific regions, with studies reporting activation in the amygdala, OFC, IFG, and the insula (210, 211).

Drug-induced reward is thought to be mediated via the mesolimbic system. This is demonstrated by increased firing of DA neurons in the VTA followed by DA release in the NAcc shell and limbic forebrain. The increase in DA in the NAcc is said to result in the feelings of euphoria induced by recreational drugs. Human imaging studies have demonstrated that this system is activated after consuming recreational drugs such as cocaine or amphetamine.

The magnitude of the reward also influences reward processing, for example, whether the monetary amount is high or low. Delgado et al. (215) investigated the effects of both valence and magnitude and found that the dorsal striatum plays a role in distinguishing the valence of stimuli and ranking reward based on magnitude—where a greater magnitude of

reward or loss reflected greater activation. Specifically, the role of the caudate has been proposed to be involved in approach behaviour, by reflecting how valuable the presented stimulus is. Furthermore, imaging research investigating the role of the VTA in memory reported that increased activation in response to larger potential rewards (331). Additionally, reward magnitude appears affect PFC activation (216).

To investigate the effects of BZP and TFMPP both alone and in combination on the anticipatory stage of reward processing and the magnitude of reward we used an event-related gambling (guessing) task and fMRI to determine regional activation.

2.2.2. Materials and Methods

Approval for this study was granted by the Northern X Regional Ethics Committee of NZ (Ethics approval number NTX/07/08/078). The trial recruited healthy participants and excluded subjects with a history of mental illness, cardiac disease, head trauma, epilepsy, endocrine disorders, or who were pregnant or breastfeeding. Participants attended an initial screening session where a questionnaire was completed by each participant, describing their medication history, recreational drug, alcohol and cigarette use, sleeping patterns and stress levels to ensure they were not drug naive or current or past heavy recreational drug users. Thirteen non-smoking healthy participants (seven female and six male; aged 18–40 years) gave written consent to participate in a double-blind placebo controlled cross-over trial. Due to errors with data collection some data sets were unusable leaving 10 subjects for BZP drug comparisons; 11 subjects for TFMPP drug comparisons and 12 for the BZP+TFMPP drug comparison group. All groups were compared to the equivalent placebo counterparts.

2.2.2.1. Drugs

Benzylpiperazine hydrochloride (200 mg), trifluoromethylphenylpiperazine (50 mg for participants weighing < 60 kg or 60 mg if weighing > 60 kg) and a combination of benzylpiperazine and trifluoromethylphenylpiperazine (100 mg + 30 mg respectively) were given in this study on separate trial days. All capsules were manufactured by the School of Pharmacy, University of Auckland, New Zealand, using good manufacturing practice. Placebo capsules containing methylcellulose and were identical in appearance to the other capsules.

2.2.2.2. Procedure

The study used a double-blind cross-over procedure where participants were tested after taking each drug or placebo in a randomised order with a minimum of 7 days between sessions. Participants fasted for 12 hours before the trial and were asked to abstain from

alcohol or caffeine from the evening prior to testing. Participants were excluded on the day of testing if their urine tested positive for the presence of recreational drugs including marijuana, cocaine, amphetamines, opiates or benzodiazepines or pregnancy where appropriate. All capsules were taken with 250 mL of water 90 minutes before imaging to allow peak plasma concentrations of BZP and TFMPP (332). During this time, participants remained in the presence of researchers in a comfortable area with minimal stimulation.

fMRI was performed at the Centre for Advanced MRI, University of Auckland. The gambling (guessing) task was undertaken during imaging and presented on a screen located 3.5 metres from the participants, at the foot of the scanner and visible via a prism built into a head restraint, used to minimise movement during imaging.

Blood oxygen level dependant functional images were acquired using a T2*-weighted echo planar imaging (EPI) sequence with a 1.5T Siemens Magnetom Avanto scanner using the following parameters: TR 2500 ms, TE 50 ms, FOV 192 mm, in-plane voxel size 3.0 mm× 3.0 mm, flip angle 90°, 29 slices, slice thickness 4.0 mm no gap. On each trial day 176 volumes were collected for each participant for each run and two runs were completed at each visit with a 30 second break between each run. For anatomical reference, a high-resolution structural MPRAGE image was acquired for each at the end of the first session.

The gambling (guessing) task allowed the investigation of drug effects on stimuli at the anticipation stage of reward processing, where there was an uncertain outcome (Figure 17; highlighted in yellow for the purposes of this paper). Participants were instructed that when the reverse side of a card was presented on the screen with a question mark (“?”), they had to guess whether the suit of the card was black or red and respond using a hand held response box, used to minimise head movements and that money could be won or lost depending upon the outcome. After completing the trial, subjects expected to receive the monetary reward representing the net win from the task however, the outcomes were programmed to have a pre-determined valence and magnitude presentations. The pre-determined stimuli presented in each sequential run were randomised within E-prime. This ensured that participants did not suspect that there was a net outcome of $0 for each trial. Eight stimuli of large, little and no rewards and eight large, little and no monetary losses were presented within each run. Each session comprised two runs of 72 stimuli, each with a selection, anticipation and reward phase. Each selection phase was presented for 2000 msec, followed by an anticipatory phase of 1500 msec and the final outcome stage was split into two (the reveal and the final outcome), each lasting a duration of 750 msec. The inter-stimulus interval was set at a mean of 500 msec, which has been shown by Dale and colleagues to ensure efficiency of estimation (333, 334).

A neutral stimulus was given by presenting an “X” on the back of a card instead of a question mark, and instructing participants *not to play that particular game*. The stimuli would progress as usual with the computer selecting the colour but the result would be “no-change”. If participants did not respond to a selection stimulus the result was also shown as “no-change”.

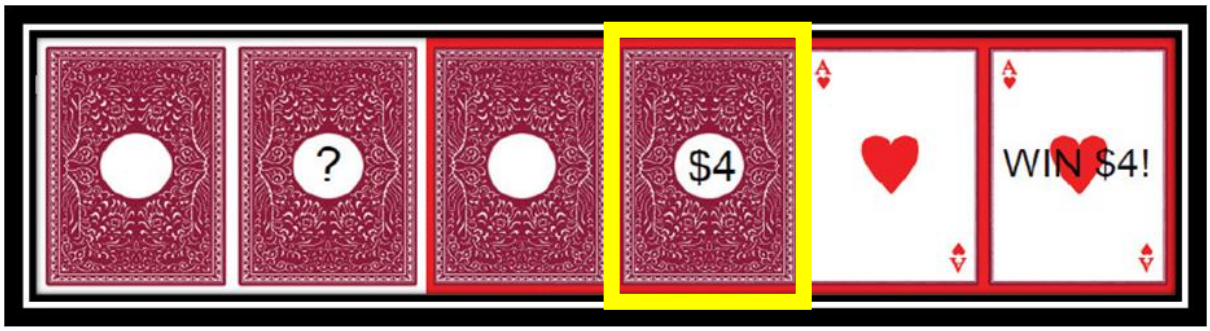

Figure 17: Progression of the gambling stimuli (from left to right), with the anticipation stage highlighted in yellow

Reaction times between drug groups were assessed in SPSS using a one way ANOVA.

Raw data were analysed using SPM8 (Wellcome Department of Cognitive Neurology, London, UK), implemented in MATLAB 7.8.0 (Mathworks, Sherborn, MA, USA). After being co-registered to the T1- weighted structural volume, the EPI images were normalised to a standard space (Montreal Neurological Institute [MNI] template). Images were spatially smoothed using an isotropic Gaussian kernel of 8 mm full-width at half-maximum (FWHM) in the x, y, and z axes.

Outliers due to movement or signal from pre-processed EPI files, using thresholds of 3 *SD* from the mean, 0.75 mm for translation and 0.02 radians rotation were removed from the data sets, using ART repair (335). Outliers were recorded to ensure fewer than 15% of scans were removed from each run (two runs per session). ART repair was also used to check for stimulus correlated motion at first level analysis. Top-down quality assurance of the ResMS, mask, beta, con and SPMT images were checked for abnormalities and artefact after both first and second level. An *F*-test across all conditions was performed by session to ensure each subject displayed activity in the visual cortex after first level analysis.

First level analysis allowed for the determination of activation during nine gambling conditions—win, lose or no-change (No-ch) (0c, 50c and $4), by constructing t-contrasts. No interaction contrasts were made at this stage to maintain maximum specificity at second level analysis.

The contrasts were subsequently used in a second level group comparison, using a flexible factorial design. The anticipation stimuli did not have any cue to predict reward or loss; therefore the reward and loss anticipation stimuli were combined and compared to the no-change anticipation stimuli in the second level contrasts. Event-related responses to anticipation of a large amount (anticipation of $4 minus No-ch anticipation of $4) and anticipation of a small amount (anticipation 50c minus No-ch anticipation 50c) were defined. Analysis was divided into three parts for each drug state: (1) BZP, (2) TFMPP, (3) BZP+TFMPP. For each drug state, inter-drug comparisons were individually made with placebo by constructing F interaction contrasts. Voxel-wise analysis was conducted using a significance threshold of $p < 0.001$ uncorrected and cluster threshold of ten voxels. Anatomical locations were derived from a customised script in SPM (336). Parameter estimates of the conditions at significant coordinates were plotted and interpreted as percentage BOLD signal change in reference to the whole brain mean to determine the direction of activation. Significant clusters were displayed on an average brain created from the structural files of participants.

2.2.3. Results

The aim of this study was to investigate whether there were any differences in reaction time and neural network activation involved in the reward circuitry after the administration of BZP, TFMPP and BZP+TFMPP, in comparison to placebo. An F-contrast was constructed to specifically examine this interaction whereby the trials for the winning and the losing where grouped and contrasted to the no-change stimuli.

Reaction times showed no significant differences between the individual drug states and placebo (BZP x placebo $p < 0.800$; TFMPP x placebo $p < 0.162$; BZP+TFMPP x placebo $p < 0.540$). After anticipation of a large amount ($4), when BZP was contrasted to placebo, there were significant clusters found in the right insula (Figure 19), right IFG (Figure 18), right precentral and left parahippocampal and mid-occipital regions. The cluster in the right IFG stemmed from a reduced activation in the BZP drug state in comparison to placebo. The right insula and occipital activation showed relative deactivations in comparison to placebo. In all the clusters of activation the BZP No-ch condition also showed greater activation than the corresponding placebo No-ch condition.

After TFMPP administration, relative to placebo, two clusters were induced in the right insula (Figure 21) and one in the right putamen (Figure 20) during anticipation of a large monetary amount. Analysis revealed that the cluster in the putamen was due to greater activation in the BZP drug state than placebo, and that in the insula BZP had a reduced activation relative to placebo.

When BZP and TFMPP were given in combination and contrasted to placebo, only one cluster was activated, which was in the rolandic operculum (Figure 22), showing a reduced activation in comparison to placebo.

Analysis of the anticipation phase in response to a smaller monetary amount (50c) was also conducted for the three drug states. After administration of BZP, small monetary outcomes (50c) resulted in six clusters: in the right cerebellum, left lingual gyrus, and four clusters in the bilateral middle temporal gyrus. While the two clusters in the right temporal gyrus stemmed from a reduction in activation by the BZP drug state, the two in the left temporal region was due to an increase in activation by BZP. The cerebellum also showed a reduction in activation after BZP, and the lingual cluster was induced by less deactivation by BZP.

The comparison of TFMPP and placebo also resulted in six clusters: one in the right mid-cingulate, right IFG, left precuneus, right pre and post central gyrus and the left medial superior frontal gyrus. The cluster in the cingulate and IFG were induced by greater activation in the TFMPP drug state than placebo.

The combined of dose of BZP and TFMPP relative to placebo, induced two clusters in the right hippocampus, one in the vermis and one in the right cerebellum, with reduced activation seen in all clusters by BZP+TFMPP.

Neural regions modulated by the drug states in comparison to placebo contrast for the reward anticipation phase. Drug (Ant$4- No-ch$4) – Placebo (Ant$4-No-ch$4)											
Anatomical region	***F* value**	**MNI coordinates**			**Directionality: Contrast estimates and SE**						
		x	**y**	**z**	**Drug No-ch $4**	**Drug Ant$4 (lose stimulus)**	**Drug Ant$4 (win stimulus)**	**Placebo No-ch $4**	**Placebo Ant$4 (lose stimulus)**	**Placebo Ant$4 (win stimulus)**	**SE**
BZP x placebo											
'Precentral_R'	18.19	56	2	28	2.1222	-0.5658	-0.3635	-0.6174	0.1203	0.7909	0.5724
'ParaHippocampal_L'	17.52	-20	-30	-12	1.6936	-0.7	-0.9654	-1.9835	0.1517	-0.1807	0.7164
'Frontal_Inf_Oper_R'	16.46	40	2	26	2.9826	0.1383	-0.0019	0.2997	0.9217	1.3152	0.6138
'Insula_R'	15.13	36	-20	12	1.7002	-2.2709	-0.6516	-0.5795	0.6908	-0.2867	0.6762
'Occipital_Mid_L'	15.08	-30	-78	30	1.3476	-0.7482	-0.763	-3.0388	-0.213	0.1461	0.8776
TFMPP x placebo											
'Putamen_R'	16.85	28	-8	4	-0.7946	0.2581	1.2976	1.6566	-0.0623	-0.6208	0.5838
'Insula_R'	13.56	35	21	-8	1.4689	2.3323	1.8265	-1.5957	3.1853	2.2541	0.6752
'Insula_R'	13.24	35	21	2	1.1058	1.7307	1.0885	-1.4374	3.2872	1.1604	0.6398
BZP+TFMPP x placebo											
'Rolandic_Oper_L'	16.57	-64	-10	12	1.0054	-1.5951	-2.3764	-0.3767	2.8698	0.2901	0.7327

Table 1: Reward anticipation activations to the $4 monetary amount for individual drug states in comparison to placebo

Note: All clusters are significant at $p < 0.001$ (uncorrected); cluster threshold of 10 voxels
The *F* value at the peak voxel within each cluster is reported.

Ant: anticipation; No-ch: no-change; SE: standard error

Neural regions modulated by the drug states in comparison to placebo contrast for the reward anticipation phase. Drug (Ant50c-No-ch50c) – placebo (Ant50c-No-ch50c)											
Anatomical region	***F* value**	**MNI coordinates**			**Directionality: Contrast estimates and SE**						
		x	**y**	**z**	**Drug No-ch 50c**	**Drug Ant 50c (lose stimulus)**	**Drug Ant 50c (win stimulus)**	**Placebo No-ch 50c**	**Placebo Ant 50c (lose stimulus)**	**Placebo Ant 50c (win stimulus)**	**SE**
BZP x placebo											
'Temporal_Mid_R'	21.94	64	-38	0	1.8649	-0.8616	-0.9921	-0.6183	3.1225	1.1608	0.7851
'Cerebelum_3_R'	15.68	8	-36	-16	-0.2361	-2.7124	-2.0299	-3.2873	0.326	1.0894	1.0253
'Lingual_L'	14.38	-6	-74	-2	-4.4456	-1.9005	-2.4242	-2.4046	-5.4228	-4.9133	0.8817
'Temporal_Mid_R'	14.01	56	-54	2	-0.0816	-1.9741	-2.2117	-1.4215	1.9898	-0.4611	0.7429
'Temporal_Mid_L'	13.69	-56	-26	-2	-1.1082	1.7609	1.2164	1.6745	0.2954	0.6834	0.6771
'Temporal_Mid_L'	12.76	-60	-20	-6	-0.586	2.0023	1.3299	2.0038	0.1963	0.9959	0.6788
TFMPP x placebo											
'Cingulum_Mid_R'	22.47	10	32	34	2.0278	0.0122	0.393	-0.9742	1.3868	0.1815	0.5371
'Frontal_Sup_Medial_L'	20.60	-14	38	29	0.2428	-0.5328	-2.0399	-0.9332	0.8514	0.7879	0.5137
'Precentral_R'	16.82	54	2	18	2.1713	0.4658	0.4104	-0.9818	0.2458	0.6774	0.5503
'Frontal_Inf_Tri_R'	15.94	53	36	8	-0.3309	1.4171	0.4892	1.3061	-0.7033	-1.2323	0.6332
'Postcentral_R'	15.43	57	-21	38	1.0885	-0.9743	-0.6652	-1.1421	1.0254	0.0523	0.6492
'Precuneus_L'	14.84	-10	-64	34	0.9128	-1.4492	-1.5235	-1.8161	-0.9519	-0.3415	0.6582
BZP+TFMPP x placebo											
'Hippocampus_R'	17.54	28	-34	-2	1.3709	-1.2407	0.0643	-0.8455	0.6253	1.235	0.5617
'Hippocampus_R'	17.20	28	-38	6	-0.9061	-2.8444	-3.2021	-1.39	0.1123	0.0059	0.5416
'Vermis_6'	13.81	0	-66	-24	0.0873	-0.5163	-1.8486	-1.5098	0.3953	1.0404	0.5928
'Cerebelum_3_R'	12.63	9	-42	-16	1.2955	-0.821	-0.8609	-1.5515	0.6994	0.6958	0.7774

Table 2: Reward anticipation activations to the 50c monetary amount for individual drug states in comparison to placebo

Note: All clusters are significant at $p < 0.001$ (uncorrected); cluster threshold of 10 voxels
The *F* value at the peak voxel within each cluster is reported.

Ant: anticipation; No-ch: no-change; SE: standard error

Neural regions modulated by the contrast during the reward anticipation phase: BZP x placebo

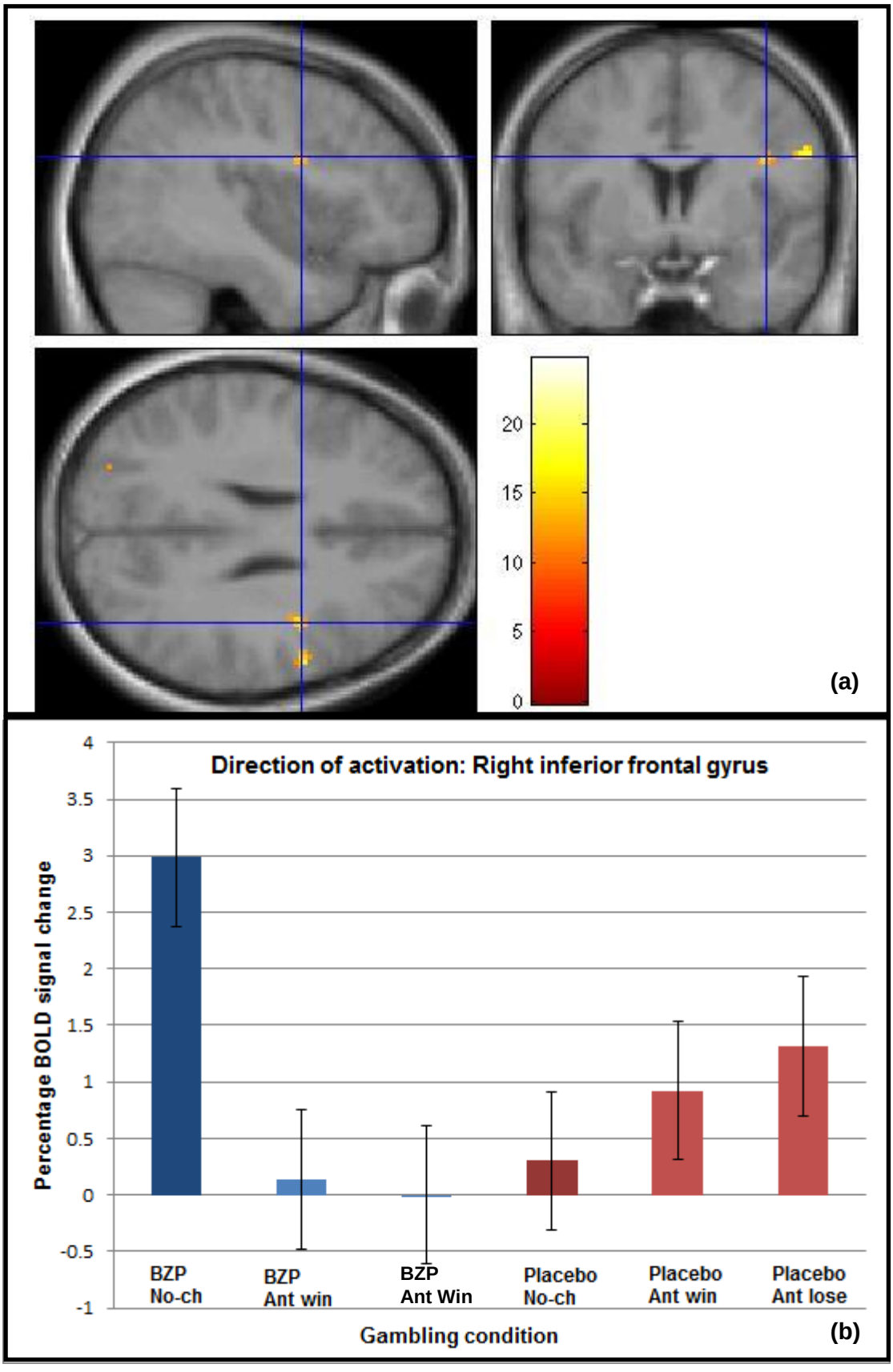

Figure 18: Activations associated with the anticipation of $4, when BZP is contrasted to placebo (Drug (Ant $4 -No-ch $4) – placebo (Ant $4 -No-ch $4)) $p < 0.001$ uncorrected; cluster threshold > 10 voxels. (a) Activation in the right inferior frontal gyrus and (b) Plot of parameter estimates, indicating the direction of activation in the right inferior frontal gyrus.

Neural regions modulated by the contrast during the reward anticipation phase: BZP x placebo

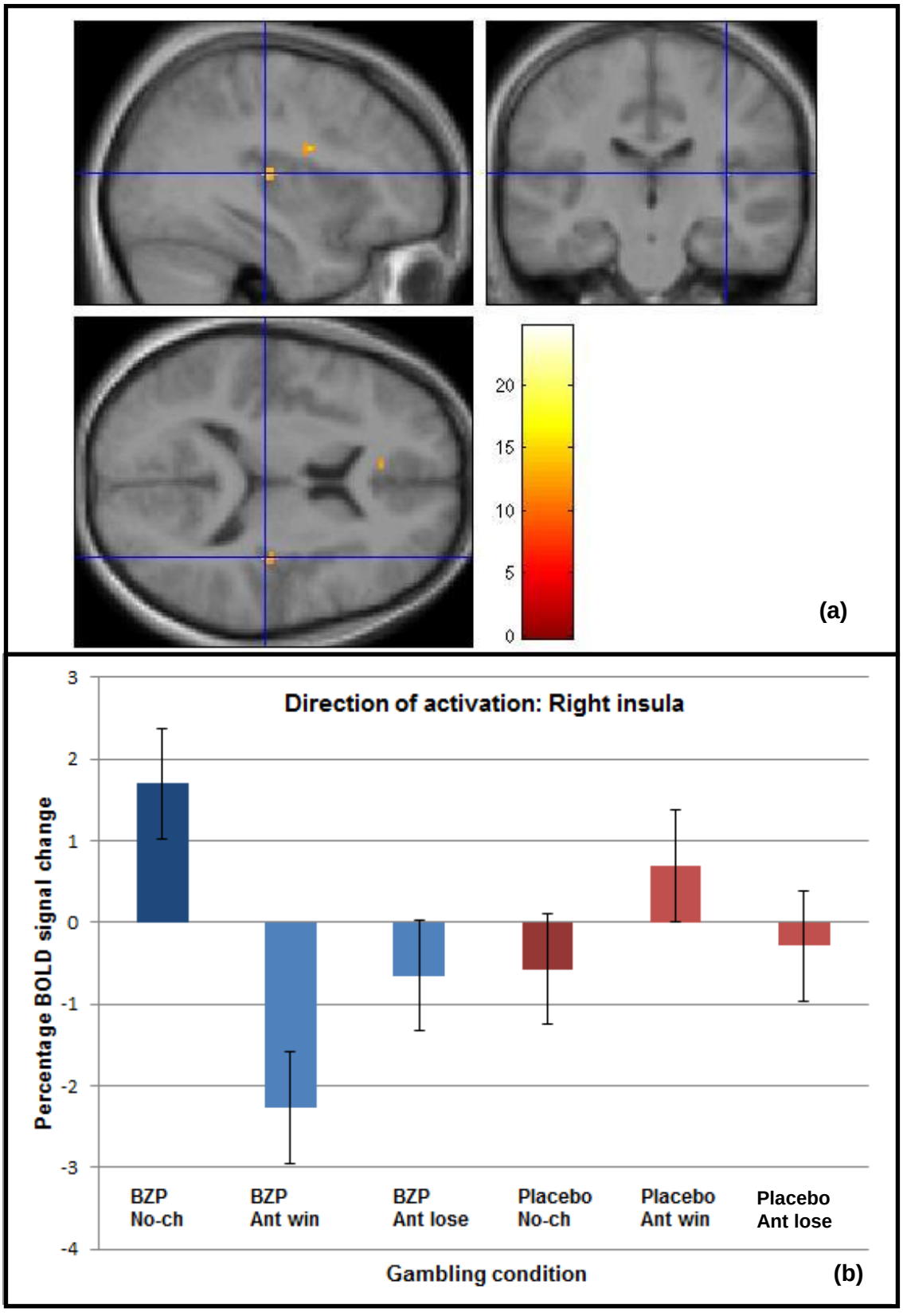

Figure 19: Activations associated with the anticipation of $4, when BZP is contrasted to placebo (Drug (Ant $4 -No-ch $4) – Placebo (Ant $4 -No-ch $4)) p <0.001 uncorrected; cluster threshold > 10 voxels. (a) Activation in the right insula and (b) Plot of parameter estimates, indicating the direction of activation in the right insula

Neural regions modulated by the contrast during the reward anticipation phase: TFMPP x placebo

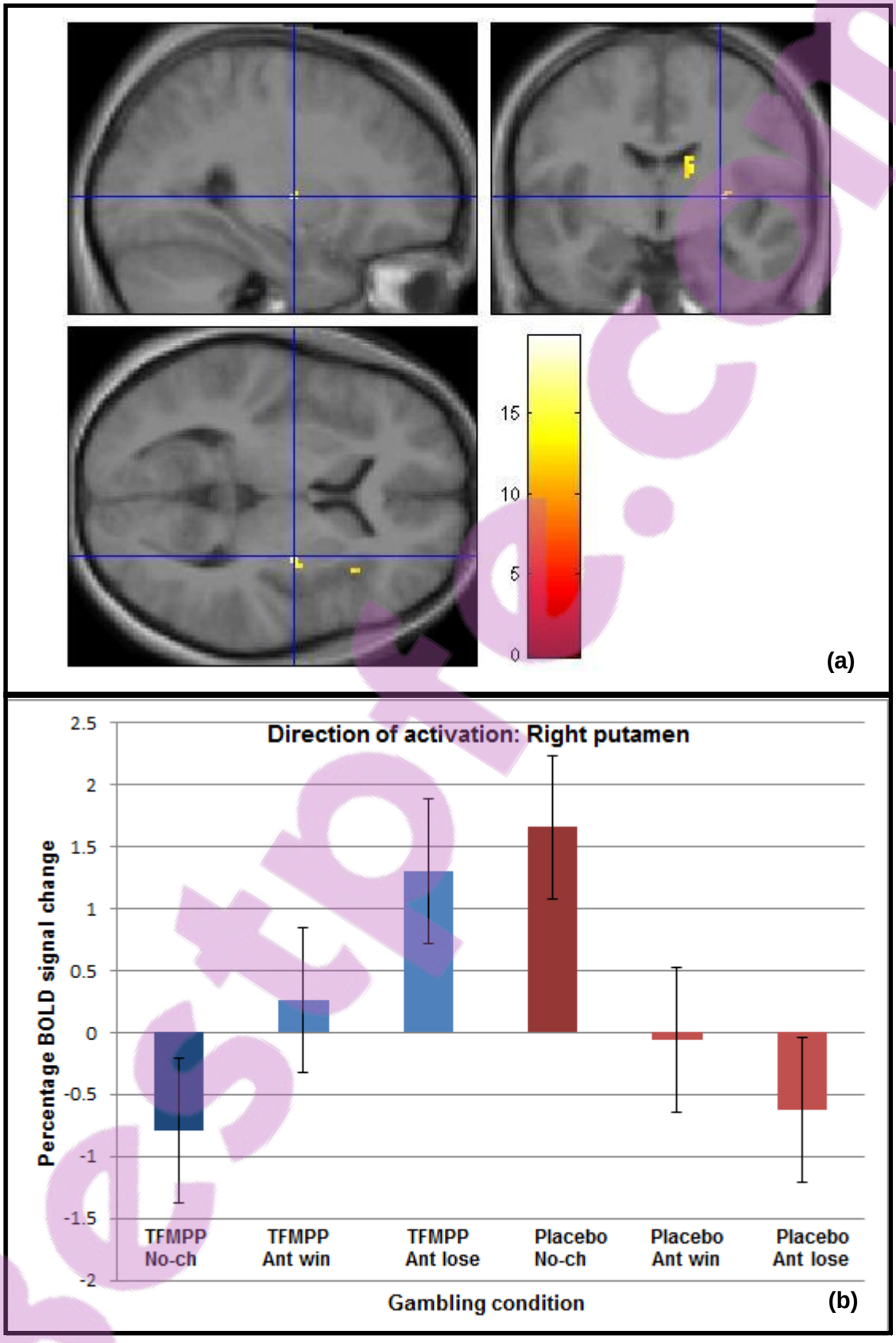

Figure 20: Activations associated with the anticipation of $4, when TFMPP is contrasted to placebo (Drug (Ant $4 -No-ch $4) – placebo (Ant $4 -No-ch $4)) *p* <0.001 uncorrected; cluster threshold > 10 voxels. (a) Activation in the right putamen and (b) Plot of parameter estimates, indicating the direction of activation in the right putamen

Neural regions modulated by the contrast during the reward anticipation phase: TFMPP x placebo

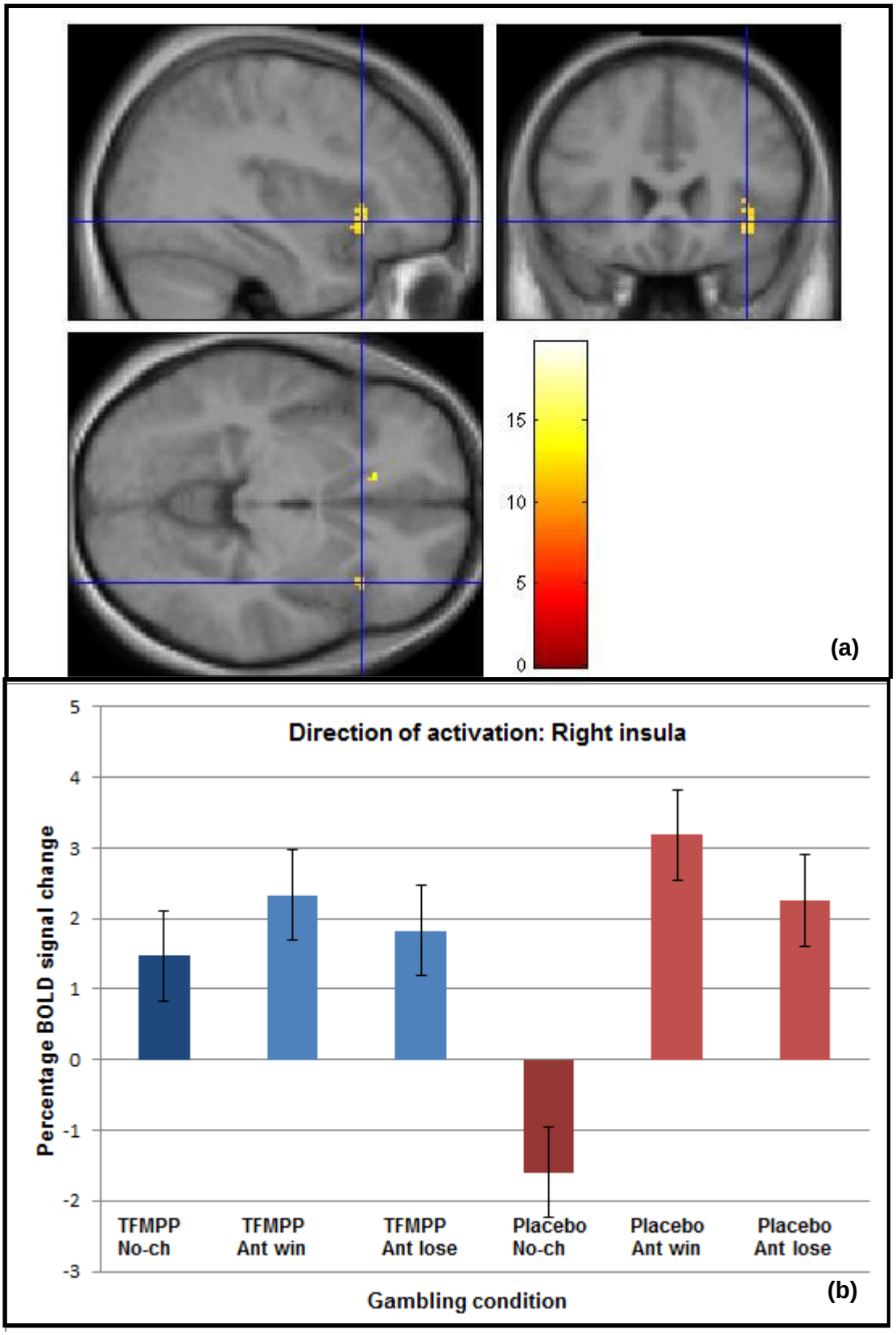

Figure 21: Activations associated with the anticipation of $4, when TFMPP is contrasted to placebo (Drug (Ant $4 -No-ch $4) – placebo (Ant $4 -No-ch $4)) *p* <0.001 uncorrected; cluster threshold > 10 voxels. (a) Activation in the right insula and (b) Plot of parameter estimates, indicating the direction of activation in the right insula

Neural regions modulated by the contrast during the reward anticipation phase: BZP+TFMPP x placebo

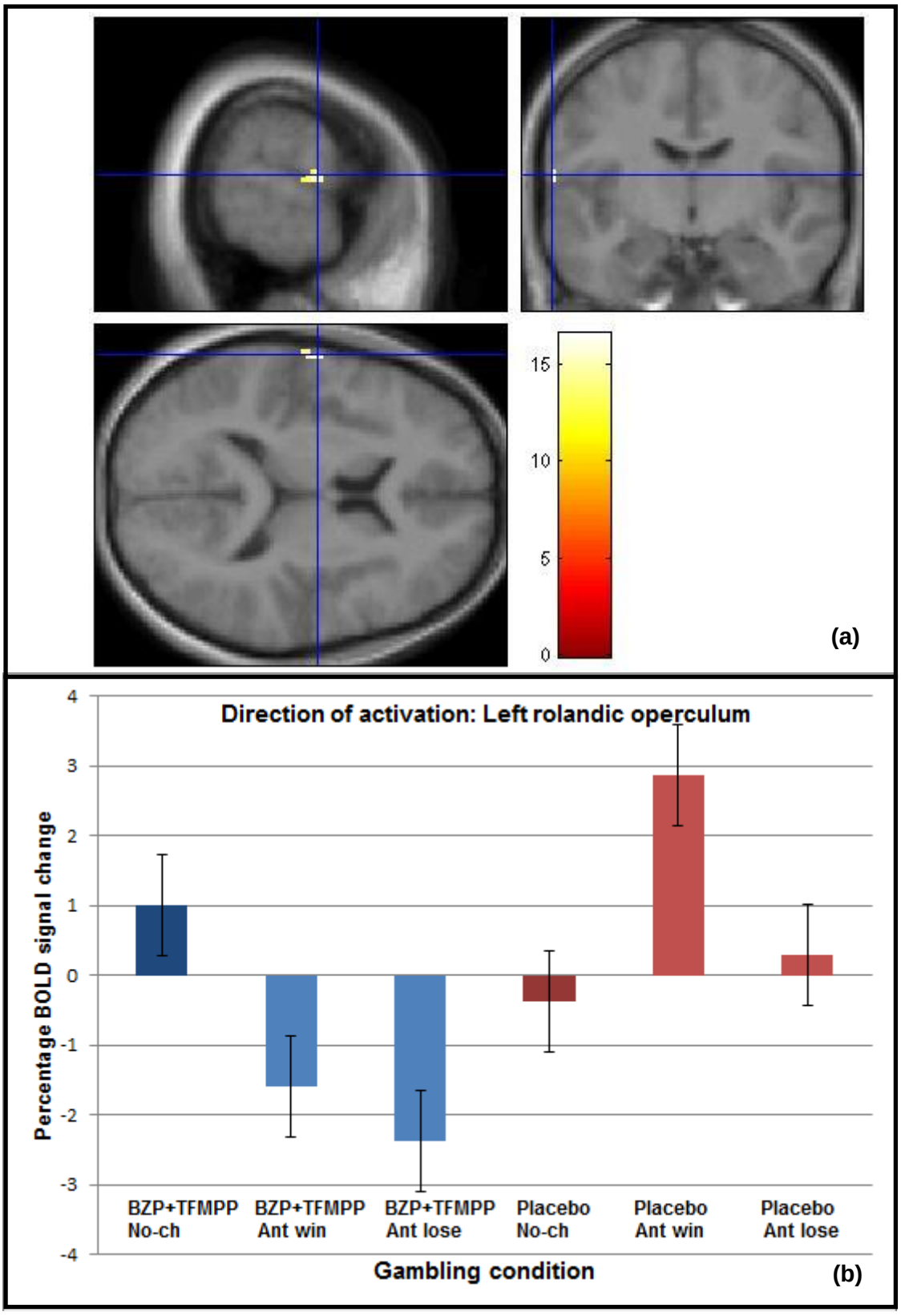

Figure 22 Activations associated with the anticipation of $4, when BZP+TFMPP is contrasted to placebo (Drug (Ant $4 -No-ch $4) –placebo (Ant $4 -No-ch $4)) *p* <0.001 uncorrected; cluster threshold > 10 voxels. (a) Activation in left rolandic operculum and (b) Plot of parameter estimates, indicating the direction of activation in the left rolandic operculum

2.2.4. Discussion

This is the first study to our knowledge to investigate the effects of BZP and TFMPP alone and in combination, relative to placebo, on anticipation of reward using fMRI. The anticipatory phase of reward is important because it has been associated with positive expectancies, and motivates an individual to behave in a way to increase the likelihood of receiving expected rewards (107, 108). We utilised an event-related gambling (guessing) task to minimise the expectancy normally observed using blocked trials (291).

Unlike prior studies using a monetary incentive delay task, participants were unable to predict the outcome of consecutive stimuli (i.e. whether the outcome would be a positive or negative valence) (110, 158). They completed a task where they were required to guess whether the suit of a presented card was going to be red or black and after their choice was made, the monetary amount that they could win or lose was presented. This was known as the anticipation stage. Participants were told that at the end of the trial they would be awarded the sum of their winnings to ensure their participation. However, the cumulative total was not displayed to ensure participants were unaware of their current monetary position and that their baseline for evaluating anticipation did not change during the progression of sessions or subsequent trial days.

In a parallel study our laboratory has found the outcome/receipt phase of reward was also modulated after giving these drugs (337). To compare the relative difference between BZP, TFMPP, a combination of BZP+TFMPP and placebo, we used an interaction contrast which incorporated a no-change anticipation condition to minimise baseline activation due to direct and indirect pharmacological effects.

In previous studies recreational drugs have been shown to activate regions similar to those activated after monetary reward (134, 338). Whilst some report that reward consumption and the anticipation stage of processing induce similar areas of regional activation others argue there are distinct differences (109, 110).

When there is an element of risk involved in the decision making, regional activation has been reported in the striatum, insula, IFG and the OFC (129, 198, 339, 340). Moreover, the presentation of stimuli with an unknown outcome provokes risk related regional activation (341). Activation in response to uncertainty has been reported in the amygdala, OFC, IFG, and insula (210, 211). Although risk was not a behavioural parameter that could be measured in this study, the regions activated are areas that have been associated with risk in prior studies (129, 198).

Reaction time was assessed between drug groups and placebo, with no significant differences found, however, relative to placebo, BZP induced clusters in the IFG, the right insula, and the occipital region in response to a large monetary amount ($4). The IFG showed reduced activation relative to placebo, and BZP caused a deactivation in the insula and occipital region in comparison to placebo.

BZP administration caused decreased activation in comparison to placebo. Studies using the Iowa gambling task, have reported activation of the IFG was more pronounced in risk aversive participants suggesting that this region is involved in the inhibition of risky choices (342). Previous studies have shown that amphetamine modulates the BOLD response to monetary incentives during a gambling task, whereby there is a reduction in amplitude, but an increase in duration of the signal (142), thought to be due to its effects on DA receptors. Schmitz et al. (343) showed that amphetamine blunts the phasic release of DA by acting as an agonist at D_2 autoreceptors while DA levels are elevated, and increases the tonic DA levels by blocking re-uptake in the ventral striatum. BZP also predominantly effects dopaminergic circuitry and its subjective effects have been reported to be similar to other psychostimulants (2). We hypothesise that the BZP-induced reduction in activity in the IFG is the result of its effects on DA and suggests there is a reduction in inhibitory control associated with risky decisions.

The insula has been associated with response to both receipt (159, 198, 344) and the expectation of aversive stimuli (345, 346) in addition to associations with uncertainty and risky decision making (342). There was a decrease in activation of the insula during anticipation after the administration of BZP, suggesting that participants are less responsive to the anticipation of uncertain risk.

It has been suggested that the functional difference between the IFG and the insula, is that the IFG is mainly associated with behavioural inhibition (239). In the study by d'Acremont and colleagues (342), the BOLD response was activated to a greater extent by risk aversion in an ambiguous situations, whereas in a separate study by Preuschoff et al. (344), there was activation of the insula, but no change in the IFG. The difference between the two studies is that the latter study did not involve a choice; the selection was out of the participants' control, and hence confirms the hypothesis that the IFG is involved in the inhibition of responses to risky or uncertain stimuli.

In a parallel investigation, we investigated BZP's effect on the outcome phase within the same gambling (guessing) task (337). There was an increase in activation of the insula following aversive stimuli. We believe that this is in line with the proposal by Ikemoto and Panksepp (157) of "safety seeking" where aversive events elicit striatal activity due to the

anticipation of a positive outcome. In a separate study the administration of amphetamine led to an increase in activation following monetary losses (142). The authors hypothesise that this increase is reflective of amphetamine's ability to maintain motivation even after aversive events. The results in the current study concur with this hypothesis, that when a risky or uncertain event was presented, neither the insula nor IFG were recruited to the extent of the placebo condition. This is in accordance with the theory that the positive feelings of arousal induced by the administration BZP, led to a reduction in neural responses to risk, and hence may promote risk taking (142, 198). It must be noted that the task given to participants to complete in this study was unable to measure behavioural responses to risk. Although the areas activated have been previously associated with risk, future trials should be conducted with a modified task, similar to that of the Iowa Gambling task, where the subject can choose a more risky but profitable stimulus or that of lesser value reward with less risk associated to further investigate whether the administration of BZP does alter these specific behavioural responses (347).

In summary after giving BZP, the participants were less likely to utilise the IFG and insula, in situations of uncertain risk. We propose that this could be due to the effect of BZP on dopaminergic transmission, promoting positive arousal. This also supports suggestions that BZP acts in a similar manner to other stimulants, such as amphetamine, and calls for direct comparisons should to be made between BZP and amphetamine.

After giving TFMPP, the putamen showed greater activation, and the insula showed less activation than placebo. TFMPP is an agonist at 5-HT_{2C} receptors; 5-HT_{2C} receptor agonists reduce the firing rate of mesolimbic DA neurons originating in the VTA which subsequently reduces DA release in terminal regions (41, 42).

We hypothesised that TFMPP would reduce activation in the striatum/putamen due to its effects on dopaminergic transmission, and its effects on 5-HT, as 5-HT has been associated with increased aversion (194, 195). The striatum has a role in the processing of uncertainty and risky decision making. In research by Kuhnen and Knutson (129), the NAcc was activated while making risky choices. The dorsal striatum has been reported to be activated when subjects had to choose between two stimuli compared to a no-choice stimulus (348). This led authors to propose that dorsal striatum may have a role in stimulus-response reward learning. Similarly this was observed in a study where the anterior caudate was differentially activated for active versus observational learning (349). The putamen has also been found to be associated with the detection of disgust, which is an emotional response to guiding avoidance (350). If these results can be extended to our

study, perhaps the administration of a 5-HT agonist caused an increase in the putamen due to an emotional response of increased avoidance/ aversion.

In summary after taking TFMPP the participants had a modulation of processing of aversion. They were less likely to recruit the insula but instead recruited the putamen in the face of an uncertain stimulus. It is possible that the reduction in the insula is due to the effects of TFMPP on dopaminergic transmission via 5-HT_{2C} receptors; however the increased activation of the putamen could reflect the serotonergic modulation of aversion, which in these results led to an increase in the emotional response to uncertainty.

When BZP and TFMPP were given as a combination the only area with significant neural changes was the rolandic operculum, where the drug state caused a reduction in activation. This region has been associated with gustatory reward (351, 352), language (353) and teeth clenching or grinding (354, 355). Furthermore, the rolandic operculum was found to be activated after the administration of levodopa (356). In addition, the activation induced by individual drugs was not shown when the two were given together. We believe this and the activation of the rolandic operculum to be due to BZP and TFMPP's opposing effects on DA: whilst BZP increases extracellular DA, conversely TFMPP may reduce DA via its effects on 5-HT_{2C} receptors.

2.2.4.1. Magnitude

After the presentation of 50c in this task, there were activations in all of the drug states relative to placebo, however, the locations were different to those activated after the presentation of a higher monetary value.

After giving BZP, the 50c anticipatory stimulus evoked activity in the middle temporal gyri, cerebellum and lingual gyrus. The clusters of activation were mixed in their direction, with activation in the right versus the left middle temporal gyri showing different patterns. The regions activated do not seem to be reflective of a change in processing of uncertainty, although, MPH has been shown in a previous study to induce activation in the middle temporal gyrus and hippocampus during a decision making task with differing uncertainty (357). The temporal activation possibly could be due to a change in the processing of learning caused by the increase in DA after the administration of BZP.

Increases in endogenous DA have been reported to modulate stages of learning and memory (358, 359). Adcock and colleagues (331), suggest that dopaminergic pathways are involved in the anticipation of reward and modulate declarative memory formation. In addition, a reduction in the expression of DA receptors and lesions in the midbrain of

rodents resulted in impaired learning (360, 361), whereas stimulation of dopaminergic receptors in the medial temporal lobes improved learning (362).

If the clusters of activation in the temporal gyri were due to a change modulation of learning, induced by the increase in extracellular DA from the administration, we would have expected to see the same pattern after the anticipation of larger stimuli, which we did not. Therefore, we hypothesise that the lack of regional activation seen in response to a 50c stimulus may be that the stimulus was insufficient to cause a differential response in the participants, and therefore there were no differences seen between the placebo and BZP condition in the networks involved in processing of uncertainty/risk.

TFMPP in comparison to placebo activated areas in the cingulate, medial superior frontal gyrus and the IFG. The clusters in the medial superior frontal gyrus showed less activity in the TFMPP drug state compared to placebo, but in the cingulate and IFG there was increased activation. This is reflective of the results from monetary stimuli of a greater magnitude, in that, there appears to be an increased response in areas affected by the anticipation of loss and uncertainty. These results further add to the hypothesis that serotonergic modulation by TFMPP increases the response to loss and/or uncertainty, due to the effect of 5-HT on aversive responding (198, 199).

When BZP and TFMPP are combined, a reduction in activation was found in the hippocampus, cerebellum and vermis. Interestingly, when combined, the increased activation evoked by BZP and TFMPP alone is diminished, and there is a lack of activation in regions associated with the anticipation. This may reflect the opposing effects of DA and 5-HT on reward processing, that has been suggested by Daw and colleagues (194).

Recent studies by Rowe (363) and D'Esposito (364) have identified that preparation for motor responses can cause activations in non-motor regions, including the PFC. However, when a subject has been instructed to make a button press rather than given the choice of a button press, these activations have not been observed. It has been hypothesised therefore that these activations are related to preparation to make a decision that does not necessarily involve an actual movement. In this study we found activations when the drug state and placebo were compared in the PFC. Therefore, despite each contrast involving a comparison to the baseline neutral condition, there is the possibility that these activations could stem from differences in preparation for motor responses.

2.2.5. Conclusion

In conclusion BZP, TFMPP and a combination of the two induce discrete differences, in comparison to placebo, at the anticipatory stage of a gambling (guessing) task. We

propose that the effects of BZP and TFMPP on the dopaminergic and serotonergic circuitry, respectively, reflect the change in activation in regions that have been previously associated with risk and uncertainty. The dopaminergic modulation by BZP appears to increase positive arousal and subsequently, reduces responses in these areas. TFMPP also appears to change neural responses to uncertainty, possibly increasing emotional responses, which may be due to 5-HTs effects on aversion.

2.3. Gambling Task: Comparing BZP, TFMPP and Combination for the Reward Outcome Stage of Reward Processing

Acute effects of the synthetic drugs benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP) alone and in combination using functional magnetic resonance imaging (fMRI) to investigate their influence on response to reward value.

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2.3.1. Introduction

BZP and TFMPP are the two major constituents in a group of relatively new synthetic drugs that have been marketed worldwide as safe and legal alternatives to illicit recreational drugs, MDMA or MA, since the late 1990's. Despite the reported popularity of these drugs there is a lack of knowledge about their acute and long term effects in humans.

Studies investigating the pharmacological effects of BZP have been carried out in rats and monkeys (19) where it has been shown to affect predominately dopaminergic neurons (14, 16, 17, 19), with additional but considerably less effect on both serotonergic (19) and noradrenergic circuitry (20). In contrast the effects of TFMPP are mainly serotonergic, with its stimulus effects mediated by 5-HT_{1B} and 5-HT_{2C} receptors (27). TFMPP also has indirect effects on both DA and NA (32-34). When BZP and TFMPP are combined and given to rats in a 1:1 ratio at low doses (3 mg/kg, i.v.) the effects were reported to mimic those of low dose MDMA (three fold less potent), while higher doses (10 mg/kg, i.v.) exhibited a synergistic effect on DA release (19).

Fantegrossi and colleagues (37) found that the combination of BZP+TFMPP (1:1) was a less effective reinforcer than BZP alone in adult rhesus monkeys, which may be due to the agonist effects of TFMPP on 5-HT_{2C} receptors, which are known to reduce the firing within the DA mesolimbic system involved in reinforcement (41, 42). In this sense the effects of the combination of BZP and TFMPP were suggested to be comparable to the serotonergic modulation by cocaine (37).

In humans the subjective effects of BZP were reportedly similar to other psychostimulants (2), such as amphetamine, in contrast, TFMPP was reported to be similar to fenfluramine and mCPP (21).

Reward plays an important role in motivating behaviour. The mesolimbic dopaminergic neurons originate in the VTA and project forward into the NAcc, within the ventral striatum,

and then into the prefrontal, frontal and anterior cingulate cortices (the limbic forebrain) (329, 365). In animal and human studies it is well recognised that structures including the vmPFC mPFC, amygdala, striatum, and dopaminergic midbrain act as an integrated highly interconnected network (366). Drug-induced reward is mediated via the mesolimbic system and demonstrated by the increased firing of dopaminergic neurons in the VTA followed by DA release in the NAcc shell and limbic forebrain. This release of DA is suggested to result in feelings of euphoria induced by recreational drugs (367).

Rewarding stimuli have been described as those that evoke positive reinforcement toward a behaviour or action. Consumption or receipt of a reward produces pleasurable consequences that initiate a learning process for the future receipt of reward. On the other hand, punishment or a negative reinforcer cause avoidance behaviour (107).

It has been reported that there is a network of multiple cortico-striatal loops modulated by DA in the midbrain. This region has been shown to increase firing in response to rewards that are unexpected and stimuli that are predictive or associated with rewarding outcomes (138). Within this network the striatum is thought to play a role in processing reward-related information (138, 158), and reward outcomes (110). Research has indicated that the dorsal striatum is an integral part of the circuit involved in decision making, specifically in different aspects of motivational and learning behaviour supporting goal-directed actions. Furthermore, the dorsal striatum has a distinct role from the ventral striatum within contingent learning, that is, the learning of actions and their associated reward consequences (368, 369). On the other hand, the ventral striatum is involved in a more passive form of learning (366).

Berridge and colleagues (109, 370) stated that there are distinct differences in valenced and non-valenced aspects of reward, and more specifically within the response of dopaminergic neurons in the VTA and NAcc. Studies investigating reward detection and the valence (winning or losing) of the stimuli implicate the involvement of both the ventral and dorsal striatum (113, 114).

The effect of negatively valenced stimuli i.e. punishment or aversive stimuli have also shown specific patterns of activation in the brain. Several studies have shown that there is an increase in DA release via phasic bursts in response to aversive stimuli in the dorsal and ventral striatum. It has been suggested that dopaminergic pathways determine the motivational salience of environmental stimuli (205). Ikemoto and Panskepp suggested this DA release is a behavioural response to “safety seeking” (157). When an aversive event is presented, this elicits DA release in the striatum in order to maintain motivation. This proposal is also used to explain the increased activation observed after monetary losses

following amphetamine administration (142). The authors suggest that this may maintain motivation even following events considered aversive.

Electrophysiological studies in monkeys who had undergone classical conditioning reported that after learning a stimulus predicts the availability of reward there is a subsequent burst of firing in DA neurons. Upon receipt of the reward the firing of DA neurons reflects the *difference* between the expected and actual reward. When the reward was greater than expected there was an increase in firing, however, when a reward was less than predicted, firing was inhibited. Furthermore, if the predicted reward was then received then there was little change in firing. This effect is known as the reward prediction error (213, 214).

In addition to dopaminergic modulation of reward and punishment, 5-HT has also been reported to modulate the response to aversion, with changes in activation shown after the administration of SSRIs (201) (203).

The magnitude of reward can also influence processing following the administration of recreational drugs. Delgado and colleagues (215) examined both the valence and magnitude of reward and found that the dorsal striatum was an integral component of both aspects of reward feedback. In addition, a greater magnitude or loss reflected a greater magnitude of subsequent activation. Furthermore, Adcock and colleagues (331) reported increased activation in the VTA in response to larger potential rewards.

In humans, similar findings have been made using imagining techniques. For example, those addicted to cocaine were given an i.v. infusion of cocaine (134) and healthy controls given low-doses of morphine (338), both displayed activation in similar areas to those observed following monetary reward.

Recreational drugs, including BZP and/or TFMPP are consumed for their rewarding effects. BZP's subjective effects have been reported to include "euphoria", "increased sociability" and "drug-liking", whilst TFMPP produced feelings of "stimulated" and "high", and induced effects if "dysphoria" and "confusion/bewilderment" (21). However, there has been no research about their effects on the reward system and what their motivational effects might be.

In order to further investigate the effects of BZP and TFMPP both alone and in combination on both pleasurable and aversive stimuli. We developed a custom-designed gambling (guessing) task which was designed to examine the anticipation and outcome stages of reward processing. We used fMRI to determine the effects that each drug had on regional responses to the outcome phase of the task relative to placebo. We specifically

investigated the effects on valence i.e. monetary reward and loss and whether the magnitude of the reward or loss affects activation. Behavioural responses in the form of reaction time were also assessed.

2.3.2. Materials and Methods

Approval for this research was granted by the Northern X Regional Ethics Committee of New Zealand (Ethics approval number NTX/07/08/078). The trial recruited healthy participants; subjects were excluded if they had a history of mental illness, cardiac disease, head trauma, epilepsy, endocrine disorders, or were pregnant or breastfeeding. Participants attended an initial screening session, and completed a custom questionnaire to detail their medication history, recreational drug, alcohol and cigarette use, sleeping patterns and stress levels. This was to ensure that participants were not drug naive, or current or past heavy users of recreational drugs and not dependent on any substances. Thirteen non-smoking healthy participants (seven female and six male; aged 18–40 years) were recruited to participate in a double-blind placebo controlled cross-over trial. Due to E-prime data file errors in some of the data sets were rendered unusable. This resulted in 10 subjects in the BZP drug comparison, 11 subjects in the TFMPP drug comparison and 12 subjects in the BZP+TFMPP drug comparison group. All drug comparisons were made to the equivalent placebo counterparts.

2.3.2.1. Drugs

Benzylpiperazine hydrochloride (200 mg), trifluoromethylphenylpiperazine (50 mg for participants weighing < 60kg or 60 mg if participants weighed > 60kg) and benzylpiperazine plus trifluoromethylphenylpiperazine (100 mg + 30 mg, respectively) were given to the participants on separate trial days. All of the capsules were manufactured by the School of Pharmacy, University of Auckland, New Zealand, using good manufacturing practice. Placebo capsules identical in appearance containing methylcellulose were also manufactured.

2.3.2.2. Procedure

The study used a double-blind cross-over procedure where participants were tested after taking each drug or placebo in a randomised order with a minimum of 7 days between sessions. Participants fasted for 12 hours before the trial and were asked to abstain from alcohol or caffeine from the evening prior to testing. Participants were excluded on the day of testing if their urine tested positive for the presence of recreational drugs including marijuana, cocaine, amphetamines, opiates or benzodiazepines or pregnancy where appropriate. All capsules were taken with 250 mL of water 90 minutes before imaging to

allow peak plasma concentrations of BZP and TFMPP (332). During this time, participants remained in the presence of researchers in a comfortable area with minimal stimulation.

fMRI was performed at the Centre for Advanced MRI, University of Auckland. The gambling (guessing) task was undertaken during imaging and presented on a screen located 3.5 metres from the participants, at the foot of the scanner and visible via a prism built into a head restraint, used to minimise movement during imaging.

Blood oxygen level dependant functional images were acquired using a T2*-weighted echo planar imaging (EPI) sequence with a 1.5T Siemens Magnetom Avanto scanner using the following parameters: TR 2500 ms, TE 50 ms, FOV 192 mm, in-plane voxel size 3.0 mm× 3.0 mm, flip angle 90°, 29 slices, slice thickness 4.0 mm no gap. On each trial day 176 volumes were collected for each participant for each run and two runs were completed at each visit with a 30 second break between each run. For anatomical reference, a high-resolution structural MPRAGE image was acquired for each at the end of the first session.

The gambling (guessing) task allowed the investigation of drug effects on stimuli at the anticipation stage of reward processing, where there was an uncertain outcome (Figure 23; highlighted in yellow for the purposes of this paper). Participants were instructed that when the reverse side of a card was presented on the screen with a question mark (“?”), they had to guess whether the suit of the card was black or red and respond using a hand held response box, used to minimise head movements and that money could be won or lost depending upon the outcome. After completing the trial, subjects expected to receive the monetary reward representing the net win from the task however, the outcomes were programmed to have a pre-determined valence and magnitude presentations. The pre-determined stimuli presented in each sequential run were randomised within E-prime. This ensured that participants did not suspect that there was a net outcome of $0 for each trial. Eight stimuli of large, little and no rewards and eight large, little and no monetary losses were presented within each run. Each session comprised two runs of 72 stimuli, each with a selection, anticipation and reward phase. Each selection phase was presented for 2000 msec , followed by an anticipatory phase of 1500 msec and the final outcome stage was split into two (the reveal and the final outcome), each lasting a duration of 750 msec. The inter-stimulus interval was set at a mean of 500 msec, which has been shown by Dale and colleagues to ensure efficiency of estimation (333, 334).

A neutral stimulus was given by presenting an “X” on the back of a card instead of a question mark, and instructing participants *not to play that particular game*. The stimuli would progress as usual with the computer selecting the colour but the result would be “no-

change". If participants did not respond to a selection stimulus the result was also shown as "no-change".

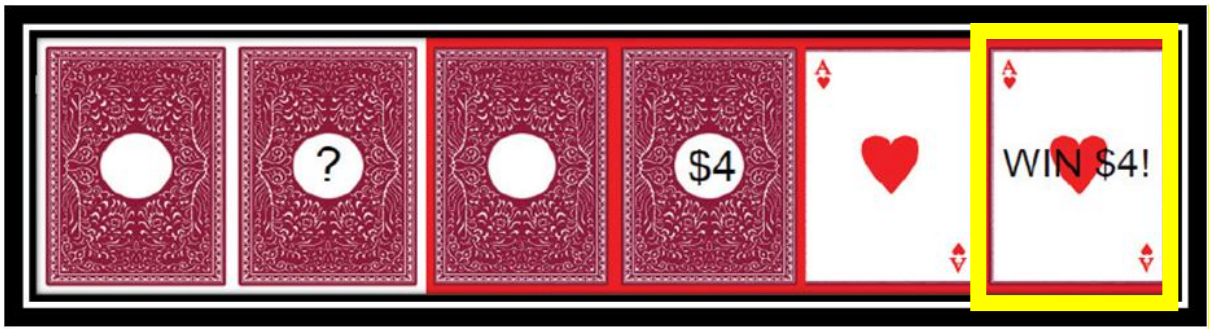

Figure 23: Progression of the stimuli (from left to right)
Includes the selection (?), anticipation (monetary amount revealed) and reward stages (suit colour and reward/punishment revealed; highlighted in yellow)

The raw imaging data were analysed using SPM8 (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB 7.8.0 (Mathworks, Sherborn, MA, USA). After being co-registered to the T1- weighted structural volume, the EPI images were normalised to a standard space (Montreal Neurological Institute [MNI] template). Images were spatially smoothed using an isotropic Gaussian kernel of 8 mm full-width at half-maximum (FWHM) in the x, y, and z axes.

Outliers due to movement or signal from pre-processed EPI files, using thresholds of 3 *SD* from the mean, 0.75 mm for translation and 0.02 radians rotation were removed from the data sets, using ART repair (335). Outliers were recorded to ensure fewer than 15% of scans were removed from each run (two runs per session). ART repair was also used to check for stimulus correlated motion at first level analysis. Top-down quality assurance of the ResMS, mask, beta, con and SPMT images were checked for abnormalities and artefact after both first and second level. An *F*-test across all conditions was performed by session to ensure each subject displayed activity in the visual cortex after first level analysis.

First level analysis allowed for the determination of activation during nine gambling conditions—win, lose or No-ch (0c, 50c and $4), by constructing t-contrasts. No interaction contrasts were made at this stage to maintain maximum specificity at second level analysis.

T-contrasts were subsequently used in a second level group comparison. Event-related responses to win and loss of a large amount (win/loss of $4 minus no-change win/loss of $4) and win or loss of a small amount (win/loss 50c minus no-change win/loss 50c) were contrasted to their no-change counterparts. Analysis was divided into three parts for each

drug state: (1) BZP, (2) TFMPP, (3) BZP+TFMPP. For each drug state, inter-drug comparisons were individually made with placebo by constructing F interaction contrasts. Voxel-wise analysis was conducted using a significance threshold of $p < 0.001$ uncorrected and cluster threshold of twenty voxels. Anatomical locations were derived from a customised script in SPM (336). Parameter estimates of the conditions at significant coordinates were plotted and interpreted as percentage BOLD signal change in reference to the whole brain mean to determine the direction of activation. Significant clusters were displayed on an average brain created from the structural files of participants.

2.3.3. Results

The aim of this study was to investigate whether administration of BZP, TFMPP or a combination of BZP+TFMPP affected regional activation during the outcome stage (reward or loss) of a guessing task compared to placebo treatment. To investigate regional activation an F-contrast was constructed to specifically examine the interaction between drug states and placebo during the outcome stage of the task (monetary reward and loss in comparison to the No-ch $4 outcome). The data was thresholded at $p < 0.001$ with clusters containing a minimum of 20 voxels. At this threshold we observed no significant activations in the Win $4 condition minus No-ch $4 for any of the drug states in comparison to placebo, however, there were significant differences in the lose $4 condition when compared to placebo.

After taking BZP, clusters were observed in the bilateral mid-cingulate (Figure 25), left inferior and superior frontal gyri, right insula (Figure 24), bilateral rolandic operculum, three clusters in the right precuneus, left postcentral and bilateral precentral gyri, cerebellum (see Table 3). The right mid-cingulate, IFG, insula, post and precentral gyri and two of the clusters in the precuneus, all showed an increase in activation by the BZP drug state compared with placebo. The remaining clusters showed a lesser deactivation by BZP.

TFMPP induced activation in the right thalamus (Figure 26) and right lingual gyrus (Figure 27), both regions showed a lesser deactivation by TFMPP than placebo.

After giving the combination of BZP and TFMPP, lose $4 induced clusters in 15 regions including three clusters in the left mid-cingulate (Figure 28), one in the left ACC, one in the right insula, one in the left superior frontal gyrus and two in the right precuneus. In addition, there was activation in the lingual, occipital, paracentral, superior motor area and temporal gyri, bilateral parietal, and post central gyri and the vermis. All regions showed an increase in activation by the BZP+TFMPP drug state.

2.3.3.1. Magnitude

We also wanted to investigate whether the magnitude of losses or wins would alter regional activation, winning 50c induced activation following BZP and the combination of BZP and TFMPP, but not following TFMPP (Table 4).

BZP induced three clusters of activation, two in the right superior frontal gyrus and one in the left medial superior frontal gyrus. Where there is a reduction in activation in the BZP versus the placebo drug state.

BZP+TFMPP induced two clusters, one in the right hippocampus and one in the right mid-frontal gyrus. The hippocampal activation stemmed from a reduced deactivation, and the cluster in the mid-frontal gyrus from a reduced activation.

After losing 50c there was only activation in the BZP+TFMPP combination condition and not when the drugs were given individually. BZP+TFMPP evoked six regions of activation, including the mid-temporal gyrus, hippocampus, superior and mid-frontal gyri, lingual and occipital gyri.

Reward outcome Drug (Lose$4- No-ch$4) – Placebo (Lose$4-No-ch$4)									
Anatomical region	***F* value**	**MNI coordinates**			**Directionality: Contrast estimates and SE**				
		x	**y**	**z**	**Drug No-ch$4**	**Drug Lose $4**	**Placebo No-ch$4**	**Placebo Lose $4**	**SE**
BZP x placebo									
'Postcentral_R'	32.95	66	-6	30	-1.0683	1.7924	1.1007	-1.4624	0.5652
'Precentral_R'	11.72	54	-4	40	-0.7099	1.0111	0.8042	-0.665	0.5573
'Cingulum_Mid_R	25.78	8	2	36	-1.0339	1.2409	0.3605	-1.4165	0.4773
'Precuneus_L'	23.71	-6	-64	42	-0.8973	0.5838	0.0341	-3.4403	0.6087
'Precuneus_L'	15.92	-4	-54	36	-1.7159	-1.6093	1.1907	-3.4355	0.7097
'Frontal_Sup_L'	19.44	-22	38	32	-1.3483	-0.1776	0.5839	-1.5233	0.4447
'Parietal_Inf_L'	18.59	-42	-52	52	-1.2899	0.8995	1.9679	-2.2099	0.8834
'Frontal_Sup_L'	17.71	-18	52	34	-2.2695	-0.3414	-0.1387	-2.5184	0.6124
'Parietal_Inf_L'	17.66	-52	-55	42	-0.2523	1.1986	3.419	-0.1306	0.7117
'Angular_L'	16.49	-45	-62	33	-1.0577	1.4652	0.294	-2.9871	0.8606
'Frontal_Inf_Orb_L'	17.10	-44	44	-4.7	-0.1727	1.1829	2.1349	-0.9682	0.6451
'Insula_R'	16.71	34	-24	12	-0.834	1.0023	-0.3932	-2.1563	0.5268
'Rolandic_Oper_R'	13.38	40	-20	16	-2.3535	-0.8093	-1.0334	-3.5514	0.6643
'Precentral_L'	16.25	-44	8	36	0.3841	1.72	0.9845	-1.3445	0.5438
'Cingulum_Mid_L'	16.07	-8	-44	52	-2.4769	-1.1627	-1.4838	-3.6896	0.5252
'Cerebelum_4_5_R'	15.50	24.7	-35.3	-25.3	-1.1399	1.3422	-0.4306	-2.0127	0.6175
'Cuneus_L'	14.60	-10	-74	34	-1.6986	-0.0021	0.2299	-1.898	0.6099
'Precuneus_L'	14.30	-16	-70	30	-2.4219	-0.3725	-0.8685	-2.9454	0.646
'Rolandic_Oper_L'	14.10	-56	-9	12	-1.2417	0.1584	0.2124	-1.8401	0.5462
TFMPP x placebo									
'Lingual_R'	22.71	8	-62	8	0.3603	1.8885	1.8816	0.2965	0.4518
'Thalamus_R'	15.02	12	-10	16	-2.5381	-1.022	1.3534	-1.4324	0.6257
BZP+TFMPP x placebo									
'Occipital_Inf_L'	23.60	-38	-82	-12	1.6189	3.9807	5.6849	2.9323	0.6195
'Postcentral_L'	22.64	-50	-8	16	-1.6879	0.3945	1.2689	-0.4674	0.4724
'Precuneus_R'	21.48	2	-51	55	-2.7428	-0.9391	-1.8978	-4.9464	0.6162
'Cingulum_Mid_L'	21.31	-10	-8	50	-1.5588	0.4587	0.5721	-0.8418	0.4375
'Postcentral_R'	20.20	65	-5	32	-1.6323	-0.6035	0.9927	-1.7615	0.4953
'Postcentral_R'	16.95	62	-10	20	-2.1196	-0.4863	0.132	-1.6829	0.4929
'Cingulum_Ant_L'	20.16	-8	46	6	-1.2116	-0.1246	1.0232	-1.5285	0.477
'Parietal_Sup_R'	19.95	20	-50	64	-3.9789	-2.2404	-2.5348	-4.7822	0.5252
'Vermis_6'	17.96	-4	-62	-24	-1.1292	0.2337	1.0029	-1.4006	0.523
'Temporal_Mid_L'	17.68	-62	-12	-4	-0.8018	0.544	0.6186	-2.1651	0.578
'Temporal_Sup_L	16.65	-63	-8	7	-2.9377	-1.713	-0.3393	-4.0174	0.7072
'Angular_R'	17.51	54	-62	30	-1.3339	-0.2177	1.3088	-2.7144	0.7228
'Lingual_R'	16.46	8	-62	8	-3.6108	-2.3134	-0.9284	-4.6115	0.7224
'Lingual_R'	13.72	6	-64	0	-4.1046	-1.9812	-0.8598	-2.7758	0.6417
'Lingual_R'	11.54	6	-74	-2	-4.6235	-1.3679	-2.4176	-3.6871	0.7841
'Paracentral_Lob_L'	16.33	-12	-14	72	-2.4991	-1.6829	-1.0346	-5.1491	0.718
'Parietal_Sup_L'	5.82	-23	-54	60	-2.5067	-0.2678	-1.3506	-2.6179	0.5188
'Insula_R'	13.68	38	-12	16	-1.5047	-0.3578	-0.6281	-2.5128	0.4824
'Supp_Motor_R'	15.20	8	2	50	-0.8622	0.5109	0.7747	-1.1273	0.4945
'Cingulum_Mid_L'	14.82	-5	10	38	-1.9541	0.2664	0.2546	-0.6744	0.4814
'Cingulum_Mid_L'	14.01	-6	1	37	-1.7851	-0.1428	-0.2167	-1.6629	0.4856
'Temporal_Mid_L'	14.09	-58	-38	4	-0.5136	0.7867	1.8814	-0.846	0.6314
'Postcentral_R'	14.00	32	-39	64	-3.8923	-2.0438	-1.7842	-3.2851	0.5269

Table 3: Reward interaction after win/loss of $4 in comparison to placebo
Note: All clusters are significant at $p < 0.001$ (uncorrected); cluster threshold of 20 voxels
The *F* value at the peak voxel within each cluster is reported.
No-ch: no-change; SE: standard error

Reward outcome

Drug (Win50c- Noch50c) – Placebo(Win50c-Noch50c

Anatomical region	F value	MNI Coordinates			Directionality: Contrast estimates and SE				
		x	y	z	Drug Noch 50c	Drug Win 50c	Placebo No-ch50c	Placebo Win 50c	SE
BZP x placebo									
'Frontal_Sup_R'	22.59	14	24	60	2.1652	-0.9089	-1.9503	-0.248	0.6062
'Frontal_Sup_R'	17.70	24	58	14	1.3338	-0.741	-1.4844	0.5746	0.5928
'Frontal_Sup_ Medial_L'	15.79	-6	28	42	1.3171	-0.0025	-0.7995	1.8148	0.5972
TFMPP x placebo									
No suprathreshold clusters > 20 voxels									
BZP+TFMPPx placebo									
'Hippocampus_R'	17.24	26	-36	4	-2.1977	-0.4339	0.5659	-1.0139	0.4497
'Frontal_Mid_R'	14.07	44	40	16	1.3602	-0.2314	0.3624	2.3969	0.5398

Drug (Lose50c- Noch50c) – Placebo(Lose50c-Noch50c

Anatomical region	F value	MNI Coordinates			Directionality: Contrast estimates and SE				
		x	y	z	Drug No-ch50c	Drug Lose 50c	Placebo No-ch50c	Placebo Lose 50c	SE
BZP x placebo									
No suprathreshold clusters > 20 voxels									
TFMPP x placebo									
No suprathreshold clusters > 20 voxels									
BZP+TFMPP x placebo									
'Temporal_Mid_R'	24.21	50	-50	4	-0.1984	0.7831	1.8033	-1.2091	0.462
'Hippocampus_R'	22.70	28	-34	0	-1.5488	1.0281	0.4384	-0.5829	0.4298
'Frontal_Sup_L'	19.64	-18	54	8	-0.9759	-0.0807	0.2165	-1.8414	0.3793
'Frontal_Mid_L'	13.94	-28	56	12	-1.537	-0.2347	0.1429	-1.7713	0.4903
'Lingual_R'	17.71	29	-68	0	-0.6543	1.5713	0.408	-0.7923	0.4633
'Occipital_Mid_L'	16.23	-26	-64	40	0.113	2.2107	2.4164	0.5416	0.5612

Table 4: Reward interaction after the win/loss of 50 cents in comparison to placebo

Note: All clusters are significant at $p < 0.001$ (uncorrected); cluster threshold of 20 voxels
The *F* value at the peak voxel within each cluster is reported.

No-ch: no-change; SE: standard error

Neural regions modulated by the contrast during a $4 monetary loss: BZP x placebo

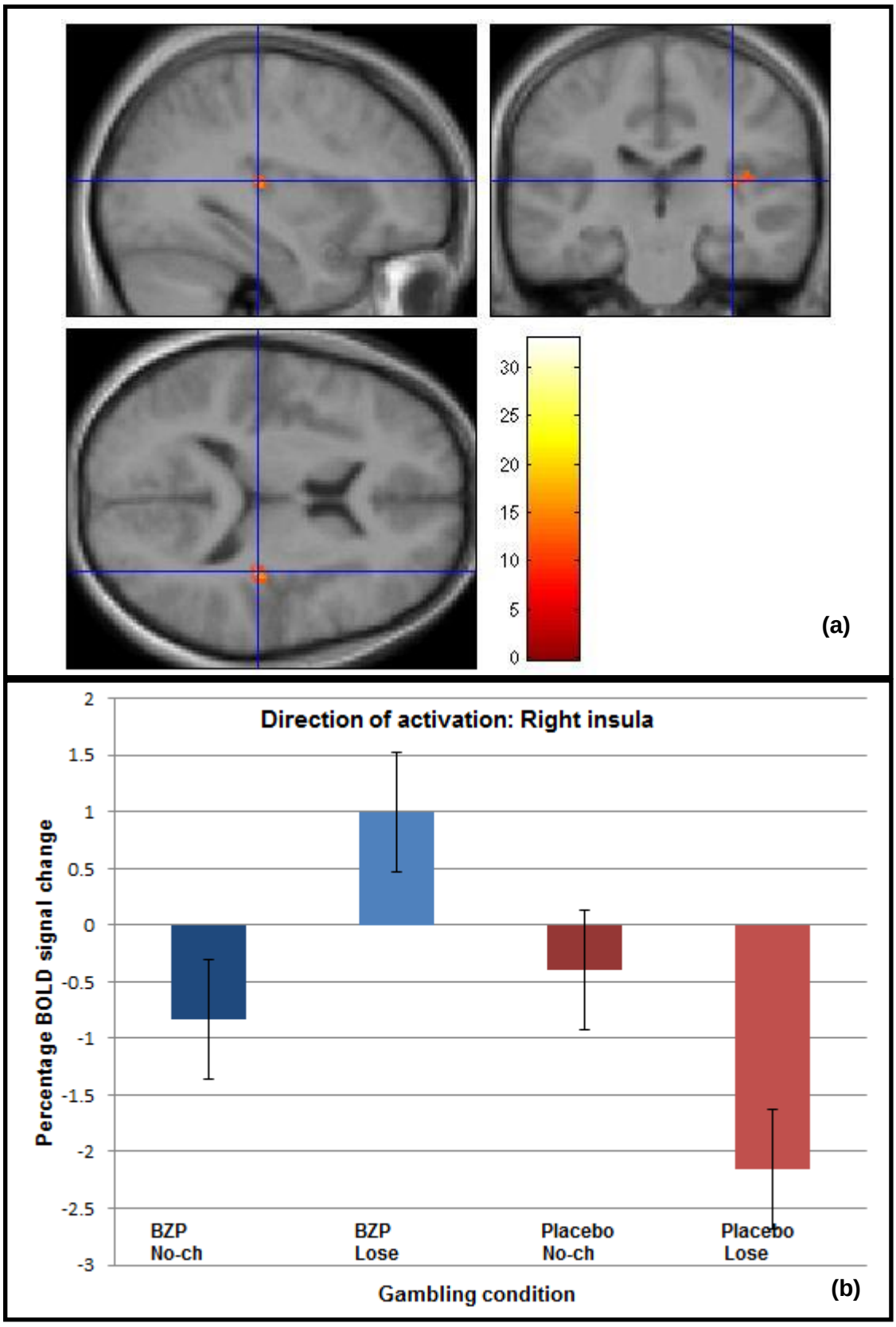

Figure 24: Activations associated with a monetary loss of $4, when BZP is contrasted to placebo (Drug (Lose $4 -No-ch $4) – placebo (Lose$4 -No-ch $4)) *p* <0.001 uncorrected; cluster threshold > 20 voxels. (a) Activation in the right insula and (b) Plot of parameter estimates, indicating the direction of activation in the right insula

Neural regions modulated by the contrast during a $4 monetary loss: BZP x placebo

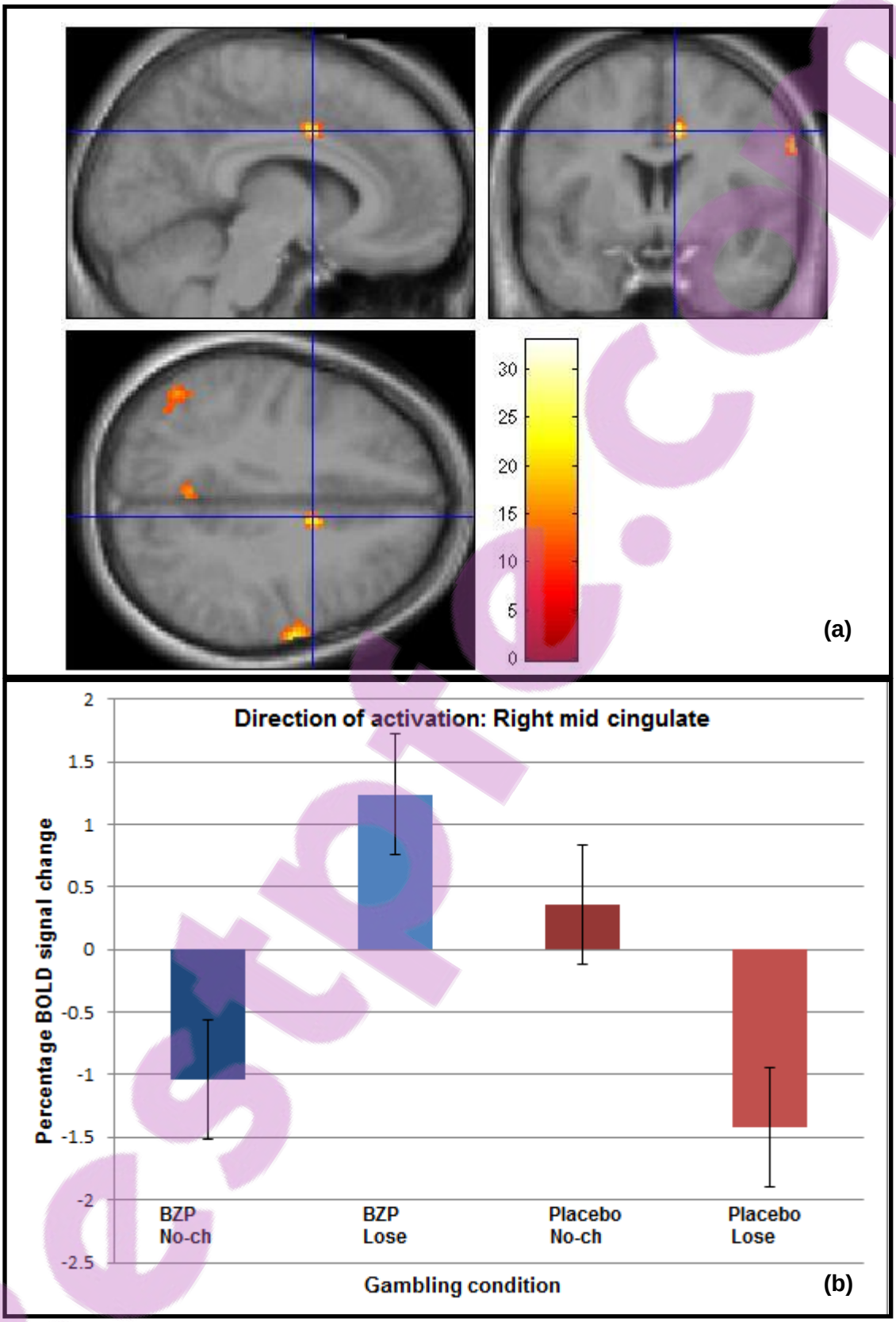

Figure 25: Activations associated with a monetary loss of $4, when BZP is contrasted to placebo (Drug (Lose $4 -No-ch $4) – placebo (Lose$4 -No-ch $4)) *p* <0.001 uncorrected; cluster threshold > 20 voxels. (a) Activation in the right mid-cingulate and (b) Plot of parameter estimates, indicating the direction of activation in the right mid-cingulate

Neural regions modulated by the contrast during a $4 monetary loss: TFMPP x placebo

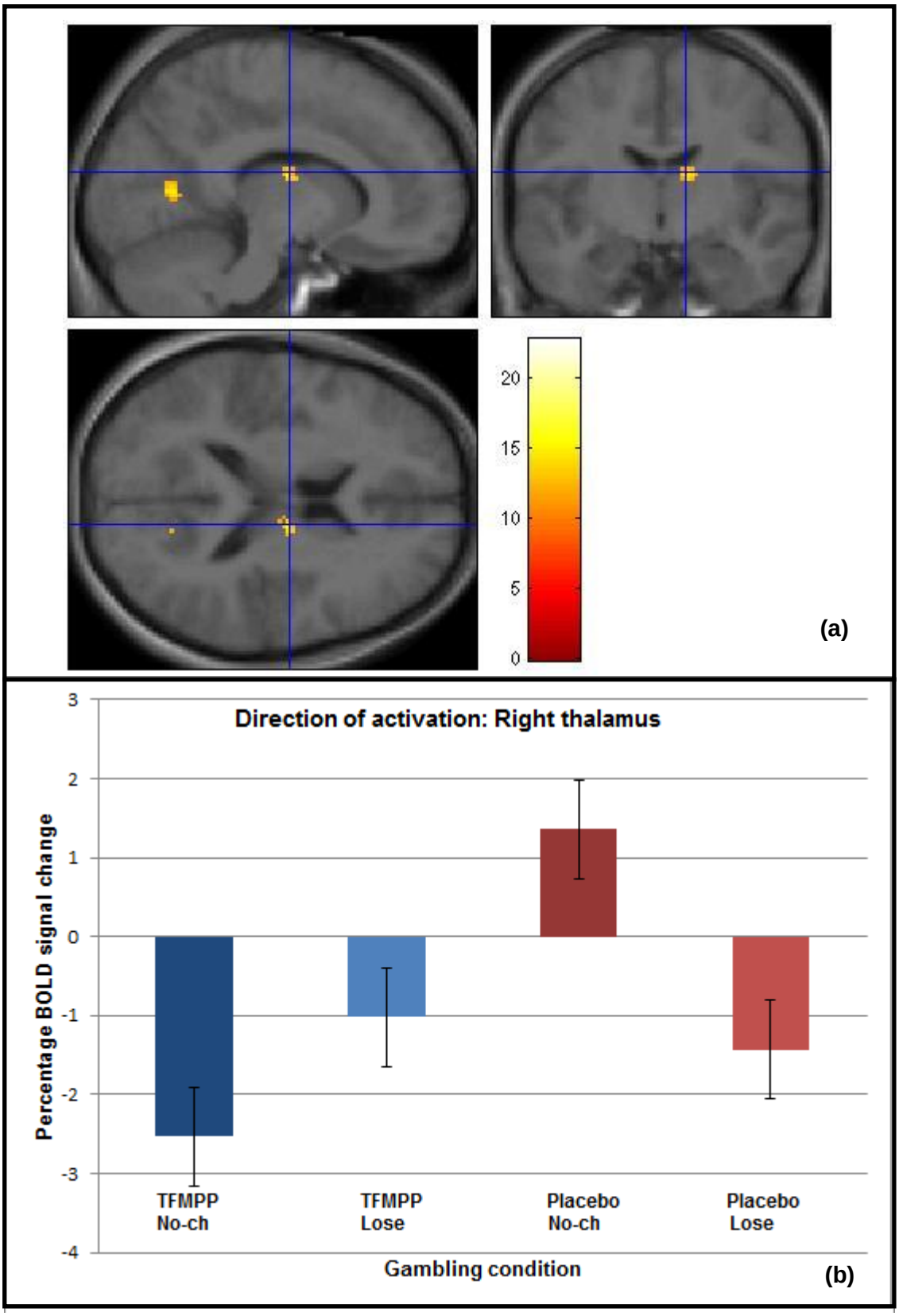

Figure 26: Activations associated with a monetary loss of $4, when TFMPP is contrasted to placebo (Drug (Lose $4 -No-ch $4) – placebo (Lose$4 -No-ch $4)) *p* <0.001 uncorrected; cluster threshold > 20 voxels. (a) Activation in the right thalamus and (b) Plot of parameter estimates, indicating the direction of activation in the right thalamus

Neural regions modulated by the contrast during a $4 monetary loss: TFMPP x placebo

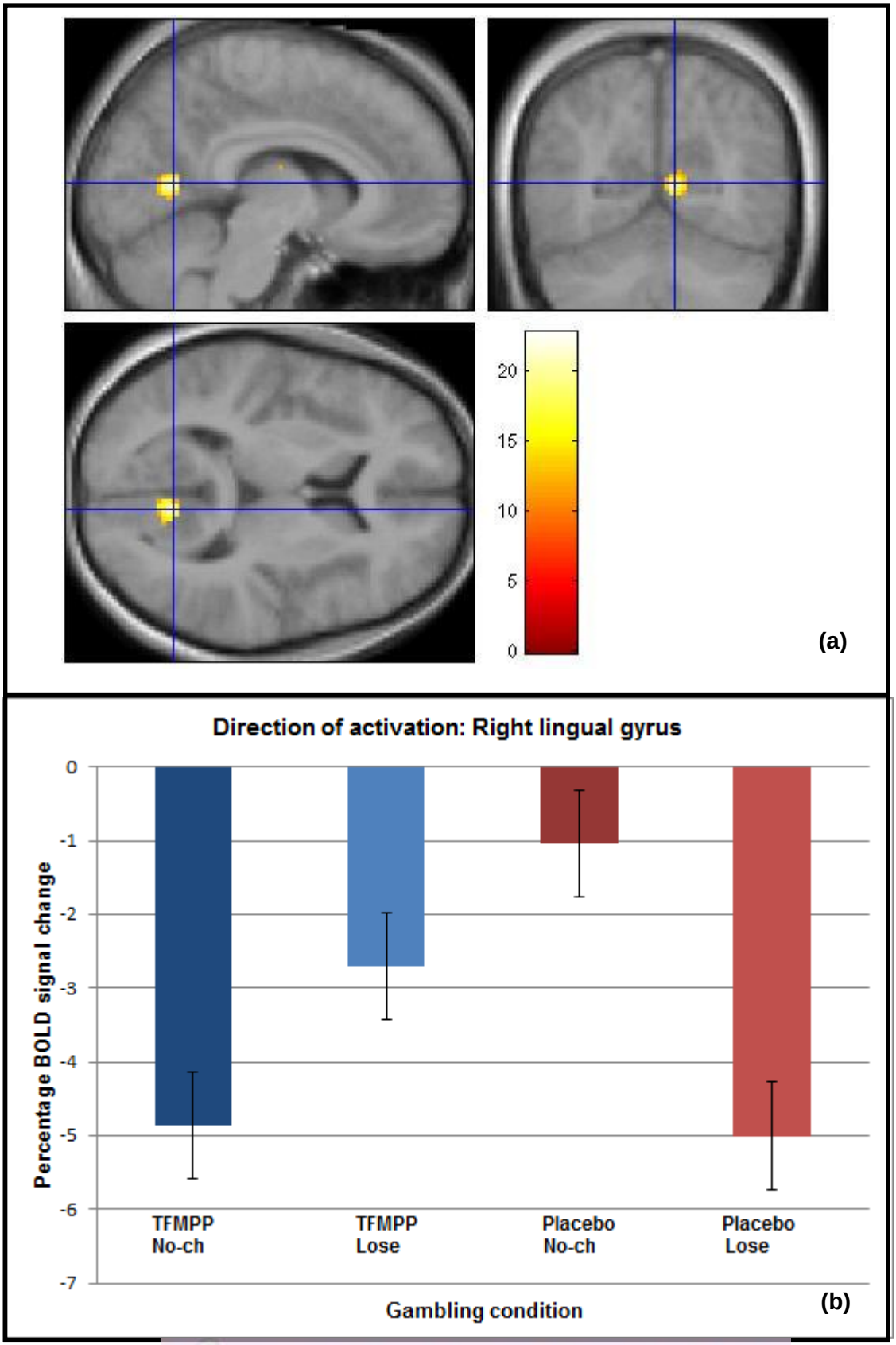

Figure 27: Activations associated with a monetary loss of $4, when TFMPP is contrasted to placebo (Drug (Lose $4 -No-ch $4) – placebo (Lose $4 -No-ch $4)) p <0.001 uncorrected; cluster threshold > 20 voxels. (a) Activation in the right lingual gyrus and (b) Plot of parameter estimates, indicating the direction of activation in the right lingual gyrus

Neural regions modulated by the contrast during a $4 monetary loss: BZP+TFMPP x placebo

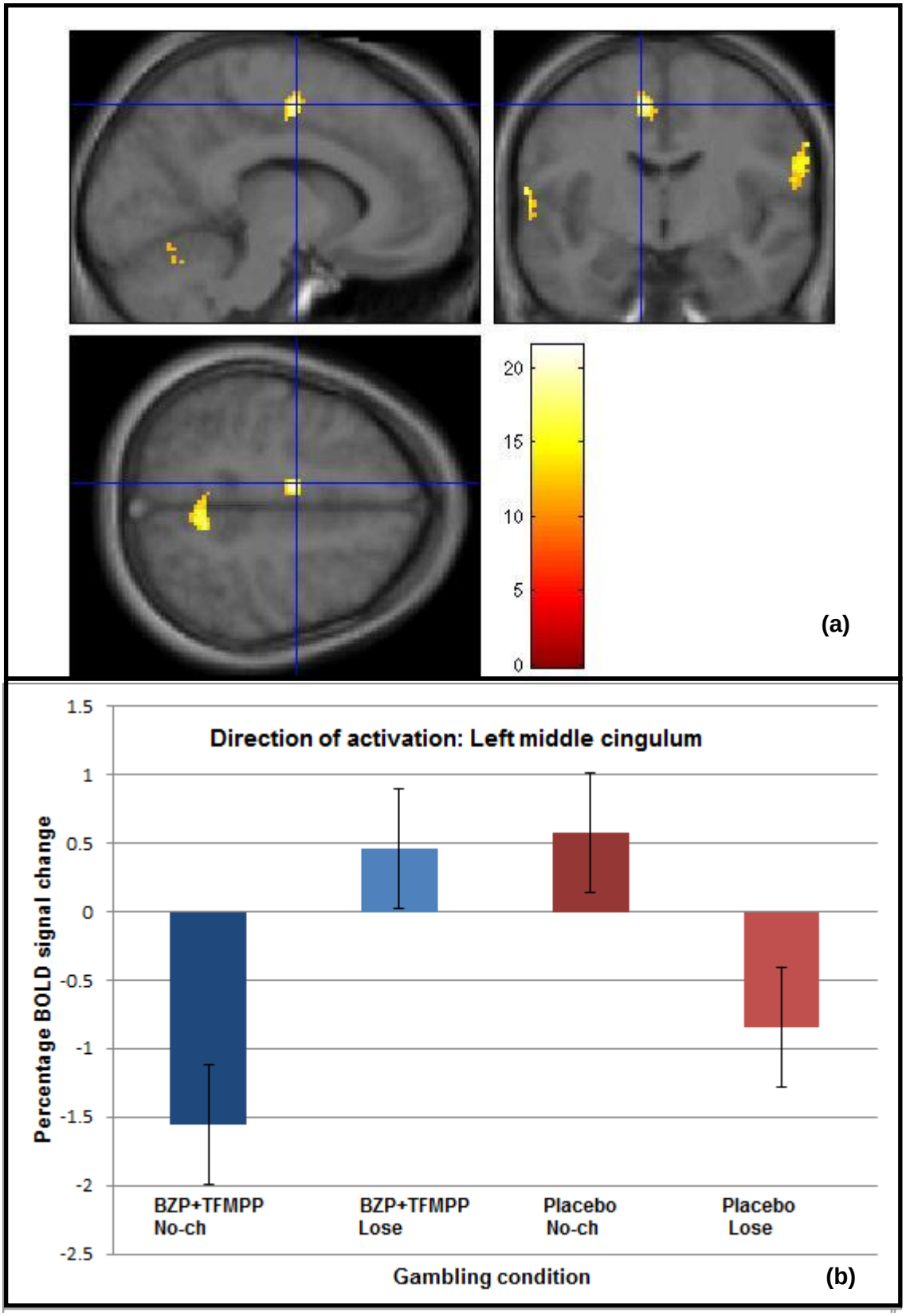

Figure 28: Activations associated with a monetary loss of $4, when BZP+TFMPP is contrasted to placebo (Drug (Lose $4 -No-ch $4) – placebo (Lose $4 -No-ch $4)) p <0.001 uncorrected; cluster threshold > 20 voxels. (a) Activation in the left middle cingulum and (b) Plot of parameter estimates, indicating the direction of activation in the left middle cingulum

2.3.4. Discussion

The aim of this study was to decipher whether BZP, TFMPP or a combination of the two, alter reward processing, and if so, to what degree. To our knowledge this is the first study to report the effects of BZP and TFMPP both alone and in combination on regional brain activation during reward processing. We used an interaction contrast to investigate the relative differences between drug states and placebo, a no-change reward condition was incorporated to remove any baseline activation due to direct or indirect pharmacological effects. This enabled the observation of the effect of each drug on reward processing after winning or losing, relative to placebo. To investigate drug effects on the reward and loss of money, the participants undertook a game of chance where they could win or lose money depending on the outcome of their guesses. The sum of their winnings was given to them at the end of the trial. The cumulative total was not displayed to participants so that subjects could not maintain a monetary position to ensure the baseline for evaluating outcomes did not change during the session progression or subsequent trial days.

The same psychological processes that underlie receipt and the anticipation of monetary rewards are activated after taking recreational drugs (134, 338). Studies investigating the effects of amphetamine on dopaminergic transmission have shown that it blunts the phasic release of DA by acting as an agonist at D_2 autoreceptors and increases tonic DA levels by blocking re-uptake in the ventral striatum (343). Using an event-related delay task during fMRI, Knutson and colleagues (142) reported that amphetamine blunted the magnitude of response but appeared to prolong the duration of the BOLD signal in the ventral striatum while anticipating gains, however monetary gain outcomes evoked activation in similar regions to placebo. Following the anticipation of gains there local effects rather than a global effect in areas such as the medial prefrontal cortex (142).

Therefore we hypothesised that the BZP and the combination of BZP and TFMPP would reduce activation within the dopaminergic reward circuitry after monetary gains and that there was likely to be no difference after giving TFMPP due to its indirect effects on DA release via 5-HT_{2C} receptors. However, our results show that BZP, TFMPP and the combination BZP+TFMPP failed to induce significant activation. It is possible that Win $4 stimuli were insufficient to induce a response. It has been suggested by several studies (215, 371, 372) that the win to loss ratio should be 2:1 to elicit the equivalent neural response, as losing outcomes therefore it might be that winning $8 would induce a visible response under these conditions.

In contrast the lose $4 condition evoked a response following each drug in comparison to placebo. Although accepted to a lesser degree, aversive and stressful experiences

reportedly cause large changes in synaptic DA concentration. Administration of DA agonists and antagonists have been shown to change the behavioural effects of these stimuli (159). Several studies have shown that aversive stimuli are associated with increased DA transmission via phasic bursts. However, some have shown the opposite (i.e. a reduction in DA transmission after aversive stimuli). Bromberg-Martin (108) suggest this may be due to individual regions influencing the resulting effect. Gray and colleagues (373) reported that DA is released in humans in response to aversive events, such as, unavoidable foot shocks, possibly due to DA release from uncontrollable mild stress (374, 375). It has been suggested that dopaminergic pathways determine the motivational salience of environmental stimuli (205). An alternative explanation is the "safety seeking" hypothesis that by Ikemoto and Panksepp, (157), who proposed that aversive events elicit striatal activity as the subject anticipates a positive outcome. This proposal is also used to explain the increased activation observed after monetary losses after receiving amphetamine (142). The authors suggest that this may cause organisms to maintain motivation even following events considered aversive.

The effects of BZP are predominantly dopaminergic therefore we hypothesised that it would increase activation in regions within the reward pathway. We observed activation in the bilateral cingulate and IFG. BZP induced greater activation in the right mid-cingulum and IFG in comparison to placebo and in contrast less deactivation in the left cingulate than placebo. Knutson and colleagues (114) observed significant activation in the dorsal striatum, ACC and the thalamus after monetary losses which supports the suggestion we would observe activation in the cingulate. The IFG has been associated with uncertainty (342) and inhibition (239) and importantly, is affected by dopaminergic transmission. Therefore it is possible that the increased activation of the IFG we observed reflects the recruitment of additional resources to ensure the maintenance of inhibitory control when the loss stimuli were presented. However inhibition itself cannot be confirmed by the reaction time data, as there were no significant changes for this task. Future research needs to be conducted to assess this finding, by modifying the gambling (guessing) task to include a measure of inhibition or risk.

Following BZP, we also observed activation in the insula known to be responsive to both receipt (198, 202, 344, 376) and the expectation of aversive stimuli (345, 346). It is also thought to play an important role in linking affective processing with motivation, decision making and behaviour in addition to being associated with frustration and losing in a gambling task (377). After administering haloperidol activation of the insula was abolished in response to learning aversive conditioned responses (176). In comparison the D_2/D_3 receptor antagonist sulpiride decreased activation in response to the taste of mouldy

strawberries in the insula (159), and in a separate study, the D_2/D_3 agonist pramipexole disrupted connectivity between the NAcc, frontal regions, and insula (207). The authors proposed that this disturbance could lead to sub-optimal decisions being made and an increase in impulsive behaviours. Our results suggest that the changes we observed were also likely due to disruption of dopaminergic transmission and an increased response to negative stimuli.

BZP also induced activation in bilateral rolandic operculum which has been associated with gustatory reward processing (351, 378) speech production (379, 380) and learning a new language (381) in addition to clenching or grinding of teeth (355). Recently levodopa, a precursor to DA, was given to healthy controls before a semantic processing task which also induced activation of the rolandic operculum (356). This also supports the hypothesis that BZP's predominant effects are on DA release.

When TFMPP was contrasted to placebo, two clusters were induced, specifically in the right lingual gyrus and right thalamus. Both of the clusters showed less deactivation in the TFMPP lose $4 condition than placebo. The lingual gyrus is activated following the presentation of visual stimuli such as the colour-word stroop task (382). The SSRI citalopram (201) and fenfluramine (383) have also been reported to enhance occipital activation which suggests that 5-HT either decreases attention or the processing of visual information.

A second cluster observed after the administration of TFMPP was in the thalamus, showing less deactivation. The thalamus is reportedly activated in response to monetary loss during gambling tasks (114) and with reward processing, and is part of the BGTC. The thalamo-cortical region links reward with specific goal directed behaviours and the thalamus has been suggested to be involved in learning, and aids in adjusting behaviour to maximise outcomes (150).

The effects of TFMPP are mainly serotonergic and 5-HT has been reported to modulate the response to aversion. Administration of citalopram decreased activation in the right OFC and the right parahippacampal/amygdala region, and increased activation in the bilateral thalamus and the fusiform gyri (201). Moreover, Marutani (203) investigated the effects of an acute dose of paroxetine on a monetary incentive task and found that brain activity induced by motivation was diminished. Therefore it is likely that the thalamic activation observed is due to the serotonergic effects of TFMPP.

When BZP and TFMPP were given together, our observations reflect those of when they were given separately, however there was an increase in the number of clusters observed.

Antia et al. (384) monitored the plasma concentrations of BZP, TFMPP and the combination of BZP and TFMPP in humans. The study reported that the peak plasma concentrations were greater following the combination than when BZP and TFMPP were given alone, using the same doses as used within this study. This increase in plasma concentration may be attributed to drug interaction as both drugs are metabolised by CYP 2D6 (384). It is therefore possible that this pharmacokinetic interaction caused increase plasma concentrations and subsequently an increase in the number of clusters activated. The no-change condition that the outcome stimuli were compared to minimised the tonic differences caused by the drug in DA and 5-HT transmission. Therefore the activations that are observed should be phasic increases and task related.

Neural responses are known to vary in response to increasing monetary wins or losses (215, 385). Therefore, in response to a lesser magnitude stimulus i.e. 50c, we expected a reduction in the number of regions activated. Interestingly, after winning 50c, BZP activated regions in the right superior and left medial superior frontal gyri, where BZP had greater activation than placebo. In addition, BZP+TFMPP induced activation in the right middle frontal gyrus and hippocampus, but had a reduction in activation in the middle frontal gyrus and less deactivation in the hippocampus. These results are reflective of the hypothesis that we see in the loss of a large monetary amount, where BZP induces positive arousal via an increase in DA transmission, and therefore activates frontal regions. Possibly, this increase in positive arousal perceives that the lesser magnitude win is still rewarding, however in the placebo condition this effect is not the perception. This would explain why there is a difference seen after a small monetary value, but not a larger one. Furthermore, this response is diminished after the administration of BZP+TFMPP, possibly due to TFMPP's opposing effects on DA transmission via 5-HT_{2C} receptors.

After losing 50c the only activation that was seen was in the combination BZP+TFMPP in comparison to placebo. This comparison resulted in greater activation in right mid temporal, right hippocampus, left superior and middle frontal gyri, right lingual and left middle occipital gyri by BZP+TFMPP than placebo. Again, this possibly reflects the pharmacokinetic interaction when the two drugs are administered together in response to aversive stimuli. The lack of differential response by the BZP and TFMPP drug states when the two are given alone may reflect that the losing 50c is insufficient to evoke a response in the task.

Unexpected non-reward events also provoke phasic firing of dopaminergic neurons i.e. unexpected alerting events. However, as both winning and losing stimuli were presented to participants with differing results, we do not believe our observations reflect non-rewarding

stimuli. Therefore we suggest that the differences we observed reflect differential effects on dopaminergic and serotonergic activity from BZP and TFMPP respectively.

Due to the task design the behavioural responses to inhibition and could not be directly measured. While some of the regions that are activated in these contrasts have been previously associated with inhibition and aversion (239, 342), we cannot directly infer that these drugs affect inhibition from our current data. Further study needs to be conducted using a task that assesses inhibition and risk to determine a direct causative effect.

An alternative explanation for activation within the PFC could be attributed to the preparation for motor responses. This preparation has been identified as a cause of activations in non-motor regions, including the PFC (363, 364).

2.3.5. Conclusion

In conclusion, at the doses we used, BZP, TFMPP and the combination of BZP and TFMPP alters the response to rewarding stimuli during a gambling (guessing) task in comparison to placebo. BZP induces similar subjective effects to the psychostimulant, amphetamine. This study suggests that it can induce a similar response following the presentation of aversive stimuli and therefore a direct comparison between BZP and amphetamine is warranted. TFMPP induced activation in only the lingual gyrus and thalamus, both of which we believe reflects the role of 5-HT in aversive responses to stimuli. When the BZP and TFMPP were given together the activation observed was greater than the clusters observed when the drugs were given separately. We believe this is the result of the pharmacokinetic properties of each drug; specifically, that they inhibit the metabolism of each other, which results in higher than expected plasma concentrations (384) and a consequent synergistic effect. When the magnitude of the winning and losing stimuli were compared the magnitude of the neural response also changed, which confirms prior studies that show that greater stimuli result in greater activation.

2.4. Gambling Task: Comparing the Effects of BZP to Dexamphetamine to Investigate the Effects of Reward Anticipation and Reward Outcome

A comparison between the acute effects of the synthetic drug benzylpiperazine (BZP) and dexamphetamine (DEX) using functional magnetic resonance imaging (fMRI) to investigate their influence on response to reward value and anticipation of reward.

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2.4.1. Introduction

BZP is one of the most commonly used constituents in a relatively new group of synthetic drugs, used in the so called party pills. Party pills have been marketed as safe and legal alternatives to illicit amphetamines such as MA and MDMA (200). There has been no evidence to support these safety claims. Although the legislation surrounding BZP and related piperazines has been changed in many countries to make them illegal, reports suggest that the use of BZP is continuing.

The reported effects of BZP in humans are similar to other psychostimulants; both physiological and subjective data being have been reported to be similar to MDMA and DEX (2). The psychostimulant effects of BZP are supported by findings from preclinical research. For example, rats trained to recognise a bupropion cue generalised to BZP, cocaine and methylphenidate (15).

Dopamine plays an important role in the modulation of reward processing and subsequently in motivational and cognitive control (108, 386). The pharmacological effects of BZP are essentially dopaminergic, with minor effects on 5-HT and NA. BZP is thought to inhibit DA reuptake in a manner similar to cocaine (16, 17) and release DA from nerve terminals in a similar fashion to amphetamine (18, 19). In addition, it acts as a direct agonist at postsynaptic dopaminergic receptors (14). Intravenously administered BZP (3 and 10 mg/kg) produced a dose-dependent elevation in extracellular DA and 5-HT concentrations in the NAcc of rats. The increase in 5-HT release was only found following the high dose of BZP (19). Moreover, BZP has also been shown to cause the peripheral release of NA by blocking synaptic reuptake in an in vitro preparation (20) using the isolated rabbit pulmonary artery (20).

Rewarding and aversive stimuli are proposed to underlie behavioural motivation. DA is reported to play an important role in motivational control (108), and is thought to mediate the association between motivational and cognitive control (386). DA neurons originating in the substantia nigra and the VTA modulate DA levels in terminal regions via phasic firing (387), and it is phasic responses that are triggered by different types of reward and reward related stimuli (388).

In humans, the effects of monetary rewards have been investigated using gambling tasks and fMRI. Responses to monetary incentives have been reported to activate a specific network of regions which include the NAcc, supplementary motor cortex, OFC, ACC, and the insular cortex (114, 150, 158). There has been a differentiation between regions responding to the anticipation of reward and those that respond to the receipt or consumption of reward. Knutson and colleagues (110) suggest that the NAcc is involved in the anticipatory stage, whereas the vmPFC is activated following the reward receipt. The NAcc also plays a role in distinguishing between reward versus no reward and also the magnitude of reward; this was described as the incentive effect (150, 158). In addition, a separate network of regions is activated in response to stimuli that have an element of risk and/or uncertainty. These regions include the amygdala, OFC, IFG, and insula (210, 211).

Pharmacological manipulation of dopaminergic neurons can modulate reinforcement learning, such as is seen with addictive drugs (389). Imaging studies have used pharmacological probes to examine the effect of DA on the reward system and the cognitive processing associated with learning, such as, the DA agonists amphetamine (142, 176) and pramipexole (207) and antagonists, such as, haloperidol (176). The response to uncertainty and risky decision making also involves dopaminergic influences; D'Ardenne and colleagues (390) reported that BOLD response in the VTA reflected a positive reward prediction error, whereas increased activation in the ventral striatum was associated with both reward-prediction errors to positive and negative valencies.

While fMRI does not directly measure DA modulation, Knutson and colleagues (288) proposed that the anticipation of stimuli is due to DA release in the NAcc. This release leads additional effects including alterations in postsynaptic membrane polarity following the activation of D_1 receptors and subsequent metabolic processes that ultimately leads to an increased BOLD signal (288, 391).

Research by our laboratory using an event-related fMRI gambling (guessing) task has shown that BZP induces significant differences in the neural responses to both anticipation of an uncertain outcome and also to the response to monetary losses, but not gains (337, 392). These responses indicate that BZP has similar effects to amphetamine. Since both

BZP and DEX effect the response to anticipation and reward outcome (142, 337, 392) and induce similar subjective effects (2) we wanted to establish whether there were also similarities in their ability to alter reward processing during both uncertain anticipation and outcome phases. In this study we directly compare the effects of BZP with DEX, and DEX to placebo during the anticipatory and outcome receipt, that is, rewards and loss, stages of processing, using the event-related gambling (guessing) fMRI task. This is the same paradigm previously used to compare the effects of BZP and/or TFMPP with placebo.

2.4.2. Materials and Methods

Approval for this research was granted by the Northern X Regional Ethics Committee of NZ (Ethics approval number NTX/07/08/078). The trial recruited healthy participants, excluding those with a history of mental illness, cardiac disease, head trauma, epilepsy, or endocrine disorders, and those who were currently pregnant or breastfeeding. Potential participants completed a custom designed questionnaire detailing their medication history, recreational drug, party pill, alcohol and cigarette use. This was to ensure participants were not drug naive and, not current or past heavy users of recreational drugs or drug dependent. Thirteen non-smoking healthy participants (seven female and six male; aged 18–40 years) were recruited to participate in this double-blind placebo controlled cross-over trial. E-prime data file errors during the data collection phase of the trial rendered three data sets unusable, which left the comparison of 10 subjects in the cross-over study.

2.4.2.1. Drugs

Benzylpiperazine hydrochloride (200 mg) and dexamphetamine (20 mg) capsules were manufactured by the School of Pharmacy, University of Auckland NZ, using good manufacturing practice. Placebo capsules contained methylcellulose and were identical in appearance to the BZP and DEX capsules.

2.4.2.2. Procedure

The study used a double-blind cross over design, participants were tested following BZP, DEX and placebo, in a randomised order with a minimum of seven days between sessions. Participants fasted for 12 hours before the trial and were asked to abstain from alcohol or caffeine from the evening prior to testing. Prior to drug administration urine analysis was undertaken for the presence of recreational drugs and pregnancy was tested. Participants were excluded from the trial if either test result was positive. All capsules were administered with 250mL of water ninety minutes before imaging; the time taken to reach peak plasma concentrations of BZP is 75 minutes (393) and the onset of action of DEX is 30 minutes (394) and peak plasma concentration is reached 2 to 3 hours after

administration (395). During this time, participants remained in the presence of researchers in a comfortable area with minimal stimulation.

2.4.2.3. fMRI data analysis and acquisition

fMRI testing was performed in the Centre for Advanced MRI at the University of Auckland. Blood oxygen level dependent functional images were acquired using a T2*-weighted echo planar imaging (EPI) sequence with a 1.5T Siemens Magnetom Avanto scanner using the following parameters: TR 2500 ms, TE 50 ms, FOV 192 mm, in-plane voxel size 3.0 mm× 3.0 mm, flip angle 90°, 29 slices, slice thickness 4.0 mm no gap. In each trial day 176 volumes were collected for each participant for each run and two runs were completed at each visit with a 30 second break between each run. For anatomical reference, a high-resolution structural MPRAGE image was acquired for each participant at the end of the first session.

A gambling (guessing) task was completed during image acquisition by presenting images of cards (as shown in Figure 29) on a screen located 3.5 metres from the participants, at the foot of the scanner and visible via a prism built into the head restraint used to minimize head movements during the scan.

Figure 29: Progression of the stimuli (from left to right)

Includes the selection (?), anticipation (monetary amount revealed; highlighted in yellow) and reward stages (suit colour and reward revealed; highlighted in orange)

Blood oxygen level dependant functional images were acquired using a T2*-weighted echo planar imaging (EPI) sequence with a 1.5T Siemens Magnetom Avanto scanner using the following parameters: TR 2500 ms, TE 50 ms, FOV 192 mm, in-plane voxel size 3.0 mm× 3.0 mm, flip angle 90°, 29 slices, slice thickness 4.0 mm no gap. On each trial day 176 volumes were collected for each participant for each run and two runs were completed at each visit with a 30 second break between each run. For anatomical reference, a high-resolution structural MPRAGE image was acquired for each at the end of the first session.

The gambling (guessing) task allowed the investigation of drug effects on stimuli at the anticipation and outcome stages of reward processing (Figure 29; highlighted in yellow and orange respectively for the purposes of this paper). Participants were instructed that when the reverse side of a card was presented on the screen with a question mark ("?"), they had to guess whether the suit of the card was black or red and respond using a hand held response box, used to minimise head movements and that money could be won or lost depending upon the outcome. After completing the trial, subjects expected to receive the monetary reward representing the net win from the task however, the outcomes were programmed to have a pre-determined valence and magnitude presentations. The pre-determined stimuli presented in each sequential run were randomised within E-prime. This ensured that participants did not suspect that there was a net outcome of $0 for each trial. Eight stimuli of large, little and no rewards and eight large, little and no monetary losses were presented within each run. Each session comprised two runs of 72 stimuli, each with a selection, anticipation and reward phase. Each selection phase was presented for 2000 msec , followed by an anticipatory phase of 1500 msec and the final outcome stage was split into two (the reveal and the final outcome), each lasting a duration of 750 msec. The inter-stimulus interval was set at a mean of 500 msec, which has been shown by Dale and colleagues to ensure efficiency of estimation (333, 334).

A neutral stimulus was given by presenting an "X" on the back of a card instead of a question mark, and instructing participants *not to play that particular game*. The stimuli would progress as usual with the computer selecting the colour but the result would be "no-change". If participants did not respond to a selection stimulus the result was also shown as "no-change".

Raw data were analysed using SPM8 (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB 7.8.0 (Mathworks, Sherborn, MA, USA). After being co-registered to the T1- weighted structural volume, the EPI images were normalized to a standard space (Montreal Neurological Institute [MNI] template). Images were spatially smoothed using an isotropic Gaussian kernel of 8 mm full-width at half-maximum (FWHM) in the x, y, and z axes.

Outliers due to movement or signal from the pre-processed EPI files, using thresholds of 3 *SD* from the mean, 0.75mm for translation and 0.02 radians rotation were removed from the data sets, using ART repair (335). Outliers were recorded to ensure no more than 15% of scans were removed from each run (two runs per session). Furthermore, ART repair was also used to check for stimulus correlated motion at first level analysis, and for top-down quality assurance purposes the ResMS, mask, beta, con and SPMT images were checked

for abnormalities and artefact after both first and second level analysis. An *F*-test across all conditions was done per session to ensure that each subject had activity in visual cortex after first level analysis.

First level analysis allowed for the neural activation of each individual to be evaluated for each of the nine gambling conditions win, lose or No-ch (0c, 50c and $4) by constructing t-contrasts. No interaction contrasts were made at this stage to maintain maximum specificity during second-level analysis.

T-contrasts were then used for a second level group comparison analyses. Event-related responses to anticipation (Ant) of a large amount (Ant $4 minus No-ch Ant $4) and the outcome of a large amount (Win/lose $4 minus No-ch Win/lose$4) were defined. Analysis was conducted in separate models for each drug state: (1) DEX in comparison to placebo, and (2) BZP in comparison to DEX. For each drug state inter-drug state comparisons were made using F interaction contrasts. Voxel-wise analysis was conducted using a significance threshold of $p < 0.001$ uncorrected and a cluster threshold of ten voxels. Anatomical locations were derived using a customised script in SPM8 (336). Parameter estimates of the conditions at significant coordinates were plotted, which can be interpreted as percentage BOLD signal change in reference to the whole brain mean, to determine the direction of the activation. Significant clusters of activation are displayed using an average brain created from the structural files of the participants.

2.4.3. Results

The aim of this study was to investigate whether there were differences in activation within the reward circuitry after giving DEX, BZP or placebo whilst participants completed a gambling task. An F-contrast was constructed to examine this interaction. During the anticipatory phase of the task the participants were unaware whether the outcome was going to be a win or a loss. Consequently, the trials for winning and losing were grouped and compared to the no-change (No-ch) stimuli. Contrasts were also made to compare the effects of outcome, that is, monetary reward (Win $4) and loss (Lose $4) following DEX, BZP or placebo. Winning and losing were individually contrasted to the No-ch reward $4 and No-ch loss of $4 stimuli.

Analysis of the anticipation stage revealed that when DEX was compared to placebo, there was reduced activation in cingulate, the thalamus and the post central gyrus. A direct comparison between BZP and DEX showed one regional difference in the thalamus (Figure 30); in this region BZP induced greater activation than DEX (Table 5).

For responses to the reward stage following DEX in comparison to placebo, after Win $4, DEX induced greater activation in the cingulate, post central gyrus and one of the clusters in the superior frontal gyrus relative to placebo. The second cluster in the superior frontal gyrus and the cluster in the temporal gyrus both caused less deactivation in the DEX drug state than placebo. When BZP and DEX were compared two clusters of activation were induced i.e. in the thalamus and cingulate. DEX induced greater activation in both regions (Table 6).

After losing $4, DEX relative to placebo, increased activation in the thalamus, cingulate, middle frontal and post central gyri. When BZP was compared to DEX, BZP evoked an increase in activation in the cingulate (Figure 32) and insula (Figure 31). However, in the thalamus DEX induced activation in comparison to BZP (Table 6).

Reward Anticipation interaction Drug A (Ant$4- Noch$4) – placebo/Drug B (Ant$4-Noch$4)											
Anatomical region	**F value**	**MNI coordinates**			**Directionality: Contrast estimates and SE**						
		x	**y**	**z**	**Drug Noch$4**	**Drug Ant$4 (lose stimulus)**	**Drug Ant$4 (win stimulus)**	**Placebo Noch$4**	**Placebo Ant$4 (lose stimulus)**	**Placebo Ant$4 (win stimulus)**	**SE**
DEX x placebo											
'Thalamus_R'	24.19	12	-30	-2	1.4434	-1.0191	-1.5509	-3.5023	-0.121	0.4103	0.8255
'Cingulum_Mid_L'	18.27	-4	-2	32	0.6642	-1.9687	-0.5348	-0.8907	0.2441	2.2798	0.6062
'Postcentral_R'	17.36	56	-6	28	1.2203	-1.9635	-1.3266	-0.9363	0.1618	0.799	0.6545
BZP x DEX											
'Thalamus_R'	18.26	20	-28	10	-1.4332	1.1521	0.3858	0.9256	-0.5381	-1.8512	0.6529

Table 5: Neural correlates of activation of DEX in comparison to placebo and BZP to DEX after anticipation of uncertain outcome

Note: All clusters are significant at $p < 0.001$ (uncorrected); cluster threshold of 10 voxels.
The *F* value at the peak voxel within each cluster is reported.

Ant: anticipation; No-ch: no-change; SE: standard error

Reward outcome interaction **Drug A (Win$4- Noch$4) – placebo/ Drug B (Win$4-Noch$4**									
Anatomical region	***F* value**	**MNI Coordinates**			**Directionality: Contrast estimates and SE**				
		x	**y**	**z**	**Drug Noch$4**	**Drug Win $4**	**Placebo No-ch$4**	**Placebo Win $4**	**SE**
DEX x placebo									
'Cingulum_Mid_L	19.09	-4	0	34	-1.9639	0.4314	0.6598	-0.8778	0.5353
'Temporal_Sup_R'	17.07	53	-28	16	-1.6802	0.4775	-1.132	-2.7261	0.54
'Postcentral_R'	15.90	56	-6	28	-2.3408	-0.0851	0.9103	-0.8185	0.5942
'Frontal_Sup_L'	15.31	-16	49	30	-2.2691	-0.8953	0.4659	-1.2327	0.4669
'Frontal_Sup_L'	13.89	-20	50	20	-0.9327	0.4186	0.2217	-1.3818	0.4714
BZP x DEX									
'Thalamus_R'	18.85	22	-28	12	0.0264	-2.6085	-1.7396	-0.151	0.5678
'Cingulum_Mid_L	18.28	-8	0	32	0.8014	-1.0415	-0.838	0.823	0.4783
Drug A (Lose$4- Noch$4) – Placebo/ Drug B (Lose$4-Noch$4									
Anatomical region	***F* value**	**MNI Coordinates**			**Directionality: Contrast estimates and SE**				
		x	**y**	**z**	**Drug No-ch $4**	**Drug Lose $4**	**Placebo No-ch $4**	**Placebo Lose $4**	**SE**
DEX x placebo									
'Postcentral_R'	19.68	58	-8	26	-2.1608	0.7125	0.3523	-1.4385	0.5922
'Thalamus_R'	19.49	12	-24	10	-1.7511	0.2367	1.1079	-0.9879	0.5211
'Postcentral_L'	17.17	-60	-6	14	-1.7044	0.4201	0.8659	-1.7674	0.6468
'Postcentral_L'	16.74	-54	-12	14	-1.4994	0.6922	0.9204	-1.1097	0.5812
'Cingulum_Mid_L'	16.57	-6	-2	34	-1.1203	0.8511	0.6557	-0.6778	0.4574
'Frontal_Mid_Orb_R'	16.17	4	52	-6	-3.6892	-1.4716	-1.0076	-4.1582	0.752
BZP x DEX									
Insula_L'	17.84	-36	18	0	-0.2651	2.7166	1.5145	0.1966	0.595
'Thalamus_R'	15.52	16	-24	8	0.5827	-0.9494	-1.2295	0.5075	0.485
'Cingulum_Mid_R	14.47	12	0	36	-0.7668	0.7211	0.2984	-1.3016	0.4744

Table 6: Neural correlates of activation of DEX in comparison to placebo and BZP to DEX after winning or losing $4 reward phase

Note: Note: All clusters are significant at $p < 0.001$ (uncorrected); cluster threshold of 10 voxels. The *F* value at the peak voxel within each cluster is reported.

No-ch: no-change; SE: standard error

Neural regions modulated by the contrast for the reward anticipation phase: BZP x DEX

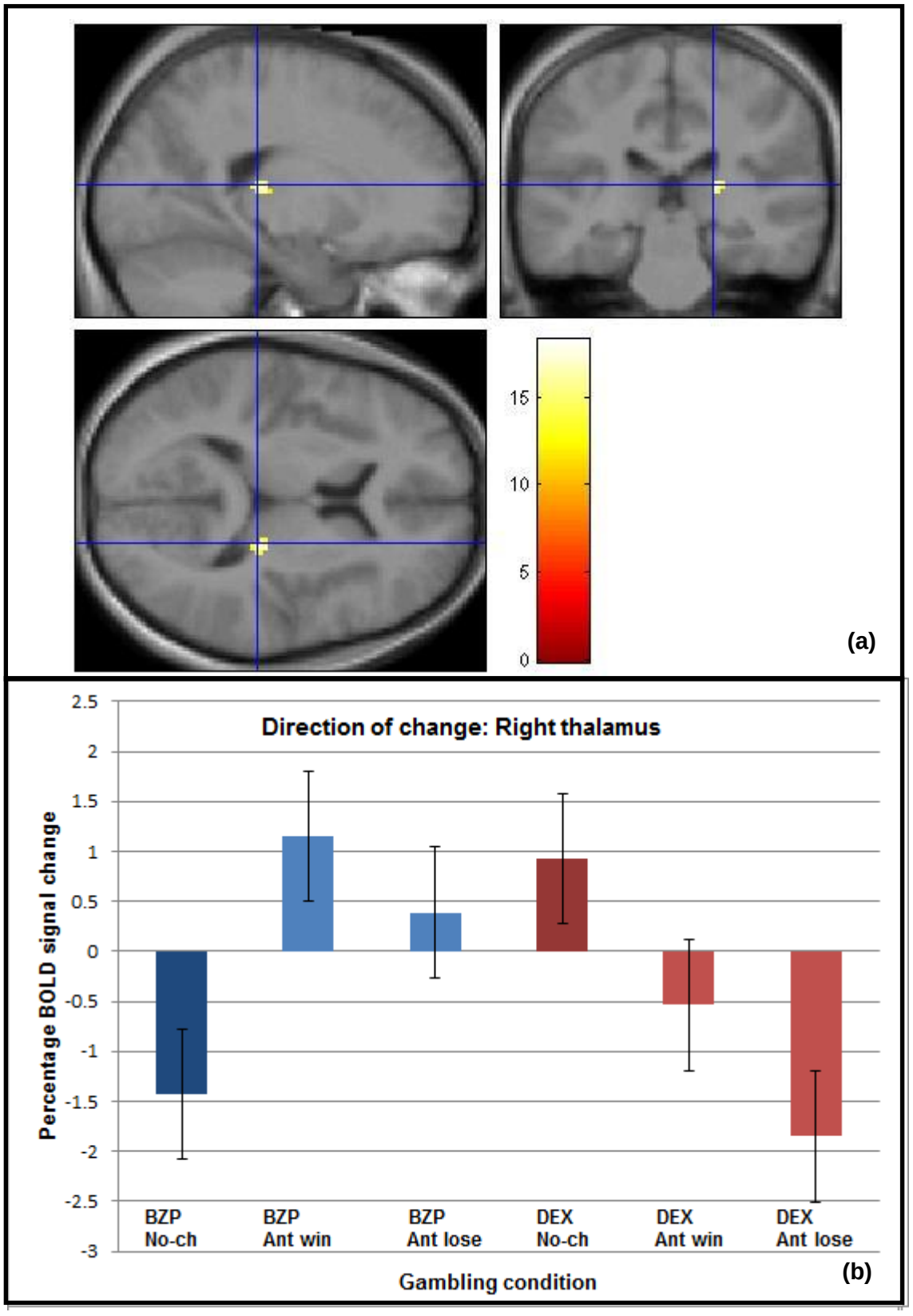

Figure 30: Activations associated the anticipation of $4, when BZP is contrasted to DEX (BZP (Ant $4 -No-ch $4) – DEX (Ant $4 -No-ch $4)) *p* <0.001 uncorrected; uncorrected; cluster threshold > 10 voxels. (a) Activation in right thalamus and (b) Plot of parameter estimates, indicating the direction of activation in the right thalamus

Neural regions modulated by the contrast for the monetary loss of $4: BZP x DEX

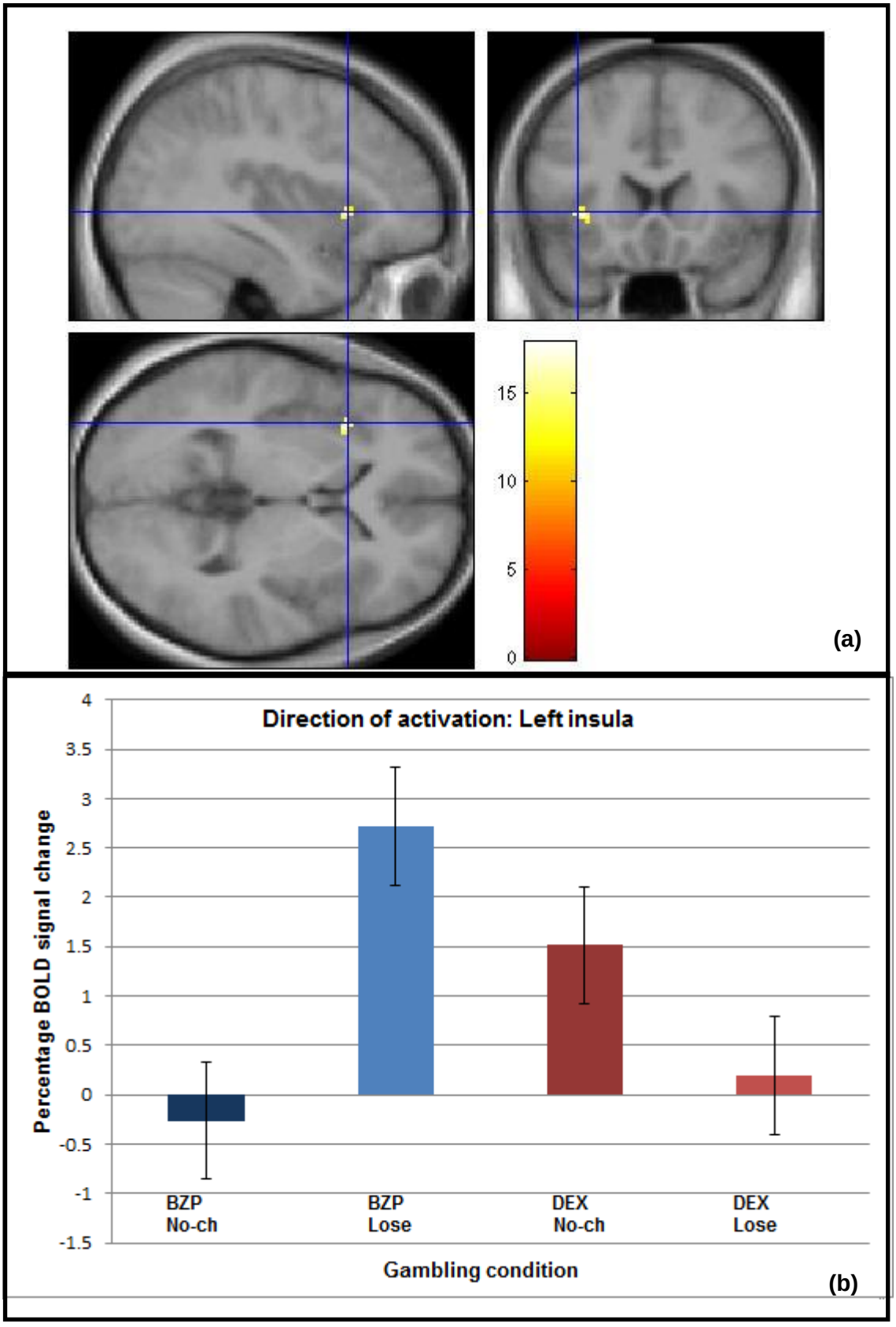

Figure 31: Activations associated with a monetary loss of $4, when BZP is contrasted to DEX (BZP (Lose $4 -No-ch $4) – DEX (Lose $4 -No-ch $4)) *p* <0.001 uncorrected; uncorrected; cluster threshold > 10 voxels. (a) Activation in the left insula and (b) Plot of parameter estimates, indicating the direction of activation in the left insula

Neural regions modulated by contrast for the monetary loss of $4: BZP x DEX

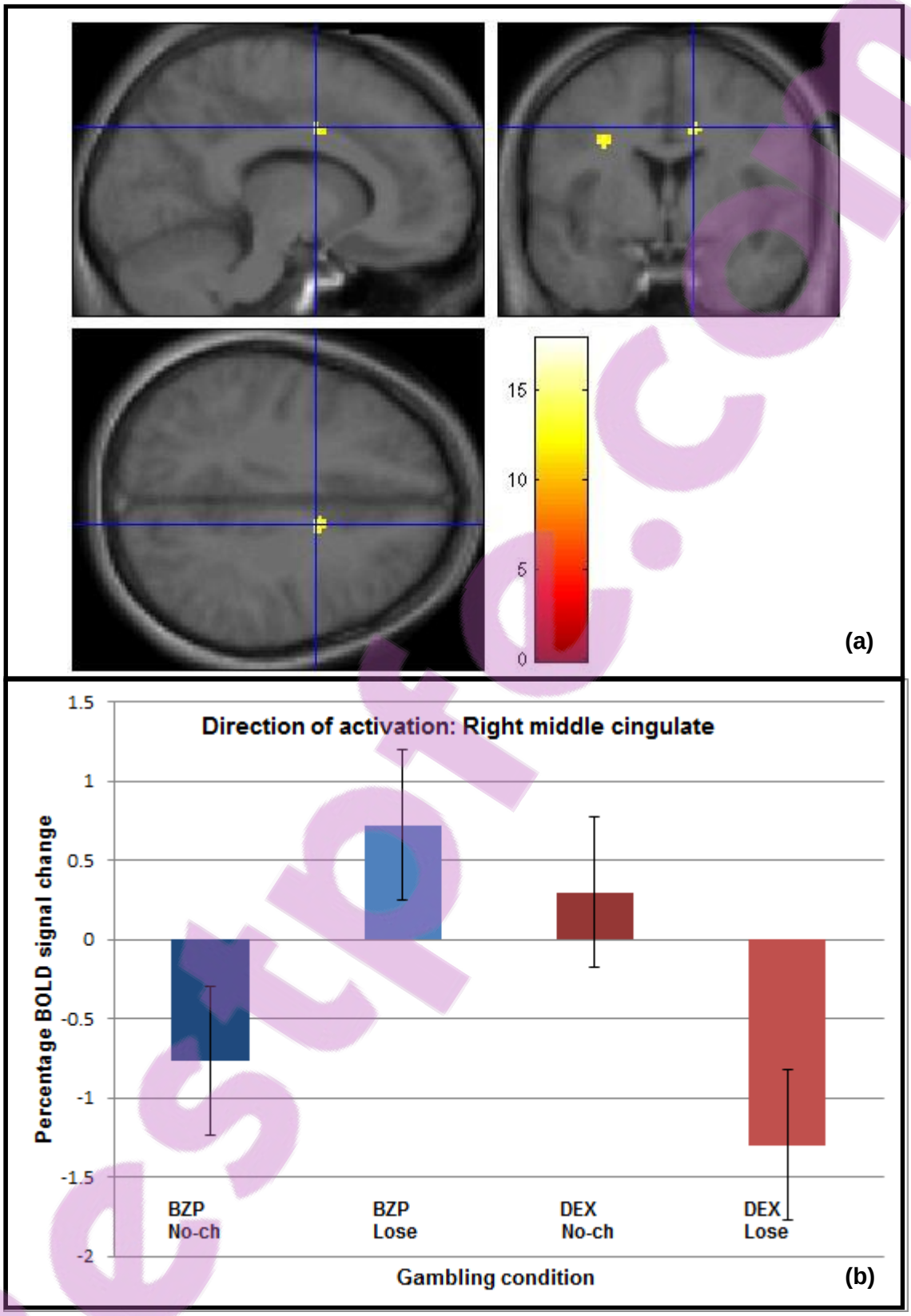

Figure 32: Activations associated with a monetary loss of $4, when BZP is contrasted to DEX (BZP (Lose $4 -No-ch $4) – DEX (Lose $4 -No-ch $4)) p <0.001 uncorrected; uncorrected; cluster threshold > 10 voxels. (a) Activation in the right middle cingulate and (b) Plot of parameter estimates, indicating the direction of activation in the right middle cingulate

2.4.4. Discussion

To our knowledge this is the first study to directly compare the effects of BZP with DEX on both anticipation and outcome stages of reward processing using fMRI. Using a custom-designed event-related gambling (guessing) task allowed the evaluated of separate stages of reward processing. This gambling (guessing) task allowed subjects to participate in a game of chance where they could win or lose money depending on the outcome of their guess. A no-change reward condition was incorporated into the task to account for baseline changes that may occur due to direct or indirect drug-induced pharmacological effects. This allowed us to determine whether DEX was affecting reward processing during anticipation and after winning or losing stimuli relative to placebo or BZP. The participants were told that they would be given the sum of their winnings at the end of the trial in the form of vouchers. Unbeknownst to the participants the stimuli were programmed to have a predetermined distribution of valence and magnitude. Due to this pre-distribution, the cumulative totals of wins and losses were not displayed to the subjects so they were unable to maintain their current monetary position. This should ensure that their baseline for evaluating the outcomes did not change over the session progression and subsequent trial days.

Gambling tasks using monetary incentive and those which involve an element of risk taking activate specific circuitry, and this circuitry can be modulated by drugs effecting dopaminergic transmission. For example, activity in the NAcc has been found to be modulated by D_2/D_3 autoreceptors (396). In addition, amphetamine blunts the phasic firing of dopaminergic neurons by acting as an agonist at D_2 autoreceptors when DA levels are elevated and enhancing tonic DA levels by blocking re-uptake in the ventral striatum (343). In a separate study which administered amphetamine and used a monetary incentive delay task, there was a reduction in the amplitude of the BOLD response, but an increased signal duration, consistent with its effects in the NAcc (142).

Further modulations have also been reported. After administration of a D_2/D_3 agonist, pramipexole, there was an increased interaction found between the NAcc and the insula, but reduced connectivity between the frontal cortex and the NAcc (207). Menon and colleagues (176) investigated the effects of DA on PE, that is the difference between the actual and the predicted reward, for drugs that affect DA transmission and reported that after administering haloperidol, a D_2 antagonist, there was no activity in any neural regions when the predicted error activation and the outcome were contrasted. These changes have important implications, as alterations in dopaminergic circuitry therefore could lead to changes in the processing of reward and aversion and subsequent changes in motivation.

For example, an imbalance between the insula, NAcc and frontal regions connectivity has been suggested to influence decision making. Specifically, disruptions in this circuitry may bias reactivity towards immediate- rather than long-term gains, and ultimately impulsive behaviour (129, 207, 397).

2.4.4.1. Anticipation

We recently reported that BZP reduced activation relative to placebo in the insula and the IFG in response to anticipation of monetary gains and losses (392). In the current study DEX reduced activation in the thalamus, mid cingulate and post central gyri. A direct comparison between BZP and DEX revealed only one regional difference i.e. the thalamus, where BZP had a greater effect.

The DEX-induced reduction in activation of the cingulate is in line with results from Knutson and colleagues (142) who showed a reduction in DA phasic firing after giving amphetamine to healthy controls, and the regions activated are consistent with locations known to be involved in the anticipation of reward. BZP however, relative to placebo, reduced activation in regions shown to be associated with responses to stimuli that are risky or have a degree of uncertainty.

Hence both DEX and BZP reduced the activation of areas sensitive to alterations in dopaminergic transmission. These regions are associated with reward based learning in the striatal-thalamo-cortical network (132, 148). However, there are distinct differences, in that BZP causes a reduction in regions sensitive to uncertainty, whereas DEX does not. This is an important finding, as it indicates that after taking BZP, the subjects were potentially more likely to have a reduction in activation in those regions that have previously be associated with risk or with uncertain outcomes. The data collected did not allow for the direct behavioural analysis of risk. Modification of the ‘gambling’ task to incorporate an element of risk and associated reward to confirm whether these activations do indeed reflect risk is an avenue for future research.

The gambling (guessing) task design used in this research had a predetermined distribution of wins and losses, and as the decision that the participants made was not one based on, for example, learning a risky deck of cards versus a non-risky deck of cards, like the Iowa gambling task. Therefore, behaviourally we could not compare the participants on their response to risk. However, this is an avenue that should be taken in future studies.

The thalamus is a region that is activated after monetary losses (114), and is part of a circuit involving the basal ganglia and prefrontal regions, which is known as the BGTC. The thalamic nuclei are known to transmit output from the basal ganglia to the frontal cortex,

forming a loop which reportedly drives motivation by communicating with parallel circuits (147). The BGTC circuit is also involved in reward related behaviour, specifically the thalamo-cortical region which is associated with linking reward and specific goal directed behaviours (148). In addition, the thalamus is also involved in learning (150). Galvan and colleagues (2005) demonstrated that thalamic activity associated with a conditioned response task decreases over time and the thalamus appears to have a role in adjusting behaviour from learning experiences in the task to maximise potential outcomes (150). In our study BZP, DEX and placebo differentially activated the thalamus, which may reflect in their effects on the processing reward based learning. Possibly, the increase in DA transmission induced by DEX enables more efficient processing, and hence a reduction in activation of the thalamus. Alternatively, thalamic activation may be due to an absence of conditioned learning in this paradigm i.e. there was no cue allowing the prediction of winning or losing, so information processing to enable learning outcomes was not established, and hence participants may have been trying to seek a pattern from the beginning of the task to the end. This also is reflective of the previous hypothesis from when BZP was contrasted to placebo, that BZP increases positive arousal; as participants may be trying to seek a pattern to the presentation of the rewarding versus losing stimuli throughout the task, despite there not being one.

2.4.4.2. Reward outcome

After being presented with the outcome of the gamble, i.e. win or loss, DEX showed distinct differences in comparison to placebo and to BZP. DEX increased activation in the cingulate, superior frontal gyrus post central and superior temporal gyrus relative to placebo. This implies that after the administration of DEX, there is increase in phasic firing in NAcc in response to rewarding stimuli.

When BZP and DEX were compared after a rewarding outcome, DEX induced greater activation in the cingulate and the thalamus than BZP. This further adds to our knowledge about the characteristics of BZP. Although it displays similar subjective effects to amphetamine (2), there are differences in its effects in DA transmission in response to reward, possibly due to the differences in phasic firing of DA. The dose of BZP may also be the reason to the difference, previously it has been reported that the relative effects of BZP to amphetamine is 10:1, this may not be the case. Alternatively, the effects on other neurotransmitter systems such as the serotonergic and noradrenergic systems may be contributing to the differences seen.

After a monetary loss, DEX relative to placebo evoked clusters in the thalamus, cingulate, medial frontal gyrus and post central gyrus. In all of these regions except the middle frontal

gyrus, DEX showed an increase in activation in comparison to placebo, and in the middle frontal gyrus DEX showed a lesser deactivation. When the BZP drug state was compared to DEX after losses, BZP evoked an increase in activation in the cingulate and the insula. However in the thalamus DEX evoked a greater activation in comparison to BZP. BZP has been reported to have a greater activation in the right middle cingulum, right insula and left IFG in comparison to placebo (337). Results from this and the previous work by our group are potentially reflective BZP and DEX's effect on neurotransmitters, and show that although BZP has a reduced response in the anticipation of risky behaviour, it has a heightened response after losses.

In accordance with Matsumoto and Hikosaka (205, 206), DA affects rewarding stimuli and the processing of aversive stimuli. They suggest that dopaminergic pathways determine the motivational salience of both types of stimuli—rewarding and aversive (205). In addition, Bromberg-Martin (108) suggest that even though rewarding and aversive events have opposite valencies, they both trigger orienting of attention, cognitive processing and increases in motivational salience. Similar regions of activation are reportedly activated in response to monetary losses and rewards with additional activation within the ACC and thalamus after losses (114).

When we consider the relative difference between DEX, placebo and BZP there are clear differences in our observations on aversive outcomes. We observe increases in activation after in areas that are associated with monetary losses after the administration of BZP. Ikemoto and Panksepp, (1999) hypothesised that aversive events would lead to an increase in NAcc activity, due to the anticipation of a positive outcome, or "safety seeking hypothesis" (157). Amphetamine administration increased the BOLD signal in anticipation of losses, which they concluded may be increasing motivation regardless of outcome (142). The DA agonist pramipexole also induced an exaggerated response to reward and a reduction in top down responses to the control of behaviours (207). The increase in activations that we see in regions responsive to monetary losses may be reflective of the safety seeking hypothesis, that is, there is an increased response to aversive stimuli following BZP administration, and increased motivation in the task regardless of the outcome, due to increases in DA. In this manner BZP seems to maintain motivation in the face of aversive outcomes. We hypothesised that we would also see an increase in activation in the striatum, as losses induce similar patterns of activation to monetary rewards (114). Although this was not seen, there was increased activation in regions downstream of the striatum, i.e. the cingulate and insula.

BZP displays attenuated responses to uncertainty and causes a more profound response to loss than DEX. These differences are likely to occur due to modulations of the dopaminergic circuit and illustrate likely differences in their pharmacological properties. It has been suggested that 5-HT might oppose the role of DA on reward processing (194, 195), with studies reporting evidence of a serotonin-dopamine gradient along the caudal-rostral axis in the striatum (196, 197). It is possible that the heightened response to negative outcomes is due to more pronounced serotonergic influences of BZP in comparison to DEX.

Alternatively, BZP could modulate dopaminergic transmission in a different manner than DEX. For example, amphetamine blunts phasic release of DA by acting as an agonist at D_2 autoreceptors when DA levels are elevated, and enhances tonic DA levels by blocking re-uptake in the ventral striatum (343). Tonic firing of DA neurons is regulated by glutamatergic projections from the PFC which modulates extracellular DA levels. The reduction in extracellular DA levels may be due to the stimulation of D_2 receptors, which may inhibit glutamate neuronal activity in the prefrontal cortex leading to a reduction in firing of NAcc neurons (398, 399). Therefore, although DEX decreases regional activation relative to placebo, this may reflect extracellular levels of DA. BZP, on the other hand, induces an increase in activation after losses and a greater motivational outlook towards the anticipatory stage leading to risky decision making and an augmented response to loss, which may be due to their differences on the effects on tonic and phasic firing of DA. To clarify this, further studies must be carried out to decipher exactly why this difference is occurring.

DEX, placebo and BZP all induced distinct differences in regional activation The differences seen between BZP and DEX will be due to differing mechanisms of action but it should be noted that different doses of the same drug may also result in differing patterns of regional activation (400, 401), therefore a range of dose-effect studies could be undertaken.

2.4.5. Conclusion

These results add to our current understanding of the effects of BZP on reward processing by demonstrating that use of BZP at this dose could induce risky behaviours and a heightened response to negative outcomes. This study demonstrates that BZP induces similar effects to psychostimulants such as DEX and also describes differences.

Chapter 3: Party Pills and the Stroop Task—Have I Got Your Attention?

3.1. Preamble

The previous chapter described the effects of these drugs on reward processing in comparison to placebo and DEX. As we know, manipulations of the dopaminergic and serotonergic pathways can affect other cognitive processes in addition to reward, including aspects of executive function–selective attention and inhibition. This next chapter will report the effects of an acute dose of BZP, TFMPP and BZP+TFMPP on executive function using an event related colour-word Stroop task. The task involves the participant responding to the colour of a presented word using a hand held response box. Participants are required to respond to one of three conditions, that is, neutral (control) words comprised of a non-colour word, congruent words where a colour word is presented in its matching colour and incongruent words where the colour of the word and the colour of its presentation do not match.

To investigate the Stroop effect (incongruent–congruent), we analysed both the behavioural and the imaging data. This investigation enabled the detection of differences in accuracy and response time and if there were regional differences in activation for the Stroop effect.

Each drug state, that is, BZP, TFMPP, combination of BZP+TFMPP and DEX were compared with placebo. In addition, to evaluate possible similarities between BZP and DEX, a direct comparison was made.

The results are presented in the form of two papers. The papers presented will be the effect of BZP, TFMPP and combination BZP+TFMPP relative to placebo on the Stroop task, and the second paper reports the effects of BZP in comparison to DEX.

All imaging data were collected on a 1.5T Magnetom Avanto Siemens scanner at CAMRI, and data pre-processing and analysed using SPM8. The group level analysis was conducted using a flexible factorial model. The behavioural data was collected using E-prime 2.0, and analysed using SPSS.

3.2. Stroop Task and BZP, TFMPP and Combination of BZP+TFMPP in Relation to Placebo

An investigation of the acute effects of the synthetic drugs benzylpiperazine (BZP) and trifluoromethylphenylpiperazine (TFMPP) alone and in combination on impulsivity and selective attention using functional magnetic resonance imaging.

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3.2.1. Introduction

Party pills containing BZP and/or trifluoromethylphenylpiperazine TFMPP have been marketed worldwide as safe and legal alternatives to illicit recreational drugs, such as MDMA and MA, since the late 1990s. These drugs are used to enhance confidence, extend hours of socialising, induce euphoria and increase energy (4). The majority of BZP and/or TFMPP users are typically in their late teens and early twenties; however, these drugs are now illegal in the majority of countries.

Despite the extensive use of BZP, its effects on the human brain have not been thoroughly investigated. Studies examining the pharmacological effects of BZP in rats and monkeys, show that it affects mainly DA release and reuptake, with additional but comparably smaller effects on both 5-HT and NA release, similar to amphetamine (18, 19). BZP is also thought to inhibit dopaminergic reuptake (16, 17), and act as an agonist on postsynaptic dopaminergic receptors (14). Intravenously administered BZP (3 and 10 mg/kg) produced a dose-dependent elevation in extracellular DA and 5-HT concentrations in the NAcc of rats, although 5-HT release was only induced following high doses (19). BZP has also been shown to cause the peripheral release of NA in the isolated rabbit pulmonary artery (20).

Behavioural studies using rodents have also reported that BZP has stimulant-like effects comparable to amphetamine and cocaine (14, 15). The reported subjective and physiological effects of BZP in humans are similar to those of other psychostimulants such as MDMA and dexamphetamine (2).

TFMPP is also a major component of many party pills, but rarely used alone and often combined with BZP. Historically, TFMPP has been extensively used as a biomarker for 5-HT activity (24). Specifically, it affects 5-HT_{1B} and 5-HT_{2C} receptors that are thought to mediate its stimulus properties (27). TFMPP, like MDMA, also stimulates 5-HT transporter-mediated release from neurons in vitro and in vivo (19, 30, 31). TFMPP also has an indirect

effect on DA release via interactions with 5-HT_{2C} receptors and GABA blockade (32-34), in addition to indirect effects on NA release via either 5-HT_{2C} or 5-HT_{1B} receptors (33, 35). Results from animal studies have shown some abuse potential because rats trained to discriminate MDMA from saline generalise to a TFMPP cue (36, 37). However, it was not self-administered by rhesus monkeys trained to self-administer cocaine or amphetamine (37).

Research using electroencephalography in human males, has shown that TFMPP speeds up inter-hemispheric information transfer across the corpus callosum (23). Importantly, when TFMPP (60 mg, oral) was given to human participants its subjective effects were similar to fenfluramine and mCPP (21).

The ratio of BZP and TFMPP in party pill preparations ranges from 2:1 to 10:1 (40). Baumann and colleagues (19) reported that when BZP+TFMPP was given as a combination (1:1) to rats, parallel increases in dialysate 5-HT and DA release were observed. Low doses of BZP/TFMPP (3 mg/kg, i.v.) mimicked the effects of low dose MDMA. However, Fantegrossi and colleagues (37) found the combination of BZP/TFMPP (1:1) was a less effective reinforcer than BZP alone in adult rhesus monkeys. The authors consequently hypothesised that could be due to the agonist effects of TFMPP at 5-HT_{2C} receptors that are known to reduce firing within the dopaminergic mesolimbic system (41, 42).

Subjective effects of the combination of BZP and TFMPP have anecdotally been compared to that of MDMA. Recent investigations into the subjective and physiological effects of these drugs reflect these reports, with data indicating that the combination shows similarities to DEX and MDMA (40, 297).

Comparing the effects of BZP and BZP+TFMPP with MA and MDMA raises concerns over their safety, because the chronic use of MDMA (402-404) and MA (309) has been associated with mood disorders, long term deficits in memory and cognitive function and neurological abnormalities. Research using the Stroop task, a task used to test selective attention and inhibition, reported that following chronic MA and cocaine use there is a reduction in performance (259, 261, 405), which reflects the hypothesis that stimulant use alters an individual's ability to selectively attend to stimuli or inhibit pre-potent responses.

The Stroop task involves the inhibition of a pre-potent response when an interference stimulus is presented to the participant. Other studies have used tasks which also assess behavioural inhibition including the stop signal task (406). The effects of acute doses of MPH and atomoxetine, agents which affect both DA and NA transmission and citalopram,

an SSRI were studied using the stop signal task, the task requires participants to inihibit the response to the pre-potent "go" response when presented with an infrequent he response. Both MPH and atomoxetine modulate NA and DA in the PFC to similar extents, however only MPH selectively increases DA within the striatum. MPH was reported to enhance response inhibition, possibly due to the increased DA transmission in the basal ganglia.

Similarly, a study by Aron and colleagues (48) demonstrated that stop signal task responses were slower in adults patients with ADHD in comparison to healthy controls , however after MPH administration the deficits were eradicated.

Both amphetamine and MPH are known to decrease impulsive decision making in healthy adults, which is thought to be the result of an increase in catecholamine release and reuptake within the synaptic cleft (407, 408). The effects on decreasing impulsive decision making are thought to be mediated through DA's effects on D_2 receptors, with a suggestion that NA also plays a strong role (409). Despite reported similarities between BZP, TFMPP and psychostimulants such as amphetamine there is to our knowledge no published data describing the acute effects of BZP and TFMPP, both as individual constituents or combined on the function of the human brain using fMRI. The aim of this study was to investigate the effects of BZP and TFMPP, both alone and in combination, on the neural networks associated with attentional control and executive function using an event related Stroop paradigm during fMRI. In this study, Stroop interference contrasts were used as a reflection of inhibitory performance whilst conducting the task.

3.2.2. Materials and Methods

Thirteen non-smoking healthy participants (seven female and six male; aged 18–40 years) were recruited to participate in a double-blind, placebo controlled cross-over trial. Approval for this study was granted by the Northern X Regional Ethics Committee of NZ (Ethics approval number NTX/07/08/078). Participants attended an initial screening session, where written consent was obtained. Participants were excluded if they had a history of mental illness, cardiac disease, head trauma, endocrine disorders, epilepsy, were pregnant or breastfeeding. Three data sets were rendered unusable due to faults in E-prime files (one from each group), which left 12 subjects in each group.

A custom designed questionnaire was completed by each participant detailing their medication history, recreational drug, alcohol and cigarette use, sleeping patterns and stress levels to ensure they were not drug naive or current or past heavy recreational drug users.

3.2.2.1. Drugs

Benzylpiperazine hydrochloride (200 mg), trifluoromethylphenylpiperazine (50 mg for participants weighing < 60 kg or 60 mg if > 60 kg) benzylpiperazine and trifluoromethylphenylpiperazine (100 mg + 30 mg, respectively) and placebo (methylcellulose) were given to participants in a randomised order. All capsules were identical in appearance and were manufactured using good manufacturing practice by the School of Pharmacy, University of Auckland, NZ.

3.2.2.2. *Procedure*

The Stroop paradigm has been used by many researchers to investigate the domains of selective attention and inhibition. Participants are required to respond to one of three conditions, that is, neutral (control) words comprised of a non-colour word, congruent words where a colour word is presented in its matching colour and incongruent words where the colour of the word and the colour of its presentation do not match. When the incongruent condition is presented there is a pre-potent response to respond to the written word rather than its colour. An inability to suppress this pre-potent response and respond to the weaker but task-relevant response is said to reflect impulsivity and impaired selective attention. When the word and its colour of presentation conflict that is, the incongruent condition, participants are slower to respond than when there is no, or less, conflict when compared to control and congruent conditions. This is known as the Stroop interference effect (410).

Participants fasted for 12 hours before the trial and were asked to abstain from alcohol or caffeine from the evening prior to testing. Participants were excluded from the trial if they were found to be positive by urinalysis test kit for recreational drug use or pregnancy where appropriate on the day of testing.

Prior to drug administration participants completed a practice version of the colour-word Stroop task to ensure a minimum accuracy of 75%. Drug or placebo capsules were given with 250 mL of water 90 minutes before imaging. The time taken to reach peak plasma concentrations of BZP is 75 minutes (393) and TFMPP is 90 minutes (332). During this time, participants remained in the presence of researchers in a comfortable area with minimal stimulation. Participants were then tested during fMRI after taking each drug or placebo using a randomised double-blind schedule with a minimum of 7 days between sessions.

3.2.2.3. fMRI data analysis and acquisition

fMRI was performed at the CAMRI at The University of Auckland. The Stroop paradigm was presented on a screen located 3.5 metres from the participants and visible via a prism built into the head restraint used to minimise head movements during imaging. Control, congruent, incongruent and rest (fixation cross) conditions were presented to the participants. Each trial consisted of 180 presentations: 36 congruent, 36 incongruent, 72 control and 36 rest fixation crosses. Each stimulus was presented for 2000 msec with a jittered inter-stimulus interval with a mean of 500 msec, which has been reported by Dale and colleagues to be sufficient to allow for efficient estimation (333, 334). Participants were instructed to respond to the colour of the presented word as soon as it appeared on the screen using two, two-buttoned hand held response boxes (one in each hand) to minimise potential head movement caused by vocalisation. Each button was assigned a colour (from left to right—red, green, blue and yellow). Incorrect responses were not used in either the reaction time or functional data to ensure that the errors did not affect the selective-attention data.

Blood oxygen level dependent functional images were acquired using a T2*-weighted echo planar imaging (EPI) sequence with a 1.5 T Siemens Magnetom Avanto scanner: TR 3000 ms, TE 50 ms, FOV 192 mm, in-plane voxel size 3.0 mm× 3.0 mm, flip angle 90°, 29 slices, slice thickness 4.0 mm no gap. On each trial day 157 volumes were collected for each participant per run and two runs were completed during each visit with a 30 second break between runs. For anatomical reference, a high-resolution structural MPRAGE image was acquired at the end of the first session on each trial day.

Raw data were analysed using SPM8 (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB version 7.8.0 (Mathworks, Sherborn, MA, USA). After co-registration to the T1- weighted structural volume, EPI images were normalised to standard space (Montreal Neurological Institute [MNI] template). Images were spatially smoothed using an isotropic Gaussian kernel of 8 mm full-width at half-maximum (FWHM) in the x, y, and z axes. Incorrect and non-responses to the Stroop paradigm were not eliminated from analysis because the accuracy was greater than 90% in all cases.

Outliers due to movement or signal from pre-processed EPI files using thresholds of 3 *SD* from the mean, 0.75 mm for translation and 0.02 radians rotation were removed from the data sets using ART repair (335). Outliers were recorded to ensure less than 15% of scans were removed from each run (two runs per session). Furthermore, ART repair was used to check for stimulus correlated motion at first level analysis. For top-down quality assurance purposes the ResMS image that shows the variance of error, the mask, beta, con and

SPMT images were checked for abnormalities and artefacts after both first and second level. An *F*-test across all conditions was carried out per session to ensure each subject displayed activity in the visual cortex following first level analysis.

First level analysis allowed for an individual's activation to be evaluated for the three conditions, that is, congruent, control and incongruent by constructing t-contrasts. No interaction contrasts were made at this stage to maintain maximum specificity for second level analysis.

T-contrasts were subsequently used in the second level group comparison. Event-related responses to the Stroop effect (congruent minus incongruent) were defined and the analysis divided into three parts for each drug state: (1) BZP, (2) TFMPP, (3) BZP+TFMPP. For these drug states inter-drug state comparisons were individually compared to placebo by constructing F interaction contrasts.

Voxel-wise analysis was conducted using fMRI data with a significance threshold of $p < 0.005$ uncorrected. Anatomical locations were derived using a customised script in SPM8 (336). Parameter estimates of the conditions at significant coordinates were plotted and interpreted as the percentage BOLD signal change in reference to the whole brain mean allowing determination of the direction of activation. Significant clusters of activation were displayed using an average brain created from the structural files of participants.

Behavioural data (accuracy [Ac] and reaction time [Rt]) were analysed using SPSS and a repeated measures ANOVA for both condition effect and group (drug state) x condition effect. Rt data were filtered to display correct responses only.

3.2.3. Results

There were no significant differences between drug state (inter-group) comparisons for both Ac and Rt. However, within each group (intra-group) differences were found between congruent (Cong) and incongruent (Incong) conditions $p < 0.0001$ (Table 1). Intra-group Rt comparisons showed the "Stroop effect" where the incongruent task took the longest time to respond to, compared to both congruent and control tasks (Figure 33-35). There were no significant differences in inter-group Ac (Table 2).

Reaction times (Rt [msec]) of response to Stroop conditions

Drug state	BZP			Placebo		
Condition	**Cong**	**Control**	**Incong**	**Cong**	**Control**	**Incong**
Mean RT (msec)	721.67	742.67	873.28	739.79	778.54	868.90
Standard error	35.39	36.15	36.09	35.39	36.15	36.09
Drug state	**BZP+TFMPP**			**Placebo**		
Condition	**Cong**	**Control**	**Incong**	**Cong**	**Control**	**Incong**
Mean RT (msec)	737.70	775.69	909.00	711.82	757.50	856.42
Standard error	23.19	24.18	21.68	23.19	24.18	21.68
Drug state	**TFMPP**			**Placebo**		
Condition	**Cong**	**Control**	**Incong**	**Cong**	**Control**	**Incong**
Mean RT (msec)	762.25	823.05	938.82	747.68	795.60	880.08
Standard error	38.07	40.54	37.34	38.07	40.54	37.34

Table 7: Mean Rt (msec) ± the SE for each Stroop condition after taking either BZP, TFMPP or BZP+TFMPP in comparison to placebo

Accuracy (Ac) of response to Stroop conditions

Drug state	BZP			Placebo		
Condition	**Cong**	**Control**	**Incong**	**Cong**	**Control**	**Incong**
Mean Ac	0.98	0.99	0.97	0.97	0.98	0.97
Standard error	0.01	0.01	0.01	0.01	0.01	0.01
Drug state	**BZP+TFMPP**			**Placebo**		
Condition	**Cong**	**Control**	**Incong**	**Cong**	**Control**	**Incong**
Mean Ac	0.98	0.98	0.97	0.97	0.98	0.96
Standard error	0.01	0.01	0.01	0.01	0.01	0.01
Drug state	**TFMPP**			**Placebo**		
Condition	**Cong**	**Control**	**Incong**	**Cong**	**Control**	**Incong**
Mean Ac	0.97	0.98	0.98	0.97	0.98	0.96
Standard error	0.01	0.01	0.01	0.01	0.01	0.01

Table 8: Mean Ac ± the SE for each Stroop condition after taking either BZP, TFMPP or BZP+TFMPP in comparison to placebo

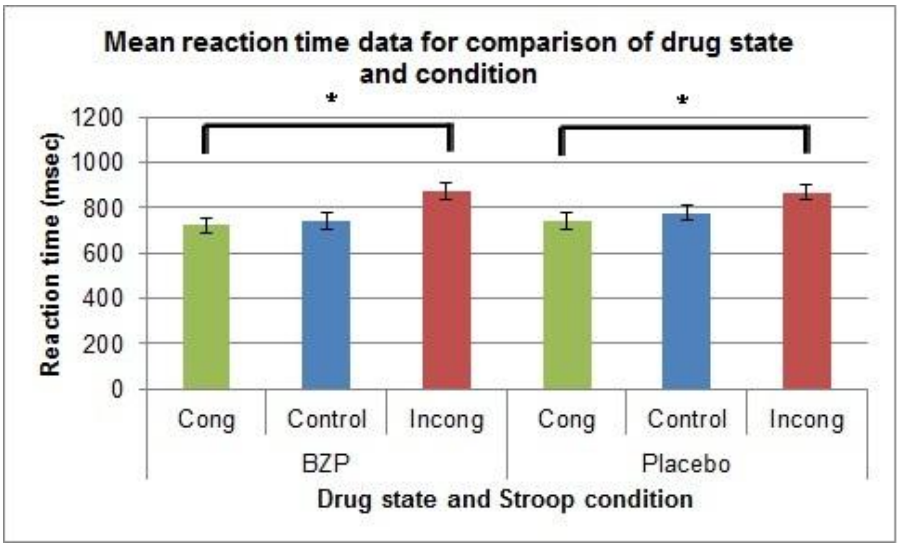

Figure 33: Mean Rt data for the comparison of Stroop conditions for BZP x placebo

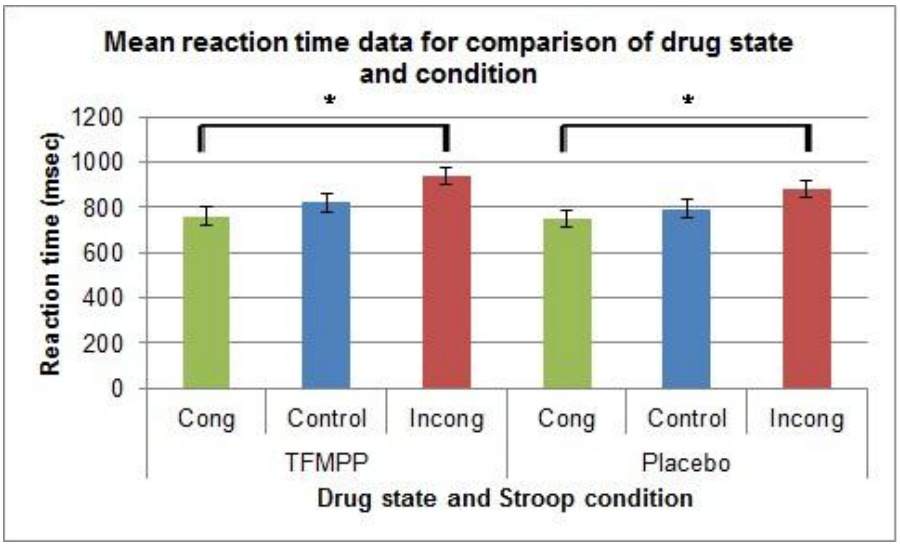

Figure 34: Mean Rt data for the comparison of Stroop conditions for TFMPP x placebo

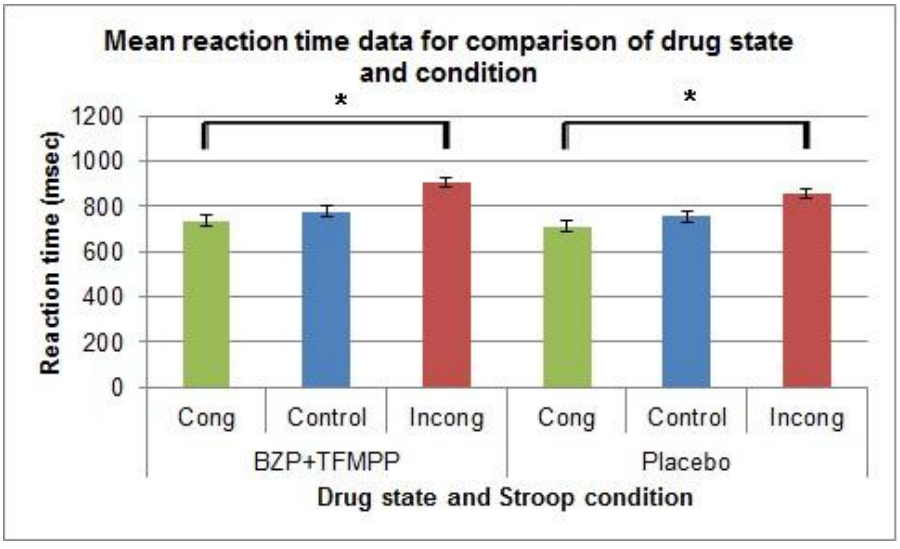

Figure 35: Mean Rt data for the comparison of Stroop conditions for BZP+TFMPPx placebo
Note: no significant differences were found between any of the groups and placebo, but within groups between the congruent (cong) and incongruent (incong) conditions (*) $p < 0.05$

Anatomical region	MNI coordinates				Directionality: Contrast estimates and standard error (SE)				
	x	y	z	*F* value	Drug Cong	Drug Incong	Placebo cong	Placebo Incong	SE
A. BZP X placebo interaction $p < 0.005$ uncorrected									
'Occipital_Sup_R'	26	-78	44	13.06	-0.46	-0.01	-0.63	-0.94	0.13
'Caudate_L'	-20	-18	24	12.09	-0.20	0.09	-0.01	-0.13	0.07
'Temporal_Inf_L'	-52	-24	-18	11.25	0.04	-0.26	0.12	0.27	0.08
'Caudate_R'	18	14	2.7	10.77	0.04	0.36	0.39	0.21	0.09
B. TFMPP X placebo interaction $p < 0.005$ uncorrected									
'Thalamus_R'	16	-24	8	25.68	-0.18	0.09	0.12	-0.14	0.07
'Thalamus_R'	18	-26	0	11.06	0.0524	0.21	0.16	-0.01	0.07
'Thalamus_R'	8	-12	2	21.03	-0.0967	0.30	0.20	-0.06	0.10
'Lingual_L'	-24	-72	-4	18.25	0.231	-0.22	-0.03	0.14	0.10
C. BZP/TFMPP X placebo interaction $p < 0.005$ uncorrected									
'Thalamus_R'	17	-14	11	13.82	-0.06	0.29	0.16	-0.02	0.09
'Thalamus_L'	-8	-10	0	12.70	0.02	0.39	0.10	0.07	0.07
'Caudate_L'	-7	7	12	12.26	-0.45	-0.20	-0.14	-0.36	0.08
'Temporal_Inf_L'	-46	-16	-24	10.07	0.08	-0.27	-0.17	0.00	0.10

Table 9: Neural correlates of activation of Drug state in comparison to placebo for the Stroop interaction-incongruent (incong)- congruent (cong)

Note: All clusters are significant at $p < 0.005$ (uncorrected); cluster threshold of 5 voxels
The *F* value at the peak voxel within each cluster is reported.

Cong: congruent; incong: incongruent; SE: standard error

The aim of this study was to investigate whether there were regional differences in neural network activations due to selective attention and/or inhibition after giving either BZP, TFMPP or BZP + TFMPP in combination, in comparison to placebo. An F-contrast was constructed to examine the Stroop interaction, specifically, by looking at an interaction between the drug state and placebo for the Stroop effect (see Table 9)

Stroop contrasts (incongruent minus congruent) for the BZP drug state compared to placebo, yielded activations at the significance level of $p < 0.005$ uncorrected with an extent threshold of 5 voxels. Parameter estimates were derived for the congruent and incongruent conditions at each significant coordinate to determine the direction of the activation change. Parameter estimates were interpreted as the percentage BOLD change in relation to the whole brain mean (411), referred to as percentage BOLD signal change.

In comparison to placebo, BZP induced four significant clusters in the bilateral caudate (Figure 36), left inferior temporal gyrus and right superior occipital gyrus (Table 9, Section A). The cluster in the caudate was found to be due to increased activation during the incongruent condition, the left inferior temporal gyrus showed decreased activation during the incongruent condition and the right superior occipital gyrus cluster is derived from the attenuation of the BZP incongruent condition to a lesser extent than following placebo.

TFMPP, in comparison to placebo, induced four clusters: three in the right thalamus (Figure 37) and one in the left lingual gyrus (Table 9, Section B). The percentage BOLD signal change indicated that all three clusters displayed greater activation following TFMPP in the incongruent condition and that lingual activation increased in the TFMPP congruent condition compared to placebo.

When BZP and TFMPP were given together and compared to placebo, activation occurred in the thalamus, right caudate, and left inferior temporal gyrus (Table 3, Section C). Percentage BOLD signal change plots identified that the cluster in the thalamus was due to increased activation following TFMPP in the incongruent condition (similar to that caused by TFMPP alone). Alternatively, the caudate showed *reduced activation* following the combination of BZP and TFMPP in the congruent condition in comparison to placebo.

Neural regions modulated by the Stroop effect: BZP x placebo

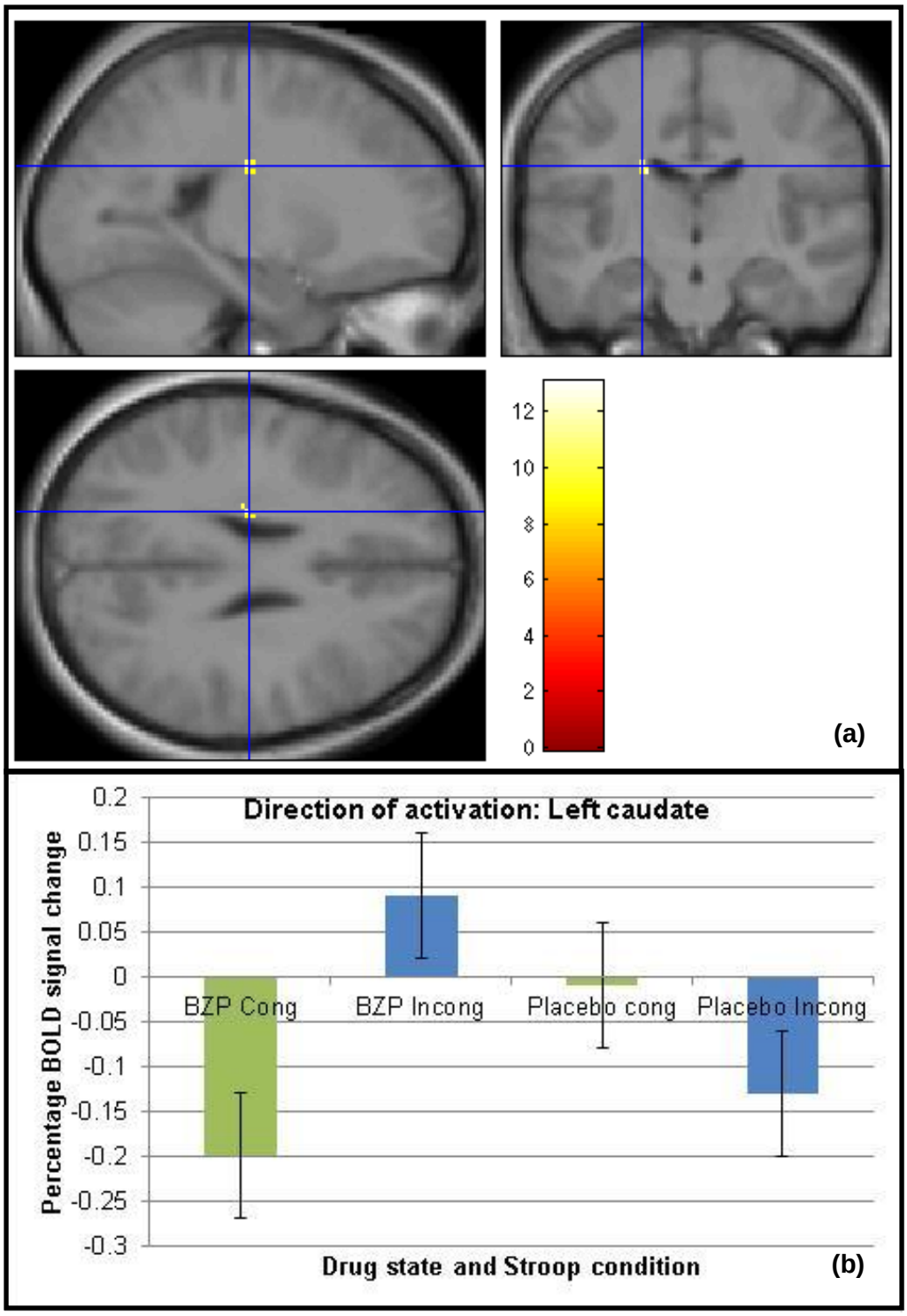

Figure 36: Activations associated with the Stroop interference contrast:, when BZP is contrasted to placebo p <0.005 uncorrected; cluster threshold > 5 voxels
(a) Activation in the left caudate and (b) Plot of parameter estimates, indicating the direction of activation in the left caudate

Neural regions modulated by the Stroop effect: TFMPP x placebo

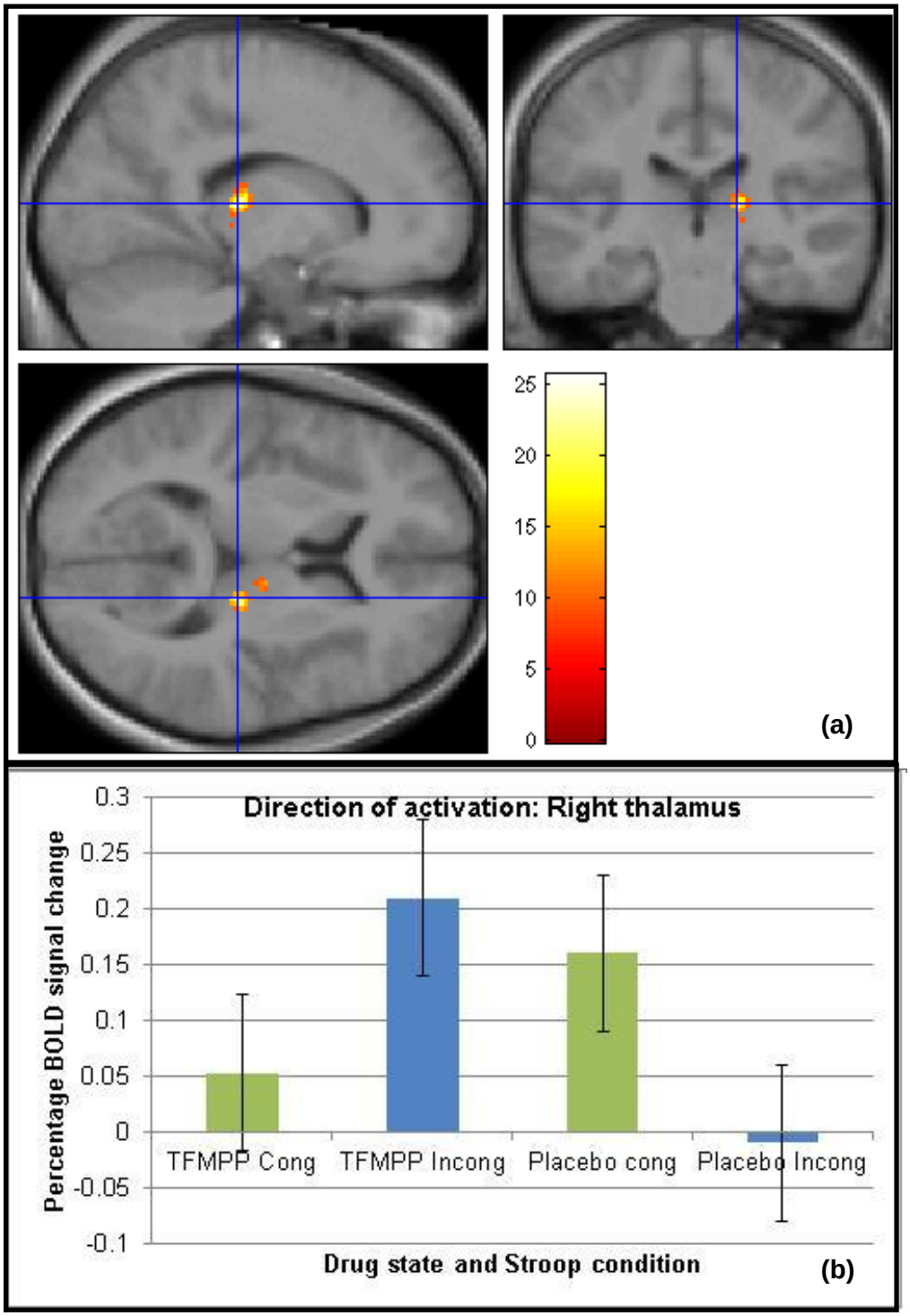

Figure 37: Activations associated with the Stroop interference contrast:, when TFMPP is contrasted to placebo p <0.005 uncorrected; cluster threshold > 5 voxels
(a) Activation in the right thalamus and (b) Plot of parameter estimates, indicating the direction of activation in the right thalamus

3.2.4. Discussion

This study investigated the acute effects of BZP and TFMPP both alone and in combination in comparison to placebo on the neural networks associated with selective attention and inhibition using an event-related Stroop paradigm during fMRI. Stroop interference contrasts were used to reflect inhibitory performance whilst conducting the task. It is generally agreed that the behavioural effects induced by the Stroop paradigm are due to a conflict between a pre-potent response and a weaker, task-relevant response.

Behavioural performance is an important aspect of this study. In our research, both Ac and Rt were not significantly affected by each drug or the combination. However, fMRI analysis shows distinct drug-induced differences. These changes in activation are a reflection of a change in processing to some degree, based on the resources allocated to task performance. Regional activation during the Stroop paradigm has been reported predominantly in the ACC and DLPFC (127, 412), although their respective roles have been disputed. The ACC is thought to play a role in the application of attentional control (413), detection of information conflict (414-416) and monitoring of performance (148, 414, 417). However, other researchers have argued that top-down attentional control itself may be mediated by structures such as the DLPFC (148, 418-420). As our study focused on drug-induced effects during the Stroop paradigm, we hypothesised that we could identify activation in additional areas that would reflect drug-induced changes in neural processing.

BZP induced regional activation in the bilateral caudate, L inferior temporal gyrus and R superior occipital gyrus when compared to placebo (Table 9, Section A). We believe reduced activation of the temporal and occipital gyri were likely due to processing visual stimuli. Dopaminergic modulation is reportedly involved in the guidance of attention toward relevant locations and in the cognitive processing of visual stimuli (421, 422). The changes induced by BZP may be a reflection of dopaminergic stimulation that subsequently requires the allocation of fewer resources than would normally occur.

In humans, bromocriptine (2.5 mg), a known D_2 agonist and amphetamine (0.25 mg/kg) decrease reaction times during the Stroop task (225, 249). The effects of BZP are mainly dopaminergic, with lesser effects on noradrenergic and serotonergic pathways. The mesocorticolimbic DA system, which includes the dorsal striatum (caudate and putamen) is implicated in reward processing. The caudate, rich in DA receptors, is reported in part to mediate the relationship between action and reward outcome (175). Dopaminergic neurons within the caudate nucleus increase firing following unexpected rewards and conditioned stimuli associated with reward (138). Imaging studies have reported this phenomenon following both primary and secondary rewards. For example, Knutson and colleagues (114)

used monetary incentive tasks to investigate reward and punishment. Elliot et al. proposed that the striatum and amygdala mediate the function of rewards in eliciting goal directed behaviour, whilst others have shown that the head of the caudate nucleus is involved in coding reward prediction errors during goal directed behaviour (138, 348, 423). While the role of the caudate involves responding to reward, it is also is said to contribute to the ability to learn through reinforcement (138). Since BZP affects dopaminergic neurons, we initially considered that the overall increase in DA release increased activation within the caudate, rather than the task itself. However, if this was the only factor, the change we observed in the bilateral caudate would also show increased activation during the congruent and incongruent conditions, which did not occur. There is only an increase during the BZP incongruent condition, which leads us to assume that it is partially a task-related change in processing causing activation.

Zink and colleagues (424) suggest that caudate activity is closely linked to the behavioural relevance of the stimuli. In our study, the bilateral caudate was activated following BZP during the incongruent condition. Therefore, this could be an aid to learning, which requires suppression of the pre-potent response and responding to the weaker task-relevant stimuli.

Alternatively, it has been suggested that the head of the caudate controls interference. An fMRI study of healthy participants completing the Stroop and Simon tasks with the aim of investigating both word and spatial interference respectively, found the head of the left caudate was activated during Stroop interference only. This suggests the caudate plays a role in the control of word but not spatial interference (231, 244). In addition, Li et al. (245) demonstrated that during a stop-signal task the caudate plays a role in the inhibitory control of pre-potent responses.

We propose BZP impairs the ability to attend to task-relevant information during the task, thus requiring recruitment of the caudate as a compensatory mechanism, either as an aid to learning or for inhibitory control, which allowed participants to perform to the same standard as they had following placebo.

TFMPP, in contrast to BZP, is a serotonin agonist that induced four clusters of activation: three in the right thalamus and one in the left lingual gyrus (Table 9, Section B). The lingual gyrus is activated following the presentation of visual stimuli. In research by Andrews and Anderson (383) fenfluramine, also a serotonergic agonist, increased flicker fusion threshold suggesting 5-HT enhances early stage visual information processing, and thus accounting for changes in activation following TFMPP administration.

Associations between 5-HT, inhibition and attention have been reported. For example, rodent studies demonstrated a modulatory role for 5-HT in inhibitory control processing (425). However, it has been proposed that different 5-HT receptor subtypes have opposing effects, that is, activation of 5-HT_{2A} receptors enhances DA release (426) and in contrast, 5-HT_{2C} activation inhibits DA release (33, 41, 42, 426). After administration of SB 242084 (a 5-HT_{2C} receptor antagonist) rodents completing the five-choice serial reaction time task showed an increase in premature responding and a decreased latency indicating that 5-HT_{2C} receptors play a role in regulating behavioural inhibition (426). TFMPP's stimulus effects are thought to be mediated by 5-HT_{2C} receptors, and thus might reduce DA release.

The effect of reducing global 5-HT content on selective attention has been investigated in humans following acute tryptophan (a precursor of 5-HT) depletion. An fMRI investigation of the effects of ATD on the Stroop task found improved performance and modulation of the BOLD response (427). The authors suggested this was due to the removal of the inhibitory influence of 5-HT on cortical arousal. As 5-HT release promotes cortical de-arousal systems (428, 429), thus decreasing 5-HT function should reduce inhibition and improve arousal and attention.

An fMRI study investigating the effects of mCPP on humans reported an enhanced response within the lateral OFC and no significant change in behavioural effects (93). mCPP, like TFMPP is a 5-HT_{2C} agonist and also found in some party pill preparations. The areas of activation observed following administration of mCPP were consistent with the hypothesis that 5-HT affects inhibitory responses. Therefore, we hypothesised that TFMPP would also impair behavioural performance during the Stroop task; however, we did not find this.

The thalamus, rich in 5-HT reuptake sites, is affected by antidepressants (430). Serotonergic pathways play an important role in modulating behavioural arousal (428, 429). After administering TFMPP we observed an increase in thalamic activation, so we propose that this region was recruited in a compensatory manner allowing participants to maintain attention on the task in the presence of altered arousal. Attention and arousal are two cognitive domains that are linked. Whilst attention is governed mainly by cortical systems, arousal is governed mainly by subcortical structures however, both domains share an important anatomical structure i.e. the thalamus (431).

There have been similar reports of compensatory recruitment mediating attentional processes. For example, clonidine, an α_1/α_2 agonist that down-regulates central noradrenergic function, also reduced sustained attention and reduced activation in the thalamus. When participants were required to complete a task requiring attention during

imaging, the thalamus showed increased activation (432). The effects of clonidine were thought to reflect its effects on cortical arousal, thus the thalamus was only recruited to complete the task.

Further to this hypothesis, the function of the thalamus has been described as a gateway for cortical signalling (433). It is part of the cortico-striato-thalamo-cortical loop, and plays a key role in controlling or "gating" information to the cortex (434) and consequently involved in regulating the level of awareness and attention attributed to specific stimuli. Studies have shown that the greatest amount of information transfer to the thalamus occurs while a person is awake (435), especially when attention is required for a task, with Crick (1984) describing the thalamus as a type of "searchlight" (436, 437).

Research has shown that psychostimulants such as amphetamine improve vigilance, attention and concentration in healthy control subjects (225, 438). Since TFMPP has inhibitory effects on both DA and NA, we expected our participants would have a lower standard of accuracy and/or a longer reaction time supporting our hypothesis that the thalamus is recruited in a compensatory manner. However this was not the case.

In subjects that have no disturbances in monoaminergic systems, DA and 5-HT have inhibitory influences on the striatum (439). GABAergic input from the striatum and the pallidum is thought to have an inhibitory effect on the neurons in the thalamus. This inhibition should active in a protective manner, as the result should be a reduction in sensory input into the cortex from the thalamus. Therefore, if there is an increase in DA or 5-HT, this may lead to a reduction in the inhibitory influence of the striatum and open this thalamic filter, possibly leading to an overload of sensory information to the cortex and potentially psychoses (433). Therefore, the thalamic activation that we observe following TFMPP may be a reflection of this gating influence being modulated by the disturbance in 5-HT and DA.

As stated previously, TFMPP predominantly induces 5-HT release, with indirect effects on dopaminergic and adrenergic transmission in the frontal cortex (33). 5-HT_{2C} agonists reduce the firing rate of mesolimbic dopaminergic neurons originating in the VTA, subsequently leading to a reduction in DA release in the NAcc and frontal cortex (33, 41, 42). Therefore, the increased thalamic activation we observed could be a result of increased serotonergic activity leading to reduced dopaminergic activity and subsequently, reduced inhibition in the thalamus.

When BZP and TFMPP were given in combination, activation of both the thalamus and the left dorsal striatum occurred (Table 9, Section C). The increased activation of the thalamus

was similar to that induced by TFMPP alone while caudal activation induced by BZP alone and in combination with TFMPP was not the same. Further analysis revealed that the activation arose from different conditions. Increased caudate activation induced by BZP occurred during the Stroop incongruent condition. In contrast, BZP combined with TFMPP induced activation resulted from *attenuation during the* congruent condition in comparison to placebo.

We hypothesised that changes in activation induced by BZP combined with TFMPP would reflect the changes we observed after giving them individually but this did not occur uniformly. The subsequent attenuated activation of the caudate suggests that when BZP and TFMPP are combined it is likely that their differential effects on dopaminergic and serotonergic neurons are responsible. TFMPP is thought to have opposing effects on dopaminergic activity because it is a 5-HT_{2C} receptor agonist and therefore inhibits firing within the dopaminergic mesolimbic system (41, 42). Consequently, a comparative reduction in DA release compared to that observed following BZP alone may be responsible. Furthermore, this is reflected by research that found a combination of BZP and TFMPP was a less effective reinforcer than BZP alone in rhesus monkeys (37).

The subjective and neurophysiological effects induced by the combination of BZP and TFMPP have been compared to MDMA (50). Following TFMPP administration both alone and combined with BZP, there was increase in thalamic activity, which we propose is due to compensatory recruitment induced by a state of altered arousal. In this sense, this does not reflect the effects of MDMA because MDMA *increases* arousal.

The event-related Stroop paradigm we used was of moderate length and the participants were asked to repeat the task every 7 days to complete the overall trial (i.e., after taking each drug/placebo), which could have led to a learned response to the Stroop effect. Therefore, the trial was designed to give each drug/placebo in a randomised order to ensure that any learned effect had limited impact.

We also chose doses of BZP and/or TFMPP based on our laboratory's past research, that is, doses known to evoke behavioural responses while avoiding drug-induced adverse effects. It is likely that higher doses than those used in this trial may result in differential effects.

3.2.5. Conclusion

This study is the first to investigate the effects of the relatively new synthetic drugs BZP and TFMPP both alone and in combination on selective attention and impulsivity using fMRI. While no significant behavioural effects during the Stroop task were observed, we

found separable drug-induced changes in regional activation. BZP increased activation in the dorsal striatum possibly due to an inability to attend to task-relevant information. TFMPP induced thalamic activation, suggesting that compensatory resources were recruited that allowed participants to perform the Stroop task to the same standard as those who had taken placebo. When the BZP and TFMPP were given together, there was activation in both the thalamus and the dorsal striatum, albeit caudate activation was attenuated by this combination.

3.3. Stroop Task and BZP in Comparison to Dexamphetamine

Comparing the effects of benzylpiperazine to dexamphetamine to assess the differences in inhibition and executive function in humans using functional magnetic resonance imaging (fMRI).

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3.3.1. Introduction

The Stroop task has been frequently used as a psychological tool to evaluate selective attention and inhibition. Recently, by combining the task with functional magnetic resonance imaging (fMRI) or pharmaco MRI (phMRI), we were able to investigate the effects that certain patient groups and drugs have on the task. The classical colour-word Stroop paradigm involves the participant responding to one aspect of the presented stimulus whilst ignoring another (220). The participant is asked to respond to the colour that the word is written in. The stimuli presented can be one of three conditions: control, where a non-colour word is presented; congruent, where the colour and the word match (e.g., red written in red); and the incongruent condition, where the colour and the word do not match (e.g., red written in blue). The Stroop effect is the difference between the congruent and the incongruent conditions on speed, accuracy (220) and in the case of imaging tasks, correlating activations in neural substrates. This effect that is seen is thought to be derived from the difficulty of suppressing the natural or pre-potent response to read the word. This action of reading and responding to the written word is thought to be a more automatic response as it is more practised (221, 222).

Executive function encompasses a range of processes including the ability to selectively attend to and inhibit responses on tasks. The prefrontal cortex has been reported to have a critical role in the mediation of executive functions. Dopaminergic circuits project from the midbrain to the PFC, with connections from the striatum to the basal ganglia. Dopamine levels have an important impact on these circuits with suggestions that cognitive performance is affected by DA in an inverted U-shaped response curve manner (246); when the levels of DA are too high or too low, due to either drug administration or dysfunctions in the DA circuitry, the result is an impairment in performance. These alterations in the dopaminergic circuitry may be due to either drug administration or dysfunctions in the DA circuitry, such as in schizophrenia and ADHD.

There is a growing body of research that has shown that DA agonists can improve cognition in healthy controls, with bromocriptine (440), amphetamine (225) and MPH (441) all showing improvements. However, there is also evidence that these improvements may be dependent on the performance prior to drug administration, that is, those with the worst performance had the greatest improvement, whilst those with already optimum performance were impaired (247, 248).

BZP is a popular constituent in a group of relatively new synthetic club drugs, which are reported to be found in similar environments to MDMA. The subjective effects of BZP are similar to the amphetamines MDMA and MA (2). BZP has been shown in animal studies to have mainly dopaminergic activity, with additional but lesser effects on both NA and 5-HT (19). Preclinical research investigating the pharmacology of BZP has shown it inhibits dopaminergic uptake in a manner similar to cocaine (16, 17), releases DA from nerve terminals in a similar fashion to amphetamine (18, 19), and acts as a direct agonist on postsynaptic dopaminergic receptors (14).

In addition, there was a dose-dependent elevation in extracellular DA and 5-HT in the NAcc of rats after BZP was intravenously administered (3 and 10 mg), although 5-HT release was only affected with the higher dose (19). Peripheral release of NA has also been reported by blocking synaptic reuptake (20), and increases both the resting and the nerve-evoked release of NA in the isolated rabbit pulmonary artery (20).

We have previously compared the effects of BZP with placebo on its effects on the Stroop task in healthy volunteers. We reported that although there were no behavioural differences, there was an increase in activation in several neural regions, including the caudate (382). The caudate has been reported to have a role in controlling interference, with prior studies shown that the left caudate was associated with Stroop interference (231). Furthermore, during a stop-signal task, designed to study the effects of behavioural inhibition, the caudate was found inhibit the control of pre-potent responses (245).

Despite reports of BZP showing subjective similarities to amphetamine and neurochemical similarities in animal studies, BZP has never been directly compared with amphetamine's effects on selective attention and inhibition. This study aims to further investigate the effects of BZP on selective attention and inhibition, using an event-related Stroop paradigm, by comparing it to DEX whilst undergoing fMRI.

3.3.2. Materials and Methods

Thirteen non-smoking healthy participants (seven female and six male; aged 18–40 years) were recruited to participate in a double-blind placebo controlled cross-over trial. Approval

for this study was granted by the Northern X Regional Ethics Committee of NZ (Ethics approval number NTX/07/08/078). Participants attended an initial screening session, where written consent was obtained, and were excluded if they had a history of mental illness, cardiac disease, head trauma, endocrine disorders, epilepsy, were pregnant or breastfeeding. Two data sets were rendered unusable due to faults in data collection, leaving 11 subjects for each drug comparison.

A custom designed questionnaire was completed by each participant, detailing their medication history, recreational drug, party pill, alcohol and cigarette use, sleeping patterns and stress levels. This was to ensure that participants were not drug naive, but also were not current or past heavy users of recreational drugs. Participants were required to produce a negative urine analysis, testing for recreational drugs, and where appropriate, a negative pregnancy test prior to the trial commencing on each test day.

3.3.2.1. Drugs

Benzylpiperazine hydrochloride (200 mg) and dexamphetamine (20 mg) capsules were manufactured by the School of Pharmacy, University of Auckland New Zealand, using good manufacturing practice. Identical placebo capsules were also manufactured and contained methylcellulose.

3.3.2.2. Procedure

The Stroop paradigm has been frequently used by researchers to investigate selective attention and inhibition. The three conditions were neutral (control) words, which comprised of a non-colour word, congruent where a colour word was presented in its matching colour and incongruent where the colour word and colour did not match. When the incongruent condition is presented there is a pre-potent response to respond to the written word rather than the colour. The inability to suppress this pre-potent response and respond to the weaker but task-relevant response is a reflection of impulsivity and impaired selective attention (442). Furthermore, when the word and the colour conflict, as in the incongruent condition, participants are slower to respond than when there is no, or less, conflict reflected by control and congruent conditions. This is known as the Stroop interference effect (410). Prior to the trial commencing participants were required to complete a practice version of the colour-word Stroop paradigm to a minimum of 75% accuracy.

Participants were tested with each drug and placebo in a randomised schedule using double-blind conditions with a minimum of 7 days between sessions. Participants fasted for 12 hours before the trial and were asked to abstain from alcohol or caffeine from the evening prior to testing. All capsules were administered with 250 mL of water 90 minutes

before imaging; the time taken to reach the peak plasma concentration of BZP is 75 minutes (393) and the onset of action of DEX is 30 minutes (394) and peak plasma concentration is reached 2 to 3 hours after administration (395). During this time, participants remained in the presence of researchers in a comfortable area with minimal stimulation.

3.3.2.3. FMRI data analysis and acquisition

Blood oxygen level dependent functional images were acquired using a T2*-weighted echo planar imaging (EPI) sequence with a 1.5T Siemens Magnetom Avanto scanner using the following parameters: TR 3000 ms, TE 50 ms, FOV 192 mm, in-plane voxel size 3.0 mm× 3.0 mm, flip angle 90°, 29 slices, slice thickness 4.0 mm no gap. On each trial day 157 volumes were collected for each participant for each run and two runs were completed at each visit with a 30 second break between each run. For anatomical reference, a high-resolution structural MPRAGE image was acquired for each at the end of the first session.

fMRI testing was performed at the Centre for advanced MRI at The University of Auckland. The Stroop paradigm was completed whilst participants were being scanned. The screen was located 3.5 metres from the participants, at the foot of the scanner and was visible via a prism built into the head restraint used to minimise head movements during scanning.

Control, congruent, incongruent and rest (fixation cross) conditions were presented to the participants. Each trial consisted of 180 presentations: 36 congruent, 36 incongruent, 72 control and 36 rest fixation crosses. Four different versions of the event-related Stroop task were used, so participants never received the same order of randomised words. Each stimulus was presented for 2000 msec with a jittered inter-stimulus interval with a mean of 500 msec, which has been reported by Dale and colleagues to be sufficient to allow for efficient estimation (333, 334). Participants were instructed to respond to the colour of a presented word, as soon as it appeared on the screen, using two, two-buttoned hand held response boxes (one in each hand) to minimise potential head movement caused by vocalisation. Each button was assigned a colour (from left to right: red, green, blue and yellow). Incorrect responses were not used in either the reaction time or functional data to ensure that the errors did not affect the selective- attention data.

Raw data were analysed using SPM8 (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB version 7.8.0 (Mathworks, Sherborn, MA, USA). After being co-registered to the T1- weighted structural volume, EPI images were normalised to a standard space (Montreal Neurological Institute [MNI] template). Images were spatially smoothed using an isotropic Gaussian kernel of 8 mm full-width at half-

maximum (FWHM) in the x, y, and z axes. Incorrect and non-responses to the Stroop paradigm were not eliminated from analysis as the accuracy was above 90% in all cases.

The first level analysis allowed for an individual subject's activation to be evaluated for the three Stroop conditions, that is, congruent, control and incongruent by constructing t-contrasts. No interaction contrasts were made at this stage to maintain maximum specificity at second level analysis.

T-contrasts were subsequently used in a second level group comparison analysis. Event-related responses to the Stroop effect (congruent minus incongruent) were defined. Analysis was divided into two parts: (1) BZP in comparison to DEX, and (2) DEX in comparison to placebo by constructing F interaction contrasts.

Voxel-wise analysis was conducted using fMRI data with a significance threshold of $p < 0.005$ uncorrected. Anatomical locations were derived using a customised script in SPM8 (336). Parameter estimates of the conditions at significant coordinates were plotted; this can be interpreted as the percentage BOLD signal change in reference to the whole brain mean, which allows the determination of the direction of activation. Significant clusters of activation were displayed using an average brain created from the structural files of participants.

Outliers due to movement or signal from the pre-processed EPI files, using thresholds of 3 *SD* from the mean, 0.75mm for translation and 0.02 radians rotation were removed from the data sets using ART repair (335). Outliers were recorded to ensure no more than 15% of scans were removed from each run (two runs per session). Furthermore, ART repair was used to check for stimulus correlated motion at first level analysis, and for top-down quality assurance purposes the ResMS, the image that shows the variance of the error, the mask, beta, con and SPMT images were checked for abnormalities and artefacts after both first and second level. An *F*-test across all conditions was carried out per session to ensure that each subject had activity in the visual cortex after first level analysis.

Behavioural data (accuracy [Ac] and reaction time [Rt]) were analysed in SPSS 19, using a repeated measures ANOVA for both condition effect and group (drug state) x condition effect. Rt data was filtered to display correct responses only.

3.3.3. Results

The aim of this study was to investigate whether there were regional differences in the activations of neural networks due to selective attention and/or inhibition after giving either DEX relative to placebo and BZP in comparison to DEX.

There were no significant differences between drug state (inter-group) comparisons for both Ac and Rt. However, within each group (intra-group) differences were found between congruent (Cong) and incongruent (Incong) conditions $p < 0.0001$ (Table 10). Intra-group Rt comparisons showed the "Stroop effect" where the incongruent task has longer response times than congruent and control tasks (Figure 38 and 39). There were no significant differences in inter-group Ac (Table 11).

Accuracy (Ac)

Drug state	**DEX**			**Placebo**		
Condition	**Cong**	**Control**	**Incong**	**Cong**	**Control**	**Incong**
Mean Ac	0.99	0.99	0.98	0.97	0.98	0.97
Standard error	0.02	0.01	0.03	0.06	0.03	0.02
Drug state	**BZP**			**DEX**		
Condition	**Cong**	**Control**	**Incong**	**Cong**	**Control**	**Incong**
Mean Ac	0.98	0.99	0.97	0.99	0.99	0.98
Standard error	0.02	0.01	0.03	0.02	0.01	0.03

Table 10: Mean Ac ± the SE for each Stroop condition after taking either BZP, DEX or placebo

Rt (msec)

Drug state	**DEX**			**Placebo**		
Condition	**Cong**	**Control**	**Incong**	**Cong**	**Control**	**Incong**
Mean RT (msec)	706.06	740.20	849.79	735.87	779.47	855.58
Standard error	126.80	128.31	117.37	135.59	135.44	94.22
Drug state	**BZP**			**DEX**		
Condition	**Cong**	**Control**	**Incong**	**Cong**	**Control**	**Incong**
Mean RT (msec)	712.22	741.35	861.21	706.06	740.20	849.79
Standard error	116.77	127.70	146.94	126.80	128.31	117.37

Table 11: Mean Rt (msec) ± the SE for each Stroop condition after taking either BZP, DEX or placebo

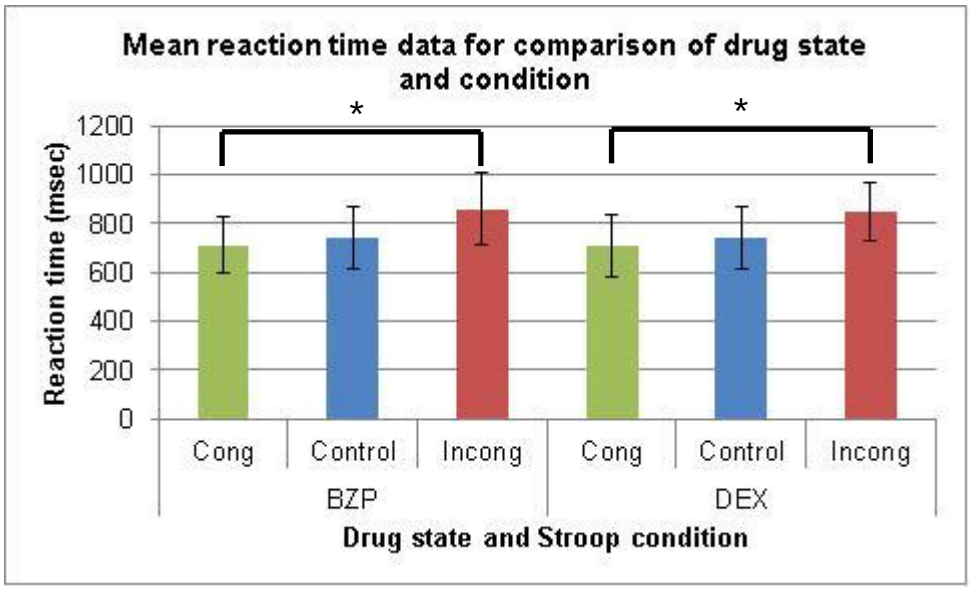

Figure 38: Mean Rt data for the comparison of Stroop conditions for BZPxDEX comparison
Note: no significant differences were found between groups, but within groups between the congruent (cong) and incongruent (incong) conditions (*) $p < 0.05$

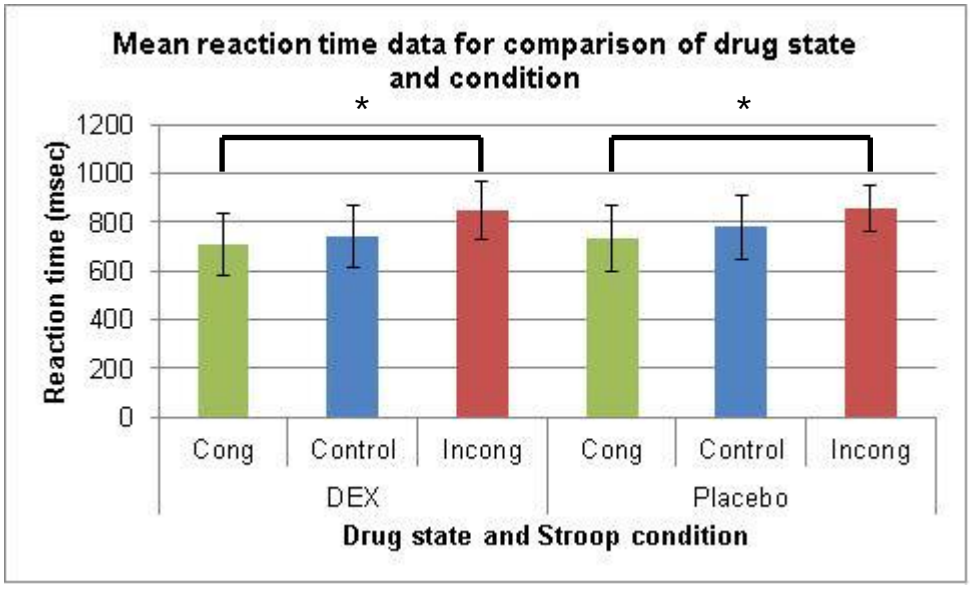

Figure 39: Mean Rt data for the comparison Stroop conditions for DEX x placebo
Note: no significant differences were found between groups, but within groups between the congruent (cong) and incongruent (incong) conditions (*) $p < 0.05$

Anatomical region	MNI coordinates			Directionality: Contrast estimates and SE					
A. DEX x placebo interaction $p < 0.005$ uncorrected									
	x	**y**	**z**	**F value**	**DEX Cong**	**DEX Incong**	**Placebo cong**	**Placebo Incong**	**SE**
'Thalamus_R'	12	-10	0	19.89	-0.0431	0.3747	0.1549	0.0386	0.075
'Precentral_R'	51	1	29	16.32	0.3603	0.2499	0.0163	0.4066	0.077
'Thalamus_R'	16	-22	10	15.83	0.3846	0.6004	0.3266	-0.0173	0.088
'Hippocampus_L'	-22	-8	-22	15.62	0.1793	-0.1566	-0.3275	-0.1364	0.083
'Occipital_Mid_L'	-34	-70	30	14.39	0.8309	0.8306	0.314	1.0699	0.124
'Fusiform_R'	32	-48	-4	13.49	-0.2027	-0.5216	-0.2885	-0.1822	0.072
'Frontal_Inf_Oper_L'	-32	16	30	12.35	0.1021	0.0406	-0.1535	0.1429	0.063
B. BZP x DEX interaction $p < 0.005$ uncorrected									
	x	**y**	**z**	**F value**	**BZP Cong**	**BZP Incong**	**DEX cong**	**DEX Incong**	**SE**
'Frontal_Inf_Tri_L'	-36	34	6	14.40	-0.2565	0.1796	0.0542	-0.0962	0.091
'Cingulum_Ant_L'	-10	47	1	11.90	-0.3207	-1.0692	-0.678	-0.7001	0.124
'Frontal_Sup_Medial_R'	8	52	4	11.24	-0.1793	-0.8635	-0.4857	-0.4001	0.135
'Frontal_Mid_Orb_L'	-2	40	-8	11.24	-0.1633	-1.1493	-0.6934	-0.6825	0.175

Table 12: Neural correlates of activation of DEX in comparison to placebo, and BZP in comparison to DEX for the Stroop interaction (incongruent (incong)- congruent (cong))
Note: All clusters are significant at $p < 0.005$ (uncorrected); cluster threshold of 5 voxels
The *F* value at the peak voxel within each cluster is reported.
Cong: congruent; incong: incongruent; SE: standard error

An F-contrast was constructed to examine the Stroop interaction, specifically by looking at an interaction between the DEX and placebo, or BZP and DEX to allow for the direct comparison (see Table 11). The clusters of activations that are reported are at $p < 0.005$ uncorrected, with an extent threshold of five voxels. Parameter estimates were derived for the congruent and incongruent conditions at each significant coordinate to determine the direction of the activation (i.e., incongruent>congruent or congruent>incongruent) induced by each interaction. Parameter estimated were interpreted as the percentage BOLD change in relation to the whole brain mean (411), referred to as percentage BOLD signal change.

Stroop interference (incongruent minus congruent conditions) for the DEX drug state compared to placebo yielded seven significant clusters. These clusters were located in the right thalamus, right precentral, left hippocampus, left middle occipital, left inferior frontal and right fusiform gyri (Table 11, Section A).

The cluster observed in the thalamus (Figure 41) is derived from the increase in activation in the DEX incongruent condition. Conversely, the cluster in the inferior frontal activation stems from a decrease in activation in the incongruent DEX drug state. The precentral and occipital clusters are caused by the increase in activation in the placebo incongruent

condition, whereas the activity DEX congruent and incongruent conditions were relatively unchanged in this region. Hippocampal activation stems from the congruent DEX condition, with a deactivation in all other conditions. Finally, the fusiform gyrus shows deactivation in all conditions, with DEX incongruent causing the greatest deactivation.

The direct comparison of BZP to DEX resulted in four clusters of activation, including left IFG (Figure 40), left ACC, right medial superior frontal and left middle frontal gyri. Left IFG activation was evoked by an increase in activation following the BZP during the incongruent condition. In the remaining three clusters, all conditions show relative deactivation, with the BZP incongruent condition causing the greatest deactivation.

Neural regions modulated by the Stroop effect: BZP x DEX

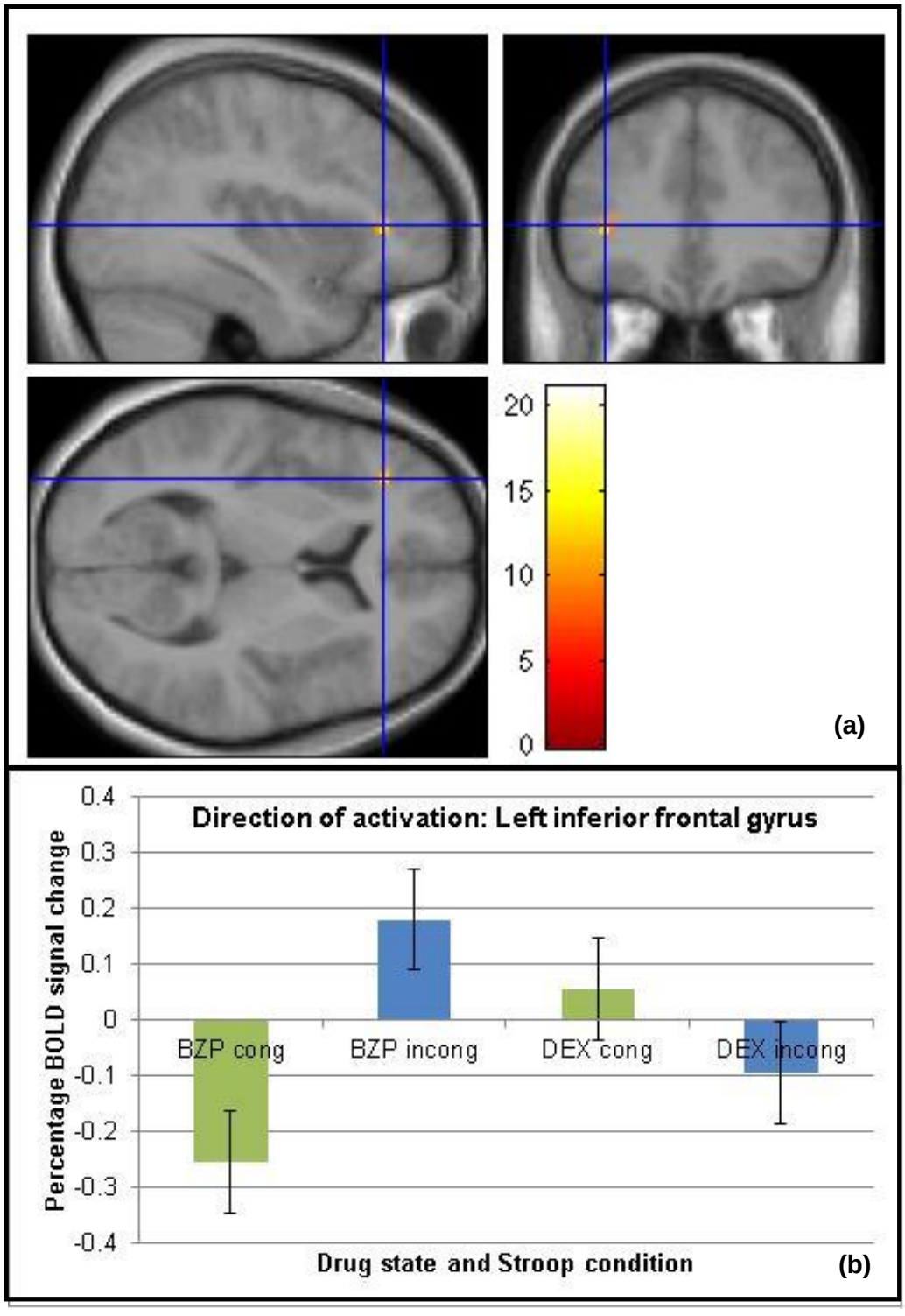

Figure 40: Activations associated with the Stroop interference contrast:, when BZP is contrasted to DEX p <0.005 uncorrected; cluster threshold > 5 voxels
(a) Activation in the left inferior frontal gyrus and (b) Plot of parameter estimates, indicating the direction of activation in the left inferior frontal gyrus

Neural regions modulated by the Stroop effect: DEX x placebo

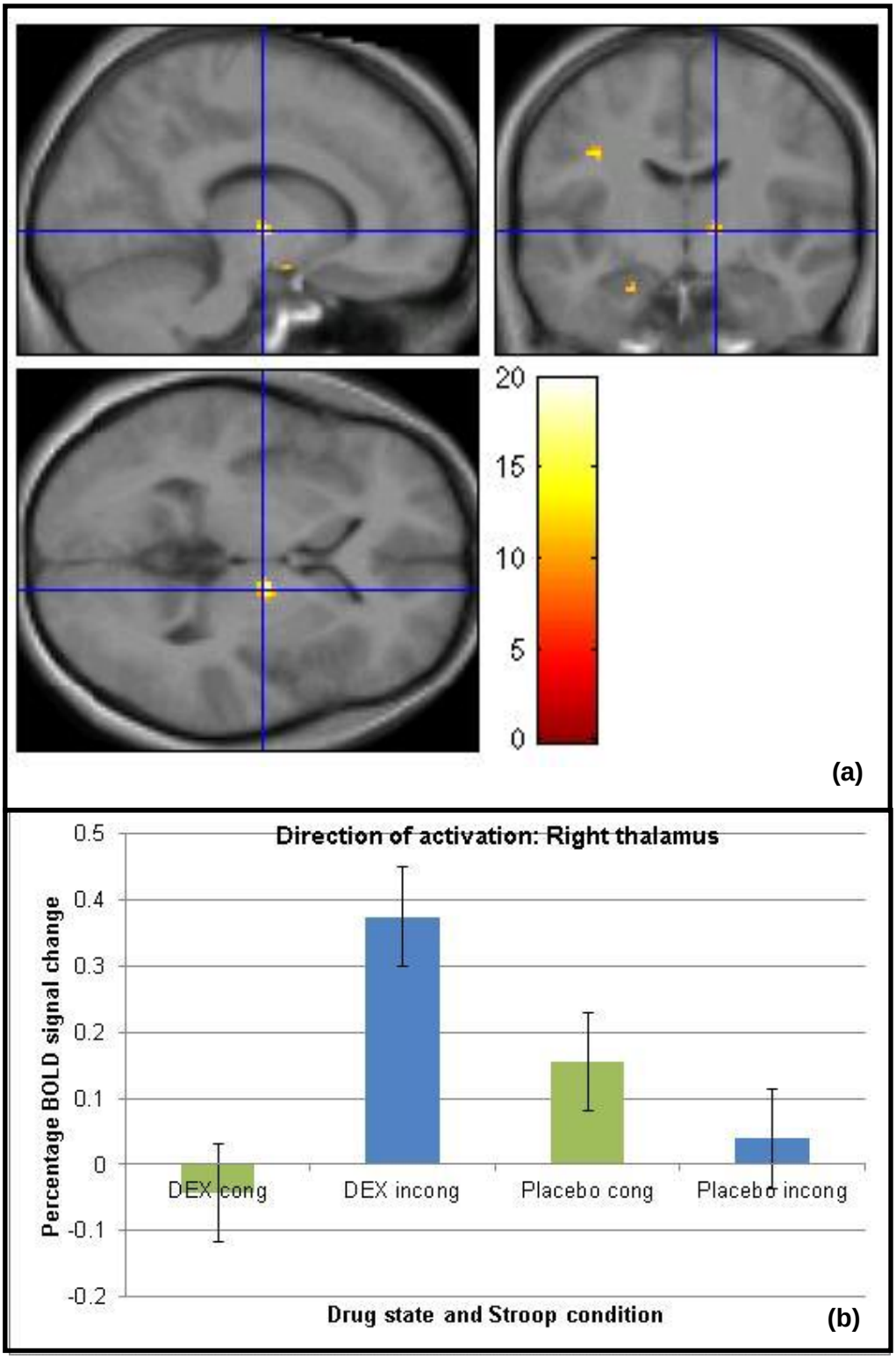

Figure 41: Activations associated with the Stroop interference contrast:, when DEX is contrasted to placebo p <0.005 uncorrected; cluster threshold > 5 voxels
(a) Activation in the right thalamus and (b) Plot of parameter estimates, indicating the direction of activation in right thalamus

3.3.4. Discussion

This is the first study to directly compare the effects of BZP with DEX, on the neural networks involved during selective attention and inhibition. This was conducted by using an event-related fMRI protocol whilst participants completed the Stroop task. Stroop interference contrasts have been used in several investigations to study cognitive control, when faced with an interference dimension. Patients with frontal lobe lesions and those with disorders such as ADHD (224) and schizophrenia (219, 225) have shown impairments in the Stroop task. In addition, the effects of recreational drugs and drug dependence modulate the Stroop effect (227).

Changes in regional activation have been reported in several studies, mainly focussing on the ACC and DLPFC. The ACCs role in cognitive control has been defined as a general monitoring system (230), but other authors specifically suggest a role in detecting any conflict (414-416). Azizian and colleagues (234) proposed that an increase in activation of the ACC may demonstrate a compensatory mechanism by which the region is recruited to ensure support for selective attention processes. In addition, the DLPFC has been proposed to be involved in the resolution of the conflict (148, 418-420).

We recently compared the effects of BZP with placebo, and found that BZP caused an increase in caudate activation. We suggest that this was due to compensatory neuronal recruitment allowing the task to be completed to the same accuracy and speed as occurred following placebo (382). The caudate has been described as a structure that is important in the mediation of attention and inhibition (231, 244, 245). Studies investigating brain function in patients with obsessive-compulsive disorder who are known to show deficits in executive function (443), have reported the abnormal function of regions including the caudate nucleus (226, 444-446).

The effects of BZP on mood have been compared to amphetamine and MDMA (2), BZP and DEX also have predominantly dopaminergic effects and DA is known to have direct effects on cognitive control (447). Therefore, we wanted to further characterise BZP by a direct comparison with DEX.

Although the comparisons between DEX and placebo, and BZP and DEX demonstrated no significant differences in accuracy and reaction time during the Stroop task, there were significant differences in the imaging data. Four clusters were observed after the comparison of BZP with DEX which included the left IFG, induced by an increase in activation during the BZP incongruent condition. In addition, significant clusters were also found in the ACC, right medial superior frontal and left middle frontal gyri, where all

conditions showed relative deactivation, with the BZP incongruent condition causing the greatest deactivation.

The increase in IFG activation following BZP, relative to placebo, reflects the results of our previous findings, as both the caudate (231, 244, 245) and IFG (239, 240) have been associated with inhibitory responses. Bernal and colleagues (228) report that the IFG is specifically involved in inhibition of response action during the Stroop task. It is possible these increases during Stroop interference are due to the recruitment of neural substrates allowing subjects to perform to the same level of ability. The difference in activation, i.e. of caudate versus IFG in these parallel studies, may reflect that following DEX there is an increase in the efficiency of processing. Previous studies have reported that amphetamine improved the Stroop effect in healthy controls (225).

BZP and DEX have mainly dopaminergic effects but each also has unique differences. Other dopaminergic agonists, for example bromocriptine (440) and levodopa (248) modulate executive function. In prior studies giving amphetamine to healthy volunteers induces a typical inverted U-shaped dose-response curve in prefrontal regions, in relation to working memory (247). However the authors reported an improvement in accuracy in people who performed poorly prior to taking the drug, and a reduction in performance in those who previously performed well (247). Aging adults and young healthy volunteers also completed a Stroop task following levodopa administration. After drug treatment, performance in the younger subjects was impaired and associated with an increase in activity of the ACC, whereas this did not occur in the older adults (248). The likely increase in extracellular DA release in prefrontal circuits following BZP, may have also induced a hyperdopaminergic state, and thus led to impaired cognitive control.

These drug-induced activations might also be due to their influence on other influencing neurotransmitters and neuronal pathways, as both amphetamine and BZP also affect 5-HT and NA release and reuptake to varying degrees.

When DEX was compared with placebo there was activation in the right thalamus and left IFG; DEX increased activation of the thalamus and decreased activation of the IFG. Change was also observed in the occipital region and thought stem from a decrease in activation during the DEX drug state. This is in line with other studies, which report that after the administration of a DA agonist such as bromocriptine, there was a reduction in activation of the occipital region (440).

Thalamic activation was found during the incongruent condition following DEX administration. The thalamus is connected to a number of cortical areas (448-450) involved

in processes including arousal, emotion and a variety of cognitive functions (451). Studies conducted in patients with thalamic lesions have found reductions executive function (452-454).

Imaging studies have reported thalamic and striatal abnormalities in patients with ADHD and obsessive-compulsive disorder, which are thought to underlie disruptions in the motivation and regulatory self-control pathways (455). Furthermore when adolescents with bipolar affective (456) and substance use disorders (457) were investigated disruptions were found in the dorsal striatum and thalamus. Patients with minor cognitive impairment also recruited the thalamus and pre/post central regions during a Stroop task, which the authors thought indicative of a more effortful response selection or possibly impaired inhibitory control (458).

Our data indicates that DEX relative to placebo increased thalamic activation, but a decrease in IFG activation. This is contrary to our prediction that DEX would aid in performance on this task, instead if our hypothesis is correct regarding the thalamic activation, it seems that there is a modulation of regions recruited to perform the task.

Some have shown that psychostimulants such as amphetamine improve vigilance, attention and concentration in healthy control subjects (225, 438), however others have reported that this is dependent on the baseline performance of participants (247). Our results could also reflect the inverted U-shaped dose response curve of dopaminergic activity. As our participants had no known dysfunction of their dopaminergic pathways, this increase in DA levels may have led to a hyperdopaminergic state, consequently the thalamus was recruited to ensure task performance.

Alternatively, the thalamus has been described as a gateway for cortical signalling, and plays a key role in controlling or “gating” information to the cortex. Disruptions past the point of normal gating could lead to an overload of sensory and cognitive information (433). DA and 5-HT have inhibitory influences on the striatum, counterbalanced by excitatory glutamatergic input derived from cortico-striatal pathways (439). GABAergic input from the striatum and the pallidum is thought to have an inhibitory effect on neurons in the thalamus. This inhibition should act in a protective manner, as the result should be a reduced sensory input to the cortex from the thalamus. However, an increase in dopaminergic activity by for example, amphetamine in this research, or an increase in 5-HT, might reduce inhibitory influences on the striatum. This could subsequently open the thalamic filter and lead to an overload of sensory information being passed to the cortex (433).

Possibly, the increased activation of the thalamus reflects this reduction in inhibition caused by an increase in DA, rather than the recruitment of compensatory resources following DEX administration. This is in line with the reduction of activation of the IFG, and reflects previous studies showing improved inhibitory control during the Stroop task.

3.3.5. Conclusion

In conclusion, this is the first study to compare the effects of BZP with DEX during fMRI. Results indicate that after administration of BZP relative to DEX there is a compensatory recruitment of neural resources required to perform the task. This reflects other research by our group, which found caudate activation when BZP was compared with placebo. When DEX was compared with placebo, an increase in thalamic activation was observed with a decrease in IFG activation. This is in line with previous work that showed that amphetamine has the ability to improve cognitive function. Although BZP has comparable effects on mood to other stimulants, its effects on selective attention and inhibition appear to have distinct differences - BZP appears to need additional recruitment of resources possibly as it reduces inhibition.

Chapter 4:Validation Task of fMRI Effects—is it Changes in Cognition or Just the Rush (of Blood)?

4.1. Preamble

This final results Chapter will present the data acquired from a validation task. Functional magnetic resonance imaging is based on the assumption that when there is an increased cognitive demand in a particular region there will be a subsequent increase in blood flow within that region. Therefore there is concern that drugs which affect vasculature may have a direct effect on the regional activation seen during experimental paradigms.

This task was used to evaluate whether fMRI is a technique that can be used to study the regional activation in cognitive tasks, despite drug effects on the blood vessels of participants. BZP and BZP+TFMPP have been shown to cause significant increases in blood pressure and heart rate (2, 40). This task involved the participant to tap their thumb and forefinger together as quickly as possible, when a checkerboard was presented on the screen. The checkerboard flashed for a duration of one second, and was presented 29 times over the run.

Each drug state, that is, BZP, TFMPP, combination of BZP+TFMPP and DEX were compared with placebo, and the results are presented in the form of one combined paper.

All imaging data were collected on a 1.5T Magnetom Avanto Siemens scanner at CAMRI, and data pre-processing and analysed using SPM8. The first level analysis was conducted using a Finite Impulse Response (FIR) basis set with 20 x 1 second time-bins, resulting in 20 observations (contrasts) per subject. The group level analysis was conducted using a flexible factorial model.

4.2. Validation Task: Comparing BZP, TFMPP, the Combination BZP+TFMPP and DEX to Placebo to Investigate vascular effects on the BOLD signal

A validation task to determine whether the vascular effects of the synthetic drugs BZP, TFMPP and combination BZP+TFMPP affect the BOLD signal in fMRI tasks.

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4.2.1. Introduction

Functional Magnetic resonance imaging is frequently used to study regions undertaking cognitive tasks. In addition, fMRI enables the exploratory analyses of drugs effects to determine their effects on regional activation during psychological paradigms.

fMRI is based on the principle that when there is an increased cognitive demand in a particular region there will be a subsequent increase in blood flow within that region. The increased flow stems from an increase in the metabolic demand for oxygen. We are able to monitor the change in flow by comparing the magnetic signal of oxygenated blood which is diamagnetic with and deoxygenated blood which is paramagnetic. Similar to contrast agents the paramagnetic signal alters the T2* weighted magnetic resonance image signal and thus deoxygenated blood can be measured (282, 283). However, many drugs have direct effects on blood vessels for example cocaine has been shown to increase blood pressure, and thus there is concern over whether these vascular effects are responsible for the activation observed during cognitive testing during fMRI studies (459). It is therefore vital that the direct effects of a drug on the brain are known, to allow the determination of whether further analytical techniques are required to allow group comparisons.

There have been conflicting reports in the literature about the extent of vascular effects on fMRI data. While some argue that there are substantial changes, others oppose this view. A recent study by Murphy and colleagues (3) compared regional changes during a finger tapping task between four groups of participants: cocaine, nicotine and cannabis users and in addition, a second part of that research cocaine or saline was administered to cocaine users to compare regional activation was compared. They reported no difference in activation of the motor cortex between the different groups and additionally, after the administration of cocaine relative to saline.

A series of investigations was conducted in this by our laboratory to determine the effects of two relatively new synthetic drugs on aspects of executive function and reward processing using fMRI. The study was a randomised double-blind crossover design to

compare the effects BZP, TFMPP, a combination of BZP+TFMPP and DEX prior to conducting Stroop and gambling (guessing) tasks, whilst the participants underwent fMRI.

The results were compared to placebo and a direct comparison was between BZP and DEX. Regional differences were found for each drug during each task. However, it is important to note that in previous studies it has been reported that BZP and the combination of BZP+TFMPP increases both blood pressure and heart rate (2, 297).

To ensure the regional activation reported in these studies was in response to the fMRI paradigms, and not a consequence of either direct or indirect effects on blood flow, a simple finger tapping task was conducted. The task used was similar to that of Murphy and colleagues (3) and compared changes in the motor cortex between each drug and placebo.

4.2.2. Materials and Methods

Approval for this research was granted by the Northern X Regional Ethics Committee of NZ (Ethics approval number NTX/07/08/078). The trial recruited healthy participants and excluded subjects with a history of mental illness, cardiac disease, head trauma, epilepsy, endocrine disorders, or who were pregnant or breastfeeding. Participants attended an initial screening session where a questionnaire was completed by each participant, describing their medication history, recreational drug, alcohol and cigarette use, sleeping patterns and stress levels to ensure they were not drug naive or current or past heavy recreational drug users. Thirteen non-smoking healthy participants (seven female and six male; aged 18–40 years) gave written consent to participate in a double-blind placebo controlled cross-over trial. Due to an error in data collection one subject was excluded which left 12 subjects for each drug comparison.

4.2.2.1. Drugs

BZP (200 mg), TFMPP (50 mg for participants weighing < 60 kg or 60 mg if weighing > 60 kg), a combination of BZP and TFMPP (100 mg + 30 mg respectively) and DEX (20 mg) were each given in on separate trial days. All capsules were manufactured by the School of Pharmacy, University of Auckland, NZ, using good manufacturing practice. Placebo capsules containing methylcellulose were also manufactured and identical in appearance to other capsules.

4.2.2.2. Procedure

The study used a randomised double-blind cross-over procedure where participants were tested after taking each drug or placebo in a randomised order with a minimum of 7 days

between sessions. Participants fasted for 12 hours before the trial and asked to abstain from alcohol or caffeine from the evening prior to testing. Participants were excluded on the day of testing if their urine tested positive for the presence of recreational drugs including marijuana, cocaine, amphetamines, opiates or benzodiazepines or pregnancy where appropriate. All capsules were taken with 250 mL of water 90 minutes before imaging to allow peak plasma concentrations of BZP and TFMPP (332). During this time, participants remained in the presence of researchers in a comfortable area with minimal stimulation.

fMRI was performed at the CAMRI, University of Auckland. The task was undertaken during imaging and presented on a screen located 3.5 metres from the participants, at the foot of the scanner and visible via a prism built into a head restraint, used to minimise movement during imaging.

Blood oxygen level dependant functional images were acquired using a T2*-weighted echo planar imaging (EPI) sequence with a 1.5T Siemens Magnetom Avanto scanner using the following parameters: TR 2500 ms, TE 50 ms, FOV 192mm, in-plane voxel size 3.0 mm× 3.0 mm, flip angle 90°, 29 slices, slice thickness 4.0 mm no gap. 165 volumes were collected for each participant at each visit. For anatomical reference, a high-resolution structural MPRAGE image was acquired for each at the end of the first session.

The task used allowed the examination of differential activation in the motor cortex following the administration of each drug. Participants were instructed to tap their thumb and forefinger together as quickly as possible, on each hand, when a checkerboard appeared on the screen. The checkerboard was presented to participants for the duration of one second per presentation, and presented to participants 29 times over 6 minutes with an inter-stimulus interval that varied between 8 and 18 seconds. To prepare the participants for the stimulus, prior to the checkerboard appearing on the screen, a countdown timer appeared.

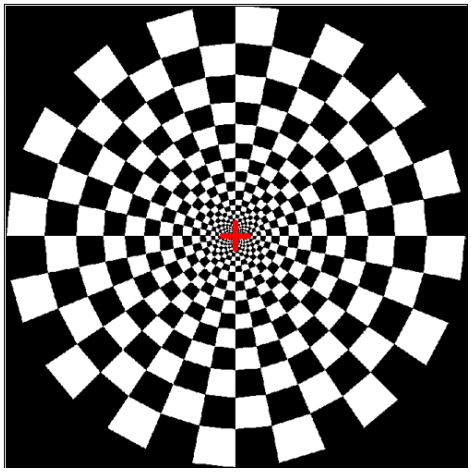

Figure 42: Checkerboard that was flashed to participants for a duration of one second

Raw data were analysed using SPM8 (Wellcome Department of Cognitive Neurology, London, UK), implemented in MATLAB 7.8.0 (Mathworks, Sherborn, MA, USA). After being co-registered to the T1- weighted structural volume, the EPI images were normalised to a standard space (Montreal Neurological Institute [MNI] template). Images were spatially smoothed using an isotropic Gaussian kernel of 8 mm full-width at half-maximum (FWHM) in the x, y, and z axes.

Outliers due to movement or signal from pre-processed EPI files, using thresholds of 3 *SD* from the mean, 0.75 mm for translation and 0.02 radians rotation were removed from the data sets, using ART repair (335). Outliers were recorded to ensure fewer than 15% of scans were removed.

First level analysis was conducted using a Finite Impulse Response (FIR) basis set with 20 x 1 second time-bins, resulting in 20 observations (contrasts) per subject. Subsequent group level analysis (i.e. drug state x placebo) was conducted using a flexible factorial model. Final contrasts were made to evaluate any changes between the drug condition and placebo, using a comprehensive interaction contrast for observations x group (drug state).

4.2.3. Results

The aim of this study was to investigate whether there were differences in the activation of the motor cortex after the administration of BZP, TFMPP, BZP+TFMPP or DEX, in comparison to placebo. An F-contrast was constructed to specifically examine the interaction between each drug and placebo.

The comparison of BZP, TFMPP, BZP+TFMPP and DEX with placebo yielded no activations in the motor cortex. A single activation was identified in the BZP+TFMPP drug state, relative to placebo, in the left middle temporal region ($p < 0.01$).

Drug state versus placebo comparison for regional activation in the validation task				
Anatomical region	MNI coordinates			F value (peak voxel)
	x	y	z	
BZP+TFMPP x placebo				
Temporal_Mid_L	-36	-64	12	4.058

Figure 43: Neural correlates of activation of BZP+TFMPP in comparison to placebo, for the finger tapping validation task

Note: The cluster is significant at $p < 0.01$ (corrected); The *F* value at the peak voxel within each cluster is reported.
Cong: congruent; incong: incongruent; SE: standard error

4.2.4. Discussion

This study aimed to investigate the effects of BZP, TFMPP, the combination of BZP+TFMPP and DEX in comparison to placebo on the regional activation of the motor cortex. The experimental design used was based on research by Murphy and colleagues (3), and involved a simple finger tapping task to elucidate whether there were changes in the motor cortex. Their comparisons of different groups and of the difference in regional activation before and after giving cocaine evoked no changes in the motor cortex.

Other studies investigating the effects of cocaine and nicotine on regional activation have also concluded that despite the effects of these drugs on the vasculature there was no effect on the BOLD signal. Luo and colleagues (460) administered cocaine to rodents and found that although there were significant effects on mean arterial blood pressure there were no significant global changes in the BOLD signal. The authors suggested that fMRI studies can be used to map drug-induced changes in neuronal activity.

In the present research, the effects of an acute dose of BZP, TFMPP, BZP+TFMPP and DEX did not induce activation of the motor cortices in these participants. This suggests that the observed effects on blood pressure of BZP and BZP+TFMPP do not alter the coupling between BOLD signal and activity in the motor cortex. These results are consistent with prior studies which have shown that drug effects on the vasculature do not lead to the regional changes observed in response to cognitive tasks. This finding adds to the literature surrounding the effects of drugs on regional activation induced during fMRI tasks.

The study that this validation task was based on used two different types of analysis techniques to compare the participant groups and cocaine users administered an acute dose of cocaine versus saline. In this current research we only used one method of analysis. In the future we could also analyse the data by comparing the shape of the hemodynamic response curve, to determine whether there were any changes induced by the drugs in comparison to placebo.

4.2.5. Conclusion

We therefore propose that despite the increase in blood pressure induced by BZP, TFMPP, their combination and DEX the changes observed in cognitive tasks such as the Stroop and gambling (guessing) task, are due to task-specific changes in regional activation, and not a direct effect of each drug on the vasculature. We can conclude that the neural activations found in the experiments we recently conducted are due to the tasks and warrant no further post-hoc analyses.

Chapter 5: Discussion and Conclusion

5.1. Preamble

This final chapter will give a summary of the research presented in this thesis. Using the knowledge from previous research, the data will be presented in the context of other drugs that affect serotonergic and dopaminergic circuitry. This chapter will attempt to combine findings from the current work, with what is already known about the effects of BZP and TFMPP and provide possible interpretations of this work.

The aim of this research was to investigate the effects of BZP and TFMPP, alone and in combination on the processing of human reward and executive function. This Chapter will also comment on the wider implication of this research, combined with previous work, with regard to policy around the availability of these drugs. This section will also outline limitations of this research and suggest areas for future study. Finally, an overall conclusion from this thesis will be provided.

5.2. Summary of Findings

Our understanding of the effects of the party pill drugs BZP and TFMPP both alone and in combination is limited. Recent studies about the subjective and pharmacokinetic properties in humans are broadening our knowledge of these compounds. However, it is unclear how they affect reward and cognitive processing. The series of studies presented in this thesis have attempted to address the paucity of data about the effects of BZP, TFMPP and the combination of BZP+TFMPP on the circuitry involved in reward and executive function.

Previous studies have shown that alterations in dopaminergic and serotonergic circuitry can lead to changes in processing of reward-related stimuli, attention and, inhibition, leading to changes in regional brain activation. Whilst people are under the influence of recreational drugs, they have been reported to make sub-optimal decisions (461). Recreational drugs influence these decisions for example by increasing positive arousal which makes users more likely to make risky decisions. Furthermore, recreational drugs affect decision by altering the processing of executive function for example, by decreasing response inhibition. Similar studies have examined the effects of both recreational and chronic drug use on memory, motor control and the response to gambling paradigms (306, 462). The experiments presented in this thesis, examined the acute effects of BZP, TFMPP and a combination of BZP+TFMPP with placebo (and DEX), using fMRI and experimental paradigms designed to target reward and executive function.

5.2.1. Reward Processing

Studies 1, 2 and 3 (presented in Chapter 2.2, 2.3, and 2.4, respectively) suggest that BZP and TFMPP alter the anticipation and outcome stages of reward.

5.2.1.1. Reward anticipation

When anticipation of a large monetary outcome was presented to participants (Studies 1 and 3), BZP caused a reduction in activation of the IFG, insula and occipital region relative to placebo. This suggests that BZP reduces the response to uncertain anticipatory stimuli and response inhibition. These effects imply that less than optimal decisions could be made by users (461).

When the effects of BZP were compared to DEX, there was a distinct difference seen in the thalamus (BZP induced greater activation), suggesting that DEX altered extracellular DA levels leading to more efficient learning. Conversely, the differences might stem from the positive arousal induced by BZP, that is, despite there being no predictors of whether the stimuli would be positive or negatively valenced, subjects continued to try and find a solution to the task, or be trying to monitor their monetary position.

In the studies on TFMPP, there was greater activation in the putamen, and less in the insula relative to placebo. This may reflect a change in the processing of uncertain anticipatory stimuli. The putamen has been associated with emotional response to aversion, and 5-HT has been linked to an increase in attention to, or response to aversive stimuli. The serotonergic effects of TFMPP may therefore be expected to increase activity in the putamen, showing a differential processing of anticipation.

When BZP and TFMPP are combined and compared to placebo, the only change in activation observed was in the rolandic operculum. This region is known to be activated in response to teeth grinding, and activation is increased after the administration of levodopa (356). Therefore, this effect and the absence of any others might be due to the opposing effects of TFMPP on BZP-induced dopaminergic activation.

5.2.1.2. Reward receipt

The effect of these drugs on the outcome of reward (Studies 2 and 3) were also examined. BZP, TFMPP and the combination of BZP+TFMPP were individually compared with placebo. Following the receipt of a $4 monetary reward there were no observable differences induced by any of the drugs. However, when DEX was compared to BZP, DEX induced greater activation in the cingulate and less deactivation in the thalamus. This might reflect the more specific effects of DEX on dopaminergic transmission in comparison to BZP. Interestingly, the effects of a $4 loss evoked differences in the regional activation

induced by each drug states in contrast to placebo, and possibly reflect the statement that “loss looms larger than gain” (463).

BZP induced greater activation in the right mid-cingulate, IFG and insula than placebo, and less deactivation in the left cingulate in response to a large monetary loss. These regions are terminal sites of DA neurons, suggesting that DA may mediate some of the activation. Knutson and colleagues (114) also reported activation of the ACC and thalamus after monetary losses while the IFG has been associated with uncertain decision making (342) and inhibition (239). Moreover, the insula is activated after the receipt of monetary losses (198, 202, 344, 376) and the expectation of aversive stimuli (345, 346). It is likely that the increased activation of the cingulate reflects an increase in DA release after the receipt of an aversive stimulus. When BZP and DEX were compared following monetary punishment i.e. a $4 loss, BZP increased activation in the cingulate and insula while DEX increased activation of the thalamus. This is consistent with the hypothesis that there was an increase in DA release following aversive stimuli, which was also observed when the effects of BZP and placebo were compared. What is evident from the direct comparison is that DEX appears to mediate a different effect on DA release than BZP.

TFMPP, on the other hand, showed very different patterns of regional activation. Only two regions were significantly affected in comparison to placebo—the thalamus and the lingual gyrus. Due to its serotonergic nature, TFMPP appears to modulate the response to anticipation. Possibly, after the administration of TFMPP, the participant expects a positive outcome to a lesser degree than after placebo, and therefore has a reduced response to the anticipation of $4. This could be due to the serotonergic nature of TFMPP; as 5-HT has been associated with heightened aversion.

When combined, BZP+TFMPP activated a number of regions following the receipt of reward, including the lingual gyrus, ACC, temporal and occipital lobes, to a greater extent than when each of the drugs were given individually. These effects are possibly due to their inhibitory effects on hepatic metabolism when the drugs are combined which results in higher plasma concentrations of BZP and TFMPP.

These studies highlight the differences between each drug on the processing of anticipation and receipt outcome. This data also adds further evidence about the activation of distinct circuitry during the anticipation and outcome phases of gambling paradigms (110).

5.2.2. Selective Attention and Inhibition

In Studies 4 and 5 (Chapter 3.2 and 3.3, respectively), BZP, TFMPP and the combination of BZP+TFMPP were found to alter neuronal recruitment during the processing of executive function. BZP appears to decrease inhibition which then requires the additional recruitment of the caudate to maintain performance during the Stroop task. Furthermore, BZP acts in a different manner to DEX, which reduces recruitment of the IFG, an area actively recruited in response to inhibition. TFMPP, on the other hand, recruits the thalamus which is known for its gating role and also for its effects on the inhibition of responses (464). When combined, BZP+TFMPP reduced activation in the caudate. We propose that this is due to the effects of TFMPP's on 5-HT_{2C} receptors and an increase in thalamic activation, similar to what was seen after the administration of TFMPP's alone.

5.3. Reward Processing: BZP, TFMPP and Combination BZP/TFMPP

5.3.1. Anticipation

5.3.1.1. BZP

Using a custom designed event-related gambling (guessing) task, we were able to concentrate our analysis on specific aspects of reward processing, including the anticipation of uncertain rewards. Berridge and colleagues (109) presented evidence of the dissociation between "wanting" and "liking", where wanting is a behavioural response to the anticipation of reward and liking refers to the receipt of the reward (i.e., reward outcome). They proposed a functional and neural dissociation between the two domains, with the mesolimbic dopaminergic system implicated in wanting. The anticipatory phase of reward is reported to be critical in reward processing, as it has been associated with positive expectancies and motivates the behaviour of the individual to increase their likelihood of receiving an expected reward (107, 108).

Experimental paradigms have shown that stimuli that were originally neutral can become conditioned and used to predict reward or loss. When this occurs, the conditioned stimuli evokes motivational properties to strive towards receiving the reward, a process thought to be controlled by limbic mechanisms (109). This wanting can be altered by the manipulation of extracellular DA levels. Wyvell and Berridge (465) administered amphetamine directly into the NAcc of rats, which caused increases in wanting of a sucrose reward, evidenced by increased sucrose-associated lever pressing independent of the rewarding aspect. In addition, the reward outcome stage was not affected by amphetamine. Similar effects have been reported in humans using PET imaging. For example, Leyton and colleagues (466) imaged healthy controls, before and after administration of DEX, and found increases in the

binding potential of DA in the ventral striatum were associated with an increase in drug wanting. Volkow and colleagues (467), in another PET study, showed striatal DA receptor occupancy correlated with the wanting of food. In addition, in a separate study, the administration of the D_2 receptor antagonist, haloperidol, reduced the number of cigarettes smoked by nicotine-dependent participants (468) which suggests DA blockade decreases wanting.

When BZP was compared to placebo during the anticipation of a $4 win/loss, regional activation was induced in the IFG, insula and occipital region. In all three regions, BZP induced less activation than placebo. This is indicative of a reduction in the responses to anticipation of uncertain stimuli. Prior imaging studies have used monetary incentive delay tasks to investigate the effect of reward anticipation. In these tasks an initial cue indicates the potential reward to be obtained and after a short delay a target appears; if the participant responds correctly, they receive the reward (113, 126, 132, 158, 330). These studies found a specific network was activated during rewards and losses which included the mPFC, dorsal striatum and insula, with additional activation in the thalamus after losses (114). When the subject is responding to stimuli predictive of a reward or loss, distinct regions have been reported, which reflect uncertainty and/or risk. These regions include the striatum, amygdala, OFC, IFG and the insula (129, 198, 210, 211, 339, 340). Therefore, it seems that BZP reduces regional responses to uncertain stimuli. This may be due to positive arousal induced by increased dopaminergic transmission following drug administration. This is in line with prior studies reporting that manipulating dopaminergic circuitry affects reward processing (109, 365, 468).

To further expand on this hypothesis, the next section will discuss each of the regions affected by BZP and the implications of its reduction in activation. In response to the anticipation of winning or losing $4, BZP reduced activation in the IFG which has been well documented as involved in response inhibition and specifically, the inhibition of risky choices (342). A study using the Iowa gambling task found an increase in activation in the IFG, which correlated with losses (342). Furthermore, this effect was more pronounced in risk aversive participants, indicating an association between the IFG and risk. The reduction in IFG activation in our study may be associated with a reduction in neural activity to risky choices. Previous studies have shown that amphetamine reduces the amplitude of the BOLD signal in response to monetary incentives during a gambling task (142), which was thought to be due to its effects on DA. BZP, like amphetamine, increases dopaminergic transmission. Possibly, the increase in extracellular DA release causes an increase in positive arousal, which subsequently dampens the response to risk/ uncertainty.

BZP also reduced activation in the insula, a region associated with uncertainty and risky decision making (342). The insula is also associated with responses to both the receipt (159, 198, 344) and the anticipation of aversive stimuli (345, 346). Patients with lesions of the insula show deficits in their ability to estimate potential risk (469, 470). Furthermore, the insula is involved in both the processing of risk and consequential decisions (207, 471). The reduction in activation of the insula induced by BZP relative to placebo, adds to the hypothesis that BZP reduces response mechanisms associated with risky or uncertain stimuli.

The hypothesis that changes induced by BZP reflect its effects on dopaminergic transmission is founded on previous studies that show modulations of dopaminergic transmission affects reinforcement learning, as seen with addictive drugs (389). Although fMRI does not directly show changes in extracellular DA levels, Knutson and colleagues (288) have reported activation in the NAcc in response to anticipation is due to DA release. Additionally, a recent study reported that amphetamine increases the ventral striatum response to the anticipation of losses (142). This increase in activation was proposed to arise from the increase in arousal or motivation induced by the drug and was based on a previous safety seeking hypotheses (157). In line with these reports BZP may also increase positive arousal by increasing dopaminergic activity to the extent that participants do not respond to the potential uncertain stimuli. Consequentially, we propose that BZP may also have a direct effect on risky behaviours.

As the effects of BZP are largely dopaminergic and its effects are comparable to amphetamine on mood, we wanted to directly compare BZP with DEX. DA agonists such as amphetamine (142, 176) and pramipexole (207) and antagonists, such as haloperidol (176) alter regional activation in response to anticipation. For example, the effects of amphetamine and haloperidol on aversive learning were compared. The PE related activity, that is, the difference in activation between the anticipated and actual outcome, after amphetamine was found to activate a wider network of structures than placebo However, haloperidol did not induce activation (176). Similarly, pramipexole, a DA agonist at D_2/D_3 receptors was proposed to reduce the top-down control on impulses by modulating the connectivity between the NAcc and insula and the NAcc and PFC. This possibly explains why patients prescribed pramipexole experience impulse control deficits (207).

The direct comparison of BZP with DEX revealed only one regional difference i.e. the thalamus where BZP induced greater activation. When DEX was compared to placebo, DEX reduced activation in the thalamus, mid-cingulate and post central gyri. This is of particular interest because it shows that BZP and DEX, relative to placebo, reduce

activation in regions linked with reward processing and reward-based learning in the striatal-thalamo-cortical network (132, 148). However, there remain distinctive differences in the exact regional activation - BZP causes a reduction in regions sensitive to uncertainty, whereas DEX does not.

The thalamus is responsive to monetary losses (114), and part of a circuit involving the basal ganglia and prefrontal regions, known as the BGTC (147). It's role has been associated with linking reward and specific goal directed behaviours (148) and learning (150). In our study, BZP, DEX and placebo differentially activated the thalamus, which may reflect their effects on the processing of reward-based learning. As DA can modulate learning, possibly the increase in dopaminergic transmission induced by DEX enables more efficient information processing. Alternatively, participants may realise that there is no "rule" or "pattern" to the winning and losing cards presented; BZP does not share this characteristic. The BZP-induced thalamic activation may be due to an absence of conditioned learning in this paradigm. That is, there was no cue allowing the prediction of a win or loss so information processing to enable learning outcomes was not established, and hence participants may have been seeking a pattern from the beginning of the task to the end. On the other hand, the participants may be trying to monitor their monetary position, and DEX enables more efficient processing in this regard.

This activity of the thalamus supports the hypothesis that the positive arousal caused by BZP-induced DA release may modulate reward processing - participants are less responsive to uncertain stimuli and continue trying to establish a pattern in winning and losing throughout the task.

Unfortunately, the gambling (guessing) task did not allow the assessment of behaviour toward risky choices. Unbeknownst to the participants the task had a pre-determined distribution of wins and losses. It would be interesting to further investigate the hypothesis that BZP promotes risky or uncertain behaviour, by repeating this research with a task such as the Iowa gambling task which would allow this to be assessed.

In summary, BZP, relative to placebo, induces regional activation of the IFG and insula, indicative that subjects are more likely to make risky decisions, or those with uncertain outcomes when under the influence of BZP. We propose that this is due to the positive arousal induced by BZP-related dopaminergic transmission.

5.3.1.2. TFMPP

From previous studies investigating the effects of reduced dopaminergic transmission, TFMPP was predicted to reduce activity in the striatum due to its effect on 5-HT_{2C} receptors

and a subsequent reduction in DA release in the VTA and striatal regions (41, 42). However, TFMPP induced activation of the putamen and reduced activation in the insula. As described earlier the striatum and insula are associated with uncertainty and risky decision making and 5-HT stimulation is associated with increased aversion (194, 195).

This change in activation of the putamen and insula may be due to TFMPP altering the way that the anticipation of rewards and losses are processed. The putamen is part of the dorsal striatum which is reportedly activated in response to anticipation of both large rewards and punishment (158), and it is thought that it may play a role in stimulus-response based reward learning (348). Evidence for this was presented in a study by Bellabaum et al. (349), where the caudate was differentially activated for active learning in comparison to observational learning. Possibly, the increased activation of the putamen is a result of potential wins or losses of large monetary value and indicative of increased risk aversion, (198, 199).

The reduction in insula and increase in putamen activation may be a differential response induced by TFMPP's effects on the serotonergic circuitry. 5-HT is known to mediate aspects of impulse control and uncertainty. Rogers et al. (472) investigated the effects of tryptophan depletion on healthy volunteers, whilst completing a decision making task in and comparison to groups of drug users. The results showed that tryptophan depletion induced deficits in performance similar to those observed in chronic MA users suggesting a role for 5-HT in decision making when there is an element of risk. In a study investigating responses to disgust, the putamen and caudate were recruited. The detection of disgust is an emotional response guiding avoidance from potential negative consequences (350). If these results are extended to our study, perhaps the administration of a 5-HT agonist such as TFMPP increased activation of the putamen due to increased avoidance.

Recently, reports of 5-HT_{2C} agonist's effects on dopaminergic transmission in the VTA have been shown to inhibit the tonic and phasic levels of DA. This is thought to by activation of a signalling cascade, whereby 5-HT_{2C} agonists increase the firing of GABA neurons which then lead to a reduction in firing of dopaminergic neurons (473). It is possible that this amplified DA release after the presentation of anticipatory stimuli in the putamen.

In summary, after taking TFMPP our participants were less likely to recruit the insula during situations of uncertain risk but instead recruited the putamen. We propose this reduced activation of the insula with a corresponding increase in activation of the putamen is due to the effects of TFMPP on serotonergic transmission, which have been linked to aversive behaviour.

5.3.1.3. BZP+TFMPP

When BZP and TFMPP were given in combination the only significant effect during the anticipation stage was in the rolandic operculum, where BZP+TFMPP reduced activation. Activation of this region is associated with gustatory reward (351, 352), language (353) and teeth clenching or grinding (354, 355). Previously the rolandic operculum showed an increase in activation after the administration of levodopa (356) probably due to its effects on dopaminergic transmission.

Interestingly, the activation induced by individual drugs did not occur when the two were given together. We believe this is possibly due to opposing effects of BZP and TFMPP on DA transmission -whilst BZP increases extracellular DA release, TFMPP may reduce it indirectly by its effect on 5-HT_{2C} receptors. This further supports the conclusion that the activation we observed when the drugs were given separately is mediated to some extent by dopaminergic mechanisms.

Alternatively, the dose of BZP+TFMPP (100 mg + 30 mg) used based on previous research within this laboratory may have been insufficient to produce the responses observed when BZP (200 mg) and TFMPP (60 mg) were given alone. Caution was used in this research because in animal research high doses of the combination produced seizures which we wanted to avoid (19). On the other hand the combined dose used has been shown to induce changes in affect in previous studies. However, this is unlikely as other stimuli in this gambling (guessing) task have evoked neural responses to reward loss, following the combination of BZP+TFMPP greater than that seen when the drugs were administered separately.

5.3.2. Reward Outcome

5.3.2.1. Wins

The effects of BZP, TFMPP and the combination BZP+TFMPP on reward outcome were subsequently investigated i.e. when the win or loss of the guess was revealed. Interestingly, when winning $4 was presented, there were no regional differences seen in any of the drug states in comparison to placebo. This could be for two reasons.

Firstly, it is possible that the win $4 stimulus was insufficient to induce a response. It has been suggested by several studies (215, 371, 372) that the win to loss ratio needs to be 2:1 to elicit the same extent of response in winning as losing. Therefore, the presentation of an $8 win may induce a visible response under these conditions. Future experiments could compare a win of $4 with $8 and compare this to placebo further investigate this effect.

On the other hand, there are possibly no differences between the drug states and placebo. Studies have shown that modulation of DA circuitry effects the anticipation phase, that is, the wanting of the reward but not the actual receipt or liking of the reward (465). This might explain why there was no change between any drug state and placebo. Despite this proposal, DEX induced greater activation in the cingulate, superior frontal gyrus and superior temporal gyrus relative to placebo. Furthermore, when BZP was directly compared to DEX, regional activation was induced by DEX in the cingulate and there was less deactivation in the thalamus.

Therefore, although BZP induced similar subjective states to amphetamine (2), there appears to be a difference in its effects on phasic DA firing in response to reward. It is also likely there are dose effects of each drug. Alternatively, the effects of BZP on other neurotransmitters such as 5-HT and NA may cause the differences.

5.3.2.2. Losses

In contrast, after the receipt of loss, each drug caused differential activations in comparison to placebo. Although accepted to a lesser degree, aversive and stressful experiences alter synaptic DA concentrations. Dopamine's role is thought to determine motivational salience despite the valence of the stimuli (205). McCabe and colleagues (159) used sulpiride a D_2 antagonist to investigate the involvement of DA in gustatory and visual reward and punishment. Sulpiride reduced activation of the OFC and insula at the sight and taste of aversive stimuli. Similarly, haloperidol abolished activation of the insula (176). Gray and colleagues (373) reported that DA is released in response to aversive stimuli such as unavoidable foot shocks, and it has also been proposed that DA is released due to uncontrollable mild stress (374, 375).

5.3.2.3. BZP

When BZP was compared with placebo, it induced greater activation in the right mid-cingulate, IFG and insula, and less deactivation in the left cingulate. Previously activation of the cingulate has been observed in response to aversive stimuli (114). In addition, the IFG is also associated with uncertain decision making (342) and more frequently with inhibition (239). Both regions are affected by dopaminergic transmission. Therefore, it is likely that the increased activation of the cingulate and IFG reflects an increase in DA release after the receipt of aversive stimuli.

Insula activation was also found after the receipt of monetary loss, which has been found to be activated in response to both receipt (198, 202, 344, 376) and the expectation of aversive stimuli (345, 346). It is also thought to play an important role in frustration, seen

after switching away from the default choice and losing in a gambling task (377). These are all potential reasons for the insula to be activated after the aversive stimuli in our study.

Alternatively it could be proposed that there is a disruption of dopaminergic transmission between the NAcc and IFG and the NAcc and insula as previously discussed (207). Therefore, BZP could also alter connectivity between these regions leading to an increased aversive response in the insula and consequently an increase in IFG recruitment to ensure the maintenance of inhibitory control following a large monetary loss. Connectivity analyses would need to be conducted to determine if this was happening.

When BZP and DEX were compared, BZP increased activation of the cingulate and insula, and DEX increased activation of the thalamus. This is in line increased DA release after the presentation of aversive stimuli, and the increased activation in these regions was also observed when the effects of BZP were compared to placebo. Therefore it can be concluded that DEX and BZP mediate DA release to differing extents.

These results demonstrate that BZP and DEX induce similar responses to aversive stimuli; however BZP has a greater effect on losses than DEX, which may be reflective of their differing potency. It is likely that different doses of the same drug induce differing patterns of regional activation (400, 401); therefore dose-effect studies should be undertaken in the future.

The only region that DEX evoked greater activation than BZP was the thalamus, which as previously discussed has been associated with motivation in reward processing and learning. Possibly, the activation seen stems a greater response to the loss of $4 than DEX. Distinctive differences were also observed when DEX was compared with placebo. DEX relative to placebo increased activation of the thalamus and cingulate and showed a greater deactivation of the middle frontal gyrus. This is a similar trend to what was seen by BZP when compared to placebo, and possibly is due to both drugs increasing DA transmission.

However, we cannot dismiss the possibility that the individual drug's effects on 5-HT may be causing these changes. It has been proposed that 5-HT opposes the effects of DA on reward processing (194, 195). Possibly, the heightened response to negative outcomes is due to more pronounced serotonergic effects induced by BZP in comparison to DEX.

5.3.2.4. TFMPP

When TFMPP was compared with placebo its effects on the outcome stage of reward processing showed differences in activation of the right lingual gyrus and right thalamus;

both of the clusters showed less deactivation in the TFMPP Lose $4 condition than placebo.

This was not expected for two reasons: firstly, TFMPP is mainly serotonergic and the effects of serotonin, as discussed in the previous section, affect aversive stimuli - it heightens the response. It was hypothesised that a 5-HT agonist such as TFMPP would lead to a wider network of regional activation following the presentation of aversive stimuli. Alternatively, TFMPP could reduce the responses to loss, due to its effects on the 5-HT_{2C} receptors and a subsequent reduction in DA release in the NAcc.

However, the results showed a less deactivation of the thalamus than that induced during the placebo condition. This could be for several reasons; firstly, PE has been reported to be the difference between the expected and the actual response. If the receipt of an outcome is more than expected, then an increase in DA firing occurs; if the outcome is less than expected a reduction in firing occurs. Possibly, after the administration of TFMPP, the participant expects a positive outcome to a lesser degree than after placebo, and therefore has a reduced response to the anticipation of $4. This could be due to the serotonergic nature of TFMPP; as 5-HT has been associated with heightened aversion. Upon realisation of a loss, a reduction in dopaminergic firing is seen, leading to reduced activation of the NAcc and other regions sensitive to reward outcome such as the thalamus. However, TFMPP has a decreased response to monetary loss. This modulated response has been shown to be the result of increased serotonergic transmission. Marutani (203) investigated the effects of an acute dose of paroxetine during a monetary incentive task and found that brain activity induced by motivation was diminished. Therefore it is likely that the lingual and thalamic activation observed is due to the serotonergic effects of TFMPP.

5.3.2.5. BZP+TFMPP

We predicted that when BZP and TFMPP were given together, the results would reflect what is seen after they were given individually. However, the results showed a wider network of activation within the lingual gyrus, ACC, temporal and occipital regions, and to a greater degree than when they were given individually. Antia et al. (384) monitored the plasma concentrations of BZP, TFMPP and the combination of BZP and TFMPP in humans and noted that the peak plasma concentrations were greater following the combination than when they were given alone using the same doses used within this study. Therefore it may be that the pharmacokinetic interaction between BZP and TFMPP is leading to an increased plasma concentration of the drugs, and subsequently, an increase in the regions activated.

5.4. Selective Attention and Inhibition: BZP, TFMPP and Combination and the Stroop Task

5.4.1.1. BZP

The previous section discussed the effects of BZP, TFMPP and the combination BZP+TFMPP on reward processing. This next section discusses the effects of these drugs on aspects of executive function, specifically selective attention and inhibition, using an event-related colour-word Stroop task.

BZP induced regional activation in the bilateral caudate, left inferior temporal gyrus and right superior occipital gyrus when compared to placebo. The activation of the temporal and occipital gyri is likely due to the processing of visual stimuli. Dopaminergic modulation is involved in the guidance of attention toward relevant locations and the cognitive processing of visual stimuli. Vitay and Hamker (474) suggest that one of the roles of DA is a modulatory influence on directing learning toward stimuli associated with potential reward. In addition, Mogami and Tanaka (422) hypothesise that reward association occurs in a feed-forward manner along the ventral pathway because information about potential rewards is carried to visual areas by dopaminergic neurons and incorporated within visual processing. The changes induced by BZP may be a reflection of this increase in dopaminergic firing, which subsequently requires the allocation of fewer resources than would normally be utilised following placebo administration.

Due to the dopaminergic nature of BZP, and previous comparisons to stimulants, it was predicted that BZP would show similarities to amphetamine (225), that is, decreased response times, or a reduction in activation in regions associated with behavioural inhibition or selective attention. However, this was not the case.

BZP caused increases in activation of the bilateral caudate. The head of the caudate is associated with the control of interference. When participants were asked to complete a Stroop and Simon task to allow for the investigation of both word and spatial interference, they found that the head of the caudate was activated during the word interference only (231, 244). In addition, Li and colleagues (245) used the stop-signal reaction task (SSRT) in two groups of participants: one group were presented short and the other long SSRT trials. In short SSRT trials, the caudate showed greater activation, which was positively correlated with motor activity in the pre-supplementary motor cortex, a region also involved in motor responses involved with cognitive control. To further corroborate the association between interference control and the caudate, studies investigating brain function in patients with obsessive-compulsive disorder who are known to show deficits in executive function (443), have reported the abnormal function of regions including the caudate

nucleus (226, 444-446). Using the Stroop task, Nakao and colleagues (475), found varied regional activation during tasks in those who had obsessive-compulsive disorder.

Furthermore, it has been suggested that the role of the caudate stretches to have an involvement in controlled movements, rather than habitual or automatic movements, as shown by Riecker et al. (476). In this study, subjects completed a finger tapping task where they had to respond to auditory stimuli presented at different temporal frequencies. The slow controlled movements evoked caudate activation.

The caudate was reported to mediate the relationship between action and reward outcome. In a recent study using α-methylparatyrosine to investigate the effect of DA depletion on the human reward system, placebo increased activation within the left caudate during the anticipation of reward, in contrast this was not observed following DA-depletion (175). In addition, there was an increase in firing within the caudate following unexpected rewards and conditioned stimuli associated with reward (138), and other studies have shown that the head of the caudate nucleus is involved in coding reward prediction errors during goal directed behaviour (138, 348, 423). In the Stroop task used in this research there was no reward as such. However, one could argue that realisation that you have made the correct response to the incongruent stimulus, due to its conflicting nature could be elicited as a reward, that is, the of accomplishing of a task-relevant response.

In summary, we propose the increase in caudate activation seen after administration of BZP in comparison to placebo, stems from a compensatory mechanistic recruitment of neural resources to ensure that the participant maintains performance on the task. This recruitment is believed to ensure adequate inhibitory control following the consumption of BZP.

Reduced inhibition following BZP was the opposite of what was thought to occur, due to reports of its similarities to amphetamine. Therefore a direct comparison was made between BZP and DEX. When BZP and DEX were compared, four clusters of activation were seen, including the IFG, evoked by an increase in activation by the BZP incongruent condition. In addition the ACC, the medial superior frontal and middle frontal gyri were deactivated, by both BZP and DEX.

The increased activation of the IFG by BZP is reflective of findings comparing BZP to placebo. The IFG has a well-documented response to inhibition (239, 240). Bernal and colleagues (228) further investigated the neural regions involved with inhibition. They found that cognitive inhibition showed left brain lateralisation, whilst the right brain lateralisation was involved with motor inhibition. These findings suggest that BZP and DEX have

dissimilar effects on the circuitry involved in response inhibition The comparison of DEX with placebo reflects previous work, whereby amphetamine improved the Stroop effect in healthy controls (225).

The possible reason underlying reduced inhibition by BZP may be due to unique effects on dopaminergic transmission. Dopaminergic agonists, including amphetamine (247), and levodopa (248) alter executive function. Drugs that affect the dopaminergic circuitry also exhibit an inverted U-shaped dose-response curve in the prefrontal regions in relation to working memory (247). An improvement in accuracy was seen in people who performed poorly prior to being given amphetamine and a reduction in performance was observed in those who had performed well prior to drug administration (247). Therefore, the difference that we see between BZP and DEX could be due to the differing amounts of extracellular DA release in prefrontal circuits which might lead to impairments in cognitive control.

Amphetamine administration in response to reward evoked a decrease in the amplitude, but an increased duration in the ventral striatum (142). It was proposed that this response was reflective of studies which have found that amphetamine reduces the phasic firing of DA, while increasing tonic levels (343). As discussed in the previous section discussing reward, the differences between BZP and DEX could be reflect differences in how each might affect dopaminergic transmission. Conversely, the activation evoked by these comparisons may be due to other influencing neurotransmitter circuitry including the effects that BZP has on 5-HT and NA.

Modulating serotonergic and noradrenergic circuitry has been found to affect different aspects of executive function. Both amphetamine and BZP also affect these neurotransmitters, so it could be that the differences in regional activation are partly due to these effects.

5-HT release inversely correlates with performance on tasks involving inhibition. Research has found that citalopram evoked activation in the lateral OFC; however decreased activation was found in the medial OFC during a Go/No-go task. In a subsequent task presenting aversive faces, citalopram reduced activation of the lateral OFC (93). This suggests that the activation was task specific, and that caudate activation, may occurred to maintain task performance. Therefore, we cannot rule out the possibility that activation of the caudate was caused by altered 5-HT transmission.

The selective noradrenaline reuptake inhibitor atomoxetine also has significant effects on inhibitory control in healthy subjects and patients with ADHD. Chamberlain and colleagues (477) reported that an acute dose of atomoxetine led to an improved behavioural response

during a stop-signal task compared to placebo in healthy participants. Furthermore, both acute administration (478) and continued therapy (479, 480) with atomoxetine has shown improved inhibitory control in patient populations with ADHD (478).

The pharmacological effects of BZP and DEX are the likely cause of the regional differences induced by each drug. DEX appears to cause more efficient processing of inhibitory stimuli, leading to a reduction in regions associated with inhibition, whereas BZP induces activation, suggestive of compensatory recruitment. Conducting further imaging studies that compare the effects of selective noradrenergic or serotonergic agonists could allow further insight into which mechanism is leading to the changes that we observe in this research. However, we can conclude that BZP has distinct differences to DEX which led to recruitment of additional regions which allowed our participants to perform to level during the Stroop task.

5.4.1.2. TFMPP

The effects of TFMPP were also compared with placebo to during the Stroop task, which resulted in four clusters of activation—three in the thalamus and one in the lingual gyrus. The lingual gyrus has been shown to be activated following the presentation of visual stimuli; however the results of this research show differences between placebo and TFMPP. Del-Ben et al. (201) reported enhanced occipital activation after giving citalopram to healthy males, and in a separate study fenfluramine increased the critical flicker fusion threshold (CFFT), whilst methysergide, a 5-HT antagonist showed the opposite effect (383). The CFFT is a test to see at what frequency an observer views the intermittent light stimulus to be completely steady in presentation. Previous studies have found similar findings with CFFT using SSRIs (481, 482) -it has been proposed that the reason for this increase is mediated via 5-HT_2 receptors. These findings suggest a role for 5-HT in the enhancement of early stage visual information processing (383), and thus may account for the change in activation following TFMPP administration.

As discussed in the discussion of BZP's effects on the Stroop task, 5-HT effects inhibition, and an acute dose of mCPP enhanced responses within the lateral OFC (93). mCPP, like TFMPP is a 5-HT_{2C} agonist and also found in some party pill preparations. The areas of activation observed following the administration of mCPP were consistent with the hypothesis that 5-HT affects inhibitory responses. Therefore, it was hypothesised that due to TFMPP's similar effects on 5-HT_{2C} receptors similar results may be found. However, the comparison between TFMPP and placebo showed increased activation in the thalamus.

Recruitment of the thalamus by TFMPP may be indicative of a recruitment of additional regions to allow the maintenance of attention. The thalamus is proposed to have a key role

in the attention given to respective stimuli, with Crick (436, 437) suggesting that the thalamus acts as a “searchlight”. In addition, studies investigating the sub thalamic nuclei have shown that it plays a role in inhibiting responses and subsequent motor responses. Aron and Poldrack (464) used the stop-signal task to further study the effects of cortical and subcortical involvement in response inhibition. The authors reported greater activation in the thalamic nuclei during stop trials compared to go trials, which was associated with short versus long trials. This suggests thalamic involvement in stop-signal performance. Furthermore, the involvement of the thalamus with short trials indicates motor response inhibition. This could be a reason for thalamic involvement in the Stroop task in our research, after the administration of TFMPP, that is, there is an increase in thalamic activation as additional recruitment of neural regions are required to ensure task performance.

Alternatively, serotonergic pathways play an important role in modulating behavioural arousal (428, 429). Attention and arousal are two domains that are linked. Whilst attention is governed mainly by cortical systems, arousal is governed mainly by subcortical structures; however, both attention and arousal share an important anatomical structure—the thalamus (431). Therefore the thalamus may have been recruited to allow participants to perform to the same standard by aiding the maintenance of attention during the task in the presence of altered arousal due to TFMPP. A similar report of compensatory thalamic recruitment was made after the administration of the α1/α2 agonist clonidine, which reduced sustained attention.

The thalamus forms part of the CSTC and has been described as a gateway for cortical signalling (433, 434). The increase in thalamic activation could result from this gating capacity. Vollenweider (433) suggested disruptions beyond the point of normal range of thalamic gating of sensory and cognitive information, for example, hallucinogens can lead to an overloading of sensory and cognitive information. The inhibitory and excitatory influences on the striatum are mediated by 5-HT and DA and glutamatergic inputs, respectively (439). GABAergic input from the striatum and the pallidum is thought to inhibit neurons in the thalamus and result in a reduction of sensory input into the cortex from the thalamus.

Modulations of DA or 5-HT levels cause a disruption in this balance, that is, the change in levels of DA or 5-HT reduces the inhibitory influence on the striatum and therefore opens the thalamic filter, subsequently leading to an overload of sensory information being passed to the cortex and potentially psychosis (433). As stated previously TFMPP predominantly induces serotonergic activity, the increased activation observed in the

thalamus could be a result of increased 5-HT levels, and subsequently reduced inhibition of the thalamus.

In summary, TFMPP induced regional activation of the thalamus during the Stroop task. This recruitment is reflective of compensatory recruitment of regional substrates allowing the maintenance of task performance however, whether this is due to effects on attention or inhibition is unclear.

5.4.1.3. BZP and TFMPP combined

The combined administration of BZP and TFMPP evoked activation in the thalamus and the dorsal striatum. Whilst the increase in thalamic activation reflected the response induced when TFMPP was given alone, the activation of the caudate did not reflect the effects of BZP alone, in fact the opposite occurred -there was attenuation of a response in the congruent condition compared to that of placebo.

These results are indicative of a difference in neurotransmitter levels when the two are administered together. The caudate is a region which has been shown to be innervated by DA neurons (483). Although BZP has been found to be mainly dopaminergic in activity, TFMPP affects DA via 5-HT_{2C} receptors which as previously discussed has an opposing effect on dopaminergic transmission. (41, 42). Consequently the reduced dopaminergic activity may be attenuating the activation seen in these data. Furthermore, this reflects animal studies that found BZP+TFMPP was a less effective reinforcer in rhesus monkeys (37).

Subjectively, the combination of BZP and TFMPP has been compared to MDMA and similar effects on DA and 5-HT release have been found in preclinical studies; however the results presented in this study show that there are distinct differences in their effects. Specifically, MDMA has been reported to have psychostimulant properties that may result in *increased* arousal, however the results from the Stroop task indicate the thalamic activation observed after giving BZP+TFMPP is a reflection of a *reduction* in arousal. It is possible that the effects in humans may not be translated from animal models and that different doses of BZP+TFMPP, may also produce different effects.

5.5. Comments on Toxicity of BZP, TFMPP and Combination

The research undertaken in this thesis, specifically aimed to determine the acute effects of BZP and TFMPP, alone and in combination on reward and executive function. Due to the lack of research about these drugs prior to this investigation, our results markedly increase the knowledge about their effects in comparison to placebo and to DEX. By comparing

what has been found in this research with similar studies using other recreational drugs, such as, cocaine and MA, we can indirectly compare their effects. Furthermore, it allows the evaluation of similarities with other stimulants and the potential for neurotoxicity to occur.

Negus and colleagues (484) studied a number of monoamine releasers as potential agonist treatments in cocaine dependence. The authors reported that BZP produced dose-dependent decreases in cocaine administration, suggested to be due to the relative selectivity of BZP on dopaminergic release. The reduction in cocaine administration, following BZP, highlights BZP's stimulant-like properties. Similarities to other stimulants have been reported in other animal and human studies. For example, in rodents, BZP and cocaine both substitute for bupropion, a NA and DA reuptake inhibitor (15), and BZP has shown amphetamine-like stimulus effects while maintaining drug self-administration in monkeys (37). When BZP and MA were compared in rodents, Herbert and colleagues (485) reported more similarities than differences -thought to be due to their similar neurochemical profiles. Combined, these studies indicate the potential for BZP to have similar abuse and dependence risks to MA (485, 486). In addition, there have been reports of behavioural similarities between BZP and stimulants by inducing hyperactivity and stereotypy (486). However, in the latter study, there were differences between MA and BZP; the potency of MA producing stereotypy was greater than its effects on hyperactivity, but BZP showed the same results on both stereotypy and hyperactivity. Stereotypy is associated with increased nigrostriatal dopaminergic activity, whereas hyperactivity is linked to the mesolimbic pathway. This highlights neurochemical differences between the two drugs.

Reports of long term deficits are even scarcer; to our knowledge only two studies have been conducted to determine effects of BZP after long-term use in animal models. Rats were administered BZP daily for 10 days during their equivalent developmental period to human adolescence. The repeated administration led to increased anxiety and in the female rats increased aggression as adults. The authors suggest these results are due to interference with maturation in regions of the brain controlling anxiety associated mechanisms modulated by 5-HT (487).

After administering psychostimulant drugs there is an increase in behavioural sensitisation, an indicator of increased motivation for seeking addictive drugs and associated with potentiation of conditioned stimulus rewards. Behavioural sensitisation is an increase in motor stimulant responses following additional doses in repeated administration studies, and has been shown to last for weeks after withdrawal. A second study compared the

effects of repeated doses of BZP with MA. Results showed potentiated locomotor response, and following withdrawal the MA pre-treated rats exhibited a sensitised locomotor and stereotypy in response to low dose MA. The BZP pre-treated rats also showed a sensitised locomotor response to low dose MA and BZP (486). Sensitisation has been considered an initial stage of drug addiction (488). These two preclinical reports combined, suggest that BZP has the potential to cause dependence. However, it must be emphasised that there have only been two reported long term studies, and to date, replication of these results has not occurred. Further studies should be conducted using different doses to examine the validity of these findings.

Changes in dopaminergic transmission lead to potentiated psychostimulant produced behaviour. Giorgi and colleagues (489) administered amphetamine to drug naïve rodents and reported results from acute and repeated doses. After acute administration, an increase in extracellular DA was found in the NAcc shell. The NAcc is involved in locomotor activity following the administration of psychostimulants, and is involved in behavioural sensitisation after repeated drug administration. The study further indicated that amphetamine induced a greater increase in extracellular DA content in the NAcc core, with a reduced content in the NAcc shell after sensitisation, suggested to be due to structural modifications of the DA neurons (489). It appears that D_1 and D_2 receptors play a role in both the acute behavioural effects of amphetamine and the behavioural sensitisation following repeated exposure. Two studies have given D_1 and D_2 antagonists prior to MA, which reduced subjective effects and behavioural sensitisation (490, 491).

D_2 receptors have been associated with dependence in humans, although it is unknown whether these changes are a cause or consequence, that is, whether drug abuse led to the alterations in D_2 levels, or whether a genetic predisposition made the person more vulnerable to drug abuse and dependence. The latter argument of vulnerability due to genetic predisposition has been shown in preclinical research. Sensitisation was examined in two groups of rats bred to be either high or low avoidance. The low avoidance group showed no behavioural sensitisation to MA, whereas the high avoidance rats did. As D_2 levels cannot be studied in humans prior to the commencement of drug use in drug users, this question remains unanswered. However, a study by Volkow et al. (174) aimed to shed light on this question. A group of non-drug taking healthy volunteers were given MPH and the subjective effects were recorded. Half of the participants reported a pleasurable experience and the remaining subjects did not. In subsequent investigations, the subjects who found the drug to be pleasant had significantly lower levels of D_2 receptors. It is thought that this difference in baseline D_2 receptor levels reflects an inverted U-shaped dose-response curve; when receptor occupancy is too low, this leads to no effect, and too

high to aversive effects. This study indirectly indicates potentially lower D_2 receptor levels could predispose people to drug abuse due to the pleasurable effects.

Based on the preclinical work, suggesting stimulant-like properties and abuse potential, it is surprising that despite the extensive use of BZP and/or TFMPP for the past decade there have been no clinical reports of dependence. There have been reports of adverse reactions; however, in many of these cases there was a lack of toxicology reports conducted to confirm BZP's involvement or the presence of other drugs. These reports include toxic reactions (7, 492, 493) , for example, renal failure after the first dose of BZP requiring medical treatment (492), and a number of often self-reported reactions including insomnia, mood swings, headaches and anxiety (494).

The lack of reports associating BZP and TFMPP in humans with dependence may be due to many reasons, which this next section will discuss.

First, Brennan and colleagues (486) reported differences between MA and BZP, evident from the degree of induced stereotypy versus hyperactivity induced. This depicts differences between the neurochemical effects of MA and BZP on nigrostriatal versus the mesolimbic pathways. Possibly, this difference negates the long term consequences of these drugs on dependence; however, this does not appear to be the case, as repeated doses of BZP actually showed similarities in cross sensitisation and the behavioural characteristics of anxiety and aggression.

Behavioural sensitisation is thought to be a key component in the addiction process, reflecting changes in the underlying dopaminergic circuitry. The role of sensitisation is postulated to be where drug taking shifts "wanting" (179). In humans, this change is thought to be reflected in craving and relapse into drug dependence (495). Moreover, the behavioural effects of increased anxiety observed in adolescent rats are mediated by the serotonergic effects of BZP in the forebrain, comparable to the long term effects of MDMA and MA on anxiety. Alternatively, BZP's effects on motor activity via modulation of the dopaminergic system may have contributed to some of the reported behaviour. While both studies illustrate the long term effects of BZP on rodents; however, whether these findings translated to humans is unknown.

The common route of administration could also be an important factor which might explain why BZP has not been linked to reports of dependence. The reinforcing effects of drugs are strongly influenced by their pharmacokinetic profiles. Preclinical studies report that the faster a drug reaches the brain, the more rewarding it's effects are (496). This has also been reported using PET imaging in humans, whereby the speed of drug entering and

leaving the brain is associated with its rewarding effects. For example, when cocaine is smoked or administrated intravenously there is a more intense high than when it is snorted intranasally. In addition, Volkow and colleagues studied the effects of cocaine (497) and MPH (498), and found that the quicker the drug reached the brain was correlated with a greater degree of “high”. The onset of action was suggested by Gorelick et al. (499) to be associated with the potential for abuse, whereby oral administration may reduce the liability (499). Therefore, since the predominant route of administration of BZP is orally via tablets and capsules, this might be the biggest contributing factor to the lack of reports of dependence.

Alternatively, it could be that regular users of party pills are also regular users of other drugs, that is, that they are poly-drug users. Consequently, the long term effects of these party pill drugs could be masked by the cognitive deficits an addiction induced by other recreational drugs used over their lifetime.

Decision making choices that involve uncertainty and reward are frequent occurrences during everyday life, and people who are under the influence of recreational drugs do not always make the right decisions (461, 500). This research aimed to investigate the acute effects of these drugs the reward process, including anticipation of an uncertain event and the outcome of reward. Moreover, we aimed to investigate the effects of these drugs on cognitive function including selective attention and inhibition, as they can also affect decision making and choices that are made. Other studies researching the effects of other recreational drugs have also found modulation of response inhibition.

Despite the unknown long term effects of BZP and TFMPP, it is clear from our results that the use of these drugs is affecting regions associated with reward and executive function relative to placebo. The increase in activation, for example, in the IFG when BZP was compared with DEX in the Stroop task, could be reflective increases seen when other drugs are given acutely, such as cocaine which increases activity in the PFC. Functional imaging studies have reported a disruption of attention and inhibition in rodents associated with the PFC. Furthermore, an acute dose of cocaine was associated with an increase in DLPFC metabolic activity, a change in task-related firing and a reduction in performance (56). This suggests that cocaine alters performance by altering PFC processing when cognitive demand is high. The chronic use of cocaine has also been shown to disrupt this region in functional imaging studies. For example, Hester and Garavan (306) found that during a Go/No-go task a reduction in recruitment of the PFC and ACC, and a shift to cerebellar activation. PET studies have also shown disruptions in this circuitry with a decrease in PFC function and brain glucose metabolism reported in cocaine dependent

subjects versus controls (188). Similarly, imaging whilst completing the Iowa gambling task revealed cocaine dependent subjects had greater activation in the OFC and less in the DLPFC and mPFC (501).

It has been proposed that the initial increase in activation seen after acute administration of a drug may occur prior to decreases observed after chronic use (56). In the case of BZP and/ or TFMPP we see increases in some of these regions. To further characterise whether this increase in activation is accompanied by an increase in glucose metabolism and not a result of task difficulty after drug administration, for example, from indirect effects, PET imaging studies should be carried out. If an increase in metabolism within the PFC was found, combined with the data presented from this research, this might imply that BZP and/ or TFMPP have the potential to cause the long term deficits induced by other psychostimulants.

To provide an informed decision about whether the change in legislation surrounding BZP and TFMPP was warranted, the data presented in this thesis suggests that both they and related piperazines should remain illegal until sufficient data are obtained to demonstrate safety. The recent debate about whether these drugs should be legal or not is a current example of the legality surrounding the use of cocaine in the 1980s where preclinical studies suggest it had similarities to other psychostimulants, and few long term studies showed an increase in anxiety and behavioural sensitisation. Human studies are scarce, and it is unsatisfactory to rely on a lack of anecdotal reports about long term effects and dependence to support continued use of these compounds.

5.6. Limitations and Future Directions for Research

This penultimate section will address the limitations of this study, and what if any measures were taken to overcome these limitations during data collection or analysis. It will also discuss potential research avenues to be pursued in future investigation of the party pill constituents BZP and TFMPP.

5.6.1. Limitations

The results that presented in this thesis did not survive the family wise corrected (FWE) significance threshold. The FWE correction is a Bonferroni correction and involves dividing the *p*-threshold by the number of tests. FWE correction is noted to be conservative, and a number of studies have published results of fMRI investigations that are uncorrected. The lack of FWE corrected results could be reflective of a limited sample size. Event-related fMRI designs are advantageous in many ways, for example, they have estimation efficiency, that is they enable researchers to estimate the HRF to a stimulus of short

duration, but they do reduce the detection power of statistical significance of the data (290). The group size was reduced in some comparisons due to errors in data collection and subsequent E-prime data files being unusable. Despite this, the results found were in regions associated with the cognitive domains we were investigating and the analysis has subsequently produced interesting results.

The OFC, as discussed earlier, is involved in reward and executive function; however, although it was hypothesised that it may be activated after the administration of BZP and/or TFMPP this was not determined in our results. The OFC has been shown in other studies to be activated during the Stroop paradigm, for example, following the administration of mCPP. As TFMPP, like mCPP, has mainly serotonergic effects it was predicted that we would see a similar effect, but this did not occur. MRI artefacts in this region have been reported in previous literature. This could be due to several factors, including close proximity to the air-filled sinuses and small movements of the head during scanning. Recently specific fMRI protocols have been designed to image this region so in time it will become easier to image. However, in this study, field maps were not collected and the protocol was not designed to specifically target this region. Future studies could adapt a data acquisition sequence specifically designed to improve imaging of the OFC (115, 294).

Comparisons of winning and losing monetary amounts were used in the gambling (guessing) task i.e. $0, 50c or $4. There is an increased neural response to the monetary losses versus monetary gains, and some gambling tasks have applied a ratio of 2:1 of wins to losses. This could be an influencing factor in our results as the losses were far greater and more widespread than wins in some cases. However, winning and losing were not directly compared. Instead a neutral no-change reward or no-change anticipation condition was used. This should ensure the validity of the results. Despite the results observed during the anticipation and aversive outcome contrasts, the outcome of winning $4 did not show any difference in relation to placebo in any of the drug conditions. Future studies could employ a Win $8 condition to ensure the intensity of the effect was a factor.

To study selective attention and inhibition we used the Stroop task, but the results only yielded significant differences in the imaging but not the behavioural data. As the study was a cross-over design, the subjects had to complete this task six times (one practice session and 5 trial days). In addition, the task was of moderate length—these factors combined could have reduced the interference effect through practice, that is, a learned response to the Stroop effect. This was known prior to the start of data collection, and so the drugs were given in a randomised schedule and there were five separate versions of the Stroop task to minimise any group effects that may occur. It would be of interest though, if this

study was repeated, to conduct a trial that was not a cross-over study to see whether a significant behavioural difference would be reported.

As indicated in other studies, dopaminergic effects on pathways are based on an inverted U-shaped response curve. Baseline dopaminergic function is one aspect of the research that was not taken into account. Measurements of specific genetic polymorphisms may be one way of attaining this information in the future.

Finally, during our laboratory's previous research, we chose doses of BZP and/or TFMPP that are known to evoke behavioural responses, while avoiding drug-induced adverse effects. Based on an inverted U-shaped dose-response curve known to represent dopaminergic modulation of circuitry (248), and has also been suggested to occur with 5-HT and NA, it is quite likely that higher or lower doses than those used in this trial would result in differential effects. This avenue warrants further investigation.

5.6.2. Future Directions for Research

This section will describe potential future research that may be conducted. It will discuss the research that could be undertaken within the data sets that have been collected that unfortunately due to time constraints were not be addressed in the current thesis, and in new experiments.

5.6.2.1. Current data set

Gambling task

Studies have reported winning or losing $0 elicits activation in regions of the opposing valence. That is, after winning $0 activations has been found in locations associated with loss of monetary incentives. Mellers (502) suggested that the way in which the participant responded emotionally to the outcome was dependent on their perceived expectation and the likelihood of the outcome and its alternative. In a subsequent study this was shown in a task involving two different spinning wheels (one good and one bad spinner) and three possible outcomes on each. When an outcome of $0 was achieved on the good spinner, a reduction in activation was seen, compared to when a $0 was achieved on the bad spinner and an increase in activation was seen (214). Using the data collected in this research, it would be interesting to determine if this also happened after giving BZP and/or TFMPP, or whether the drug states altered the effects of relief and/or disappointment.

Stroop task

Whilst the typical Stroop interference effect is the difference between incongruent and congruent conditions, some have reported differences in regional activation between the

incongruent and control conditions and the congruent and control conditions. Again using the same data set, contrasting the effects of the incongruent with the control words would provide insight into the Stroop task, and may show different results to that of contrasting with the congruent state.

5.6.2.2. Future research directions

Research about the effects of BZP and/or TFMPP is currently limited, which allows a great opportunity for continued research. Based on known research about similar psychostimulants, such as MA and MDMA, future avenues could be tailored to make further comparisons with these drugs which have an associated greater depth of research about their effects, to make more learned conclusions about the safety of BZP and TFMPP.

Acute effects of BZP, TFMPP and combination

As mentioned in the previous section, there have been reports of increased glucose utilisation after an acute dose of cocaine in non-human primates. PET studies to evaluate the effect of BZP, TFMPP and the combination have on these regions and glucose metabolism could be undertaken in the future to further address these questions.

Further studies could also use different tasks during fMRI. They could include the Iowa Gambling Task, to elucidate whether the effects observed in anticipation of uncertain outcomes are translated into risky decisions. Furthermore, tasks known to test memory could be carried out. In addition, it would be interesting to investigate whether there were any differences in prediction error. Studies have shown that prediction error has a direct influence on neuronal firing in the NAcc. That is, when there is a difference between what is expected and what is given, there is a change in DA firing.

Long term consequences of BZP, TFMPP and combination

To date there has only been two studies that have investigated the effects of BZP, and neither studied the long term effects of TFMPP or the combination of BZP+TFMPP. The studies that conducted in rodents could be replicated using different doses to evaluate the role of these compounds. In addition, in humans, comparing long term users of BZP, TFMPP and combination BZP+TFMPP with controls who were matched for age, gender, education and other drug use, could be one way of testing possible long term effects. Although the difficulty is that drug use is reported by the poly-drug users based on their memory and reliant on self-report. In addition, when comparing drug use via this method it does not take into account the effects of drug combinations.

5.6.3. Overall Conclusion

Party pills containing BZP and/or TFMPP have been used worldwide for more than a decade, despite limited knowledge about their effects in humans. This thesis has investigated the effects of BZP, TFMPP and the combination BZP+TFMPP relative to placebo, and BZP in comparison to DEX, on aspects of reward processing and executive function, using fMRI. The results have shown that these drugs induce marked differences when compared with placebo and DEX.

The gambling (guessing) task allowed insight into distinct aspects of reward processing to evaluate anticipation of an uncertain reward and reward outcome. Regional differences were found in each of the drug conditions, in relation to anticipation, and in response to reward outcome or loss. In addition, there appears to be differences between BZP and DEX in all aspects of the task. The Stroop task allowed the investigation of selective attention and inhibition, where we found activation of specific neural regions, which we proposed were recruited to ensure adequate performance during the task. It also exemplified distinct differences between BZP and DEX, as even though BZP has been suggested to have similar characteristics to DEX, it showed compensatory recruitment in regions of response inhibition. These changes reflect the direct effects that these drugs have on dopaminergic and serotonergic pathways.

Despite the number of people reported to have taken combinations of these drugs there have been no reports of long term consequences, for example dependence. Based on the findings from this research and preclinical work, there are similarities seen to other stimulants. These similarities show that there is still a distinct possibility that there may be long term consequences in regular users, such as dependence. In addition, this study shows the possibility of using fMRI as an exploratory tool after the administration of drugs, providing a unique insight into the regional effects of drugs.

Appendices

1. **Participant information form**

2. **Participant consent form**

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PARTICIPANT INFORMATION SHEET
fMRI Study

Title of Project: Determination of the effects of Party Pills on regional brain activation in humans using fMRI.

Researchers: Bruce Russell, Michelle Gordon, Louise Curley, Rob Kydd and Ian Kirk
Contact Details

Dr Bruce Russell, School of Pharmacy Ph 09 3737599 Ext 86429
Michelle Gordon, School of Pharmacy Ph 09 3737599 Ext 82329
Louise Curley, School of Pharmacy Ph 09 3737599 Ext 82329
Associate Professor Ian Kirk, Dept. of Psychology, 09 373 7599 Ext 88524
Professor Robert Kydd, Dept. of Psychological Medicine 09 373 7599 Ext 83774 at the University of Auckland

You are invited to participate in this research. This will involve six sessions. The first of which will take approximately one hour and is to determine your suitability to participate in the following five sessions, it will not involve taking a drug. The following sessions will involve you taking an oral dose of placebo, BZP, TFMPP, BZP+TFMPP combination and dexamphetamine, in a random order, each on a different study session with at least five days between sessions. Approximately one hour after taking the dose, an fMRI scan will be performed while you carry out simple tests of your memory.

During the experiments you will be placed in the MRI scanner, up to your shoulders, in the scan room, and the investigator will continuously monitor you through the window from the control room next door. It takes about ½ hour to set you up in the MRI scanner; the total time involved per testing session is approximately two and a half hours.

This experiment is testing for changes in brain function that are thought to take place after a single dose of BZP and/or TFMPP, the active constituents of the so called “Party Pills” or “Legal Herbal Highs”. The experiment uses a method of measuring human brain function called functional magnetic resonance imaging. This involves placing you in an MRI scanner. The scanner is able to detect the changes in blood oxygen level in specific regions of the human brain indicating activation of that region. We hope to measure these changes, while carrying out a test of memory, one hour after taking a single dose of placebo, BZP (250mg), TFMPP (60-80mg), BZP combined with TFMPP (100mg BZP, 30mg TFMPP) or dexamphetamine (25mg).

Professor Rob Kydd will prescribe BZP, TFMPP and dexamphetamine for participants when required.

Dexamphetamine has some well known side effects which you might experience e.g. sleeplessness, restlessness, decreased appetite, euphoria, dizziness, headache, dry mouth, sweating, palpitations and very rarely convulsions. BZP and/or TFMPP may also produce these side-effects. You may also experience a so-called “come down” (irritability, lethargy, depressed mood) after the effects of the drugs wear off. If any of these side effects occur after testing you are expected to report these to Dr Russell.

Dr Russell will make follow-up contact you by either phone or email within 24 hours of completing each session.

It is not expected that you will obtain any personal benefit from taking part in this study.

In April 2008 BZP and TFMPP were classified as C1 controlled drugs. This makes them illegal and puts them into the same class as cannabis. There is a small chance that prolonged and frequent use of these substances may lead to dependence. We recommend that you do not continue to use any of these substances after completion of this study.

The effects of combining BZP, TFMPP or dexamphetamine with other recreational substances, such as alcohol and marijuana, or other prescribed and non-prescribed medicines are unknown. You should not use any other recreational substances for the duration of this study. **You should not take part in this study if you take any prescribed or non-prescribed medicines.**

You must only participate in this study if you are willing to return home in a taxi provided by the researchers following completion of each session and then remain in the company of another responsible adult for the remainder of the day/evening.

If you have had any history of epilepsy or seizures (fits), mental illness or significant head trauma you must not participate in this study.

There is a remote possibility that the BZP and/or TFMPP will result in a seizure if you have undiagnosed epilepsy.

We realize that pregnancy will not occur for all women for a variety of reasons however, because of safety issues, one of the requirements for taking part in the study is that you are not pregnant. Therefore we are offering you a pregnancy test which is not compulsory but you can only take part in the study if you are certain you are not pregnant or the test returns a negative result.

Before participation you will be asked to estimate your prior use of drugs (legal and illegal). All personal information is strictly confidential and no material that could personally identify you will be used in any reports on this study. Your name will only appear on the Participation Consent Form. These forms will be coded with a unique number that will be used to identify individual subjects’ performances in all other data records. The Participation Consent Forms will only be seen by you and the investigator and will be kept in a secure filing cabinet. After completion of the study all data will be kept for the required period of ten years and will then be destroyed.

On the day of each session you will be asked to give a urine sample to ensure you have not been using any other drugs recently. **This information will not be made available to anyone outside the research group.**

In the unlikely event that a condition which is assessed to be a clinical abnormality is detected through performing a scan on you, you will be informed of this and referred to an appropriate medical specialist.

Because the images are not routinely reviewed by a radiologist we are unable to perform diagnostic scans for medical purposes of areas where you have known abnormalities.

Your participation is entirely voluntary (your choice). You do not have to take part in this study. If you do agree to take part, you are free to withdraw at any stage of the testing (between now and the end of the study (1/12/10)). You do not have to give any reason for your decision and there will be no penalty of any sort for withdrawing. There are no repercussions (academic or otherwise) to students who do withdraw or do not wish to participate. If you choose to withdraw from the study your data will be destroyed.

Any participants in this research may have access to the information collected about them including the results of the testing and the final published report of the study. You may contact the researchers if you wish to receive a summary of the findings at the end of the study period.

In the unlikely event of a physical injury as a result of your participation in this study, you may be covered by ACC under the Injury Prevention, Rehabilitation and Compensation Act. ACC cover is not automatic and your case will need to be assessed by ACC according to the provisions of the 2002 Injury Prevention Rehabilitation and Compensation Act. If your claim is accepted by ACC, you still might not get any compensation. This depends on a number of factors such as whether you are an earner or non-earner. ACC usually provides only partial reimbursement of costs and expenses and there may be no lump sum compensation payable. There is no cover for mental injury unless it is a result of physical injury. If you have ACC cover, generally this will affect your right to sue the investigators.

If you have any questions about ACC, contact your nearest ACC office or the investigator.

The experiment will be carried out in Room 312, Human Sciences Building, University of Auckland, Symonds Street.

Please feel free to contact the researchers if you have any questions about this study. Further information can be obtained from Associate Professor Ian Kirk at the Dept. of Psychology, (Ph 373-7599 ext 88524) or Dr Bruce Russell, School of Pharmacy, or Professor Rob Kydd (Ph 373-7599 ext 83774) Department of Psychological Medicine, University of Auckland.

The Head of Department is Prof. John Shaw ext 83778

If you have any queries or concerns regarding your rights as a participant in this study, you can contact an independent Health and Disability Advocate. This is a free service provided under the Health and Disability Commissioner Act:
Telephone: 0800 555 050
Free Fax: 800 2787 7678 (0800 2 SUPPORT)
Email: advocacy@hdc.org.nz

This study has received ethical approval from the Northern X Regional Ethics Committee.
Reference NTX/07/08/078

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Consent Form
fMRI Study

Title of Project: Determination of the effects of Party Pills on regional brain activation in humans using fMRI.

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Professor Robert Kydd, Dept. of Psychological Medicine 09 373 7599 Ext 83774 at the University of Auckland

Name of Subject: _____ **Age**: _____ years

Subject Number: _____

I have read and I understand the information sheet dated April 2008 for volunteers taking part in the study designed to determine the effects of BZP and/or TFMPP on regional brain activity and compare them with dexamphetamine. I have had the opportunity to discuss this study, and I am satisfied with the answers I have been given. I have also had time to consider whether to take part.

I understand my right to receive a copy of the results of this study and that the results may be used for future research related to the effects of BZP and TFMPP for which further consent will be obtained by an accredited New Zealand ethics committee.

I understand that taking part in this study is voluntary (my choice) and that I may withdraw from the study at any time up until the end of the study (1/12/10).

I have agreed to return home and remain in the company of a responsible adult for the remainder of the day/evening following the completion of each study session.

I understand that if I am a student and wish to withdraw from, or not participate in the study this will have no consequences (academic or otherwise).

I understand that my participation in this study is confidential and that no material which could identify me will be used in any reports on this study.

I understand that the testing procedure will be stopped if I am in any discomfort.

I understand that the effects of BZP and/or TFMPP on an unborn child are unknown and I have been offered a pregnancy test.

I understand that if there is any chance that I might be pregnant, I must not take part.

I understand that I am not eligible to participate if I have any history of epileptic seizures or fits.

I understand that there is a remote possibility of BZP or TFMPP eliciting a seizure (fit) if I have undiagnosed epilepsy.

I understand that in the unlikely event that a potential abnormality is detected in my fMRI scan, I will be advised of this and referred to an appropriate medical specialist.

I have agreed to report any drug-induced side effects to the Principal Investigator by either text, phone or email within 24 hours of my participation in each session.

I _____ (full name) hereby consent to take part in this study.

Signature: _____

Date: _____

Project Explained By: Bruce Russell/Michelle Gordon

Project Role: Principal Investigator/Co-investigator

Signature: _____

Date: _____

This study has received ethical approval from the Northern X Regional Ethics Committee.
Reference NTX/07/08/078

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